BIOATLANTIS OMRI PETITION – SULFURIC ACID UTILIZATION IN THE PREPARATION OF MICRONUTRIENT CHELATES

Item A

Synthetic substances allowed for use in organic crop production, § 205.601.

Item B

1. **Substance's Chemical Name** - Sulfuric Acid (already considered in "(j) As plant or soil amendments" in subcategory "(7) Liquid fish products" of the § 205.601 of NOP).
2. **Manufacturer's Data** –
   Company Name: BioAtlantis, Ltd.
   Contact Person 1: John T. O'Sullivan
   Contact Person 2: Carlos Cardoso
   Address: Kerry Technology Park, Tralee, Kerry County, Ireland.
   Phone number: 00353 667 11 84 77
   E-mail address: jtos@bioatlantis.com; chemistry@bioatlantis.com.
3. **Intended Use** - Sulfuric acid as a solubilizing agent.
4. **Handling/Processing Activities** – Sulfuric acid will be used as an efficient pH adjusting substance, since it has a low impact on the final product's solids content due to its low pKa and does not participate or promote complexation and other reactions that may alter the optimal combination of non-synthetic bioactive substances or the intended amino acid chelation of micronutrients (Fe, Cu, Zn, etc.), as is otherwise the case for several organic acids such as citric acid, etc. The produced chelates will be applied according to the method and dose rate described below and to the indicated crops:
   - Mannitol chelated boron for application in fruit crops, tree nuts, field crops, cole crops, cucurbits, legumes, pulses, leafy vegetable, fruiting vegetables, tuber, root & corm vegetable, green house and shade house crops and grasses. Chelated boron application can prevent or correct iron deficiencies that may reduce crop growth, quality or yield (Will *et al.*, 2011). Apply 0.5 L to 1 L per hectare as foliar spray during vegetative growth period for nutrient maintenance in crops. During deficiency, application dosage should be increased based on the tissue analysis.
   - Amino acid complexed magnesium for application in fruit crops, tree nuts, field crops, cole crops, cucurbits, legumes, pulses, leafy vegetable, fruiting vegetables, tuber, root & corm vegetable, green house and shade house crops and grasses. Chelated magnesium application can prevent or correct iron deficiencies that may reduce crop growth, quality or yield (Salama A.S.M. *et al.*, 2014). Apply 0.5 L to 1 L per hectare as foliar spray during vegetative growth period for nutrient maintenance in crops. During deficiency, application dosage should be increased based on the tissue analysis.
   - Amino acid complexed calcium for application in fruit crops, tree nuts, field crops, cole crops, cucurbits, legumes, pulses, leafy vegetable, fruiting vegetables, tuber, root & corm vegetable, green house and shade house crops and grasses. Foliar calcium application can prevent or correct iron deficiencies that may reduce crop growth, quality or yield (Taylor & Brannen, 2008; Jeppsen, 1991). Apply 0.5 L to 1 L per hectare as foliar spray

during vegetative growth period for nutrient maintenance in crops. During deficiency, application dosage should be increased based on the tissue analysis.

- Amino acid chelated manganese for application in fruit crops, tree nuts, field crops, cole crops, cucurbits, legumes, pulses, leafy vegetable, fruiting vegetables, tuber, root & corm vegetable, green house and shade house crops and grasses. Chelated manganese application can prevent or correct iron deficiencies that may reduce crop growth, quality or yield (Datir *et al.*, 2012). Apply 0.5 L to 1 L per hectare as foliar spray during vegetative growth period for nutrient maintenance in crops. During deficiency, application dosage should be increased based on the tissue analysis.
- Amino acid chelated iron for application in fruit crops, tree nuts, field crops, cole crops, cucurbits, legumes, pulses, leafy vegetable, fruiting vegetables, tuber, root & corm vegetable, green house and shade house crops and grasses. Chelated iron application can prevent or correct iron deficiencies that may reduce crop growth, quality or yield (Ghasemi *et al.*, 2012; Yuan *et al.*, 2011; Datir *et al.*, 2010; Koksal *et al.*, 1999). Apply 0.5 L to 1 L per hectare as foliar spray during vegetative growth period for nutrient maintenance in crops. During deficiency, application dosage should be increased based on the tissue analysis.
- Amino acid chelated copper for application in fruit crops, tree nuts, field crops, cole crops, cucurbits, legumes, pulses, leafy vegetable, fruiting vegetables, tuber, root & corm vegetable, green house and shade house crops and grasses. Chelated copper application can prevent or correct iron deficiencies that may reduce crop growth, quality or yield (Datir *et al.*, 2010; Datir *et al.*, 2012). Apply 0.5 L to 1 L per hectare as foliar spray during vegetative growth period for nutrient maintenance in crops. During deficiency, application dosage should be increased based on the tissue analysis.
- Amino acid chelated zinc for application in fruit crops, tree nuts, field crops, cole crops, cucurbits, legumes, pulses, leafy vegetable, fruiting vegetables, tuber, root & corm vegetable, green house and shade house crops and grasses. Chelated zinc application can prevent or correct iron deficiencies that may reduce crop growth, quality or yield (Ghasemi *et al.*, 2013a; Ghasemi *et al.*, 2013b; Datir *et al.*, 2010; Koksal *et al.*, 1999). Apply 0.5 L to 1 L per hectare as foliar spray during vegetative growth period for nutrient maintenance in crops. During deficiency, application dosage should be increased based on the tissue analysis.

5. **Source of the Substance and its Processing** – Sulfuric acid is attained from mined elemental sulfur. This sulfur is oxidized to sulfur dioxide and, then, to sulfur trioxide, which, in turn, reacts with water to form the sulfuric acid (more details are provided in Appendix I).
6. **Substance Reviews by State/Private Certification Programs or other Organizations** – The GRAS Substances (SCOGS) Database of the Food and Drug Adminsitration (FDA) contains a scientific opinion on sulfuric acid (SCOGS report number: 33), where it can be read that "Sulfates are natural constituents of foods and normal products of sulfur metabolism in animals. In much of the published literature, toxicity evaluation of the sulfates has not been the primary objective of the work conducted, but it is evident that the toxic manifestations following oral administration of the sulfates considered in this report appear only at levels that are many times greater than those to which man is exposed in his daily diet. In light of the information contained in this report the Select Committee concludes that: There is no evidence in the available information on sulfuric acid, and on ammonium, calcium, potassium, and sodium sulfates that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might

reasonably be expected in future". Moreover, an Organization for Economic Cooperation and Development (OECD) report has concluded that "sulfuric acid is a candidate for further work".

There are also two previous technical reports asked by NOSB for the issue of sulfuric acid in organic crop production, the Technical Advisory Panel Report from 2006 (Appendix II) and the Technical Evaluation Report from 2012 (Appendix III).

Moreover, there are previous petitions to the National List for sulfuric acid, but concerning the preparation of different products, namely for the allowance of sulfuric acid as a pH adjuster for the stabilization of digested poultry manure (Appendix IV) and for its use as a processing aid in the production of organic seaweed extract (Appendix V).

7. **Regulatory Authority Registrations –** The substance (sulfuric acid) has already been registered with different state regulatory authorities:
    - *Environmental Protection Agency (EPA):* Internal tracking number 152405.
      FDA: Sulfuric acid is specifically addressed in section 184.1095 of Subpart B "Listing of Specific Substances Affirmed as Generally Regarded As Safe (GRAS)" of Part 184 "Direct Food Substances Affirmed as (GRAS)" of Subchapter B "Food for Human Consumption" of Chapter I "Food and Drug Administration" of the Title 21 "Food and Drugs" (volume 3) of the Code of Federal Regulations. Moreover, it is stated in this section that "(c) The ingredient is used as a pH control agent as defined in 170.3(o)(23) of this chapter and processing aid as defined in 170.3(o)(24) of this chapter."
8. **Chemical Abstract Service (CAS) Number and Product Labels**– CAS number is 7664-93-9 and the micronutrient chelate products' labels are attached as Appendix VI.
9. **Substance's Physical Properties and Chemical Mode of Action** – Sulfuric acid is a liquid (thick oily liquid, specific gravity: 1.84) substance at room temperature (b.p. ~340 °C) and it is considered a strong acid (pKa1: -3, pKa2: 2), which is totally miscible in water or with aqueous solutions.
    (a) Chemical Interactions with other Substances – Reacts strongly with water and alkaline substances. In this latter case, it leads to the formation of sulfate salts. Moreover, in a pH range of 3.5-5.5 (as intended for BioAtlantis Ltd. products) sulfuric acid is completely dissociated, thereby forming sulfate anions. Dilute sulfuric acid reacts with metals via a displacement reaction as with other typical acids, producing hydrogen gas and salts (the metal sulfate). It attacks reactive metals (metals at positions above copper in the reactivity series) such as iron, aluminium, zinc, manganese, magnesium and nickel. However, concentrated sulfuric acid is a strong oxidizing agent and does not react with metals in the same way as other typical acids. Sulfur dioxide, water and sulfate ions are formed instead of the hydrogen and salts. It can oxidize non-active metals such as tin and copper, depending upon the temperature. Lead and tungsten, however, are resistant to sulfuric acid. Hot concentrated sulfuric acid oxidizes non-metals such as carbon and sulfur (it should be remarked that sulfuric acid used by BioAtlantis Ltd. in the preparation of micronutrient chelates is diluted with water, so these oxidation reactions will not occur). It reacts with sodium chloride, and gives hydrogen chloride gas and sodium bisulfate.

    (b) Toxicity and Environmental Persistence – Sulfuric acid: Oral Exposure (LD50): Acute: 2140 mg/kg (in Rat). Vapour Exposure (LC50): Acute: 510 mg/m^3 2 hours (in Rat); 320 mg/m^3 2 hours (in Mouse). Products of Biodegradation in the Environment: Possibly hazardous short term degradation products are not likely. Toxicity of the Products of

Biodegradation: The products of degradation are less toxic than the product itself. More detailed information is given in Appendix VII.

(c) Environmental Impacts from its Use and Manufacture – Ecotoxicity: Ecotoxicity in water (LC50): 49 mg/l 48 hours (for bluegill/sunfish). BOD5 and COD: Not available. The environmental impacts can be minimized if best practices are used as described in Appendix I. More detailed information is given in Appendix VII.

(d) Effects on Human Health – Chronic Effects on Humans: May cause damage to the following organs: kidneys, lungs, heart, cardiovascular system, upper respiratory tract, eyes, and teeth. Other Toxic Effects on Humans: Extremely hazardous in case of inhalation (lung corrosive). Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (corrosive), of ingestion. However, it should be noted that such risks only apply to concentrated sulfuric acid under acidic pH conditions. In the final products developed by BioAtlantis, Ltd. pH is always over 3.5 and the level of sulfate will be low on a dry matter basis, so no such risks are expected. More detailed information is given in Appendix VII.

(e) Effects on Crops – The sulfuric acid is used only as a solubilizing agent and it will not exist as an individual separate substance in the final chelated micronutrient products, which is intended to be used in crops. Particularly, given the only slightly acidic pH (>3.5) of these products, only low amounts of sulfate ions will be present and no effect on the crops is to be expected.

10. **Safety Information about the Substance** – A Material Safety Data Sheet (MSDS) for sulfuric acid as well as a report by the National Institute of Environmental Health Sciences (NIEHS) can be found appended to this petition document in Appendix VIII and Appendix IX, respectively.
11. **Research Information about the Substance** – The available research information concerning sulfuric acid can be divided in the following categories:
    - Chemical and other properties of sulfuric acid:
      National Institute of Environmental Health Sciences (2011). Strong inorganic acid mists containing sulfuric acid (CAS N. 7664-93-9). .In: Report on carcinogens, 12th Edition. URL: http://ntp.niehs.nih.gov/pubhealth/roc/roc12/index.html (Accessed on 19th July 2014).
    - Advantages of sulfuric acid compared to organic acids for a successful amino acid chelation process:
      Bassi, R.; Prasher, S. O.; & Simpson, B. K. (2000). Extraction of metals from a contaminated sandy soil using citric acid. Environmental Progress, 19(4), 275-282.
      Kpomblekou, A. K.; & Tabatabai, M. A. (1994). Effect of organic acids on release of phosphorus from phosphate rocks1. Soil Science, 158(6), 442-453.
    - Health impacts and toxicological information:
      Hawley, G. G. (1987). Material safety data sheet emitted by: la Commission de la Santé et de la Sécurité du Travail du Québec. The Sigma-Aldrich Library of Chemical Safety Data, Edition II. The Condensed Chemical Dictionary, 11e ed. Van Nostrand Reinold: New York, USA.
      The "Screening Information Data Set" (SIDS) program, Organization for Economic Cooperation and Development (OECD) (2001). Sulfuric acid (CAS N. 7664-93-9). In: SIDS initial assessment report for 11th SIAM. United Nations Environment Publications (UNEP) Publications: Nairobi, Kenya, p. 89-132.
    - Environmental impacts:

Hawley, G. G. (1987). Material safety data sheet emitted by: la Commission de la Santé et de la Sécurité du Travail du Québec. The Sigma-Aldrich Library of Chemical Safety Data, Edition II. The Condensed Chemical Dictionary, 11e ed. Van Nostrand Reinold: New York, USA.

- Fate of sulfuric acid/sulfates in the environment:
  The "Screening Information Data Set" (SIDS) program, Organization for Economic Cooperation and Development (OECD) (2001). Sulfuric acid (CAS N. 7664-93-9). In: SIDS initial assessment report for 11th SIAM. United Nations Environment Publications (UNEP) Publications: Nairobi, Kenya, p. 89-132.
- Effect of micronutrient chelates on crops and their mode of action:
  Datir R.B, Laware S.L, & Apparao B.J. (2010) Effect of Organically Chelated Micronutrients on Growth and Productivity in Okra. *Asian J. Exp. Biol. Sci.*, 115-117.
  Datir, R.B., Apparao B. J., & Laware S.L. (2012). Application of amino acid chelated micronutrients for enhancing growth and productivity in chili (*Capsicum annum* L.). *Plant Sci. Feed, 2(7),* 100-105.
  Ghasemi, S., Khoshgoftarmanesh, A. H., Hadadzadeh, H., & Jafari, M. (2012). Synthesis of iron-amino acid chelates and evaluation of their efficacy as iron source and growth stimulator for tomato in nutrient solution culture. *J. Plant Growth Regul., 31(4),* 498-508.
  Ghasemi, S., Khoshgoftarmanesh, A.H., Afyuni, M., & Hadadzadeh, H. (2013a). The effectiveness of foliar applications of synthesized zinc-amino acid chelates in comparison with zinc sulfate to increase yield and grain nutritional quality of wheat. *Eur. J. Agron., 45*, 68-74.
  Ghasemi, S., Khoshgoftarmanesh, A. H., Hadadzadeh, H., & Afyuni, M. (2013b). Synthesis, characterization, and theoretical and experimental investigations of zinc (II)–amino acid complexes as ecofriendly plant growth promoters and highly bioavailable sources of zinc. *J. Plant Growth Regul., 32(2),* 315-323.
  Jeppsen, R.B. (1991). Mineral supplementation in plants via amino acid chelation. In *Biological Trace Element Research*; Subramanian K., et al, ACS Symposium Series, American Chemical Society, Washington, DC.
  Koksal A.I., Dumanoglu H., & Gunes N.T (1999). The effects of different amino acid chelate foliar fertilizers on yield, fruit quality, shoot growth and Fe, Zn, Cu, Mn content of leaves in Williams pear cultivar (*Pyrus communis* L.). *Turk. J. Agric. Forestry, 23(6),* 651-658.
  Taylor, K., & Brannen, P. (2008). Effects of foliar calcium application on peach fruit quality, shelf-life and fruit rot. In *Albion Conference on Plant Nutrition* (pp. 1-11).
  Will, S., Eichert, T., Fernández, V., Möhring, J., Müller, T., & Römheld, V. (2011). Absorption and mobility of foliar-applied boron in soybean as affected by plant boron status and application as a polyol complex. *Plant and soil, 344(1-2),* 283-293.
  Yuan, L., Wu, L., Yang, C., & Lv, Q. (2013). Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. *J. Sci. Food Agric., 93(2),* 254-261.
  Cited studies are grouped in Appendix X.

12. **Petition Justification Statement**:

BioAtlantis Ltd. has developed innovative micronutrient chelates of B, Mg, Ca, Mn, Fe, Cu, and Zn by a new route, which involves solubilisation of oxides of these elements —as foreseen in "(j) As plant or soil amendments" in subcategory "(6) Micronutrients" item "(ii) Sulfates, carbonates, oxides, or silicates of zinc, copper, iron, manganese, molybdenum, selenium, and cobalt" of the § 205.601 of NOP with extension to magnesium and calcium—

under moderately acidic conditions and its complexation by amino acids from a natural plant source.

The utilization of acidic conditions enables BioAtlantis Ltd. to solubilize to a very high level the mineral oxide substances and ensure very rich micronutrient content in the end product. Of course, this leads to a more efficient action of the micronutrient-containing products as natural plant strengthening materials. Accordingly, these products would fit into category "(j) As plant or soil amendments" in "(6) Micronutrients", specifically item "(ii) Sulfates, carbonates, oxides, or silicates of zinc, copper, iron, manganese, molybdenum, selenium, and cobalt" as mentioned in the § 205.601 of NOP, being the only difference the chelation of the elements. It should be noted that this chelation makes plant absorption easier and much more efficient. This means that only a fraction of the typical micronutrient applications to the fields is required. In this way, instead of dispersing significant amounts of micronutrients to the soil where they remain idle without being used by plants, micronutrients are channelled to the plants, given the great affinity between plant cell transport systems and the amino acids which chelate the micronutrients.

In order to solubilize the micronutrient oxides under acidic conditions, BioAtlantis Ltd. tested different acids and most effective was sulfuric acid. Given its strength, a small amount of this synthetic substance is required for ensuring a complete oxide solubility and establishing ideal pH for the chelation reactions to occur.

Furthermore, as new information not included in previous sulfuric acid petitions (Appendix IV and Appendix V), it must be remarked that sulfuric acid does not itself promote complexation and other reactions that may hinder chelation by the amino acids, as is the case of several organic acids such as citric acid (see above papers in section **11. Research Information about the Substance**, under title "Advantages of sulfuric acid compared to organic acids for a successful amino acid chelation process"). Therefore, sulfuric acid provides for a maximal micronutrient availability for plants through its indirect effect as enhancer of amino acid chelation.

Though sulfuric acid is not allowed in "(6) Micronutrients", specifically item "(ii) Sulfates, carbonates, oxides, or silicates of zinc, copper, iron, manganese, molybdenum, selenium, and cobalt", it is allowed under the same broad category "(j) As plant or soil amendments" in subcategory "(7) Liquid fish products" of the § 205.601 of NOP. What is more, the end result of the addition of sulfuric acid are sulfate anions, which are allowed and emphatically mentioned in item "(ii) Sulfates, carbonates, oxides, or silicates of zinc, copper, iron, manganese, molybdenum, selenium, and cobalt" and in subcategory "(6) Magnesium sulfate". There is no substantial chemical difference between the sulfate anions of zinc and other micronutrients —assuming that they were mined from nature as such— and the sulfates generated by BioAtlantis Ltd. process. Taking all this into account, this petition for action "A. Inclusion of a Synthetic on a National List, §§ 205.601, 205.603, 205.605(b)" does not relate to a new inclusion in the national list.

This reasoning is further supported by the fact that BioAtlantis Ltd. will use sulfuric acid prepared from elemental sulfur, which is also set in a specific subcategory, "(2) Elemental sulfur", of "(j) As plant or soil amendments" and its use would be restrained to pH adjustment for ensuring the correct acidic pH during micronutrient oxide solubilization. Hence, its role

would be only to adjust pH, a solubilizing agent, and to ensure a final stable micronutrient chelate product with a pH higher than 3.5.

The used amounts of sulfuric acid would not cause any significant chemical transformation of the bioactive substances (all non-synthetic) in the amino acid chelated micronutrient products. These substances either amino acids or mineral oxides would maintain their non-synthetic nature. The resulting chelates if stable act as an invaluable nutrition source for the plants, thus fulfilling the intent of materials listed under category "(j) As plant or soil amendments" of the § 205.601 of NOP.

BioAtlantis, Ltd. further stresses that no other synthetic substance besides sulfuric acid (as set above) will be used during the micronutrient chelate preparation process and that no formation of synthetic substances is foreseeable under applied conditions.

13. **Confidential Business Information (CBI)–** None.

APPENDIX I - SULFURIC ACID PRODUCTION PROCESS FROM ELEMENTAL SULFUR

(taken from: Ashar, N. G., & Golwalkar, K. R. (2013). A practical guide to the manufacture of sulfuric acid, oleums, and sulfonating agents. Springer Publishing: New York, USA, 146 p., ISBN 978-3-319-02041-9)

Elemental Sulfur

Process description for sulfuric acid 98.5% plant (commercial grade)

A typical sulfuric acid plant operating on sulfur as the main raw material consists of the following main sections:
1. Sulfur feeding section
2. Waste heat recovery section
3. SO2 conversion section
4. Acid towers section
5. Acid cooling and storage/handling section
6. Plant infrastructure (electrical/civil/water treatment, etc.)

2.1.1.1 Sulfur Burning

Solid sulfur is dumped on the grids of the melter and is melted by means of the heat provided through steam coils in the melter. An agitator installed in the melter helps to melt the sulfur at a faster rate. Liquified sulfur is pumped to the pressure leaf filter and the purified sulfur stored in a separate compartment equipped with steam coils. Sulfur pumps are used to feed the liquid sulfur to the sulfur burner at a predetermined constant rate. The sulfur burner is preheated to a high temperature by an earlier oil firing and hence the sulfur being fed in ignites instantaneously, producing sulfur dioxide. Dried air is supplied to the burner by an air blower through a drying tower. The gases coming out from the burner are at temperature of 950–1,000 °C and contain 10.0–10.5% sulfur dioxide. They are passed through a waste heat recovery boiler where high pressure steam is produced while the gases are cooled to 390–410 °C depending on the plant design and gas duct layout. The cooled gases are now passed through a multistage (four or five stage) conversion system (having three passes in the first converter and one/two passes in the second).

2.1.1.2 Conversion of SO2 into SO3

Both thermodynamic and stoichiometric considerations are taken into account in maximizing the formation of SO3. The Le Chatelier-Braun principle is usually taken into account in deciding how to optimize the equilibrium. This states that when an equilibrium system is subjected to stress, the system will tend to adjust itself in such a way that part of the stress is relieved. These stresses are, for example, variations of temperature, pressure, or concentration of a reactant.
For SO2/SO3 systems, the following methods are available to maximize the formation of SO3:
• Removal of heat—a decrease in temperature will favor the formation of SO3 since this is an exothermic process.
• Increased oxygen concentration in the input side.
• Removal of SO3 (double contact double absorption process) from the reaction zone.
• Raised system input pressure.
• Selection of the catalyst to reduce the working temperature (equilibrium).
• Increased reaction time.
• Increase in pressure of converter.

Optimum overall conversion of SO2 in the system requires a balance between velocity of the forward and backward reactions (equilibrium achieved). However, this optimum also depends on the SO2 concentration in the raw gas and on its variability with time. Consequently, each method is more or less specific for a particular SO2 source.
Modern converter systems have cesium promoted ring type vanadium pentoxide as catalyst in the first and the last (fourth/fifth) passes and conventional vanadium pentoxide catalyst (also ring type) in the other two/three passes. A second waste heat boiler is provided to recover additional heat after the first pass of catalyst.
Gases from the second pass of the catalyst are passed through a Hot Heat Exchanger (HHE) before entering the third pass of catalyst. Gases from the outlet of the third pass are passed through a Cold Heat Exchanger (CHE) and then through an economiser. The inter-pass absorption tower comes next and absorbs all the sulphur trioxide produced by the first three passes of the converter. Highly efficient candle type demisters completely remove all the acid mist generated in the tower so as to protect the catalyst in the later passes. Provision is made in the design of the gas ducting layout so that the economiser can be in commission or can be bypassed. A dry air injection facility is occasionally provided at the outlet of the fourth pass to cool the gases before entering the fifth pass to about 380–390 °C. This is done with a view to maximize the overall conversion of SO2 to SO3 since the last pass can be operated at as low a temperature as possible.
Gases from the outlet of the fifth pass are taken through another economiser for preheating of boiler feed water before going into the final absorption tower. The circulating sulfuric acid in the DT (Drying Tower), IPAT (Inter Pass Absorption Tower), and FAT (Final Absorption Tower) is cooled by passing through plate heat exchangers where it exchanges heat with the cooling water. The water is then cooled by an adequately sized cooling tower.

2.1.1.3 Absorption of SO3

Sulfuric acid is obtained by the absorption of SO3 into H2SO4 (with an optimum concentration of at least 98%) with the addition of appropriate amounts of water to maintain the concentration.
The efficiency of the absorption depends on the following:
• H2SO4 concentration of the absorbing liquid (98.3–98.7%)
• Range of temperature of the liquid (normally 70 °C–120 °C)
• The heat of absorption being removed by Plate Heat Exchanger (PHEs) for anodically passivated sulfuric acid coolers
• Moisture content in the raw gas which can produce fine acid mist particles, which are very difficult to absorb
• An acid mist filter to arrest the mist of sulfuric acid in the system
• Temperature of incoming gas
• SO3 emissions from the plant depend on:
• The construction and operation of the final absorber
• The acid mist formed upstream of the absorber through the presence of water vapor
• The device for separating H2SO4 aerosols

In modern plants, the strength of absorbing acid is automatically maintained at the optimum set point on the strength controller. This is done by controlling the addition of dilution water.

2.1.1.4 Tail Gas Scrubber

A two-stage alkali scrubber is provided for use during plant start-up to take care of any disturbed process conditions after any long stoppage. The concentration of alkali in the scrubbing liquor is maintained automatically. However, the scrubber will not be required during steady running of the plant when a cesium promoted catalyst is used in adequate amounts in the last pass of the converter which is operated at 385–390 °C.

Sulfuric Acid

Crops

Identification of Petitioned Substance

Chemical Names:
Sulfuric acid

Other Names:
battery acid
dihydrogen sulfate
dipping acid
dithionic acid
electrolyte acid
hydrogen sulfate
mattling acid
pyrosulphuric acid
vitriol
spirit of vitriol
sulphine acid
sulphuric acid
oil of vitriol
vitriol brown oil

Trade Names:
None

CAS Numbers:
7664-93-9

Other Codes:
X1002217-4 (ACX number)
2310 (OSHA IMIS Code Number)
WS5600000 (RTECS number)
4930040 (STCC number)
078001 (USEPA PC Code)
UN 1830 137 (DOT number; corrosive material)

Characterization of Petitioned Substance

Composition of the Substance:
Sulfuric acid (H_2SO_4) is a strong mineral acid that is highly soluble in water at all concentrations (HSDB 2005). The chemical structure of sulfuric acid is shown in Figure 1.

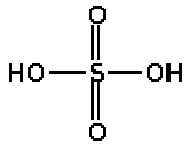

Figure 1. Chemical Structure of Sulfuric Acid

Properties of the Substance:

Sulfuric acid is a colorless to dark brown, oily, dense liquid (Chemfinder 2006). It is very corrosive and has a sharp, acrid odor. Although it is not combustible, concentrated sulfuric acid mixed with water generates a large amount of heat (HSDB 2005). Fire may result from the heat generated by contact of concentrated sulfuric acid solution with particulate combustible materials. Sulfuric acid reacts strongly with organic materials, chlorates, carbides, fulminates, water, and powdered metals. When heated, sulfuric acid emits highly toxic fumes that include sulfur trioxide. Sulfuric acid is most commonly marketed in four grades: commercial, electrolyte (high purity for batteries), textile (low organic content), and chemically pure or reagent grades (ATSDR 1998).

Sulfuric acid is one of the primary chemical agents of "acid rain" (ATSDR 2004). Because it is not very volatile, sulfuric acid from sources of air pollution can often be found in the air as microscopic liquid droplets or attached to other small particles in the air (NSC 2005). Atmospheric deposition of sulfuric acid from air pollution can lower the pH of surface waters and have a corrosive effect on living and non-living components of the aquatic and terrestrial environment.

Specific Uses of the Substance:

Sulfuric acid, along with phosphoric acid and citric acid, currently are approved for use as processing aids for pH adjustment in organically processed liquid fish products for use in crop production (NOP §205.601(j)(7)). The current approval allows for pH adjustment of liquid fish products to as low as 3.5. Sulfuric acid is petitioned to be used for the same purpose (i.e., processing aid for pH adjustment) in the production of dehydrated manure for subsequent use in organic crop production. For the petitioned use, the pH would not be lowered below 5.0.

Sulfuric acid is the world's largest volume industrial chemical in terms of production (ADEH 2003, EPA 1993); more sulfuric acid is produced in the United States than any other chemical (NSC 2005). The main use is in the production of phosphate fertilizers that convert phosphate rock to phosphoric acid, which consumes the sulfuric acid (ATSDR 1998). It is also used to manufacture explosives, other acids, dyes, glue, wood preservatives, and automobile batteries. It is used in the purification of petroleum, the pickling of metal, copper smelting, electroplating, metal work, the production of rayon and film, and as a laboratory reagent. In many of these applications, the sulfuric acid is recovered and reused. There also are numerous household products that contain sulfuric acid (HPD 2004).

Approved Legal Uses of the Substance:

Sulfuric acid is regulated as a pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (EPA 1993). It is exempt from the requirement of a tolerance for residues when used as a pH control agent in accordance with good agricultural practices as an ingredient in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest (HSDB 2005). It is also exempt from the requirement of a tolerance for residues when used in accordance with good agricultural practice as an herbicide in the production of garlic and onions and as a potato vine desiccant in the production of potatoes (EPA 1993, HSDB 2005). The U.S. Food and Drug Administration (FDA) has determined under 21 CFR §184.1095 that sulfuric acid is a "Generally Recognized as Safe" (GRAS) substance in food.

Several other regulations apply to the transport, disposal, and accidental release of sulfuric acid. The U.S. Department of Transportation (DOT) forbids spent (i.e., used) sulfuric acid from being transported on passenger-carrying aircraft or railcars (NSC 2005). Under the Federal Water Pollution Control Act, sulfuric acid is considered a hazardous substance when discharged to surface waters; it is further regulated by the Clean Water Act (CWA) Amendments of 1977 and 1978 (HSDB 2005). Sulfuric acid is regulated under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA), under which releases of more than one pound of sulfuric acid into the air, water, or land must be reported annually and entered into the Toxic Release Inventory (TRI) (EPA 2005a). In 1993, EPA delisted non-aerosol forms of sulfuric acid (EPA 2005b); thus, aqueous solutions of sulfuric acid are no longer covered under TRI. Sulfuric acid (in all forms) is included on Canada's 2004 National Pollutant Release Inventory (EC 2005).

Several U.S. governmental and non-governmental organizations have published regulations and guidance regarding occupational exposure limits to airborne sulfuric acid; these are summarized in NSC (2005), OSHA (2003), and NIOSH (2000, 2005a, 2005b). NIOSH (2005b) also summarizes international standards and regulations concerning occupational exposure to sulfuric acid.

Action of the Substance:

According to the petition, liquid sulfuric acid would be added to adjust the pH of livestock manures prior to dehydrating the solids for final use as a soil amendment in organic crop production. More specifically, sulfuric acid would be used within livestock manures to keep biologically-derived nitrogen compounds in solution as opposed to being volatilized during the manure-drying process. The pH of some excreted manures tends to be alkaline (pH 7.8-8.3) due to the use of limestone as a calcium source for bone mass in the animal feed and due to the natural generation of uric acids and ammonium in the urine and feces of the animal. Adding a small amount of sulfuric acid to the manure lowers the pH and slows the biological breakdown of the uric acids and ammonium into more volatile forms of nitrogen and organic compounds (e.g., fatty acids) , thereby greatly decreasing the release of odorous compounds (McCrory and Hobbs 2001). For the petitioned use, the pH would not be lowered below 5.0.

Status

International

Sulfuric acid is not specifically listed for the petitioned use or other uses in the following international organic standards:

- CODEX Alimentarius Commission
- European Economic Community (EEC) Council Regulation 2092/91
- International Federation of Organic Agriculture Movements

The Canadian General Standards Board permits the use of fish emulsions to amend and improve soil fertility (CGSB 1999). Liquid fish products can be pH-adjusted using sulfuric acid, but the amount of acid used cannot exceed the minimum amount needed to lower the pH to 3.5 (CGSB 2004).

Sulfuric acid is listed in the Japan Agricultural Standard for Organic Production where it is allowed for use in adjusting pH of the extracted water in producing sugar (i.e., a pH adjustment agent) (JMAFF 2000).

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

Evaluation Question #1: **Is the petitioned substance formulated or manufactured by a chemical process? (From 7 U.S.C. § 6502 (21))**

According to the petition, sulfuric acid is produced from sulfur dioxide (SO_2) collected by pollution control devices (scrubbers) during the smelting of various metal ores and ore concentrates. The sulfur dioxide is captured in the scrubbers to reduce emissions that would otherwise contribute to acid rain. The resulting "scrubber feedstock" is further purified, concentrated, and used for the subsequent production of sulfuric acid.

There are two major processes that have been used to produce commercial quantities of sulfuric acid: the "contact process" and the "chamber process" (ATSDR 1998). The contact process was developed in the early 1900s and has become the primary means of sulfuric acid production worldwide (IARC 1992). In brief, sulfur dioxide forms sulfuric acid in the presence of oxygen, water, and a catalyst (most commonly vanadium complexes), by a two-step chemical reaction shown in Figure 2 (EFMA 1997, HSDB 2005).

$$(1)\ 2SO_2 + O_2 \rightarrow 2SO_3$$
$$(2)\ SO_3 + H_2O \rightarrow H_2SO_4$$

Figure 2. Formulation of Sulfuric Acid via the Contact Process

This reaction can produce 98-99 percent pure sulfuric acid, which is stable for storage and is considered the usual form of "concentrated" sulfuric acid (ATSDR 1998, EFMA 1997). The petition includes a detailed summary of the production process, which is derived from information provided by the sulfuric acid manufacturer and sulfuric acid supplier.[1]

The other major sulfuric acid production process, the "chamber process," was once the predominant method for sulfuric acid production in the United States and western Europe, but it has dropped to virtually zero use since 1960 (ATSDR 1998).

Evaluation Question #2: Is the petitioned substance formulated or manufactured by a process that chemically changes the substance extracted from naturally occurring plant, animal, or mineral sources? (From 7 U.S.C. § 6502 (21).)

The starting point for commercial sulfuric acid manufacturing is sulfur dioxide, which is a byproduct of industrial pollution control systems (EFMA 1997). The manufacturing process involves a two-step chemical reaction using oxygen, water, and a vanadium oxide catalyst (HSDB 2005). See Evaluation Question #1 for further explanation of the manufacturing process.

Evaluation Question #3: Is the petitioned substance created by naturally occurring biological processes? (From 7 U.S.C. § 6502 (21).)

Commercial sulfuric acid is chemically synthesized. See Evaluation Question #1 for further explanation of the manufacturing process.

Evaluation Question #4: Is there environmental contamination during the petitioned substance's manufacture, use, misuse, or disposal? (From 7 U.S.C. § 6518 (m) (3).)

Globally, sulfuric acid is of environmental and regulatory concern as a result of acid rain deposition resulting from the burning of sulfur-containing fuels (ATSDR 2004). As described in Evaluation Questions #1 and #2, the feedstock for sulfuric acid manufacturing is a beneficial byproduct from the use of air pollution control devices during the smelting of various naturally occurring metal ores. Thus, according to the petition, if not turned into a commercial product, this byproduct would ultimately contribute to the formation of acid rain.

Manufacturing

Facilities that manufacture sulfuric acid are among the primary sources of sulfuric acid releases to the environment (ATSDR 1998). These releases are mainly emissions to the air. In the air, some sulfuric acid reacts with other chemicals (e.g., ammonia, magnesium, calcium), which act to neutralize the acid. Sulfuric acid droplets and particles that are not neutralized may dissolve in clouds, fog, rain, or snow, resulting in very dilute acid solutions which may impact the environment as acid precipitation.

When acid precipitation reaches surface water, the sulfuric acid dissociates to hydrogen and sulfate ions (H^+ and SO_4^{2-}); sulfate anions may combine with other metal cations, such as calcium and magnesium, to form particulate sulfate salts (ATSDR 1998). Aquatic sulfur may be oxidized to sulfuric acid by sulfur bacteria (Thiobacilli) that use sulfur to obtain energy for growth. Sulfate levels in water are highly dependent on nearby emissions of sulfur-containing compounds, which can be converted to sulfuric acid. Background sulfate concentrations in North American lakes are estimated at 20-40 µeq/L. In eastern North America where acid deposition occurs, sulfate concentrations are 80-100 µeq/L. Surface waters closer to sources of emission can have even higher concentrations.

[1] Additional information also is available from the web site of NorFalco LLC, one of the largest marketers of sulfuric acid in North America (http://www.norfalco.com/production+process.htm).

Use and Handling

The petition indicates that the method of sulfuric acid handling and addition to manure would vary between animal species, diet formulation, and respective farm manure handling facilities. Typically, small amounts of liquid sulfuric acid would be added on a continuous basis via a metering valve or pump connected to a supply tank. Addition of sulfuric acid would take place during manure transport, mixing, and storage to diminish odor generation. In cases of long storage times or noncontinuous mixing and transport of manure, sulfuric acid may be added in batch mode, but the volume of acid needed in such cases would be consistent with the continuous feed method.

According to the petition, following addition of sulfuric acid to manure, the acid is subsequently neutralized by the manure. The remaining sulfur is in the form of sulfate ions (SO_4^{2-}). Sulfate is an essential nutrient in the formation of chlorophyll and amino acids within plants (Baird 1997).

Misuse

No information sources reviewed for this report specifically address the issue of misuse of sulfuric acid during addition to manure. Accidental spills or improper disposal of liquid sulfuric acid or wastes containing sulfuric acid could result in environmental contamination. The presence of water in the soil or precipitation at the time of an accidental spill or release of liquid sulfuric acid will influence the rate of chemical movement in the soil and the likelihood that it will reach groundwater (HSDB 2005).

Disposal

As noted previously, when used as petitioned to adjust the pH of livestock manure, sulfuric acid is neutralized to sulfate, which is eventually taken up by crops as a nutrient. Disposal of unused sulfuric acid and wastes containing sulfuric acid in the United States is controlled by a number of federal regulations (e.g., EPCRA, CWA) intended to prevent environmental contamination.

Evaluation Question #5: Is the petitioned substance harmful to the environment? (From 7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i).)

Sulfuric acid is a component of acid rain, which is a well-known pollution problem of global concern (ATSDR 1998). Although sulfuric acid has been characterized as only slightly toxic to crustaceans and fish by the Pesticide Action Network (PAN 2005), the National Institute for Occupational Safety and Health (NIOSH) warns occupational users of sulfuric acid not to let it enter the environment and states that sulfuric acid is harmful to aquatic organisms (NIOSH 2000). EPA (1993) concluded that the use of registered pesticide products containing sulfuric acid in accordance with approved labeling "will not pose unreasonable risks or adverse effects to humans or the environment" except when it is used as a desiccant on potato vines. The use of sulfuric acid as a desiccant on potato vines poses significant hazards to birds and other terrestrial wildlife.

Evaluation Question #6: Is there potential for the petitioned substance to cause detrimental chemical interaction with other substances used in organic crop or livestock production? (From 7 U.S.C. § 6518 (m) (1).)

No information was identified to suggest that sulfuric acid applied to manure would cause detrimental chemical interaction with other substances used in organic crop production. If the acid is added to manure in the manner described in the petition, it is unlikely to be available to chemically interact with other substances used in organic crop or livestock production. This is because the acid is neutralized by the manure and converted to sulfate ions (see Evaluation Question #4).

Evaluation Question #7: Are there adverse biological or chemical interactions in the agro-ecosystem by using the petitioned substance? (From 7 U.S.C. § 6518 (m) (5).)

No information was identified to suggest that sulfuric acid applied to manure would result in adverse biological or chemical interactions in the agro-ecosystem. If the acid is added to manure in the manner described in the petition, it is unlikely to reach the greater agro-ecosystem in significant amounts and thus is unlikely to result in adverse chemical or biological interactions in the agro-ecosystem. This is because the acid is neutralized by the manure and converted to sulfate ions (see Evaluation Question #4).

In the event of a major spill of liquid sulfuric acid to soil, especially during a precipitation event, ions from liquid sulfuric acid (i.e., hydrogen and sulfate) can adsorb to soil particles, be converted to gases, or leach into surface water and groundwater, removing important nutrients such as ions of calcium, magnesium, potassium, and other metals attached to the clay and humus particles in the soil (Virtual Chembook 2003). Normally, the attractive forces of positive metal ions to negatively charged clay particles are sufficient to keep the metal ions in the soil despite the passage of water through the soil. However, the presence of sulfuric acid allows the hydrogen ions to trade places with the metal ions, which has two negative effects. First, the hydrogen ions are retained, which can lower the pH of the soil thereby slowing the growth of or even killing vegetation in the immediate area of the contaminated soil. Second, the metal ions are leached or washed out of the top soil into lower inaccessible subsoil, thereby making them unavailable as nutrients or fertilizers for tree and plant growth.

Evaluation Question #8: Are there detrimental physiological effects on soil organisms, crops, or livestock by using the petitioned substance? (From 7 U.S.C. § 6518 (m) (5).)

If the acid is added to manure according to the petitioned use, it is unlikely to reach the greater agro-ecosystem in significant amounts and thus is unlikely to be available to cause detrimental physiological effects on soil organisms, crops, or livestock. This is because the acid is neutralized by the manure and converted to sulfate ions (see Evaluation Question #4).

In the event of a major spill of large quantities of liquid sulfuric acid to soil, especially during a precipitation event, the pH of the soil would be lowered, which could slow the growth of or even kill vegetation in the immediate area of the contaminated soil (Virtual Chembook 2003). Lowered soil pH can also inhibit plant growth by its effect on activity of beneficial soil microorganisms. For example, bacteria that decompose soil organic matter are hindered in strongly acidic soils, which can prevent organic matter from breaking down, resulting in an accumulation of organic matter and tying up nutrients, particularly nitrogen, that are held in the organic matter (Bickelhaupt 2005).

Evaluation Question #9: Is there a toxic or other adverse action of the petitioned substance or its breakdown products? (From 7 U.S.C. § 6518 (m) (2).)

Sulfuric acid is very corrosive and irritating and can cause direct harmful effects on the skin, eyes, and respiratory and gastrointestinal tracts when there is direct exposure to sufficient amounts of concentrated acid (NSC 2005, EPA 1993). Exposure to sulfuric acid mist can irritate the eyes, nose, throat and lungs, and at higher levels can cause a buildup of fluid in the lungs (pulmonary edema) (ADEH 2003). Although liquid sulfuric acid is not absorbed through the skin, it is a corrosive chemical and can severely burn unprotected skin and eyes, causing third degree burns and blindness on contact (ATSDR 2004). Oral ingestion of concentrated sulfuric acid can burn the mouth, throat, and stomach, and can result in death (ATSDR 2004). EPA has placed sulfuric acid in Toxicity Category I (on a scale of I to IV) for eye and dermal irritations as well as inhalation effects in humans; it is in Toxicity Category II for acute oral toxicity (EPA 1993).

There are no human dietary concerns from the use of sulfuric acid as a pesticide on potato vines (EPA 1993). For this use, sulfuric acid was granted an exemption from tolerance requirements because it "is rapidly degraded in the environment to sulfate salts, which are of no toxicological concern and are Generally Recognized as Safe (GRAS) by the Food and Drug Administration."

The American Conference of Governmental Industrial Hygienists (ACGIH) has classified aerosol sulfuric acid as a suspected human carcinogen because it is carcinogenic in laboratory animals under conditions that are considered relevant to worker exposure (CCOHS 2003). However, available human studies are considered conflicting or insufficient to confirm an increased risk of cancer in exposed humans. The International Agency for Cancer Research (IARC) has determined that there is sufficient evidence that occupational exposure to strong-inorganic-acid mists containing sulfuric acid is carcinogenic to humans (IARC 1992, 1997).

From an occupational health perspective, inhalation and dermal exposure resulting from commercial production, industrial uses, and agricultural uses of sulfuric acid are of concern and subject to various exposure standards and guidance (NSC 2005, OSHA 2003, and NIOSH 2000, 2005a, 2005b). NIOSH recommends that workers wear appropriate personal protective clothing and eyewear to prevent skin and eye contact and use ventilation and breathing protection to prevent inhalation (NIOSH 2000, 2005a). Labels for pesticide products containing sulfuric acid must require use of personal protective equipment and clothing, as specified in the Worker Protection Standard, and workers must also wait 5 days before re-entering treated potato fields (EPA 1993).

Evaluation Question #10: Is there undesirable persistence or concentration of the petitioned substance or its breakdown products in the environment? (From 7 U.S.C. § 6518 (m) (2).)

According to the petition, sulfuric acid added to manure is subsequently neutralized by the manure leaving behind sulfate ions. Sulfate is an essential nutrient in the formation of chlorophyll and amino acids within plants (Baird 1997).

In the event of a spill of liquid sulfuric acid, the persistence of sulfuric acid in soil would be dependent on the extent to which soils can neutralize it, which in turn depends on several factors such as type of soil, thickness, weather, and water flow patterns (Virtual Chembook 2003). For example, if the ground is frozen, natural soil processes cannot function and the acid is not neutralized. If the soil is mainly quartz, such as those having a lot of sand, it is resistant to weathering and no bases are present to neutralize the acid. If the soil has very little base such as limestone, the acid is neutralized only slightly or with the passage of time, not at all. Sulfuric acid ions (i.e., hydrogen and sulfate) that do not adsorb to soil particles can be converted to gas and volatilize (ATSDR 1998).

Evaluation Question #11: Is there any harmful effect on human health by using the petitioned substance? (From 7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4).)

The toxic effects of sulfuric acid were summarized above in Evaluation Question #9. From an occupational perspective, sulfuric acid is unlikely to have harmful effects on human health if it is properly handled by workers during its addition to manure (i.e., use of protective equipment and ventilation). Once added to manure, sulfuric acid is unlikely to reach the environment in significant amounts and thus is unlikely to be available to cause harmful effects on human health. This is because the acid is neutralized by the manure and converted to sulfate ions (see Evaluation Question #4).

Evaluation Question #12: Is there a wholly natural product which could be substituted for the petitioned substance? (From 7 U.S.C. § 6517 (c) (1) (A) (ii).)

There are a variety of substances that can be added to livestock manure to reduce ammonia production and odor emissions (McCrory and Hobbs 2001). Additives to decrease ammonia production include acidifying agents, bacterial–enzymatic preparations, plant extracts, oxidizing agents, disinfectants, urease inhibitors, masking agents, and adsorbents. Additives to reduce odor nuisance include digestive additives, disinfecting additives, oxidizing agents, adsorbents, and masking agents. The majority of these additives cannot be considered natural products, and their effectiveness is not well established. Some additives that can be considered natural product alternatives to the use of sulfuric acid are discussed below.

The application of unreacted carbon sources (e.g., potato starch, milled wheat) is often a less hazardous alternative to sulfuric acid and induces a reduction in livestock manure pH by stimulating the naturally-occurring microorganisms to produce organic acids (McCrory and Hobbs 2001). At present, the quantity of carbon material required to induce a significant pH decline is economically prohibitive. However, if the production of acid can be optimized, possibly by using suitable lactic acid bacteria, it would offer an effective and safe means to prevent ammonia production.

A variety of natural absorbents can be use to reduce ammonia production; some of the most commonly employed are peat and clinoptilolite (a naturally occurring alumino-silicate mineral with high cation exchange capacities). The advantages associated with the use of either clinoptilolite or peat are that they are nonhazardous and act as good soil conditioners when spread with manure.

Several additives to reduce ammonia production in livestock manure are based on saponins that are extracted from the sap of the yucca plant (McCrory and Hobbs 2001). Saponins are high-molecular-weight glycosides that are believed to be responsible for the yucca's capability to conserve ammonia. The exact mechanism of ammonia reduction is unclear mechanism, and commercial use of these products has yielded mixed results.

More broadly, the use of chemically-treated animal manure can be replaced by use of composted or raw manure (the latter with restrictions) and/or composted or non-composted plant materials, which are allowed under NOP §205.203(c). Hall and Sullivan (2001) provide a review of alternative soil amendments to agricultural fertilizers and manure, including several that can be considered wholly natural, such as various plant byproducts (e.g., composted leaves), rock and mineral powders (e.g., granite dust), and seaweed products.

Evaluation Question #13: Are there other already allowed substances that could be substituted for the petitioned substance? (From 7 U.S.C. § 6518 (m) (6).)

Various acids have been proven effective in reducing ammonia volatilization; these include sulfuric, hydrochloric, nitric, phosphoric, and lactic acid (McCrory and Hobbs 2001). Of these, sulfuric acid and phosphoric acid are currently approved for use as processing aids for pH adjustment in organically processed liquid fish products for use in crop production (NOP §205.601(j)(7)). Phosphoric acid is also allowed as an equipment cleaner in livestock production (NOP §205.203 (a)(14)) and in the cleaning of food-contact surfaces and equipment (NOP §205.605 (b)). Thus, phosphoric acid is an alternative to sulfuric acid as a processing aid in the production of dehydrated manure for subsequent use in organic crop production. However, phosphoric acid is not as cost-effective in reducing ammonia production in livestock manure (McCrory and Hobbs 2001).

As noted in the response to Evaluation Question #12, the use of chemically-treated animal manure can be replaced by use of (non-chemically-treated) composted or non-composted animal and/or plant materials, which are allowed under NOP §205.203(c).

Evaluation Question #14: Are there alternative practices that would make the use of the petitioned substance unnecessary? (From 7 U.S.C. § 6518 (m) (6).)

As specified under NOP §205.203(b): "The producer must manage crop nutrients and soil fertility through rotations, cover crops, and the application of plant and animal materials." Thus, the need to use manure (whether composted, non-composted, or chemically-treated) or plant materials could be replaced through crop rotation and use of cover crops. A cover crop is any crop grown to provide soil cover for a subsequent crop and which are grown primarily to prevent soil erosion by wind and water. Sullivan (2003) provides a review of these "green manuring" practices. Other alternative practices to improve soil health and sustainability, such as tillage reduction (i.e., intentional disruption and mixing of topsoil), are reviewed in Sullivan (2004).

References

ADEH (Australia Department of Environment and Heritage) 2003. National Pollutant Inventory: Sulfuric Acid. http://www.npi.gov.au/database/substance-info/profiles/78.html.

ATSDR (Agency for Toxic Substances and Disease Registry). 1998. Toxicological Profile for Sulfur Trioxide and Sulfuric Acid. http://www.atsdr.cdc.gov/toxprofiles/tp117.pdf.

ATSDR 2004. ToxFAQs™ for Sulfur Trioxide and Sulfuric Acid. http://www.atsdr.cdc.gov/tfacts117.html.

Baird, J. 1997. SoilFacts: Sulfur as a Plant Nutrient. North Carolina Cooperative Extension Service Publication AG-439-15. http://www.soil.ncsu.edu/publications/Soilfacts/AG-439-15/#Sources_and_Amounts_of_Sulfur_for_North_Carolina_Crops.

Bickelhaupt, D. 2005. Soil pH: What it Means. SUNY College of Environmental Science and Forestry. http://www.esf.edu/pubprog/brochure/soilph/soilph.htm.

CCOHS (Canadian Centre for Occupational Health & Safety). 2003. Working Safely with Sulfuric Acid. http://www.ccohs.ca/oshanswers/chemicals/chem_profiles/sulfuric_acid/working_sa.html.

Canadian General Standards Board (CGSB). 2004. Organic Production Systems, Part 3 – Permitted Substances lists – FIRST COMMITTEE DRAFT. Available at: http://www.cog.ca/documents/OrganicStandard2004.3.pdf.

Canadian General Standards Board (CGSB). 1999. Organic Agriculture. CAN/CGSB-32.310-99. Available at: http://www.pwgsc.gc.ca/cgsb/032_310/32.310epat.pdf.

Chemfinder 2006. http://chemfinder.cambridgesoft.com/result.asp?mol_rel_id=7664-93-9.

EC (Environment Canada) 2005. Alphabetical Listing of NPRI Substances for 2004. http://www.ec.gc.ca/pdb/npri/2004Guidance/Substance_list2004_e.cfm.

EFMA (European Fertilizer Manufacturers Association) 1997. Booklet No 3 of 8: Production of Sulphuric Acid. http://www.efma.org/Publications/BAT%202000/Bat03/booklet3.pdf.

EPA (U.S. Environmental Protection Agency). 1993. R.E.D. FACTS: Mineral Acids. EPA-738-F-93-025. Office of Prevention, Pesticides and Toxic Substances. http://www.epa.gov/oppsrrd1/REDs/factsheets/4064fact.pdf.

EPA 2005a. Table II. EPCRA Section 313 Chemical List For Reporting Year 2004 (including Toxic Chemical Categories). http://www.epa.gov/tri/chemical/RY2004ChemicalLists.pdf.

EPA 2005b. TRI Chemical List Changes (1987-2005). http://www.epa.gov/tri/chemical/ChemListChanges05.pdf.

Hall, B, and Sullivan, P. 2001. Alternative Soil Amendments: Horticulture Technical Notes. ATTRA - National Sustainable Agriculture Information Service. http://attra.ncat.org/attra-pub/PDF/altsoil.pdf.

HPD (Household Product Database). 2004. http://householdproducts.nlm.nih.gov/cgi-bin/household/brands?tbl=chem&id=577&query=sulfuric+acid.

HSDB (Hazardous Substances Data Bank). 2005. Sulfuric Acid. http://toxnet.nlm.nih.gov/.

IARC (International Agency for Research on Cancer). 1992. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 54: Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and Other Industrial Chemicals. Lyons, France.

IARC 1997. Occupational Exposures to Mists and Vapours From Sulfuric Acid and Other Strong Inorganic Acids http://www-cie.iarc.fr/htdocs/monographs/vol54/01-mists.htm.

JMAFF (Japanese Ministry of Agriculture, Forestry and Fisheries) 2000. Japanese Agricultural Standard of Organic Agricultural Product Processed Foods (Notification No. 60). http://www.maff.go.jp/soshiki/syokuhin/hinshitu/organic/eng_yuki_60.pdf.

McCrory DF, Hobbs PJ. 2001. Additives to Reduce Ammonia and Odor Emissions from Livestock Wastes. Journal of Environmental Quality 30:345-355. http://intl-jeq.scijournals.org/cgi/content/full/30/2/345.

NIOSH (National Institute for Occupational Safety and Health). 2000. International Chemical Safety Cards: Sulfuric acid. October 2000. http://www.ilo.org/public/english/protection/safework/cis/products/icsc/dtasht/_icsc03/icsc0362.htm

NIOSH (National Institute for Occupational Safety and Health). 2005a. Pocket Guide to Chemical Hazards: Sulfuric acid. NIOSH Publication No. 2005-151. September 2005. http://www.cdc.gov/niosh/npg/npgd0577.html.

NIOSH (National Institute for Occupational Safety and Health). 2005b. The Registry of Toxic Effects of Chemical Substances: Sulfuric acid. May 2005. http://www.cdc.gov/niosh/rtecs/ws557300.html#L.

NSC 2005. Sulfuric Acid. http://www.nsc.org/library/chemical/index.htm.

OSHA 2003. Safety and Health Topics: Sulfuric Acid. http://www.osha.gov/dts/chemicalsampling/data/CH_268700.html.

PAN (Pesticide Action Network). 2005. PAN Pesticides Database - Pesticide Registration Status: Sulfuric Acid. http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC39#Regulatory.

Sullivan, P. 2003. Overview of Cover Crops and Green Manures: Fundamentals of Sustainable Agriculture. ATTRA - National Sustainable Agriculture Information Service. http://www.attra.org/attra-pub/PDF/covercrop.pdf.

Sullivan, P. 2004. Sustainable Soil Management . ATTRA - National Sustainable Agriculture Information Service. http://www.attra.org/attra-pub/PDF/soilmgmt.pdf.

Virtual Chembook (Elmherst College) 2003. Acid Rain - Soil Interactions. http://www.elmhurst.edu/~chm/vchembook/196soil.html.

Formal Recommendation
From: National Organic Standards Board (NOSB)
To: the National Organic Program (NOP)

Date: April 11, 2013

Subject: Petition to add sulfuric acid to 205.605(b)

Chair: Mac Stone

The NOSB hereby recommends to the NOP the following:

Rulemaking Action: ☐

Guidance Statement: ☐

Other: ☐

Statement of Recommendation: (Motion # 1) Passed

Motion to classify sulfuric acid (CAS 7664-93-9) as petitioned as synthetic.

Rationale Supporting Recommendation (including consistency with OFPA and NOP):

Based on the technical Report, line 262, Sulfuric acid, including food-grade sulfuric acid, is chemically synthesized.

Committee Vote:

Moved: Joe Dickson

Seconded: Harold Austin

Yes: 15 No: 0 Abstain: 0 Absent: 0 Recuse 0

Statement of Recommendation: (Motion # 2) Failed

Motion to add sulfuric acid (CAS 7664-93-9) as petitioned to section 205.605(b) of the National List.

Rationale Supporting Recommendation (including consistency with OFPA and NOP):

The petition and TR do not provide sufficient information to fully evaluate this material. However, based on limited information, it appears that this material fails several evaluation criteria, including 1. Impact on Humans and Environment, 2. Essentiality & Availability, and 3. Compatibility & Consistency. Additionally, there were few or no comments from potential users indicating a need for this substance, not were there comments from those supporting it's addition.

Committee Vote:

Moved: Joe Dickson

Seconded: Jean Richardson

Yes: 0 No: 15 Abstain: 0 Absent: 0 Recuse: 0

National Organic Standards Board
Handling Sub Committee
Petitioned Material Proposal
Sulfuric Acid

January 9, 2013

Summary of Proposed Action:
The petition is for the listing of sulfuric acid on 205.605(b) for use as a processing aid in the production of seaweed extract. Sulfuric acid is used as a pH adjuster in the extraction water for the production of seaweed extracts, particularly a class of seaweed extracts called fucoidans, which are largely used as ingredients in dietary supplements.

For a number of reasons, the Handling Subcommittee recommends that sulfuric acid not be added to the national list as petitioned:

- The redaction of substantial amounts of confidential business information (CBI) from the petition makes it impossible to evaluate the use of sulfuric acid in the manufacturing process, and impossible to establish whether the resulting seaweed extract undergoes sufficient chemical change as to render it a synthetic substance.

- The petition and TR fail to demonstrate the essentiality of this substance in the production of organic food, or the absence of viable alternatives. The petition provides little economic data or market narrative to demonstrate that this substance might play a compelling role in the production of organic products, and the redacted CBI makes it impossible to even understand how sulfuric acid is used in seaweed extract production.

- The TR clearly documents negative environmental impacts of the production of this substance, suggests negative health effects in its production and industrial use, and overwhelmingly demonstrates the substance's incompatibility with a system of organic agriculture.

Evaluation Criteria

(Applicability noted for each category; Documentation attached)	**Criteria Satisfied?**		
1. Impact on Humans and Environment	☐ Yes	X No	☐ N/A
2. Essential & Availability Criteria	☐ Yes	X No	☐ N/A
3. Compatibility & Consistency	☐ Yes	X No	☐ N/A
4. Commercial Supply is Fragile or Potentially Unavailable as Organic (only for § 205.606)	☐ Yes	☐ No	X N/A

Substance Fails Criteria Category: [1, 2 and 3] **Comments:**

Proposed Annotation (if any):

Basis for annotation: ☐ To meet criteria above ☐ Other regulatory criteria ☐ Citation
Notes:

Recommended Committee Action & Vote, including classification recommendation (state actual motion):

Classification Motion: Motion to classify sulfuric acid (CAS 7664-93-9) as petitioned as synthetic:
Motion by: Joe Dickson
Seconded by: John Foster
No further discussion

Yes: 8 No: 0 Abstain: 0 Absent: 0 Recuse: 0

Listing Motion: List sulfuric acid (CAS 7664-93-9) as petitioned on 205.605(b)
Motion by: Joe Dickson
Seconded by: Tracy Favre
No further discussion
Yes: 0 No: 8 Abstain: 0 Absent: 0 Recuse: 0

Crops	☐	**Agricultural**	☐	**Allowed**[1]	☐
Livestock	☐	**Non-synthetic**	☐	**Prohibited**[2]	☐
Handling	**X**	**Synthetic**	**X**	**Rejected**[3]	**X**
No restriction	☐	**Commercial unavailable as organic**	☐	**Deferred**[4]	☐

[1]Substance voted to be added as "allowed" on National List to § 205. with Annotation (if any):
[2]Substance to be added as "prohibited" on National List to § 205. with Annotation (if any):
Describe why a prohibited substance:
[3]Substance was rejected by vote for amending National List to § 205.605(b). Describe why material was rejected:
[4]Substance was recommended to be deferred because
If follow-up needed, who will follow up:

Approved by Subcommittee Chair to Transmit to NOSB

John Foster, Subcommittee Chair **January 9, 2013**

NOSB Evaluation Criteria for Substances Added To the National List

Category 1. Adverse impacts on humans or the environment? Substance: Sulfuric Acid

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Are there adverse effects on environment from manufacture, use, or disposal? [§205.600 b.2]	x			The TR notes that sulfuric acid is a substantial source of acid rain, and that the manufacture of this material presents adverse environmental impact (lines 327-353)
2. Is there environmental contamination during manufacture, use, misuse, or disposal? [§6518 m.3]	x			TR lines 327-353
3. Is the substance harmful to the environment and biodiversity? [§6517c(1)(A)(i);6517(c)(2)(A)i]	x			TR lines 327-353
4. Does the substance contain List 1, 2 or 3 inerts? [§6517 c (1)(B)(ii); 205.601(m)2]			x	
5. Is there potential for detrimental chemical interaction with other materials used? [§6518 m.1]	x			
6. Are there adverse biological and chemical interactions in agro-ecosystem? [§6518 m.5]			x	
7. Are there detrimental physiological effects on soil organisms, crops, or livestock? [§6518 m.5]			x	
8. Is there a toxic or other adverse action of the material or its breakdown products? [§6518 m.2]	x			TR lines 327-353
9. Is there undesirable persistence or concentration of the material or breakdown products in environment? [§6518 m.2]	x			TR lines 327-353
10. Is there any harmful effect on human health? [§6517 c (1)(A)(i); 6517 c(2)(A)i; §6518 m.4]	x			While there is no documented detrimental effect on human health from dietary sources of the material as petitioned, the manufacture and industrial use of the material present harmful effects on health. “Sulfuric acid is considered very toxic and may be fatal if inhaled or swallowed. It is corrosive to the eyes, skin, and respiratory tract, and exposure may cause blindness and permanent scarring.” –TR Lines 41-42
11. Is there an adverse effect on human health as defined by applicable Federal regulations? [205.600 b.3]		x		Not from dietary sources.
12. Is the substance GRAS when used according to FDA’s good manufacturing practices? [§205.600 b.5]	x			It is not clear from the TR that sulfuric acid is GRAS for the petitioned use; the TR does list a number of other GRAS uses (TR Lines 276-282)
13. Does the substance contain residues of heavy metals or other contaminants in excess of FDA tolerances? [§205.600		x		The petition and TR provide insufficient information to satisfy this criterion. “While residues and impurities (i.e., copper, iron,

b.5]				zinc, arsenic, mercury, lead, and selenium) have been reported in manufactured sulfuric acid product, no information was found to indicate the levels of these substances in sulfuric acid used for pH adjustment. Therefore it is unknown if these contaminants are in excess of FDA tolerances in sulfuric acid. " – TR Lines 318-321

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

NOSB Evaluation Criteria for Substances Added To the National List

Category 2. Is the Substance Essential for Organic Production? Substance: Sulfuric Acid

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Is the substance formulated or manufactured by a chemical process? [6502 (21)]	x			TR lines 262-263
2. Is the substance formulated or manufactured by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral, sources? [6502 (21)]		x		
3. Is the substance created by naturally occurring biological processes? [6502 (21)]		x		
4. Is there a natural source of the substance? [§205.600 b.1]		x		TR lines 268-269
5. Is there an organic substitute? [§205.600 b.1]		x		Because the manufacturing process is redacted from the petition, it is impossible to determine whether the use of other pH adjusters such as citric or lactic acid is viable or appropriate. TR lines 392-398
6. Is the substance essential for handling of organically produced agricultural products? [§205.600 b.6]		x		TR lines 392-398
7. Is there a wholly natural substitute product? [§6517 c (1)(A)(ii)]			x	Again, the petition and TR do not provide sufficient information to determine the necessity of the material, or if the resulting seaweed extract has undergone sufficient chemical change to be rendered synthetic.
8. Is the substance used in handling, not synthetic, but not organically produced? [§6517 c (1)(B)(iii)]		x		
9. Is there any alternative substances? [§6518 m.6]			x	
10. Is there another practice that would make the substance unnecessary? [§6518 m.6]			x	

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

NOSB Evaluation Criteria for Substances Added To the National List

Category 3. Is the substance compatible with organic production practices? Substance: Sulfuric Acid

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Is the substance compatible with organic handling? [§205.600 b.2]		x		
2. Is the substance consistent with organic farming and handling? [§6517 c (1)(A)(iii); 6517 c (2)(A)(ii)]		x		
3. Is the substance compatible with a system of sustainable agriculture? [§6518 m.7]		x		
4. Is the nutritional quality of the food maintained with the substance? [§205.600 b.3]			x	CBI redacted from petition makes it impossible to establish how the substance impacts the food.
5. Is the primary use as a preservative? [§205.600 b.4]		x		It is not clear that the petitioned use is as a preservative per se, but the TR notes a number of preservative uses of the substance (lines 288-298)
6. Is the primary use to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law, e.g., vitamin D in milk)? [205.600 b.4]		x		TR line 305
7. Is the substance used in production, and does it contain an active synthetic ingredient in the following categories: a. copper and sulfur compounds;			x	
b. toxins derived from bacteria;			x	
c. pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals?			x	
d. livestock parasiticides and medicines?			x	
e. production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners?			x	

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

NOSB Evaluation Criteria for Substances Added To the National List
Category 4. Is the commercial supply of an agricultural substance as organic, fragile or potentially unavailable? [§6610, 6518, 6519, 205.2, 205.105 (d), 205.600 (c) 205.2, 205.105 (d), 205.600 (c)]
Substance Name: Sulfuric Acid

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Is the comparative description provided as to why the non-organic form of the material /substance is necessary for use in organic handling?			x	
2. Does the current and historical industry information, research, or evidence provided explain how or why the material /substance cannot be obtained organically in the appropriate **form** to fulfill an essential function in a system of organic handling?			x	
3. Does the current and historical industry information, research, or evidence provided explain how or why the material /substance cannot be obtained organically in the appropriate **quality** to fulfill an essential function in a system of organic handling?			x	
4. Does the current and historical industry information, research, or evidence provided explain how or why the material /substance cannot be obtained organically in the appropriate **quantity** to fulfill an essential function in a system of organic handling?			x	
5. Does the industry information provided on material / substance non-availability as organic, include (but not limited to) the following: a. Regions of production (including factors such as climate and number of regions);			x	
b. Number of suppliers and amount produced;			x	
c. Current and historical supplies related to weather events such as hurricanes, floods, and droughts that may temporarily halt production or destroy crops or supplies;			x	
d. Trade-related issues such as evidence of hoarding, war, trade barriers, or civil unrest that may temporarily restrict supplies; or			x	
e. Are there other issues which may present a challenge to a consistent supply?			x	

Formal Recommendation
From: National Organic Standards Board (NOSB)
To: the National Organic Program (NOP)

Date: October 16, 2012

Subject: Petitioned to add sulfuric acid to §205.601

Chair: Barry Flamm

The NOSB hereby recommends to the NOP the following:

Rulemaking Action: ☐ Petition Failed

Guidance Statement: ☐

Other: ☐

Statement of Recommendation: (Motion # 1) Passed

Motion to classify sulfuric acid as synthetic.

Rationale Supporting Recommendation (including consistency with OFPA and NOP):

The starting point for commercial sulfuric acid manufacturing is sulfur dioxide, which is a byproduct of industrial pollution control systems. The manufacturing process involves a two-step chemical reaction using oxygen, water, and a vanadium oxide catalyst.

Committee Vote:

Moved: John Foster

Seconded: Colehour Bondera

Yes: 15 No: 0 Abstain: 0 Absent: 0 Recuse: 0

Statement of Recommendation: (Motion # 2) Failed

Motion to add sulfuric acid as petitioned to §205.601 for stabilization of digested poultry manure to a pH under 4.5 but not below 3.5.

Rationale Supporting Recommendation (including consistency with OFPA and NOP):

Sulfuric acid, when used in livestock manure, is changed to sulfate, which is in this case is a synthetically derived plant nutrient. The Board concurs with the Crops Subcommittee votes in 2012 and 2006 which recommended denying the petition because of adverse environmental and health impacts, lack of essentiality, and incompatibility with organic principles, as supported by the checklist.

Committee Vote:

Moved: John Foster

Seconded: Harold Austin

Yes: 3 No: 12 Abstain: 0 Absent: 0 Recuse: 0

National Organic Standards Board
Crops Subcommittee
Petitioned Material Proposal
Sulfuric Acid

August 7, 2012

Introduction:
A petition was submitted requesting the addition of sulfuric acid to the National List (7 CFR §205.601) for stabilization of digested poultry manure to a pH under 4.5 but not below 3.5.

Background:
In 2006, a similar petition was submitted for use in digested livestock manure. The Crops Committee voted unanimously to reject the petition because "Sulfuric acid, when used in livestock manure, is changed to sulfate, which is in this case a synthetically derived plant nutrient. Additionally, it is an important air pollutant, e.g. acid rain. Other wholly natural materials can be used." After some discussion by the NOSB at the October 18, 2006 meeting, and at the request of the petitioner, the vote on the petition was deferred.

Discussion:
The listing of sulfuric acid is not the only hurdle that petitioners need to clear in order to use their products. OMRI so far restricts the use of byproducts of anaerobic digestion of animal manures --used for generating methane-- to the uses allowed for raw manure. They say that these byproducts do not meet the NOP temperature and moisture criteria for processed manure. (They also do not contain the beneficial aerobic organisms that are an important benefit to the soil from composted manure.) Additional action by the NOSB and/or NOP will be needed to allow the full use of anaerobically-digested waste. (OMRI Materials Review, Summer 2012)

The Crops Subcommittee agrees with the 2006 vote and recommends denying the petition because of adverse environmental and health impacts, lack of essentiality, and incompatibility with organic principles, as supported by the checklist.

Evaluation Criteria:

(Applicability noted for each category; Documentation attached)	**Criteria Satisfied? (see "B" below)**		
1. Impact on Humans and Environment	☐ Yes	☒ No	☐ N/A
2. Essential & Availability Criteria	☐ Yes	☒ No	☐ N/A
3. Compatibility & Consistency	☐ Yes	☒ No	☐ N/A
4. Commercial Supply is Fragile or Potentially Unavailable as Organic (only for § 205.606)	☐ Yes	☐ No	☒ N/A

Substance Fails Criteria Category: [1,2,3]

Subcommittee Comments:
Adverse environmental and health impacts, lack of essentiality, and incompatibility with organic principles, as supported by the TR and checklist.

Recommended Subcommittee Action & Vote, including classification recommendation (state actual motion):

Classification Motion:
Sulfuric Acid is synthetic.

Motion by: Colehour Bondera Seconded by: Nick Maravell
Yes__**6**___ No__**0**__ Abstain__**0**__ Recuse__**0**__ Absent__**2**_

Listing Motion:
To list on §205.601, sulfuric acid for stabilization of digested poultry manure to a pH under 4.5 but not below 3.5.

Motion by: Harold Austin Second: Barry Flamm
Yes__**0**___ No__**6**__ Abstain__**0**__ Recuse__**0**__ Absent__**2**_

Crops	☒	**Agricultural**	☐	**Allowed**[1]	☐
Livestock	☐	**Non-synthetic**	☐	**Prohibited**[2]	☐
Handling	☐	**Synthetic**	☒	**Rejected**[3]	☒
No restriction	☐	**Commercial unavailable as organic**	☐	**Deferred**[4]	☐

[1]Substance voted to be added as “allowed” on National List to § 205. 601(h) with Annotation (if any):

[2]Substance to be added as “prohibited” on National List to § 205 with Annotation (if any):

Describe why a prohibited substance:

[3]Substance was rejected by vote for amending National List to § 205. Describe why material was rejected:

[4]Substance was recommended to be deferred because

If follow-up needed, who will follow up:

Approved by Subcommittee Chair to Transmit to NOSB

Jay Feldman, Subcommittee Chair August 7, 2012

NOSB Evaluation Criteria for Substances Added To the National List

Category 1. Adverse impacts on humans or the environment? Substance: Sulfuric Acid

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Are there adverse effects on environment from manufacture, use, or disposal? [§205.600 b.2]			X	
2. Is there environmental contamination during manufacture, use, misuse, or disposal? [§6518 m.3]	X			One of the primary sources of human sourced sulfuric acid in the environment is its manufacture. (TR, lines 187-191) According to the TRI, in 1996, releases of sulfuric acid to the air from 7 14 large processing facilities totaled 8,929,868 kg (19,690,359 pounds) (TR196 1998). (ATSDR, 1998. Toxicological Profile for Sulfur Trioxide and Sulfuric Acid. http://www.atsdr.cdc.gov/toxprofiles/tp117.pdf.)
3. Is the substance harmful to the environment? [§6517c(1)(A)(i);6517(c)(2)(A)i]	X			Sulfuric acid can kill organisms. Air borne sulfuric acid can cause pulmonary edema (TR lines 187-200 & lines 234-242 & lines 296-297) If sulfuric acid comes in contact with bodies of water the bioavailability of heavy metals increases. (Ostiguy). The International Agency for Cancer Research (IARC) has determined that there is sufficient evidence that occupational exposure to strong-inorganic-acid mists containing sulfuric acid is carcinogenic to humans (IARC 1992, 1997). (TR lines 313-316)
4. Does the substance contain List 1, 2, or 3 inerts? [§6517 c (1)(B)(ii); 205.601(m)2]		X		
5. Is there potential for detrimental chemical interaction with other materials used? [§6518 m.1]	X			Sulfuric acid can interact with other chemicals used if it comes in contact with the other materials. (TR lines 187-191) Sulfuric acid, when used as a pH adjustor for livestock manure, is changed to sulfate, which is plant nutrient. (TR lines 226-227) Sulfuric acid is corrosive..
6. Are there adverse biological and chemical interactions in agro-ecosystem? [§6518 m.5]		X		Sulfuric acid, when used in livestock manure, is changed to sulfate. (TR lines 226-227)..
7. Are there detrimental physiological effects on soil organisms, crops, or livestock? [§6518 m.5]		X		Sulfuric acid is corrosive and, at high concentrations, can kill organisms. No detrimental physiological effects on soil organisms, crops or livestock are expected for this usage. Detrimental impacts from manufacture, misuse, disposal. (TR lines 178-229)
8. Is there a toxic or other adverse action of the material or its breakdown products?[§6518 m.2]	X			Sulfuric acid is corrosive; it can harm eyes, skin, and respiratory and gastrointestinal tracts. (TR lines 294-308.) . The International Agency for Cancer Research (IARC) has determined that there is sufficient evidence that occupational exposure to strong-inorganic-acid mists containing sulfuric acid is carcinogenic to humans

				(IARC 1992, 1997). (TR lines 313-316)). (ATSDR, 1998. Toxicological Profile for Sulfur Trioxide and Sulfuric Acid. http://www.atsdr.cdc.gov/toxprofiles/tp117.pdf.)
9. Is there undesirable persistence or concentration of the material or breakdown products in environment?[§6518 m.2]	X			Sulfuric acid is not persistent. Its breakdown products are sulfate ions. It can persist in the environment if the soil is unable to neutralize it. (TR lines 330-341)..
10. Is there any harmful effect on human health? [§6517 c (1)(A)(i) ; 6517 c(2)(A)i; §6518 m.4]	X			Skin, eye respiratory and gastrointestinal tract irritation; EPA Category I toxicity; aerosol is a suspected human carcinogen (ACGIH); H_2SO_4 mist is a human carcinogen (IARC); protective clothing, eyewear & breathing protection are needed (TR lines 294-325). Sulfuric acid exposure also occurs when it is manufactured… The National Occupational Exposure Survey (NOES), conducted by NIOSH from 1981 to 1983, estimated that 56,103 and 775,348 U.S. workers may be exposed to sulfur trioxide and sulfuric acid, respectively (NOES 1990)."
11. Is there an adverse effect on human health as defined by applicable Federal regulations? [205.600 b.3]			X	
12. Is the substance GRAS when used according to FDA's good manufacturing practices? [§205.600 b.5]			X	
13. Does the substance contain residues of heavy metals or other contaminants in excess of FDA tolerances? [§205.600 b.5]			X	

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

Category 2. Is the Substance Essential for Organic Production? Substance _ Sulfuric Acid _

Question	Yes	No	N/A[1]	Documentation (TR; petition; regulatory agency; other)
1. Is there a natural source of the substance? [§205.600 b.1]			X	
2. Is there an organic substitute? [§205.600 b.1]			X	
3. Is the substance essential for handling of organically produced agricultural products? [§205.600 b.6]			X	
4. Is there a wholly natural substitute product? [§6517 c (1)(A)(ii)]	X			At present, the quantity of carbon material required to induce a significant pH decline is economically prohibitive. However, if the production of acid can be optimized, possibly by using suitable lactic acid bacteria, it would offer an effective and safe means to prevent ammonia production. (TR367-369) A variety of natural absorbents can be used to reduce ammonia production; some of the most commonly employed are peat and clinoptilolite (a naturally occurring alumino-silicate mineral with high cation exchange capacities). The advantages associated with the use of either clinoptilolite or peat are that they are nonhazardous and act as good soil conditioners when spread with manure. (TR371-374)
5. Is the substance used in handling, not synthetic, but not organically produced? [§6517 c (1)(B)(iii)]			X	
6. Is there any alternative substances? [§6518 m.6]	X			Unreacted carbon, citric acid, lactic acid bacteria or materials such as clay, peat, and clinoptilote. (TR lines 356-387).
7. Is there another practice that would make the substance unnecessary? [§6518 m.6]	X			Composting animal manure can also be used. Stabilization of animal manures can also be accomplish with unreacted carbon, lactic acid bacteria or materials such as clay, peat, and clinoptilote.(TR lines 356-387) Other types of approved composted materials and dehydrated manure can be used.). Hall and Sullivan (2001) provide a review of alternative soil amendments to agricultural fertilizers and manure, including several that can be considered wholly natural, such as various plant byproducts (e.g., composted leaves), rock and mineral powders (e.g., granite dust), and seaweed products. (TR382-387) As specified under NOP §205.203(b): “The producer must manage crop nutrients and soil fertility through rotations, cover crops, and the application of plant and animal materials.” Thus, the need to use manure (whether composted, non-composted, or chemically-treated) or plant materials could be replaced through crop rotation and

				use of cover crops. (TR 409-412)

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b)are N/A—not applicable.

Category 3. Is the substance compatible with organic production practices?

Substance __Sulfuric Acid __

Question	Yes	No	N/A[1]	Documentation (TR; petition; regulatory agency; other)
1. Is the substance compatible with organic handling? [§205.600 b.2]			X	
2. Is the substance consistent with organic farming and handling? [§6517 c (1)(A)(iii); 6517 c (2)(A)(ii)]	X			Sulfuric acid is the primary agent of acid rain, it is an air pollutant TR lines 46-50) Sulfuric acid, when used in livestock manure, is changed to sulfate, which is in this case a synthetically derived plant nutrient.TR lines 226-227). It has been allowed in similar uses for materials presently on the National List.
3. Is the substance compatible with a system of sustainable agriculture? [§6518 m.7]	X			Sulfuric acid is the primary agent of acid rain, it is an air pollutant TR lines 46-50) Sulfuric acid, when used in livestock manure, is changed to sulfate, which is in this case a synthetically derived plant nutrient. TR lines 226-227) It has been allowed in similar uses for materials presently on the National List.
4. Is the nutritional quality of the food maintained with the substance? [§205.600 b.3]			X	
5. Is the primary use as a preservative? [§205.600 b.4]			X	
6. Is the primary use to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law, e.g., vitamin D in milk)? [205.600 b.4]			X	
7. Is the substance used in production, and does it contain an active synthetic ingredient in the following categories: a. copper and sulfur compounds;	X			Sulfur compounds.
b. toxins derived from bacteria;		X		
c. pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals?		X		
d. livestock parasiticides and medicines?		X		

e. production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners?		X		

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

2006 recommendation: “Describe why material was rejected: Sulfuric acid, when used in animal manure, is changed to sulfate, which is in this case a synthetically derived plant nutrient. Additionally, it is an important air pollutant, e.g., acid rain. Other wholly natural materials can be used. (See Category 2, questions 4, 6, and 7.”

Sulfuric Acid
Handling

Identification of Petitioned Substance

Chemical Name:
Sulfuric acid

Other Names:
Dihydrogen sulfate
Hydrogen sulfate
Oil of vitriol
Battery acid
Dipping acid
Electrolyte acid
Matting acid

Trade Names:
None

CAS Number:
7664-93-9

Other Codes:
U.S. EPA PC Code: 078001
EC Number: 231-639-5
RTECS number: WS5600000
DOT number; corrosive material: UN 1830 137
OSHA IMIS Code Number: 2310

Characterization of Petitioned Substance

Composition of the Substance:
Sulfuric acid (H_2SO_4) is a strong acid that is highly soluble in water (HSDB, 2010). A molecule of sulfuric acid consists of two atoms of hydrogen, one atom of sulfur and four atoms of oxygen. The chemical structure of sulfuric acid is provided below as Figure 1.

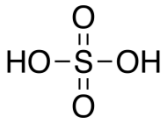

Figure 1. Chemical Structure of Sulfuric Acid (HSDB, 2010)

Properties of the Substance:
Pure H_2SO_4 is a solid with a melting point of 10.31°C (HSDB, 2010). In general, sulfuric acid is used in an aqueous solution and is a colorless to dark brown, oily, odorless liquid. While sulfuric acid itself is not flammable, contact with many organic and inorganic chemicals may cause fire or explosion and contact with metals liberates flammable hydrogen gas. When heated, sulfuric acid can decompose and form toxic gases such as sulfur oxides. Sulfuric acid is highly reactive in water, releasing toxic, corrosive, or flammable gases (HSDB, 2010; CCOHS, 1999).

Sulfuric acid is considered very toxic and may be fatal if inhaled or swallowed. It is corrosive to the eyes, skin, and respiratory tract, and exposure may cause blindness and permanent scarring. Some strong inorganic acid mists containing sulfuric acid are classified as carcinogenic (CCOHS, 1999). As described further under "Specific Uses of the Substance," sulfuric acid is manufactured in a variety of grades for use in a wide variety of applications (ATSDR, 1998).

Sulfuric acid is one of the primary chemical agents of "acid rain" (ATSDR, 2004). Because it is not very volatile, sulfuric acid from sources of air pollution are often found in the air as microscopic liquid droplets or are attached to other small particles in the air (HSDB, 2010). Atmospheric deposition of sulfuric acid from air pollution can lower the pH of surface waters and other environmental media and has a corrosive effect on living and nonliving components of the aquatic and terrestrial environments (USDA, 2006).

Physicochemical properties of sulfuric acid are provided in Table 1.

Table 1. Physical and Chemical Properties of Sulfuric Acid

Physical or Chemical Property	Value
Physical state	Solid below 10.5°C; prepared as aqueous solution
Appearance	Colorless to dark brown, oily
Odor	None
Taste	Marked acid taste
Molecular weight (g/mol)	98.1
Boiling point (°C)	337
Melting point (°C)	10.31
Solubility in water (mg/L at 25°C)	1×10^6; miscible
Corrosivity	Very corrosive
Vapor pressure (mm Hg at 25°C)	5.93×10^{-5}
Density (g/cm^3)	1.8302
pH (in solution of water)	1 N[a] sol. = 0.3; 0.1 N[a] sol. = 1.2; 0.01 N[a] sol. = 2.1

[a]N = normality; normality is equal to molarity multiplied by the valence (or ionic charge) of the anion or cation

Source: HSDB, 2010

Specific Uses of the Substance:

Non-food uses

In the United States, nearly 100 billion pounds of sulfuric acid is manufactured annually. Its production amount is nearly twice that of any other chemical. Sulfuric acid is sold or used commercially at varying concentrations, including technical grades (78–93%) and other grades (96, 98–99, and 100%). In these commercial products, impurities may include metals such as copper, iron, zinc, arsenic, mercury, lead, and selenium; sulfurous acid (as SO_2); nitrates; and chlorides (CCOHS, 1999). The four most common grades of sulfuric acid are commercial, electrolyte (high purity for batteries), textile (low organic content), and chemically pure or reagent grades (ATSDR, 1998). Commercial, electrolyte, textile, and reagent grades contain approximately 98%, 98%, 70%, and 95-98% sulfuric acid, respectively.

Nearly two thirds of the sulfuric acid produced in the United States is used in the manufacture of chemical fertilizers. For example, sulfuric acid is used to treat phosphate rock, an insoluble material containing phosphorous in the form of calcium phosphate (Stoker, 2007). The treatment of phosphate rock with sulfuric acid yields phosphorus acid in the following reaction:

$$Ca_3(PO_4)_2 + 3H_2SO_4 \longrightarrow 3CaSO_4 + 2H_3PO_4$$

The resulting phosphorus acid is used to produce soluble phosphate that acts as a source of phosphorus, which is necessary for plant growth (Stoker, 2007).

Sulfuric acid is also used in explosives, glue, dyestuffs, rayon, film, parchment paper, batteries, electronic chips, electroplating baths, nonferrous metallurgy, and ore processing (e.g., copper leaching). It can also be used to purify petroleum and to remove impurities from metals (i.e., pickling). In laboratories, sulfuric acid acts as a common reagent (ATSDR, 1998; HSDB, 2010). In many of these applications, the sulfuric acid is recovered and reused. There also are numerous household products (e.g., cleaners, detergents, rust dissolvers) that contain sulfuric acid (HHS, 2011).

Sulfuric acid is also considered a pesticide and is used in sprayable potato vine desiccant products. The use of potato vine desiccants benefits tuber appearance, limits tuber size, and improves tuber release from the vine at harvest (University of Florida, 2012).

Food Handling Uses

Sulfuric acid is considered a general purpose food additive and is used in the production of food acids (i.e., citric and lactic acids) and to directly control pH during the processing of foods (particularly packaged foods) and beverages, including seaweed extracts, alcoholic beverages, and cheeses. In the production of citric acid, calcium oxide is added to form an insoluble precipitate, calcium citrate. Citric acid is recovered by adding sulfuric acid to dissolve the precipitate (Kragl, 2005). A small amount of sulfuric acid is used in the production of high fructose corn syrup (Watson, 2002).

Sulfuric acid is used as a food additive to adjust the pH in order to create a more acidic environment that discourages the growth of bacteria and spoilage microbes. The use of sulfuric acid as a pH adjuster is a common practice in the processing of alcoholic beverages and cheese (Watson, 2002). Sulfuric acid washes or sprays are often applied to the surface of meat or poultry products to prevent the growth of spoilage microbes (FDA, 2011).

In its petition to the National Organic Program (NOP), Marinova (an Australian biotechnology company) described the use of sulfuric acid in the process of seaweed extraction. Specifically, sulfuric acid is used to adjust the pH of water used to extract fucoidans from brown algae or brown seaweed. Fucoidan is a sulfated polysaccharide that has been used as an ingredient in food supplements, function foods[1], beverages, and cosmetics. The manufacturer also claims that fucoidans have the ability to act as a viral attachment inhibitor, enzyme inhibitor, and receptor blocker, which makes them useful in many pharmaceutical and nutraceutical applications. The petitioner states that, "sulfuric acid does not impact on the seaweed extract, rather it is used solely as a processing aid," asserting that no residual sulfuric acid remains in the seaweed extraction product. In addition, the petitioner claims that liquid formulations would be overtaken by bacterial growth without this step to reduce pH (Marinova, 2010).

The petitioner manufactures seaweed extracts using sulfuric acid by a method it calls the Maritech® process. Marinova claims this method as proprietary and confidential business information because it was developed in-house by Marinova. Therefore the existence of any chemical changes that may occur during the production process is unknown. Marinova states that this method used to manufacture seaweed extracts is unique in the marketplace (Marinova, 2010).

Approved Legal Uses of the Substance:
Sulfuric acid is regulated as a pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (USEPA, 1993). It is exempt from the requirement of a tolerance for residues when used in accordance with good agricultural practices as a pH control agent in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest (HSDB, 2010). It is also exempt from the requirement of a tolerance for residues when used in accordance with good agricultural practice as an herbicide in the production of garlic and onions and as a potato vine desiccant in the production of potatoes (USEPA, 1993; HSDB, 2010).

Under the NOP Final Rule, sulfuric acid, along with phosphoric acid and citric acid, are approved for pH adjustment in liquid fish products, not to exceed the minimum needed to lower the pH to 3.5 (7 CFR 205.601(j)(7)). Sulfuric acid is petitioned to be used for the same function (i.e., pH adjustment) in the production of seaweed extracts, specifically fucoidans (Marinova, 2010). Sulfuric acid is not permitted in organic livestock production and organic handling/processing, and is not included on the National List at 205.603 and 205.605, respectively.

[1] A function food is a food where a new ingredient(s) (or more of an existing ingredient) has been added to a food and the new product has a new function (often one related to health-promotion or disease prevention) (IFIS, 2009).

Sulfuric acid is categorized by the U.S. Food and Drug Administration (FDA) as generally recognized as safe (GRAS) when used in food according to FDA's good manufacturing practices. According to 21 CFR 184.1095, sulfuric acid may be used as a pH control agent as defined in 21 CFR 170.3(o)(23)[2] and as a processing aid as defined in 21 CFR 170.3(o)(24)[3]. Sulfuric acid is allowed at a maximum amount of 0.014% in alcoholic beverages (as defined in 21 CFR 170.3[n][2]) and 0.0003% in cheeses (as defined in 21 CFR 170.3[n][5]). Sulfuric acid is regulated as a food additive used to manufacture modified hop extract (21 CFR 172.560[b][6]). It is also permitted as a pH reducer for modified food starch (21 CFR 172.892[a]). Additionally, sulfuric acid is permitted for use as an indirect food additive as a component of paper and paperboard in contact with dry food (21 CFR 176.180) and aqueous and fatty foods (21 CFR 176.170).

Action of the Substance:
Sulfuric acid is a strong acid that acts as a pH adjuster. The addition of sulfuric acid lowers the pH of a solution and prevents the growth of spoilage microbes or other bacteria.

Combinations of the Substance:
The process described by the petitioner for the manufacture of fucoidans using sulfuric acid as a handling and processing aid is considered confidential business information. Therefore no mixtures of sulfuric acid have been identified specifically for the petitioned use.

Mixtures of substances including sulfuric acid have been identified for use during common food production practices other than the petitioned use. For example, multiple mixtures have been identified for use in the processing of meat, poultry, and egg products, and these mixtures are primarily used to adjust or control the pH of water used in the processing. Aqueous solutions may combine sulfuric acid with a variety of other components, including copper sulfate, ammonium sulfate, water, sodium bisulfate, citric acid, phosphoric acid, or hydrochloric acid. Substances including peroxyacetic acid, hydrogen peroxide, acetic acid, and 1-hydroxyethylidene-1, 1-diphosphonic acid may be combined with sulfuric acid to create antimicrobial solutions. These antimicrobial mixtures may be added to process water or ice used for washing, rinsing, cooling, or processing whole or cut meat and poultry including parts, trim, and organs (FSIS, 2012).

Status

Historic Use:
Vitriols (i.e., acids, including sulfuric acid) were first discovered in ancient times, and the origin and properties of these substances were first explored by the Greeks. The contact process, the primary means of manufacturing sulfuric acid used in the production of seaweed extracts, was patented in 1831 by Peregrine Phillips (Friedman and Friedman, undated).

OFPA, USDA Final Rule:
Sulfuric acid is currently included on the National List of Allowed and Prohibited Substance (hereafter referred to as the National List) for pH adjustment in liquid fish products, not to exceed the minimum needed to lower the pH to 3.5 (7 CFR 205.601[j][7]). Sulfuric acid is not permitted in organic livestock production and organic handling/processing, and is not included on the National List at 205.603 and 205.605, respectively.

Sulfuric acid is petitioned to be used for pH adjustment in the production of seaweed extracts, specifically fucoidans, a product not included on the National List (Marinova, 2010).

[2] According to 21 CFR 170.3(o)(23), pH control agents are defined as substances added to change or maintain active acidity or basicity, including buffers, acids, alkalies, and neutralizing agents.
[3] According to 21 CFR 170.3(o)(24), a processing aids are defined as Substances used as manufacturing aids to enhance the appeal or utility of a food or food component, including clarifying agents, clouding agents, catalysts, flocculents, filter aids, and crystallization inhibitors, etc.

International:
The Canadian General Standards Board (CGSB) permits the use of fish emulsions to amend and improve soil fertility. Sulfuric acid can be used to adjust pH in liquid fish products, but the amount of acid used cannot exceed the minimum amount needed to lower the pH to 3.5 (CGSB, 2011).

The use of sulfuric acid in the production of organic sugar and gelatin products is permitted by the following international groups/agencies.

- The European Economic Community (EEC) (EEC 889/2008, 2008)
- The International Federation of Organic Agriculture Movements (IFOAM, 2008)
- The Codex Alimentarius Commission (Codex Alimentarius Commission, 2010)
- The Australian National Standard for Organic and Bio-dynamic Produce (AQIS, 2009)
- The Japan Agricultural Standard for Organic Production (JMAFF, 2006)

In 2008, the Australian Quarantine and Inspection Service ruled that fucoidans are to be considered a sugar-based product. Therefore, the use of sulfuric acid for fucoidan processing in Australia would be permitted (Marinova, 2010).

Evaluation Questions for Substances to be used in Organic Handling

Evaluation Question #1: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

Generally, sulfuric acid is manufactured by burning sulfur or a metallic sulfide in oxygen or air to create sulfur dioxide (SO_2), followed by the oxidation of SO_2 to sulfur trioxide (SO_3) and the addition of water to SO_3 to form sulfuric acid (H_2SO_4). There are two processes used to produce sulfuric acid—the nitration or "chamber" process and the catalytic or "contact" process (ATSDR, 1998; Friedman and Friedman, undated).

The chamber process was introduced in 1746 and is sometimes referred to as the nitration process because nitrogen compounds are used to improve the gas-phase reaction of sulfur dioxide with oxygen. The chemical reactions involved in the chamber process are complex and include formation of the intermediate nitrosylsulfuric acid (HNO_5S). This intermediate is then decomposed by water to form sulfuric acid and nitrogen oxide (NO). Nitrogen oxide is regenerated by oxygen or air to nitrogen dioxide (NO_2) and a combination of nitrogen compounds (NO and NO_2 or N_2O_3) is recycled to the sulfur dioxide oxidation step. While this process was once the primary method for sulfuric acid production, it has rarely been used in the United States and Western Europe after 1960 (ATSDR, 1998).

The contact process was first patented in 1831, but was not used to produce commercial quantities of sulfuric acid until the early 1900s. The principal steps in the contact process are: (1) oxidation of sulfur to SO_2 using dry air; (2) cooling of the gases; (3) conversion or oxidation of the SO_2 to SO_3; (4) cooling of the SO_3 gas; and (5) absorption of the SO_3 gas in water to produce sulfuric acid. A key component of the contact process is when sulfur dioxide is converted catalytically to sulfur trioxide. Acceptable catalysts include oxides of iron, chromium, copper, manganese, titanium, vanadium, and other metals (Friedman and Friedman, undated).

The basic three-step reaction used to produce sulfuric acid is shown below:

$$S + O_2 \rightarrow SO_2$$
$$2SO_2 + O_2 \rightarrow 2SO_3$$
$$SO_3 + H_2O \rightarrow H_2SO_4$$

The solution can be diluted with water to obtain the desired concentration of sulfuric acid (ATSDR, 1998).

Sulfuric acid can also be produced from sulfur dioxide collected by pollution control devices (scrubbers) during the smelting of various metal ores and ore concentrates. The sulfur dioxide is captured in the scrubbers to reduce emissions that would otherwise contribute to acid rain. The resulting "scrubber feedstock" is further purified, concentrated, and used for the subsequent production of sulfuric acid (USDA, 2006).

As described in Specific Uses of the Substance, the petitioner manufactures seaweed extracts using sulfuric acid by employing a method called the Maritech® process. The Maritech® process is a cold-water, ethanol-free process to extract fucoidans. This process does not degrade the product unlike alternative processes that are ethanol based. Marinova claims this method as proprietary and confidential business information because it was developed in-house by Marinova over multiple years. Marinova states that this method used to manufacture seaweed extracts is unique in the marketplace. The petitioner provides a Material Safety Data Sheet that specifies that sulfuric acid with a concentration of 50% is used in the Maritech® process (Marinova, 2010).

Evaluation Question #2: Is the substance synthetic? Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

Sulfuric acid, including food-grade sulfuric acid, is chemically synthesized. See Evaluation Question #1 for a description of its manufacturing processes.

Evaluation Question #3: Provide a list of non-synthetic or natural source(s) of the petitioned substance (7 CFR § 205.600 (b) (1)).

Sulfuric acid is chemically synthesized. See Evaluation Question #1 for a description of its manufacturing processes. Nonsynthetic forms of sulfuric acid are not commercially available.

Evaluation Question #4: Specify whether the petitioned substance is categorized as generally recognized as safe (GRAS) when used according to FDA's good manufacturing practices (7 CFR § 205.600 (b)(5)). If not categorized as GRAS, describe the regulatory status. What is the technical function of the substance?

Sulfuric acid is considered GRAS when used in food according to FDA's good manufacturing practices, which allows a maximum of 0.014% in alcoholic beverages (as defined in 21 CFR 170.3[n][2]) and 0.0003% in cheeses (as defined in 21 CFR 170.3[n][5]) (21 CFR 184.1095). Sulfuric acid is permitted for use as a food additive used to manufacture modified hop extract (21 CFR 172.560[b][6]). It is also permitted as a pH reducer for modified food starch (21 CFR 172.892[a]). Additionally, sulfuric acid is permitted for use as an indirect food additive as a component of paper and paperboard in contact with both dry food (21 CFR 176.180) and aqueous and fatty foods (21 CFR 176.170).

Evaluation Question #5: Describe whether the primary function/purpose of the petitioned substance is a preservative. If so, provide a detailed description of its mechanism as a preservative (7 CFR § 205.600 (b)(4)).

Sulfuric acid is not specifically added to food as a preservative. However, in its role as a pH reducer, sulfuric acid creates a more acidic environment that discourages the growth of bacteria and spoilage microbes (e.g., in alcoholic beverages, cheese) and helps maintain the quality of the food (Watson, 2002). Similarly, sulfuric acid washes or sprays are often applied to the surface of meat or poultry products to prevent the growth of spoilage.

Marinova's petition describes the use of sulfuric acid as a pH adjuster during the seaweed extraction process. Marinova asserts that the adjustment of pH is required for the prevention of the growth of spoilage bacteria in liquid formations (i.e., seaweed extraction water). The function of sulfuric acid as a preservative is never specifically discussed in the petition and specific details on the use of sulfuric acid in the manufacturing process are withheld as confidential business information (Marinova, 2010).

Evaluation Question #6: Describe whether the petitioned substance will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law) and how the substance recreates or improves any of these food/feed characteristics (7 CFR § 205.600 (b)(4)).

Sulfuric acid is not used to recreate or improve flavors, colors, textures, or nutritive values lost during processing.

Evaluation Question #7: Describe any effect or potential effect on the nutritional quality of the food or feed when the petitioned substance is used (7 CFR § 205.600 (b)(3)).

No information was found to indicate that sulfuric acid has any potential effect on the nutritional quality of food when used as a food processing and handling aid.

Evaluation Question #8: List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in the petitioned substance (7 CFR § 205.600 (b)(5)).

While residues and impurities (i.e., copper, iron, zinc, arsenic, mercury, lead, and selenium) have been reported in manufactured sulfuric acid product, no information was found to indicate the levels of these substances in sulfuric acid used for pH adjustment. Therefore it is unknown if these contaminants are in excess of FDA tolerances in sulfuric acid.

Evaluation Question #9: Discuss and summarize findings on whether the manufacture and use of the petitioned substance may be harmful to the environment or biodiversity (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

During the manufacturing of sulfuric acid, emissions of sulfuric acid may be released to the air. ATSDR affirms that sulfuric acid manufacturing facilities are among the primary sources of sulfuric acid releases to the air (ATSDR, 1998). In the air, some sulfuric acid reacts with other chemicals (e.g., ammonia, magnesium, calcium), which act to neutralize the acid. Sulfuric acid droplets and particles that are not neutralized may dissolve in clouds, fog, rain, or snow, resulting in very dilute acid solutions that could impact the environment as acid precipitation (ATSDR, 2004). Runoff containing wet and dry acid deposition may impact farming environments and ecosystems. Many lakes and streams examined in a National Surface Water Survey suffer from chronic acidity, a condition in which water has a consistently low pH level. Runoff may combine with existing sources of irrigation and cause contamination on farms. Acid rain causes a large number of effects that harm or kill individual fish, reduce fish population numbers, completely eliminate fish species from a water body, and decrease biodiversity. As lakes and streams become more acidic, the numbers and types of fish and other aquatic plants and animals that live in these waters decrease due to the interdependence of the entire ecosystem (USEPA, 2007). Acid deposition adds hydrogen ions to the soil, which displace nutrients including calcium, magnesium, and potassium. Ions are washed deeper into the subsoil or washed out of the top soil and this process called leaching. If ions are leached from the soil, they are no longer available to the roots of trees and plants and growth is prevented (Ophardt, 2003).

Sulfuric acid contributes to the formation of acid rain and is considered a regulatory and environmental concern.

For the extraction of fucoidan in seaweed, the petitioner uses sulfuric acid in small quantities to lower the pH of the extraction water. The petitioner states that the volume of sulfuric acid used is small (1% by weight; food grade sulfuric acid 50%) and the creation of vapors or mists containing sulfuric acid that could be released into the atmosphere is unlikely. Marinova also notes that the Maritech® process includes a neutralization step, which minimizes the release of sulfuric acid concentrations into the environment (Marinova, 2010).

Evaluation Question #10: Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

Sulfuric acid is very corrosive and irritating and can cause harmful effects on the skin, eyes, and respiratory and gastrointestinal tracts of humans (ATSDR, 1998). Exposure to sulfuric acid mist can irritate the eyes, nose, throat and lungs, and, at higher levels, can cause a buildup of fluid in the lungs (pulmonary edema) (ADEH, 2003). Although liquid sulfuric acid is not absorbed through the skin, it is a corrosive chemical that can severely burn unprotected skin and eyes, causing third degree burns and blindness on contact (ATSDR, 2004). Oral ingestion of concentrated sulfuric acid can burn the mouth, throat, and stomach, and can result in death (ATSDR, 2004). EPA has placed sulfuric acid in Toxicity Category I (on a scale of I to IV) for eye and dermal irritations as well as inhalation effects in humans; it is in Toxicity Category II for acute oral toxicity (USEPA, 1993).

The American Conference of Governmental Industrial Hygienists (ACGIH) has classified aerosol sulfuric acid as a suspected human carcinogen because it is carcinogenic in laboratory animals under conditions that are considered relevant to worker exposure (CCOHS, 2003). However, available human studies are considered conflicting or insufficient to confirm an increased risk of cancer in exposed humans. The International Agency for Cancer Research (IARC) has determined that there is sufficient evidence that occupational exposure to strong-inorganic-acid mists containing sulfuric acid is carcinogenic to humans (IARC, 1992). When working with sulfuric acid, it is advised that all workers use appropriate personal protective equipment, including protective gloves and eye protection to avoid dermal exposure and respiratory protection in cases where ventilation is inadequate (CCOHS, 2003).

There are no human dietary concerns from the use of sulfuric acid as a pesticide on potato vines (USEPA, 1993). For this use, sulfuric acid was granted an exemption from tolerance requirements because it "is rapidly degraded in the environment to sulfate salts, which are of no toxicological concern and are GRAS by the FDA." Sulfuric acid is also considered GRAS by FDA for its use as a food additive and processing aid (see Approved Legal Uses of the Substance and Evaluation Question #4).

In its petition, Marinova indicates that the sulfuric acid it uses as a processing aid for seaweed extraction products is neutralized to sulfate salts prior to isolation and purification of the extracts. Marinova asserts that no residual sulfuric acid is present in its final product (Marinova, 2010).

Evaluation Information #11: Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR § 205.600 (b)(1)).

No organic agricultural products have been identified as appropriate alternatives for sulfuric acid used in the production of seaweed extracts. The petitioner noted that citric and lactic acids have been used in the process of adjusting the pH of liquid formations produced in seaweed extraction, but concluded that their use was ineffective and impractical for fucoidan extraction (Marinova, 2010). The method used by Marinova is claimed as proprietary and confidential business information because it was developed in-house by Marinova over multiple years. Marinova states that this method used to manufacture seaweed extracts is unique in the marketplace.

References:

ADEH (Australia Department of Environment and Heritage). 2003. National pollutant inventory: Sulfuric acid. Retrieved February 8, 2012 from http://www.npi.gov.au/substances/sulfuric-acid/health.html

AQIS (Australian Quarantine and Inspection Service). 2009. National Standard for Organic and Bio-dynamic Produce (Ed 3.4). Retrieved January 19, 2012 from http://www.daff.gov.au/__data/assets/pdf_file/0018/126261/national-standard.pdf

ATSDR (Agency for Toxic Substances and Disease Registry). 1998. Toxicological profile for sulfur trioxide and sulfuric acid. Retrieved January 9, 2012 from http://www.atsdr.cdc.gov/toxprofiles/tp117.pdf

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. ToxFAQs™ for sulfur trioxide and sulfuric acid. Retrieved January 9, 2012 from http://www.atsdr.cdc.gov/tfacts117.html

CCOHS (Canadian Centre for Occupational Health and Safety). 1999. Basic information on sulfuric acid. Retrieved January 9, 2012 from http://www.ccohs.ca/oshanswers/chemicals/chem_profiles/sulfuric_acid/basic_sa.html

CCOHS (Canadian Centre for Occupational Health and Safety). 2003. Working safely with sulfuric acid. Retrieved February 8, 2012 from http://www.ccohs.ca/oshanswers/chemicals/chem_profiles/sulfuric_acid/working_sa.html

CGSB (Canadian General Standards Board). 2011. Organic Production Systems Permitted Substances List. CAN/CGSB-32.311-2006. Amended October 2008, December 2009, and June 2011. Retrieved April 5, 2012 from http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/norms-standards/internet/bio-org/documents/032-0311-2008-eng.pdf

Codex Alimentarius Commission. 2010. Guidelines for the Production, Processing, Labelling, and Marketing of Organically Produced Foods. GL-32-1999. Retrieved January 11, 2011 from http://www.codexalimentarius.net/web/more_info.jsp?id_sta=360

EEC (European Economic Community). 2008. Commission Regulation (EC) No 889/2008. Retrieved January 11, 2012 from http://eur-lex.europa.eu/JOHtml.do?uri=OJ:L:2008:250:SOM:EN:HTML

FDA (U.S. Food and Drug Administration). 2011. GRAS Notification for Sulfuric Acid and Sodium Sulfate Blend Used as an Anti- Microbial in Meat and Poultry Processing. Retrieved January 10, 2012 from http://www.accessdata.fda.gov/scripts/fcn/gras_notices/GRN000408.pdf

Friedman, L.J. and S.J. Friedman. Undated. The history of the contact sulfuric acid process. Acid Engineering and Consulting, Inc., Boca Raton: FL. Retrieved February 7, 2012 from http://www.aiche-cf.org/Clearwater/2008/Paper2/8.2.7.pdf

FSIS (Food Safety Inspection Services). 2012. FSIS Directive: Safe and suitable ingredients used in the production of meat, poultry, and egg products. Retrieved January 19, 2012 from http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1.pdf

HHS (U.S. Department of Health and Human Services). 2011. Household products database: Sulfuric acid. Retrieved January 19, 2012 from http://householdproducts.nlm.nih.gov/cgi-bin/household/brands?tbl=chem&id=1576&query=sulfuric+acid&searchas=TblChemicals

HSDB (Hazardous Substances Data Bank). 2010. Sulfuric acid. Retrieved January 9, 2012 from http://toxnet.nlm.nih.gov

IARC (International Agency for Research on Cancer). 1992. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 54: Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and Other Industrial Chemicals. Lyons, France.

IFOAM (International Federation of Organic Agriculture Movements). 2008. IFOAM Indicative List of Substances for Organic Production and Processing. Retrieved January 11, 2012 from http://www.ifoam.org/about_ifoam/standards/pdfs/20080423_IFOAM_Indicative_List.pdf

IFIS (International Food Information Service) 2009. IFIS Dictionary of Food Science and Technology. Wiley-Blackwell.

JMAFF (Japanese Ministry of Agriculture, Forestry and Fisheries). 2006. Japanese Agricultural Standard for Organic Processed Foods, Notification No.1464.

Kragl, U. 2005. Technology transfer in biotechnology. Springer, Berlin, Germany.

Marinova, 2010. Petition for non-agricultural substances allowed in or on processed products labeled as organic: Sulfuric acid (food grade 30-50%). Retrieved January 10, 2012 from http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5086279

OMRI (Organic Materials Review Institute). 2012. Online Database of OMRI Lists. Available at http://www.omri.org/omri-lists.

Ophardt, C.E. 2003. Elmhust College Virtual Chembook: Acid rain soil interactions. Retrieved April 30, 2012 from http://www.elmhurst.edu/~chm/vchembook/196soil.html

Stoker, S.H., 2007. General, organic, and biological chemistry. Houghton Mifflin Company, Boston, MA. Retrieved January 10, 2012 from http://books.google.com/books?id=6vy8MZeIcowC&pg=PA141&dq=sulfuric+acid,+fertilizer&hl=en&sa=X&ei=C1gMT-qsCYPMhAfom7nHBA&ved=0CE4Q6AEwAQ#v=onepage&q=sulfuric%20acid%2C%20fertilizer&f=false

University of Florida. 2012. Potato vine killing or desiccation. Retrieved April 27, 2012 from http://edis.ifas.ufl.edu/hs181

USDA (U.S. Department of Agriculture). 2006. Technical report for sulfuric acid, crop use. Retrieved January 10, 2012 from http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5057639

USEPA (U.S. Environmental Protection Agency). 1993. R.E.D. FACTS: Mineral Acids. EPA-738-F-93-025. Office of Prevention, Pesticides and Toxic Substances. Retrieved January 10, 2012 from http://www.epa.gov/oppsrrd1/REDs/factsheets/4064fact.pdf

USEPA (U.S. Environmental Protection Agency). 2007. Effects of Acid Rain. Retrieved February 8, 2012 from http://www.epa.gov/acidrain/effects/

Watson, D.H. 2002. Food Chemical Safety: Volume 2, additives. Woodhead Publishing Limited and CRC Press, Boca Raton, FL. Retrieved January 10, 2012 from http://books.google.com/books?id=Kpwqehy6hawC&pg=PA55&dq=sulfuric+acid,+food+additive&hl=en&sa=X&ei=uIQMT9fMMM6eOqXxpJYH&ved=0CE8Q6AEwAzgK#v=onepage&q=sulfuric%20acid%2C%20food%20additive&f=false

BioAtlantis

Nature working Naturally ™

BoroBee

A mannitol chelated boron for nutrient deficiencies

Batch No:

Net Contents: 1L

Net Weight: 1.18 kg

Specific Gravity: 1.18

Guaranteed minimum analysis:

Boron (B): 3%

Shake well before use

Manufactured by: BioAtlantis Ltd., Kerry Technology Park, Tralee, Co. Kerry, Ireland

Approval No.: IEC236629

Web: www.bioatlantis.com e-mail: info@bioatlantis.com

Tel: 00 353(0)66 7118477 Fax: 00 353(0)66 7119802

Application Rates and timings

Crops		Foliar application rates and timings	
	Rate (L/ha)	1st application	2nd application
Table Grape, Winegrapes	2	Apply at 10-30 cm shoot growth	Apply 14 days prior to bloom
Pears, Apple	1.5-2	Apply at tight cluster to first pink	Apply at start of flowering
Almonds	1.5-2	Apply at tight cluster to first pink	Apply at petal fall
Cherries	2	Apply at pink to open cluster	Repeat 14 days later
Stonefruits	0.75-1	Apply at pink bud stage	Apply at shuck on to shuck fall stage
Walnut, Pistachios, Pecan	0.75-1	Apply at the beginning of active growth	Apply at initial fruiting stage
Caneberries, Kiwis	1.5-2	Apply at 15-20 cm new growth	Apply just prior to bloom
Citrus	0.75	Apply at pre bloom	Apply at fruit set to fruit expansion stage
Strawberries	2	Apply first sign of bloom	Apply twice, at fruit set & fruit fill stage
Avocados	2-3	Apply at early bud development	Repeat 14 days later
Mangos	1.5-2	Apply at foliar bud swell stage	Apply at pre-flowering
Lychee	1.5-2	Apply at vegetative flush	Apply prior to flowering & early fruit set
Pineapple	2	Apply at pre-flowering	Apply twice, at fruit set & fruit finishing stage
Peppers, Capsicum, Chilli, Eggplant, Okra	2	Apply at first sign of bloom	Apply at fruit fill stage
Oilseed rape	1	Apply at 4 to 5 leaf stage	Apply at first sign of bloom
Wheat	1.5	Apply just before tillering & 14 days later	Apply at booting stage
Sunflower	2	Apply at 10 leaf stage	Apply at first sign of bloom
Potatoes	1.5-2	Apply at 6-8 leaf stage	Apply at tuber initiation stage
Lettuce, Spinach, Parsley, Celery	1	Apply at 2-3 true leaf unfolded stage	Apply at early rosette & 50% expected rosette diameter
Carrot, Beets, Ginger, Radish, Turnip	2	Apply at early root differentiation stage	Apply at active root bulking
Onions, Garlic, Shallots, Leeks	1-2	Apply at 2-3 leaf stage	Repeat 10 days later
Beans, Lentils, Peas, Soybean	2	Apply when side shoots develops	Apply at flower bud emergence stage
Cotton	2	Apply at early squaring	Apply at early flowering
Banana	2	Apply after 4-6 months of planting	Repeat one month later
Sugarcane	2	Apply at 3' to 4' plant height	Apply prior to canopy closure
Sugarbeets	1.5-2	Apply at 4-8 leaf stage	Apply 14 days later

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 1-2 litres BoroBee diluted in 250 litres of water per hectare are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis**. Mixing:** Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of BoroBee. Diluted solution of BoroBee should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: BoroBee is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41°F / 5°C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

Statement of warranty or Conditions for Sale: Seller's and Manufacture's obligation is limited to replacement of defective product. Neither seller nor manufacturer shall be liable for any injury, loss or damage directly or consequently arising out of the misuse or inability to use the product. Manufacturer or reseller makes no warranty, whether expressed or implied, concerning the use of this product other than for the purposes indicated on the label.

Application Rates and timings:

Crops		Foliar application rates and timings	
	Rate(Pints/acre)	1st application	2nd application
Table Grape, Winegrapes	2	Apply at 10-30 cm shoot growth	Apply 14 days prior to bloom
Pears, Apple	1.5-2	Apply at tight cluster to first pink	Apply at start of flowering
Almonds	1.5-2	Apply at tight cluster to first pink	Apply at petal fall
Cherries	2	Apply at pink to open cluster	Repeat 14 days later
Stonefruits	0.75-1	Apply at pink bud stage	Apply at shuck on to shuck fall stage
Walnut, Pistachios, Pecan	0.75-1	Apply at the beginning of active growth	Apply at initial fruiting stage
Caneberries, Kiwis	1.5-2	Apply at 15-20 cm new growth	Apply just prior to bloom
Citrus	0.75	Apply at pre bloom	Apply at fruit set to fruit expansion stage
Strawberries	2	Apply first sign of bloom	Apply twice, at fruit set & fruit fill stage
Avocados	2-3	Apply at early bud development	Repeat 14 days later
Mangos	1.5-2	Apply at foliar bud swell stage	Apply at pre-flowering
Lychee	1.5-2	Apply at vegetative flush	Apply prior to flowering & early fruit set
Pineapple	2	Apply at pre-flowering	Apply twice, at fruit set & fruit finishing stage
Peppers, Capsicum, Chilli, Eggplant, Okra	2	Apply at first sign of bloom	Apply at fruit fill stage
Oilseed rape	1	Apply at 4 to 5 leaf stage	Apply at first sign of bloom
Wheat	1.5	Apply just before tillering & 14 days later	Apply at booting stage
Sunflower	2	Apply at 10 leaf stage	Apply at first sign of bloom
Potatoes	1.5-2	Apply at 6-8 leaf stage	Apply at tuber initiation stage
Lettuce, Spinach, Parsley, Celery	1	Apply at 2-3 true leaf unfolded stage	Apply at early rosette & 50% expected rosette diameter
Carrot, Beets, Ginger, Radish, Turnip	2	Apply at early root differentiation stage	Apply at active root bulking
Onions, Garlic, Shallots, Leeks	1-2	Apply at 2-3 leaf stage	Repeat 10 days later
Beans, Lentils, Peas, Soybean	2	Apply when side shoots develops	Apply at flower bud emergence stage
Cotton	2	Apply at early squaring	Apply at early flowering
Banana	2	Apply after 4-6 months of planting	Repeat one month later
Sugarcane	2	Apply at 3' to 4' plant height	Apply prior to canopy closure
Sugarbeets	1.5-2	Apply at 4-8 leaf stage	Apply 14 days later

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 2-3 pints Iron edge diluted in 25 gallons of water per acre are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of BoroBee. Diluted solution of BoroBee should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: BoroBee is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41°F / 5°C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

Statement of warranty or Conditions for Sale: Seller's and Manufacture's obligation is limited to replacement of defective product. Neither seller nor manufacturer shall be liable for any injury, loss or damage directly or consequently arising out of the misuse or inability to use the product. Manufacturer or reseller makes no warranty, whether expressed or implied, concerning the use of this product other than for the purposes indicated on the label.

BioAtlantis

Nature working Naturally ™

MagniGreen

An amino acid complexed magnesium for nutrient deficiencies

Batch No:

Net Contents: 1L

Net Weight: 1.26 kg

Specific Gravity: 1.26

Guaranteed minimum analysis:

Magnesium (Mg): 7%

Shake well before use

Manufactured by: BioAtlantis Ltd., Kerry Technology Park, Tralee, Co. Kerry, Ireland

Approval No.: IEC236629

Web: www.bioatlantis.com e-mail: info@bioatlantis.com

Tel: 00 353(0)66 7118477 Fax: 00 353(0)66 7119802

Application Rates and timings

Crops		Foliar application rates and timings	
	Rate (L/ha)	1st application	2nd application
Apple	2-3	Apply at fruit development stage	Repeat 7 days later
Kiwis	2-3	Apply at 10-30 cm shoot growth	Apply when flower buds are just visible
Avocados	3	Apply at early new growth	Repeat 14 days later
Mangoes	3	Apply at early new growth	Repeat 14 days later
Lychee	1.5	Apply at early flush & 14 days later	Apply at fruit set, repeat twice at monthly intervals.
Pineapple	3	Apply at pre-flowering & Early flowering	Twice, post-harvest at 14 days intervals (optional)
Tea	2	Apply at early season growth	Twice, at mid-season at monthly intervals.
Rice	3	Apply at tillering stage	Apply at stem elongation stage
Tomatoes	3	Apply at 4-5 leaf stage	Apply at first sign of bloom (1.5L/ha)
Peppers, Capsicum, Chilli, Eggplant, Okra	3	Apply at 4-5 leaf stage	Apply at first sign of bloom (1.5L/ha)
Wheat	3	Apply at active tillering stage	Repeat 10-15 days later
Leaf lettuce, Spinach, Parsely, Celery	3	Apply at 2-3 true leaf unfolded stage	Twice, at early rosette & 50% expected rosette diameter
Head lettuce	3	Apply at 2.3l true leaf unfolded stage	Twice, at early rosette & early head formation
Cabbage, Broccoli, Kale, Cauliflower	3	Apply at 9 leaf stage	Apply at early head formation stage
Cucumber, Squash, Pumpkin, Melons	3	Apply at pre-flowering	Apply at early fruit expansion stage
Onions, Garlic, Shallots, Leeks	2	Apply at 2.3 leaf stage & 10 days later	Apply at early bulb development stage
Alfalfa, Sod production, Pasture, Forage	2-3	Apply after each cutting to new re-growth	Apply when plants reach 6-8 inch height
Sugarcane	4	Apply when plant height is 3 to 4 inch	Repeat 20 days later
Sugarbeets	3	Apply at 4.8 leaf stage	Apply at 9 or more leaf stage
Cotton	2	Apply at 4-5 leaf stage	
Banana	2	Apply during magnesium deficiency	
Turfs	2	Apply at monthly intervals during spring and summer periods	

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 2-3 litres MagniGreen diluted in 250 litres of water per hectare are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis

Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of MagniGreen. Diluted solution of MagniGreen should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: MagniGreen is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41°F / 5°C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

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Application Rates and timings:

Crops		Foliar application rates and timings	
	Rate(Pints/acre)	1st application	2nd application
Apple	2-3	Apply at fruit development stage	Repeat 7 days later
Kiwis	2-3	Apply at 10-30 cm shoot growth	Apply when flower buds are just visible
Avocados	3	Apply at early new growth	Repeat 14 days later
Mangoes	3	Apply at early new growth	Repeat 14 days later
Lychee	1.5	Apply at early flush & 14 days later	Apply at fruit set, repeat twice at monthly intervals.
Pineapple	3	Apply at pre-flowering & Early flowering	Twice, post-harvest at 14 days intervals (optional)
Tea	2	Apply at early season growth	Twice, at mid-season at monthly intervals.
Rice	3	Apply at tillering stage	Apply at stem elongation stage
Tomatoes	3	Apply at 4-5 leaf stage	Apply at first sign of bloom (1.5L/ha)
Peppers, Capsicum, Chilli, Eggplant, Okra	3	Apply at 4-5 leaf stage	Apply at first sign of bloom (1.5L/ha)
Wheat	3	Apply at active tillering stage	Repeat 10-15 days later
Leaf lettuce, Spinach, Parsely, Celery	3	Apply at 2-3 true leaf unfolded stage	Twice, at early rosette & 50% expected rosette diameter
Head lettuce	3	Apply at 2.3l true leaf unfolded stage	Twice, at early rosette & early head formation
Cabbage, Broccoli, Kale, Cauliflower	3	Apply at 9 leaf stage	Apply at early head formation stage
Cucumber, Squash, Pumpkin, Melons	3	Apply at pre-flowering	Apply at early fruit expansion stage
Onions, Garlic, Shallots, Leeks	2	Apply at 2.3 leaf stage & 10 days later	Apply at early bulb development stage
Alfalfa, Sod production, Pasture, Forage	2-3	Apply after each cutting to new re-growth	Apply when plants reach 6-8 inch height
Sugarcane	4	Apply when plant height is 3 to 4 inch	Repeat 20 days later
Sugarbeets	3	Apply at 4.8 leaf stage	Apply at 9 or more leaf stage
Cotton	2	Apply at 4-5 leaf stage	
Banana	2	Apply during magnesium deficiency	
Turfs	2	Apply at monthly intervals during spring and summer periods	

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 2-3 pints MagniGreen diluted in 25 gallons of water per acre are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of MagniGreen. Diluted solution of MagniGreen should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: MagniGreen is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41^{o}F / 5^{o}C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

BioAtlantis

Nature working Naturally ™

AtlantiCal

An amino acid complexed calcium for nutrient deficiencies

Batch No:

Net Contents: 1L

Net Weight: 1.19 kg

Specific Gravity: 1.19

Guaranteed minimum analysis:

Calcium (Ca): 7%

Shake well before use

Manufactured by: BioAtlantis Ltd., Kerry Technology Park, Tralee, Co. Kerry, Ireland

Approval No.: IEC236629

Web: www.bioatlantis.com e-mail: info@bioatlantis.com

Tel: 00 353(0)66 7118477 Fax: 00 353(0)66 7119802

APPLICATION RATES AND TIMINGS:

Crops		Foliar application rates and timings	
	Rate (L/ha)	1st application	2nd application
Table Grapes	3	Apply at fruit set	Apply twice, at pea size berry stage & 7 days later
Wine Grapes	2	Apply when active growth begins	Repeat at weekly intervals at the vegetative period
Pears	2-4	Apply twice at pre-flowering & petal fall	Apply at fruit set
Cherries,	3-5	Apply at straw colour fruit stage	Repeat thrice before harvest
Plums, Peaches, Nectarines, Apricots, Prunes	3	Apply at stone hardening stage	Apply twice, at fruit filling stage and 7 days later
Walnut, Pistachios, Pecan	3-4	Apply at bud break	Repeat in 4 weeks
Apple	3-5	Apply when active growth begins (1.5L)	Apply thrice at fruit development stage at 7 days intervals
Blueberry, Current, Gooseberry, Citrus	3-4	Apply at early spring new growth	Repeat twice at 7 days intervals before flowering
Kiwis	3	Apply 30 days before bud break	Apply twice at 14 days after fruit set and 7 days later
Strawberries	3	Apply at first sign of bloom (1.5L)	Apply twice, at fruit set and fruit fill stage
Avocados	3	Apply at new flush	Apply twice, at fruit set and fruit fill stage
Mangoes, Lychee	2-3	Apply 10-14 days prior to bloom	Apply twice, at fruit set and fruit bulking
Tomatoes (Fresh, process & canning)	2-3	Apply at early fruit set	Repeat 14 days later
Peppers, Capsicum, Chili, Egg Plant, Okra	3	Apply at early fruit set	Repeat 14 days later
Potatoes	3	Apply at 4-6 leaf stage	Repeat 14 days later
Head lettuce	2	Apply at early leaf rosette stage	Apply at early head formation
Cabbage, Broccoli, Kale, Cauliflower	2	Apply at early head formation	Apply at full head stage
Cucumber, Squash, Pumpkin, Melons	2-3	Apply at pre-flowering stage	Apply at early fruit expansion stage
Carrot, Beets, Ginger, Radish, Turnip	2	Apply at active root bulking stage	Repeat 7 days later
Onions, Garlic, Shallots, Leeks	2	Apply at bulb development stage	Repeat 7 days later
Alfalfa, Sod production, Pasture, Forage	1.5	Apply at each cutting to new re-growth	Apply when plants reach 6-8 inch
Cotton	2-3	Apply at later flowering stage	Apply at ball set
Carrot, Beets, Ginger, Radish, Turnip	2	Apply at early leaf stage	Apply at mid vegetative growth
Onions, Garlic, Shallots, Leeks	1.5	Apply at 2.3 leaf stage	Repeat 10 days later
Beans, Lentils, Peas, Soybean	1.5	Apply 4-6 leaf stage	Apply just before flowering
Cotton	1.5	Apply at 4-5 leaf stage	Early squaring stage
Turfs	3-4	Apply at monthly intervals during spring and summer periods.	

Application recommendations: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 1.5-2.0 litres AtlantiCal diluted in 250 litres of water per hectare are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of AtlantiCal. Diluted solution of AtlantiCal should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: AtlantiCal is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41^{o}F / 5^{o}C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

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APPLICATION RATES AND TIMINGS:

Crops		Foliar application rates and timings	
	Rate (Pints/acre)	1st application	2nd application
Table Grapes	3	Apply at fruit set	Apply twice, at pea size berry stage & 7 days later
Wine Grapes	2	Apply when active growth begins	Repeat at weekly intervals at the vegetative period
Pears	2-4	Apply twice at pre-flowering & petal fall	Apply at fruit set
Cherries,	3-5	Apply at straw colour fruit stage	Repeat thrice before harvest
Plums, Peaches, Nectarines, Apricots, Prunes	3	Apply at stone hardening stage	Apply twice, at fruit filling stage and 7 days later
Walnut, Pistachios, Pecan	3-4	Apply at bud break	Repeat in 4 weeks
Apple	3-5	Apply when active growth begins (1.5L)	Apply thrice at fruit development stage at 7 days intervals
Blueberry, Current, Gooseberry, Citrus	3-4	Apply at early spring new growth	Repeat twice at 7 days intervals before flowering
Kiwis	3	Apply 30 days before bud break	Apply twice at 14 days after fruit set and 7 days later
Strawberries	3	Apply at first sign of bloom (1.5L)	Apply twice, at fruit set and fruit fill stage
Avocados	3	Apply at new flush	Apply twice, at fruit set and fruit fill stage
Mangoes, Lychee	2-3	Apply 10-14 days prior to bloom	Apply twice, at fruit set and fruit bulking
Tomatoes (Fresh, process & canning)	2-3	Apply at early fruit set	Repeat 14 days later
Peppers, Capsicum, Chili, Egg Plant, Okra	3	Apply at early fruit set	Repeat 14 days later
Potatoes	3	Apply at 4-6 leaf stage	Repeat 14 days later
Head lettuce	2	Apply at early leaf rosette stage	Apply at early head formation
Cabbage, Broccoli, Kale, Cauliflower	2	Apply at early head formation	Apply at full head stage
Cucumber, Squash, Pumpkin, Melons	2-3	Apply at pre-flowering stage	Apply at early fruit expansion stage
Carrot, Beets, Ginger, Radish, Turnip	2	Apply at active root bulking stage	Repeat 7 days later
Onions, Garlic, Shallots, Leeks	2	Apply at bulb development stage	Repeat 7 days later
Alfalfa, Sod production, Pasture, Forage	1.5	Apply at each cutting to new re-growth	Apply when plants reach 6-8 inch
Cotton	2-3	Apply at later flowering stage	Apply at ball set
Carrot, Beets, Ginger, Radish, Turnip	2	Apply at early leaf stage	Apply at mid vegetative growth
Onions, Garlic, Shallots, Leeks	1.5	Apply at 2.3 leaf stage	Repeat 10 days later
Beans, Lentils, Peas, Soybean	1.5	Apply 4-6 leaf stage	Apply just before flowering
Cotton	1.5	Apply at 4-5 leaf stage	Early squaring stage
Turfs	3-4	Apply at monthly intervals during spring and summer periods.	

Application recommendations: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 1.0-1.5 pint AtlantiCal diluted in 25 gallons of water per acre are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of AtlantiCal. Diluted solution of AtlantiCal should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: AtlantiCal is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41^{o}F / 5^{o}C and away from direct sunlight. **CAUTION**: Keep out of reach of children. **Statement of warranty or Conditions for Sale: Seller's** and Manufacture's obligation is limited to replacement of defective product. Neither seller nor manufacturer shall be liable for any injury, loss or damage directly or consequently arising out of the misuse or inability to use the product. Manufacturer or reseller makes no warranty, whether expressed or implied, concerning the us of this product other than for the purposes indicated on the label.

BioAtlantis

Nature working Naturally ™

MangaMax

An amino acid chelated manganese for nutrient deficiencies

Batch No:

Net Contents: 1L

Net Weight: 1.24 kg

Specific Gravity: 1.24

Guaranteed minimum analysis:

Manganese (Mn): 7%

Shake well before use

Manufactured by: BioAtlantis Ltd., Kerry Technology Park, Tralee, Co. Kerry, Ireland

Approval No.: IEC236629

Web: www.bioatlantis.com e-mail: info@bioatlantis.com

Tel: 00 353(0)66 7118477 Fax: 00 353(0)66 7119802

Application Rates and timings for crops: MangaMax can be used on Fruit crops, Tree nuts, Field crops, Cole Crops, Cucurbits, legumes, Pulses, leafy vegetable, Fruiting vegetables, tuber, root & corm vegetable, Green house and Shade house crops and Grasses to prevent or correct copper deficiencies that may reduce crop growth and yield. Make the application during active growth or nutritional stress. Application may be repeated twice, every 14 days during the vegetative growth period. Apply 0.5 L to 1L per hectare.

Application Rates and timings on specific crops

Crops		Foliar application rates and timings	
	Rate (L/ha)	1st application	2nd application
Kiwis	2-3	Apply at 10-30 cm shoot growth	Apply when flower buds are just visible
Strawberries	2	Apply at first sign of bloom	Repeat 14 days later
Avocados	2-3	Apply at early spring flush	Repeat one month later
Barley, Rye, Sorghum, Oats	2	Apply at early tillering & 15 days later	Apply at boot stage
Peppers, Capsicum, Chili, Eggplant, Okra	2	Apply at first sign of bloom	Apply at fruit fill stage
Maize and Sweet corn	1-2	Apply at 6-8 leaf stage and 10 days later	Apply at early silk stage
Alfalfa, Sod production, Pasture, Forage	1-2	Apply after each cutting to new re-growth	Apply when plants reach 6-8 inch height
Sugarbeets	2	Apply at 4-8 leaf stage	Apply at 9 or more leaf stage
Pears, Apple, Citrus	2-3	Apply at spring during flush of new growth	

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 2-3 litres MangaMax diluted in 250 litres of water per hectare are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis

Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of MangaMax. Diluted solution of MangaMax should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: MangaMax is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41^{o}F / 5^{o}C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

Statement of warranty or Conditions for Sale: Seller's and Manufacture's obligation is limited to replacement of defective product. Neither seller nor manufacturer shall be liable for any injury, loss or damage directly or consequently arising out of the misuse or inability to use the product. Manufacturer or reseller makes no warranty, whether expressed or implied, concerning the use of this product other than for the purposes indicated on the label.

Application Rates and timings for crops: MangaMax can be used on Fruit crops, Tree nuts, Field crops, Cole Crops, Cucurbits, legumes, Pulses, leafy vegetable, Fruiting vegetables, tuber, root & corm vegetable, Green house and Shade house crops and Grasses to prevent or correct copper deficiencies that may reduce crop growth and yield. Make the application during active growth or nutritional stress. Application may be repeated twice, every 14 days during the vegetative growth period. Apply 0.5 L to 1L per hectare.

Specific crop Application Rates and timings

Crops		**Foliar application rates and timings**	
	Rate (Pints/acre)	**1st application**	**2nd application**
Kiwis	2-3	Apply at 10-30 cm shoot growth	Apply when flower buds are just visible
Strawberries	2	Apply at first sign of bloom	Repeat 14 days later
Avocados	2-3	Apply at early spring flush	Repeat one month later
Barley, Rye, Sorghum, Oats	2	Apply at early tillering & 15 days later	Apply at boot stage
Peppers, Capsicum, Chili, Eggplant, Okra	2	Apply at first sign of bloom	Apply at fruit fill stage
Maize and Sweet corn	1-2	Apply at 6-8 leaf stage and 10 days later	Apply at early silk stage
Alfalfa, Sod production, Pasture, Forage	1-2	Apply after each cutting to new re-growth	Apply when plants reach 6-8 inch height
Sugarbeets	2	Apply at 4-8 leaf stage	Apply at 9 or more leaf stage
Pears, Apple, Citrus	2-3	Apply at spring during flush of new growth	

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 2-3 pints MangaMax diluted in 25 gallons of water per acre are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of MangaMax. Diluted solution of MangaMax should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: MangaMax is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41^{o}F / 5^{o}C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

Statement of warranty or Conditions for Sale: Seller's and Manufacture's obligation is limited to replacement of defective product. Neither seller nor manufacturer shall be liable for any injury, loss or damage directly or consequently arising out of the misuse or inability to use the product. Manufacturer or reseller makes no warranty, whether expressed or implied, concerning the use of this product other than for the purposes indicated on the label.

BioAtlantis

Nature working Naturally ™

IronEdge

An amino acid chelated iron for nutrient deficiencies

Batch No:

Net Contents: 1L

Net Weight: 1.19 kg

Specific Gravity: 1.19

Guaranteed minimum analysis:

Iron (Fe): 7%

Shake well before use

Manufactured by: BioAtlantis Ltd., Kerry Technology Park, Tralee, Co. Kerry, Ireland

Approval No.: IEC236629

Web: www.bioatlantis.com e-mail: info@bioatlantis.com

Tel: 00 353(0)66 7118477 Fax: 00 353(0)66 7119802

Application Rates and timings for crops: IronEdge can be used on Fruit crops, Tree nuts, Field crops, Cole Crops, Cucurbits, legumes, Pulses, leafy vegetable, Fruiting vegetables, tuber, root & corm vegetable, Green house and Shade house crops and Grasses to prevent or correct iron deficiencies that may reduce crop growth and yield. Make the application during active growth or nutritional stress. Application may be repeated twice, every 14 days during the vegetative growth period. Apply 0.5 L to 1L per hectare.

Application Rates and timings for specific crops:

Crops		**Foliar application rates and timings**	
	Rate (L/ha)	**1st application**	**2nd application**
Almonds	2-3	Apply at early nut development stage	Apply at hull split
Barley, Rye, Sorghum, Oats	3	Apply at early tillering and 15 days later	Apply at booting stage
Wheat	2-3	Apply just before tillering & post tillering	Apply at booting stage
Maize & Sweet corn	2-3	Apply at 6-8 leaf stage & 10 days later	Apply at early silk stage
Beans, Lentils, Peas, Soybean	2	Apply 4-6 leaf stage	Apply just before flowering
Cotton	3	Apply at 4-5 leaf stage	
Turfs	2	Apply at monthly intervals during spring, summer and strong winter months.	

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 2-3 litres IronEdge diluted in 250 litres of water per hectare are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis

Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of IronEdge. Diluted solution of IronEdge should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: IronEdge is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41^{o}F / 5^{o}C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

Application Rates and timings for crops: IronEdge can be used on Fruit crops, Tree nuts, Field crops, Cole Crops, Cucurbits, legumes, Pulses, leafy vegetable, Fruiting vegetables, tuber, root & corm vegetable, Green house and Shade house crops and Grasses to prevent or correct iron deficiencies that may reduce crop growth and yield. Make the application during active growth or nutritional stress. Application may be repeated twice, every 14 days during the vegetative growth period. Apply 0.5 pint to 1 pint per acre.

Application Rates and timings for specific crops:

Crops		**Foliar application rates and timings**	
	Rate (Pints/acre)	**1st application**	**2nd application**
Almonds	2-3	Apply at early nut development stage	Apply at hull split
Barley, Rye, Sorghum, Oats	3	Apply at early tillering and 15 days later	Apply at booting stage
Wheat	2-3	Apply just before tillering & post tillering	Apply at booting stage
Maize & Sweet corn	2-3	Apply at 6-8 leaf stage & 10 days later	Apply at early silk stage
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Turfs	2	Apply at monthly intervals during spring, summer and strong winter months.	

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 2-3 pints Iron edge diluted in 25 gallons of water per acre are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of IronEdge. Diluted solution of IronEdge should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

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BioAtlantis

Nature working Naturally ™

CopperFasten

An amino acid chelated copper for nutrient deficiencies

Batch No:

Net Contents: 1L

Net Weight: 1.22 kg

Specific Gravity: 1.22

Guaranteed minimum analysis:

Copper (Cu): 7%

Shake well before use

Manufactured by: BioAtlantis Ltd., Kerry Technology Park, Tralee, Co. Kerry, Ireland

Approval No.: IEC236629

Web: www.bioatlantis.com e-mail: info@bioatlantis.com

Tel: 00 353(0)66 7118477 Fax: 00 353(0)66 7119802

Application Rates and timings for crops: CopperFasten can be used on Fruit crops, Tree nuts, Field crops, Cole Crops, Cucurbits, legumes, Pulses, leafy vegetable, Fruiting vegetables, tuber, root & corm vegetable, Green house and Shade house crops and Grasses to prevent or correct copper deficiencies that may reduce crop growth and yield. Make the application during active growth or nutritional stress. Application may be repeated twice, every 14 days during the vegetative growth period. Apply 0.5 L to 1L per hectare.

Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 0.5 litre CopperFasten diluted in 250 litres of water per hectare are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis

Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of CopperFasten. Diluted solution of CopperFasten should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: CopperFasten is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

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Directions: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 0.5 pints CopperFasten diluted in 25 gallons of water per acre are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of CopperFasten. Diluted solution of CopperFasten should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

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BioAtlantis

Nature working Naturally ™

ZenMaster

An amino acid chelated zinc for nutrient deficiencies

Batch No:

Net Contents: 1L

Net Weight: 1.22 kg

Specific Gravity: 1.22

Guaranteed minimum analysis:

Zinc (Zn): 7%

Shake well before use

Manufactured by: BioAtlantis Ltd., Kerry Technology Park, Tralee, Co. Kerry, Ireland

Approval No.: IEC236629

Web: www.bioatlantis.com e-mail: info@bioatlantis.com

Tel: 00 353(0)66 7118477 Fax: 00 353(0)66 7119802

APPLICATION RATES AND TIMINGS:

Crops		Foliar application rates and timings	
	Rate (L/ha)	1st application	2nd application
Table Grapes, Wine Grapes	2	Apply at 10-30 cm new shoot growth	Repeat 14 days later, before flowering
Pears	2	Apply at green tip to half inch green	Apply before leaf senescence after harvest
Almonds	1	Apply at pre-bloom	At petal fall
Cherries, Plums, Peaches, Nectarines, Apricots, Prunes	2	Apply at pink to open cluster stage	Repeat 7 days after first application
Walnut, Pistachios	2	Apply at mid to late spring flush	At petal fall
Apple	1-2	Apply 10-14 days prior to bloom	At petal fall and 2 weeks later
Blueberry, Current, Gooseberry	2	Apply 4 weeks Prior to bloom	Apply 2 weeks prior to bloom
Caneberries	1.5	Apply at 15-20 cm new growth	Just prior to bloom
Citrus	1	Apply 1—14 days prior to bloom	At petal fall and 2 weeks later
Avocados	1.5	Apply at spring flush	Apply at pre-bloom and at petal fall
Mangoes	2	Apply during vegetative flush	Repeat thrice before flowering at 14 days intervals
Lychee	2	Apply during vegetative flush	Repeat twice at 14 days interval
Pineapple	2	Apply at early flowering	14 days later
Tea	1.5	Apply at early season	Repeat twice, during mid-season at monthly intervals
Rice	1	Apply at panicle initiation	7 days later
Barley, Rye, Sorghum, Oats	2	Apply at early tillering and 15 days later	Apply at booting stage
Peppers, Capsicum, Chili, Egg Plant, Okra	1	Apply 10-15 days after emergence	Apply at first sign of bloom
Oilseed rape	1	Apply at 4-5 leaf stage	Repeat 14 days later
Wheat	2	Apply just before tillering & 14 days later	Apply at booting stage
Potatoes	1.5	Apply at 4-6 leaf stage	Repeat 14 days later
Lettuce, Spinach, Parsley, Celery	1	Apply at 2-3 leaf unfolded stage	Twice, at early rosette & 50% of expected rosette diameter
Carrot, Beets, Ginger, Radish, Turnip	1	Apply at early leaf stage	Apply at mid vegetative growth
Onions, Garlic, Shallots, Leeks	1.5	Apply at 2.3 leaf stage	Repeat 10 days later
Beans, Lentils, Peas, Soybean	1.5	Apply 4-6 leaf stage	Apply just before flowering
Cotton	1.5	Apply at 4-5 leaf stage	Early squaring stage
Banana	2	Apply after 3 months of planting	Repeat 15 days later
Sugarbeets	1	Apply at 4-8 leaf stage	Apply at 9 or more leaf stage
Turfs	1.5	Apply at monthly intervals during spring and summer periods.	

Application recommendations: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 1.5-2.0 litres ZenMaster diluted in 250 litres of water per hectare are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of ZenMaster. Diluted solution of ZenMaster should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

COMPATIBILITY: ZenMaster is compatible with most insecticides, fungicides and fertilizers. Determine the compatibility by utilizing a small quantity test (e.g. bucket test) prior to tank mixing.

STORAGE: Store above 41^{o}F / 5^{o}C and away from direct sunlight. **CAUTION**: Keep out of reach of children.

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Turfs	1.5	Apply at monthly intervals during spring and summer periods.	

Application recommendations: Dilute the product in sufficient quantity of water and apply by spray method that will provide complete coverage of the plants. Dilutions below 1.0-1.5 pint ZenMaster diluted in 25 gallons of water per acre are not recommended as this may decrease the effectiveness of the product. Rates are given as guidelines only. A tissue test is highly recommended as a basis for correct nutrient need and rates of application. During deficiency, application dosage should be increased based on the tissue analysis. Mixing: Shake the product well before opening. Fill the sprayer tank with half the required volume of water. Begin agitation and slowly add the required amount of ZenMaster. Diluted solution of ZenMaster should be sprayed promptly. Ensure more plant coverage to optimise results. Application should be made at cool conditions for best results. Avoid extreme daytime temperature or extremely strong sunshine during the application. Application of adjuvant is not required. Contact BioAtlantis for specific use recommendation.

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FOREWORD INTRODUCTION

SULFURIC ACID

CAS N°: 7664-93-9

SIDS Initial Assessment Report for 11th SIAM

(Orlando, Florida, 23-26 January, 2001)

Chemical Name : Sulfuric acid

CAS no: 7664-93-9

Sponsor Country : France

National SIDS Contact Point in Sponsor Country:

Mme. Laurence Musset
Bureau des substances et préparations
Ministère de l'environnement
20 avenue de Ségur
75302 Paris 07 SP
France

History: The national peer review consisted of a presentation and critical discussion at a national panel of experts in toxicology and ecotoxicology from administration, university and industry and nominated by the ministry of environment. In parallel, a review was performed by the national institute on environmental and industrial risk (INERIS) by request from the ministry of environment. For this particular substance, only the verification of the most relevant underlying study reports or publications was performed.

Testing completed : none

Comments:

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	7664-93-9
Chemical Name	Sulfuric acid
Structural Formula	H_2SO_4

RECOMMENDATIONS

The chemical is a candidate for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

The LC50 values for sulfuric acid aerosol observed in acute inhalation studies conducted in different species are low and are most likely due to the corrosive/irritant effect of this chemical. For guinea pigs, the LC50 (8 hours; particle size approximately 1µm) ranges from 0.018 to 0.050 mg/l, depending on the age of the animals. Depending on the duration of exposure, the LC50 ranges from 0.37 to 0.42 mg/l in rats, 0.6 to 0.85 mg/l in mice and 1.47 to 1.61 mg/l in rabbits. Only one acute oral toxicity study was available. This study indicated an LD50 of 2140 mg/kg in the rat.

Sulfuric acid is corrosive to the skin, eyes and mucous membranes. 10% solutions of sulfuric acid appear not to be irritating to the skin in difference species. Conflicting results (not irritating or severely irritating) are observed in eye irritation studies using 10% sulfuric acid, depending on the protocol used (OECD/EU or US). Sulfuric acid is not considered as an allergen by skin contact in humans.

In numerous repeated inhalation studies with sulfuric acid aerosol, toxicity was confined to changes in the structure and function of the respiratory tract, suggesting that it has a local effect and no systemic effects. The observed changes are related to the irritant properties of sulfuric acid and are most likely due to the H+ ion. In a 28-day inhalation study in the rat exposed to sulfuric acid aerosol, minimal squamous metaplasia was observed in the laryngeal epithelium following exposure to the lowest concentration used (0.3 mg/m3). This effect was fully reversible. Exposure to 1.38 mg/m3 caused more severe metaplasia accompanied by cell proliferation.

Sulfuric acid has been shown to be without effect in genetic toxicity studies *in vitro* (bacterial test). It has been shown to cause chromosomal aberrations in a non-bacterial test *in vitro*. The chromosomal effects are well known to be a consequence of reduced pH, being seen using any strong acid. There are no *in-vivo* mutagenicity studies available.

No carcinogenic effect was observed in carcinogenicity studies conducted by inhalation with sulfuric acid aerosol using 3 different animal species. Small increases in tumor incidence were reported in rats and mice after chronic gastric intubation or intratracheal instillation of sulfuric acid solution, but no clear conclusion can be drawn from these studies.

Several epidemiological studies have suggested a relationship between exposure to inorganic acid mists containing sulfuric acid and an increased incidence of laryngeal cancer. IARC has concluded that "occupational exposure to strong inorganic mists containing sulfuric acid is carcinogenic for humans (Group

1).” Concerns have been raised that confounding factors could not be fully excluded.

Because sulfuric acid is a direct-acting toxicant, and because it is unlikely to reach the reproductive organs, reproductive effects in mammals are not likely to occur following exposure to sulfuric acid by any route. In a developmental toxicity/teratogenicity study conducted by inhalation with sulfuric acid aerosol, the NOAEL for maternal toxicity appears to be 20 mg/m3 in mice and rabbits. No evidence of foetotoxicity or teratogenicity was seen in either species.

Environment

Sulfuric acid is a strong mineral acid that dissociates readily in water to sulfate ions and hydrated protons, and is totally miscible with water. Its pKa is 1.92 at 25 °C. At pH 3.92, for example, the dissociation is 99 %, and sulfate ion concentration is 1.2 x 10^{-4} moles = 11.5 mg/l. So at environmentally relevant concentrations, sulfuric acid is practically totally dissociated, sulfate is at natural concentrations and any possible effects are due to acidification. This total ionisation will imply also that sulfuric acid, itself, will not adsorb on particulate matters or surfaces and will not accumulate in living tissues.

The NOECs selected were obtained on a natural (cold water) lake artificially contaminated by the controlled addition of sulfuric acid:

- NOEC in phytoplankton community structure = pH 5.6 = 0.13 mg/l sulfuric acid
- NOEC in zooplankton population repartition = pH 5.6 = 0.13 mg/l sulfuric acid.
- NOEC in fish population recruitment = pH 5.93 = 0.058 mg/l sulfuric acid

There is only one validated NOEC available for warm water fish (*Jordanella floridae*), 0.025 mg/l, which is derived from the LOEC/2.

Exposure

Estimated worldwide production of sulfuric acid is 160 million ton/year. The main uses are non dispersive (industrial uses). In some countries, sulfuric acid is approved for agricultural use. The occurrence of sulfuric acid in the environment comes mainly from the hydrolysis of sulfur oxides produced by combustion processes (natural and anthropogenic), wet deposition, generally as a mixture with nitrogen oxides and nitric acid and not from the manufacturing and use of the acid. The emissions to the aquatic environment generally occur from manufacturing industrial locations after neutralisation and are mainly in the form of sulfate ions. Alternatively, following manufacturing and use, it can enter the terrestrial environment as stable gypsum (calcium sulfate).

NATURE OF FURTHER WORK RECOMMENDED

Environment: the collection of information about exposure during agricultural use should be considered.

Health: the collection of information about occupational exposure to sulfuric acid mist should be considered due to the carcinogenic potential.

FULL SIDS SUMMARY

CAS N° 7664-93-9		SPECIES	PROTOCOL	RESULTS
PHYSICO-CHEMICAL				
2.1	Melting point			10.4-10.5 °C (sulfuric acid 100 %) 3 °C (sulfuric acid 98 %) -32 °C (sulfuric acid 93 %) -38 °C (sulfuric acid 78 %) -44 °C (sulfuric acid 74 %) -64 °C (sulfuric acid 65 %)
2.2	Boiling point			290 °C at 1013 hPa (sulfuric acid 100 %) 310-335 °C at 1013 hPa (sulfuric acid 98 %)
2.3	Density			1.835 at 20 °C (sulfuric acid 93-100 %)
2.4	Vapour pressure			< 0.001 hPa at 20 °C 0.004 hPa at 50 °C 1.3 hPa at 145.8 °C
2.5	Partition coefficient			Not relevant for ionisable compounds
2.6	Water solubility			Miscible pKa = 1.92
2.7	Density			1.835 at 20 °C (sulfuric acid 93-100 %)
2.11	Oxidising properties			Powerful acidic oxidizer which can cause ignition or explosion in contact with many materials.
2.12	Additional remarks			Vigorous reaction when water added to sulfuric acid.
ENVIRONMENTAL FATE AND PATHWAY				
3.1.2	Stability in water			Strong acid : dissociates in water to sulfate and hydrated proton
3.3.1	Transport between environmental compartments			Very mobile in soil. Mobility increases with the dilution in water. Wet acidic deposition on soils are 75 % sulfuric acid
ECOTOXICOLOGY				
4.1	Acute/prolonged toxicity to fish	*Lepomis macrochirus* *Brachydanio rerio*	pH decreasing each 96 hours ISO 7346/1	LC50 96h = 16-28 mg/l (pH 3.25 to 3.5) LC50 24h = 82 mg/l
4.2	Acute toxicity to aquatic invertebrates	*Daphnia magna*	ISO 6341	EC50 24h = 29 mg/l
4.3	Toxicity to aquatic plants e.g. algae	Epilimnetic phytoplankton in a natural lake	Phytoplankton community structure study	NOEC = 0.13 mg/l (pH 5.6)

4.4	Toxicity to micro-organisms e.g. bacteria	*Pseudomonas fluorescens* Protozoan community	Test solutions neutralized Substrate colonization	EC0 = 6900 mg/l NOEC = pH 6.61 (from original pH 8.36)
4.5.1	Chronic toxicity to fish	*Salvelinus fontinalis* *Salvelinus fontinalis* *Salvelinus fontinalis* *Jordanella floridae* Lake fish populations	Embryo survival and time hatching Weight of young fish 26 °C, fry growth Population decrease, recruitment	NOEC = 0.31 mg/l (pH 5.2) NOEC = 0.15 mg/l (pH 5.5) NOEC = 0.13 mg/l (pH 5.56) LOEC 20 % = pH 6.0 = 0.049 mg/, NOEC = LOEC/2 = 0.025 mg/l NOEC = 0.058 mg/l (pH 5.93)
4.5.2	Chronic toxicity to aquatic invertebrates	*Tanytarsus dissimilis* Lake zooplankton population	Reproduction Population repartition	NOEC = 0.15 mg/l(pH 5.5) NOEC = 0.13 mg/l (pH 5.59)
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat	Other	LD50 = 2140 mg/kg
5.1.2	Acute Inhalation Toxicity	Guinea pig Guinea pig Guinea pig Rat Rat Rat Mouse Mouse Mouse Rabbit	Other Other Other Other Other Other Other Other Other Other	LC50 = 0.030 mg/1/8h (particle size: 0.8 µ) LC50 > 0.109 mg/1/8h (particle size:0.4 µ) LC0 (old animal) = 0.020 mg/1/8h LC50 (old animal) = 0.050 mg/1/8h LC0 (young animal) = 0.008 mg/1/8h LC50 (young animal) = 0.018 mg/1/8h LC100= 0.087 mg/1/2.75 LC50 = 0.375 mg/1/4h LC50 = 0.425 mg/1/8h LC0 = 0.461 mg/1/7h LC100 = 0.699 mg/1/7h LC0 = 0.718 mg/1/3.5h LC100 = 1.470 mg/1/3.5h LC50 = 0.510 mg/1/2h LC50 = 0.850 mg/1/4h LC50 = 0.600 mg/1/8h LC0 = 0.461 mg/1/7h LC40 = 0.699 mg/1/7h LC50 = 0.320 mg/1/2h LC0 = 0.699 mg/1/7h LC50 = 1.610 mg/1/7h

				LC0 = 0.718 mg/1/3.5h LC50 = 1.470 mg/1/3.5h
5.2.1	Skin irritation/corrosion	Rabbit, Guinea-pig, Human	FDA, FSHA, Federal Register V37, 1972	Not irritating
		Rabbit, Human	CFR, DOT 1986 (rabbit) and 1988 (human) + Hill top Chamber	Not irritating
5.2.2	Eye irritation/Corrosion	Rabbit	OECD TG 405	Sulfuric acid 10%: not irritating
		Rabbit	Directive 79/831/EEC, Annex V, part B	Sulfuric acid 10%: not irritating
		Rabbit	US, FHSA (CFR, 1979) and NAS 1138 Committee (1977)	Sulfuric acid 10% (0.01 ml): slightly irritating Sulfuric acid 10% (0.05 ml): severely irritating Sulfuric acid 10% (0.1 ml): severely irritating
		Rabbit	US.FHSA Fed. Reg. Vol 38 (187) Part II and 16 CFR 1500.42 (1973) and Draize method (1944)	Sulfuric acid 10%: severe irritant Sulfuric acid 5%: moderate irritant
5.4	Repeated Dose Toxicity by Inhalation	Rat (réf. 74)	OECD TG 412	a NOEL/NOAEL can not be identified
		Rat (réf. 106)	Other	NOEL/NOAEL not indicated
		Rat (réf. 111)	Other	NOEL/NOAEL not indicated
		Rat (réf. 26)	Other	NOEL/NOAEL not indicated
		Rat (réf. 25)	Other	NOEL/NOAEL not indicated
		Guinea pig (réf. 111)	Other	NOEL/NOAEL not indicated
		Guinea pig (réf. 26)	Other	NOEL/NOAEL not indicated
		Guinea pig (réf. 25)	Other	NOEL/NOAEL not indicated
		Guinea pig (réf. 184)	Other	NOEL/NOAEL not indicated
		Guinea pig (réf. 168)	Other	NOEL/NOAEL not indicated
		Guinea pig (réf. 2)	Other	NOEL/NOAEL not indicated
		Guinea pig (réf. 3)	Other	NOEL/NOAEL not indicated
		Rabbit (réf. 165)	Other	NOEL/NOAEL not indicated
		Rabbit (réf. 64)	Other	NOEL/NOAEL not indicated
		Rabbit (réf. 63)	Other	NOEL/NOAEL not indicated
		Rabbit (réf. 155)	Other	NOEL/NOAEL not indicated
		Rabbit (réf. 160)	Other	NOEL/NOAEL not indicated

	Repeated Dose Toxicity by Inhalation (continued)	Rabbit (réf. 154)	Other	NOEL/NOAEL not indicated
		Rabbit réf. 156)	Other	NOEL/NOAEL not indicated
		Rabbit (réf. 167)	Other	NOEL/NOAEL not indicated
		Monkey (réf. 2)	Other	NOEL/NOAEL not indicated
		Monkey (réf. 3)	Other	NOEL/NOAEL not indicated
		Mouse (réf. 168)	Other	NOEL/NOAEL not indicated
		Hamster (réf. 105)	Other	NOEL/NOAEL not indicated
		Dog (réf. 110)	Other	NOEL/NOAEL not indicated
5.5	GENETIC TOXICITY *IN VITRO*			
A.	Bacterial test (Gene mutation)	*S. typhimurium*	Other	- (with metabolic activation) - (without metabolic activation)
		E. coli	Other	- (without metabolic activation)
B.	Non-bacterial *In Vitro* test (Chromosomal aberrations)	Developing embryos of Sphaerechinus granularis and Paracentrotus lividus	Other	+ (without metabolic activation)
		Chinese hamster Ovary (CHO) K1 cells	Other	+ (with metabolic activation) + (without metabolic activation
5.7	Carcinogenicity	Rat (réf. 187)	Other	Local and weak carcinogen, (gastric intubation)
		Rat (réf. 187)	Other	Local and weak carcinogen, (intratracheal instillation)
		Mouse (réf. 187)	Other	Local and weak carcinogen, (gastric intubation)
		Hamster (réf. 105)	Other	No evidence of carcinogenic potential (inhalation, mist)
		Rat (réf. 55)	Other	No carcinogenic effect, (inhalation, mist)
		Guinea pig (réf. 54)	Other	No carcinogenic effect (inhalation, mist)
5.9	Developmental toxicity / Teratogenicity	Mouse	Similar to OECD TG 414 (inhalation)	NOAEL maternal = 20 mg/m3 NOEL teratogenicity = 20 mg/m3
		Rabbit	Similar to OECD TG 414 (inhalation)	NOAEL maternal = 20 mg/m3 NOEL teratogenicity = 20 mg/m3
5.10	Other data	49 articles/reviews included in the IUCLID dossier for additional information		
5.11	Experience with human exposure	50 articles/epidemiological studies included in the IUCLID dossier		

SIDS Initial Assessment Report

1. IDENTITY

Name (OECD) : Sulfuric acid

CAS number : 7664-93-9

Molecular formula : H_2SO_4

Molecular weight : 98

Other names : Dihydrogen sulphate
Oil of vitriol

Sulfuric acid is a colourless and odourless viscous liquid crystallising at 3 to 10 °C depending on its water content (from 0 to 2 %). Water content is generally up to 8 %. Other impurities (sulfur dioxide, nitrogen compounds and heavy metals) are < 0.1 %. Its density is 1.834 to 1.836 at 20 °C

Sulfuric acid is a strong mineral acid that dissociates readily in water to sulfate ions and hydrated protons, and is totally miscible with water. Its pKa is 1.92 at 25 °C. So at pH 3.92, for example, the dissociation is 99 %, and the sulfate ion concentration is 1.2×10^{-4} moles = 11.5 mg/l. So at environmentally relevant concentrations, sulfuric acid is practically totally dissociated, sulfate is at natural concentrations, and possible effects are due to acidification.

This total ionisation also implies that sulfuric acid will not adsorb on particulate matters or surfaces and will not accumulate in living tissues.

The dissolution/dissociation in water is strongly exothermic, so a vigorous reaction occurs when water is added to sulfuric acid. It is a powerful acidic oxidizer which can cause ignition or explosion in contact with many materials.

Sulfuric acid has a low vapour pressure (< 0.001 hPa at 20 °C). However mists and aerosols can be formed in some industrial applications.

2. GENERAL INFORMATION ON EXPOSURE

Estimated world-wide production of sulfuric acid is 160 million tonnes/year. The continental repartition is 40 million tonnes/year in Europe, 60 in America and 60 in Asia-Pacific. The production in the sponsor country (France) was 2.05 million tonnes / year in 1999.

The main uses are non dispersive :

- 32 % for phosphoric acid and fertilisers production
- 58 % as basic chemical for chemical synthesis, pigment, oil industries
- 2 % for metal extraction, refining and processing of metals
- 0.8 % batteries
- about 7 % for other industrial uses (pulp and paper …)

A very minor agricultural use (about 0.025 %) is as desiccant for potato crops.

In the workplace, sulfuric acid can exist as an acid mist. This situation can occur because sulfur trioxide generates very dense sulfuric acid mists with atmospheric humidity. However, this occurs only in the event of accidental leakage of sulfur trioxide, and is not a result of normal activity.

Other sulfuric acid uses that are important sources of sulfuric acid mists in the workplace are:
- car and industrial batteries loading
- metal sheets cleaning for surface treatment
- electro-chemical production of zinc and copper : sulfuric acid is driven off as fine droplets by evolved hydrogen.
- Loading and discharging of sulfuric acid

Occupational exposure limit values for different countries are presented in Annex I. For most of the countries (e.g. USA, France, Japan, Finland) the limit value for an 8 hour-exposure is 1 mg/m3 except for Germany : MAK value, 8 hours : 0.1 mg/m3.

Sulfuric acid occurrence in the environment mainly comes from hydrolysis of sulfur oxides produced by combustion processes (natural and anthropogenic) wet deposition, generally as mixture with nitrogen oxides and nitric acid and not from manufacturing. The emissions to the aquatic environment generally occur from manufacturing industrial locations after neutralisation and are mainly in the form of sulfate ions. Alternatively, following manufacturing and use, it can enter the terrestrial environment as stable gypsum (calcium sulfate).

Sulfuric acid use in agriculture as desiccant for potato crops is reported in UK (Food and Environment Protection Act, 1985, Part III, Control of Pesticides Regulations 1986, Evaluation of Fully Approved or Provisionally Approved Products, Evaluation on Sulphuric Acid, April 1998). In 1992, 90 685 ha of potato crops were treated with 77% w/w sulfuric acid. Doses ranged from 112 l/ha to 335 l/ha, which means a total consumption of about 40 000 t sulfuric acid in this agricultural use.

3. ENVIRONMENT

3.2. Effects on the aquatic environment

Preliminary remarks

Quality criteria: The principal quality criteria for acceptance of data are that the test procedure should be well described (with reference to an official guideline) and that the toxicant concentrations must be measured with an adequate analytical method.

Four situations can be distinguished and are summarised in the following table according to criteria defined in IUCLID system.

Table: Quality criteria for acceptance of ecotoxicity data

Case	Detailed description of the test	Accordance with scientific guidelines	Measured concentration	Conclusion: reliability level
I	+	+	+	[1] : valid without restriction

II	±	±	±	[2] : valid with restrictions; to be considered with care
III	insufficient or -	-	-	[3] : invalid
IV	the information to give an adequate opinion is not available			[4] : not assignable

Publications were assigned validity 4 when they could not be checked directly. Validity 3 was assigned systematically when no clear description was given of the test substance. This approach is important for sulfuric acid, as sources for sulfuric acid production can be recovery from many processes leading to various impurities.

Analytical monitoring reported in the IUCLID file refers to pH measurements. At concentrations reported in publications and study reports, the toxicity has been assumed to be due to acidity only, because at these low concentrations, sulfate quantities added are below most of natural medium concentrations. So the sulfuric acid environmental risk assessment is in fact acidity risk assessment.

3.2.1 Aquatic effects

3.2.1.1. Effects in fish

The acute toxicity of sulfuric acid in fish has been reported in 10 different publications, leading to 8 LC50 values in 24, 48 or 96 hours duration. Only two references were assigned validity 2 : one study performed according to the international standard ISO7346/1, in a 24 hours static test in *Brachydanio rerio*, not under GLP, giving an LC50 24 hours of 82 mg/l. The other one was obtained in a study where *Lepomis macrochirus* were exposed successively 96 hours to each pH tested (from pH 7.5 original water to pH 5.0, 4.5, 3.5, 3.25 and 3.0. However the LC50 48 hours was retained as a worst case one and measured as being from pH 3.25 to pH 3.5, which gives a value of 16 to 28 mg/l sulfuric acid. No LC50 was found lower than *Lepomis macrochirus* one in all publications assigned validity 3 or 4.

The chronic toxicity of sulfuric acid in fish was assessed in 6 publications reporting laboratory tests. 5 validity 2 NOEC values were derived, 3 of them being in the same range: NOECs for embryo survival and time for hatching of *Salvelinus fontinalis* (pH 5.2 and pH 5.5 giving substance concentrations 0.31 mg/l and 0.15 mg/l), and a NOEC for weight of young *Salvelinus fontinalis* produced in 10 month (pH 5.56, giving 0.13 mg/l). The fourth NOEC is far lower, being derived from a LOEC on fry growth of *Jordanella floridae* in 45 days of pH 6.0 (0.049 mg/l) giving 20 % inhibition, which, divided by 2 can give a NOEC of 0.025 mg/l.

The difference between *Salvelinus fontinalis* and *Jordanella floridae* is their optimal temperature : *Salvelinus* is a cold water fish (Brook trout), and *Jordanella* a warm water fish. The difference in physiology could explain the difference in sensitivity.

Table of validated fish toxicity results

		SPECIES	PROTOCOL	RESULTS
4.1	Acute/prolonged toxicity to fish	*Lepomis macrochirus* *Brachydanio rerio*	pH decreasing each 96 hours ISO 7346/1	LC50 96h = 16-28 mg/l (pH 3.25 to 3.5) LC50 24h = 82 mg/l
4.5.1	Chronic toxicity to fish	*Salvelinus fontinalis* *Salvelinus fontinalis* *Salvelinus fontinalis* *Jordanella floridae* Lake fish populations	Embryo survival and time hatching Weight of young fish 26 °C, fry growth Population decrease, recruitment	NOEC = 0.31 mg/l (pH 5.2) NOEC = 0.15 mg/l (pH 5.5) NOEC = 0.13 mg/l (pH 5.56) LOEC 20 % = pH 6.0 = 0.049 mg/, NOEC = LOEC/2 = 0.025 mg/l NOEC = 0.058 mg/l (pH 5.93)

Remark : the original results as published are underlined. Other values were calculated.

3.2.1.2. Effects in invertebrates

The acute toxicity of sulfuric acid in aquatic invertebrates is reported in 8 different publications, leading to 7 LC 50 values in 24, 48 or 96 hours duration. Only one reference describing a *Daphnia magna* test in 24 hours was assigned validity 2. This test was performed according to the international standard ISO 6341, and gave a LC50 24 hours of 29 mg/l. It is the lowest LC50 published.

The chronic toxicity in invertebrates was assessed in 4 publications, one only giving a validity 2 result. It is a laboratory test in the midge *Tanytarsus dissimilis* giving a NOEC 35 days on reproduction success of pH 5.5 (0.15 mg/l).

3.2.1.3. Effects in aquatic plants / algae

No standard algae growth inhibition study could be found. Nevertheless a NOEC in phytoplankton is available from field studies with data on *Chlorella mucosa* (chlorophyte), *Dinobryon sertularia, Mallomonas* sp., *Stichogloea* sp., *Uroglena* sp. (chrysophycean species), *Asterionella ralfsii* (diatom), *Gymnodinium* sp., *Peridinium inconspicuum* (dinoflagellates) *Chroococus minutus, Merismopedia* sp. (cyanophyte) (see chapter 3.2.1.4).

3.2.1.4 Studies on an experimentally acidified lake

The effect of sulfuric acid addition for several years (1976 to 1983) in a natural (cold water) Canadian “Lake 223” was assessed in aquatic species populations. From an initial level of about 6.7, the pH was lowered at a pH rate of about 0.5 pH units a year (6.49 – 6.13 - 5.93 – 5.64 – 5.59) until it reached an average pH 5.1 and was held there for 3 years. This lake was one of the lakes of “ELA” (Experimental Lake Area) in Canada, where a set of natural lakes was selected as representative for a natural non-polluted environment.

Fish population was analysed during these years. A NOEC for the most sensitive fish species, fathead minnow (*Pimephales promelas*) and slimy sculpin (*Cottus cognatus*) recruitment was pH 5.93, giving 0.058 mg/l. This NOEC in recruitment integrates not only reproductive success, but also prey/predator relationships (presence/lack of suitable food as smaller fish, invertebrates or

aquatic plants/algae, presence/lack of predators for smaller fish). Moreover it integrates effects of successive one-year exposures to pH 6.49 and 6.13, which models a progressive acidification by sulfuric acid deposition.

The zooplankton community study was also analysed by identifying the species and counting the organisms. A NOEC for population repartition (from copepod to cladoceran dominance) was pH 5.59 (0.13 mg/l). This NOEC integrates not only reproductive success, but also prey/predator relationships (presence/lack of suitable food as smaller invertebrates or aquatic plants/algae, presence/lack of fish predators). Here also it integrates effects of successive one-year exposures to pH 6.49, 6.13, 5.93 and 5.64.

The phytoplankton community structure was also studied, giving a NOEC of pH 5.6 (0.13 mg/l) (chlorophyte increase and species shift to large inedible *Gymnodium* sp.). This NOEC integrates not only algae growth rate, but also consumption by invertebrates and fish, and also effects of successive one year exposures to pH 6.49, 6.13, 5.93.

3.2.1.5 Toxicity in micro-organisms

A multispecies-microcosm test was performed : the structure and function of naturally derived periphytic communities on polyurethane foam artificial substrates were monitored. The artificial substrates were suspended at 1m depth in a man-made outdoor ponds. After 21 days substrates were collected. pH was set in different ponds to 8.34-7.61-6.90-6.61-5.34-3.33. The control pond was pH 8.36.

Significant effects on protozoan species richness were observed in this test at a pH = 5.33. Therefore the NOEC for species richness was 6.61. In this experiment, the sulfuric acid concentration calculation is more problematic, because the initial pH in the ponds is far from neutrality, and alkaline (pH 8.36). So the assumption that pH is only the result of sulfuric acid dilution in water, which was an approximation in pH 6.7 Canadian Lake 223 experiments, is here completely false. Ignoring the buffering capacity of the pond water, it is therefore impossible to derive a NOEC as sulfuric acid mg/l.

Discussion

It is remarkable that sensitivity to pH is not universal among species and related ecosystems : for example at pH 6.0, *Jordanella floridae* fry growth already begins to be inhibited.

Some interesting examples are also salamanders : *Ambystoma jeffersonianum* eggs have hatching success > 90 % only at pH 6 at 10 °C, and at pH 5 to 6 at 5 °C. Eggs do not hatch successfully above pH 6. And *Ambystoma maculatum* eggs hatch only from pH 7 to 9.

The sulfuric acid hazard assessment is in fact hazard assessment of acidity. All the observations made and the results derived would be the same for any strong acid, provided the anion has no toxicity in any species at environmentally relevant strong acid concentrations.

4. HUMAN HEALTH

4.2 Effects on Human Health

Preliminary remarks:

- ✓ Reliability of the studies was evaluated using the criteria for reliability categories adapted from Klimisch *et al.* (1997) and Rosner (1994). Reliability is differentiated and thus classified into 4 categories/codes as described below. In this scoring system, studies conducted and reported according to internationally accepted test guidelines and in compliance with GLP have the highest grade of reliability and should be used as reference standards.

  - *1 : Reliable without restriction* :
    - 1a GLP guideline study (OECD, EC, EPA, FDA, etc…)
    - 1b Comparable to guideline study
    - 1c Test procedure in accordance with national standard methods (AFNOR, DIN, etc)
    - 1d Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

  - *2: Reliable with restrictions*
    - 2a Guideline study without detailed documentation
    - 2b Guideline study with acceptable restrictions
    - 2c Comparable to guideline study with acceptable restrictions
    - 2d Test procedure in accordance with national standard methods with acceptable restrictions
    - 2e Study well documented, meets generally accepted scientific principles, acceptable for assessment
    - 2f Accepted calculation method
    - 2g Data from handbook or collection of data

  - *3: Not reliable*
    - 3a Documentation insufficient for assessment
    - 3b Significant methodological deficiencies
    - 3c Unsuitable test system

  - *4: Not assignable*
    - 4a Abstract
    - 4b Secondary literature
    - 4c Original reference not yet available
    - 4d Original reference not translated (e.g. Russian)
    - 4e Documentation insufficient for assessment

- ✓ Studies selected for discussion are identified in the following tables by a black bullet (•).

4.2.1 Mode of action of the chemical, toxicokinetics and metabolism

Sulfuric acid is corrosive and irritating and causes direct local effects on the skin, eyes and gastrointestinal tracts after direct exposure to sufficient concentrations. Small droplets of sulfuric acid (aerosol/mist) can also be inhaled and cause direct local effects on respiratory tract. The effects of inhaled sulfuric acid aerosols will depend on many factors: - exposure concentrations; - exposure time; - particle size of the aerosol, which determines the location in the respiratory tract where sulfuric acids aerosols will deposit; - humidity, both in the environment and in the respiratory tract, which determines the particle size; - endogenous ammonia that can neutralize sulfuric acid; - pattern of respiration and the inhalation route (oral or nasal); - buffering capacity of the airways; - species studied (e.g. respiratory tract dimension and architecture) (see ref. 10, 102, 144).

The effects of sulfuric acid are the result of the H+ ion (local deposition of H+, pH change) rather than an effect of the sulfate ion. Sulfuric acid per se is not expected to be absorbed or distributed throughout the body. The acid will rapidly dissociate and the anion will enter the body electrolyte pool, and will not play a specific toxicological role (102, 144). This is supported by experiments which have studied the active component in inorganic acids on various endpoints, using different

acids or salts (HCl, NH4HSO4, $(NH4)_2SO4$, Na2SO4). In these studies, the authors have concluded that the observed effects seemed to be due to the H+ ion while the anion appeared to have no effect (157, 161, 162, 166, 202). In an experiment studying the clearance via the blood of radiolabeled sulfuric acid aerosol in different species, the authors have observed that sulfur from sulfuric acid was rapidly cleared (from 2 to 9 minutes) from the lungs of animals into the blood following inhalation exposure (45). Sulfate is a normal constituent of the blood and is a normal metabolite of sulfur-containing amino acids, and excess sulfate is excreted in the urine. The body pool of this anion is large, and it is therefore unlikely that occupational aerosol exposures significantly modify the normal body load (102, 144).

4.2.2 Acute toxicity

The acute toxicity studies conducted with sulfuric acid that could be checked are summarized in the following tables. None of these studies have been carried out recently, under national or international guidelines, and according to GLP. Collectively, however, these studies show effects in the similar range of doses for given animal species.

4.2.2.1 Acute oral toxicity

Acute Oral Toxicity studies with sulfuric acid						
	Species, strain	**Ref. (year)**	**Protocol**	**Administration**	**Endpoint**	**Value (mg/kg)**
●	RAT (NS)	172 (1969)	Other	Oral (Intubation) 0.25 g/ml of diluted sulfuric acid	LD_{50}	2140 mg/kg

Only one acute oral toxicity study is available. This study indicates an LD50 = 2140 mg/kg in the rat. However, due to irritant and/or corrosive effects of sulfuric acid, the oral route of exposure is not appropriate for testing possible toxic endpoints. Gavage dosing of animals will not represent oral exposures in humans, which itself will be limited. Toxic signs of oral exposure in human are of irritation/corrosion of the gastrointestinal tract.

4.2.2.2 Acute inhalation toxicity

Acute Inhalation Toxicity studies with animals exposed to sulfuric acid aerosol/mist or oleum								
	Species (strain)	**Ref. (year)**	**Protocol**	**Source of mists**	**Exposure Time**	**Particle size (µm)**	**Endpoint**	**Value**
●	GUINEA PIG, (HARTLEY)	200 (1979)	Inhalation, whole body	SO3 + H2O	8 h 8 h	0.8 0.4	LC_{50} LC_{50}	0.030 mg/l/8h >0.109 mg/l/8h
●	GUINEA PIG (NS)	9 (1952)	Inhalation, whole body	NS	8 h	1	LC_0 (old animal) LC_{50} (old animal) LC_0 (young animal) LC_{50} (young animal)	0.020 MG/L/8H 0.050 mg/l/8h 0.008 MG/L/8H 0.018 MG/L/8H
	Guinea pig (NS)	185 (1950)	Inhalation, whole body	diluted SA (10-60% w/v)	2.75 h	1-2	LC_{100}	0.087 mg/l/2.75
●	(FISCHER-344)	150 (1976)	Inhalation, whole body	SO3 + humid air	4 h 8 h	1	LC_{50} LC_{50}	0.375 mg/l/4h 0.425 mg/l/8h

•	Rat (NS)	185 (1950)	Inhalation, whole body	diluted SA (10-60% w/v)	7 h 3.5 h	1-2	LC_0 LC_{100} LC_0 LC_{100}	0.461 mg/l/7h 0.699 mg/l/7h 0.718 mg/l/3.5h 1.470 mg/l/3.5h
	RAT (NS)	93 (1982)	Inhalation	NS	2 h	NS	LC_{50}	0.510 mg/l/2h
•	MOUSE (CD-1)	150 (1976)	Inhalation, whole body	SO3 + humid air	4 h 8 h	1	LC_{50} LC_{50}	0.850 mg/l/4h 0.600 mg/l/8h
•	Mouse (NS)	185 (1950)	Inhalation, whole body	diluted SA (10-60% w/v)	7 h	1-2	LC_0 LC_{40}	0.461 mg/l/7h 0.699 mg/l/7h
	Mouse (NS)	93 (1982)	Inhalation	NS	2h	NS	LC_{50}	0.320 mg/l/2h
•	Rabbit (NS)	185 (1950)	Inhalation, whole body	diluted SA (10-60% w/v)	7 h 3.5 h	1-2	LC_0 LC_{50} LC_0 LC_{50}	0.699 mg/l/7h 1.610 mg/l/7h 0.718 mg/l/3.5h 1.470 mg/l/3.5h

NS: Not specified, SA: sulfuric acid

In rats, mice and rabbits, as well as in guinea pigs, concentration of acid aerosol, time of exposure and particle size are important factors in determining lethality by inhalation. Among the different species tested, the guinea pigs appear to be the most sensitive to the acute inhalation effects of sulfuric acid mist/aerosol. For the guinea pig, the apparent LC50 for an 8 hour-exposure period to sulfuric acid mist/aerosol with a particle size of about 1µm, ranges from 0.018 to 0.050 mg/l depending on the age of the animals. Younger guinea pigs seem to be more sensitive to sulfuric acid aerosol than older animals.

According to the duration of exposure, the LC 50 appear to be about 0,375 - 0,425 mg/l in rats, 0.600 - 0.850 mg/l in mice, and 1.470 - 1.610 mg/l in rabbits, when taking into account the more reliable/relevant studies.

The sensitivity of the guinea pig may be caused by its tendency for bronchoconstriction and laryngeal spasm compared to other small laboratory animals.

The main macroscopic and/or microscopic alterations observed in respiratory tract after acute exposure to sulfuric acid aerosol were hemorrhage, edema, atelectasis and thickening of the alveolar wall in the lung of guinea pigs, hemorrhage and edema of the lungs and/or ulceration of the turbinate, trachea and larynx in rats and mice. These lesions are related to the corrosive/irritant effect of sulfuric acid.

No data are available on the acute dermal toxicity or on acute toxicity by other routes for sulfuric acid.

4.2.3 Irritation and Corrosiveness

4.2.3.1 Skin irritation

According to Annex I of the Directive 67/548/EEC, sulfuric acid is classified as C; R 35: Corrosive; Causes severe burns. Specific concentration limits are: C; R35 for concentration ≥ 15 % and Xi; R36/38 when concentrations are ≥ 5%, and < 15 %.

The skin irritation studies, that could be checked, were performed using diluted sulfuric acid and are summarized in the following table.

Skin irritation testing with sulfuric acid					
	Species, Test Type	**Ref. (year)**	**Protocol**	**Doses**	**Result**
●	RABBIT, GUINEA-PIG, HUMAN, SKIN IRRITATION TEST ON ABRADED AND INTACT SKIN	135 (1975)	FDA, FSHA, Federal register V37, 1972	0.5 ml of sulfuric acid, 10 %	Not irritating
●	RABBIT, HUMAN, STANDARD SKIN IRRITATION TEST AND HILL TOP CHAMBERS TEST	134 (1990)	CODE OF FEDERAL REGULATION, DOT 1986 (RABBIT) AND 1988 (HUMAN) + HILL TOP CHAMBER	0.4 or 0.5 ml of sulfuric acid 10 % in standard test 0.2 ml of sulfuric acid 10 % in Chamber	Not irritating

Sulfuric acid 10 % appears not to be irritating to the skin in rabbit, guinea pig and human.

4.2.3.2 Eye irritation

The eye irritation studies conducted with diluted sulfuric acid are summarized in the following table. Only available studies are presented.

Eye irritation testing with sulfuric acid					
	Specie, Test type	**Ref. (year)**	**Protocol**	**Doses**	**Result**
●	RABBIT	95 (1992)	OECD Guideline 405	0.1 ml of sulfuric acid 10 %	Not irritating
●	RABBIT	94 (1989)	Directive 79/831/EEC, Annex V, part B	0.1 ml of sulfuric acid 10 %	Not irritating
●	RABBIT	68 (1980)	US.FHSA (CFR, 1979) and NAS 1138 Committee (1977)	0.01 ml, 0.05 ml, 0.1 ml of sulfuric acid 10 %	0.01ml: slightly irritating 0.05ml: severely irritating 0.1 ml: severely irritating
●	RABBIT, WASHED AND UNWASHED EYE	128 (1982)	US.FHSA Fed. Reg. Vol. 38 (187) Part II and 16 CFR 1500.42 (1973) and Draize method (1944)	0.1ml of sulfuric acid 10 % or 5 %	10% : SEVERE IRRITANT 5%: MODERATE IRRITANT

Conflicting results are observed in eye irritation studies according to the protocol used (OECD/EU or US). However, buffering and dilution effects of tears could explain the different conclusions since sulfuric acid was instillated into the conjunctival sac of the eye in studies n° 95 and 94 while acid was administered directly to the central corneal surface in experiments 68 and 128. In this last study, the authors have observed that the washing procedure (eye washed 2 min. with tap water 30 sec. after exposure) reduced the time to onset of opacity induced by 5% sulfuric acid and slightly decreased the severity of the iritis induced by 10 % sulfuric acid.

4.2.4 Skin sensitization

No study was identified for skin sensitization potential with sulfuric acid.

Sulfuric acid has been in industrial use for many decades, and skin burns due to concentrated sulfuric acid are well documented (ILO Encyclopedia of Occupational Health and Safety, 1985). However, skin sensitisation secondary to skin irritation or burns has never been described, despite the fact that severe chemical irritation and burns are known to create favorable conditions for the induction of contact allergy (this is a strategy employed in routine skin sensitisation testing such with the Magnusson-Kligmann test).

Repeated contact with more diluted sulfuric acid is known to cause skin dessication, ulceration and chronic purulent inflammation around the nails (ILO Encyclopedia of Occupatioal Health and Safety, 1985). These symptoms are quite different from those seen in acute or chronic allergic dermatitis.

Skin contact with weak solutions of sulfuric acid (about 10%) has been quite common in the viscose rayon industry for nearly a century. Yet sulfuric acid allergy has never been noted.

Sulfate ions are unlikely to cause allergy, since the body contains large amounts of sulfate ions (~0.33 mmol/L in serum and about 50 times higher concentration intracellularly). Various metal sulfates (e.g. nickel sulfate, cobalt sulfate) are used in routine allergy testing, but positive reactions are related to the metal ion, not to the sulfate, as can be deduced from the definitely non-allergenic zinc sulfate (ECETOC Technical Report n° 77, 1999).

Based on the above, it may be concluded that sulfuric acid is not an allergen in humans, and that animal testing for sensitisation potential would not provide any information relevant for risk assessment.

4.2.5 Repeated dose toxicity

Repeated dose toxicity studies with sulfuric acid are summarized in the following tables. All of them have been realized by inhalation of sulfuric acid aerosol/mist, in several animal species. However, among them, only one study has been conducted using methodology in accordance with relevant inhalation guidelines for a 28-day study (OECD guideline n° 412 and Directive 67/548 EEC, Annex V, test method B8) and according to GLP.

NOTE: this study is not a full OECD protocol – only the respiratory tract was subject to pathology.

Repeated dose toxicity studies by inhalation conducted with sulfuric acid aerosol										
	Species (strain, sex)	**Ref. (year)**	**Protocol**	**Duration, frequency**	**Administration**	**Doses**	**Particle size (µm)**	**T°(C/F) RH (%)**	**End-point**	**Value (unit)/ results**
•	RAT (ALPK:AP$_F$SD, FEMALE)	74 (IN PREP.)	OECD N° 412 / DIR. 67/548/EEC ANN. V, B8 GLP	28 DAYS 6H/D, 5D/WK	Inhalation, nose only	0.00, 0.30, 1,38, 5.58 mg/m3	 0.62 0.83 0.94	~19.5°C ~50 %	Death: Body and lung weight: Histopathology: Cell proliferation:	No death due to SA No alteration Alteration in larynx only Alteration in larynx only
•	Rat (Sprague-Dawley, male)	106 (1997)	other	30 or 90 days 23.5 h/d, 7d/wk or intermittent (12 h/d)	Inhalation, whole body	0, 20, 100, 150 µg/m3 (SA) ± 0.12, 0.20 ppm (O3)	0.4 - 0.8	22°C 80%	Lung histopathology: Lung biochemical analyses: Morphometric analyses of alveolar tissues: Body and lung weight: +O3:	No alterations No alteration No change due to SA alone No alteration No interaction
	Rat (Sprague-Dawley, male)	111 (1979)	other	from 6 to 14 weeks, continuous	Inhalation, whole body	from 2.37 to 15 mg/m3	0.3 - 0.5	70/77°F 35-50%	Spontaneous locomotor activity: Blood gas parameters: Learning ability: Pulmonary functions: Food/water intake; body weight:	Alteration (at 2.49 mg/m3) Alteration (at 6.5 mg/m3 No alteration Alteration (at 4.05 mg/m3) No alteration
•	Rat (Fischer, male/female)	26 (1978)	other	6 months, 6h/d, 5d/wk	Inhalation, whole body	0, 10 mg/m3 (SA) ± 0.5 ppm (O3)	~ 1	82°F 60%	Hematology/blood chemistry: Lung histopathology: Body and lung weight: +O3:	No alteration Alteration (slight) No alteration No interaction
•	Rat (Fischer, male)	25 (1977)	Other	2 to 7, 14, 21 or 28 days, frequency: NS	Inhalation, whole body	0, 5, 10, 20, 30, 100 mg/m3 (SA) ± 1, 2 ppm (O3)	~ 1	70°F 55%	Death Hematology/blood chemistry: Lung histopathology: Body and lung weight: Lung lavage fluids: +O3:	No death No alteration No alteration No alteration No alteration No interaction

Repeated dose toxicity studies by inhalation conducted with sulfuric acid aerosol (continued)

	Species (strain, sex)	Ref. (year)	Protocol	Duration, frequency	Administration	Doses	Particle size (µm)	T°(C/F) RH (%)	End-point	Value (unit)/ results
	Guinea pig (NS, NS)	111 (1979)	other	from 6 to 14 weeks, continuous	Inhalation, whole body	from 6.56 to 15 mg/m3	0.2 - 0.5	70/77°F 35-50 %	Pulmonary functions:	No alterations
•	Guinea pigs (Hartley, male/female)	26 (1978)	other	6 months, 6h/d, 5d/wk	Inhalation, whole body	0, 10 mg/m3 (SA) ± 0.5 ppm (O3)	~ 1	82°F 60 %	Hematology/blood chemistry: Lung histopathology: Body and lung weight: +O3:	No alteration Alteration (slight) No alteration No interaction
•	Guinea pigs, (Hartley, female)	25 (1977)	other	2 to 7, 14, 21 or 28 days, frequency: NS	Inhalation, whole body	0, 5, 10, 20, 30, 100 mg/m3 (SA) ± 1, 2 ppm (O3)	0.53, 1, 1.66	70°C 55%	Death Hematology/blood chemistry: Lung histopathology: Body and lung weight: Lung lavage fluid: +O3:	Death at > 20mg/m3 No alteration Alteration at > 20mg/m3 No alteration No alteration No interaction
	Guinea pig (NS, NS)	184 (1958)	other	from 18 to 140 days, continuous	Inhalation whole body	0, 1 to 4 mg/m3 (medium or coarse) up to 26 mg/m3 (fine aerosol)	3.6-4.3 or 0.9 or 0.6	NS NS	Respiratory tract histopathology:	Alterations (slight); medium size (0.9µm) aerosol was the most active
	Guinea pig (Harley, female)	168 (1979)	other	7 days, continuous	Inhalation, whole body	38 to 220 mg/m3	0.32 - 0.4	NS NS	Mortality (LD50):	100 mg/m3
•	Guinea pig, (Hartley, male/female)	2 (1973)	other	12 months 23 h/d	Inhalation, whole body	0.00, 0.08, 0.10 mg/m3	0.84, 2.78	22°C 50 %	Body weight: Survival: Hematology/blood chemistry: Pulmonary function: Histopathology:	Alteration (small in female) No death No alteration No alteration No alteration
•	Guinea pig, (Hartley, male/female)	3 (1975)	other	12 months 22-23 h/d	Inhalation, whole body	0, 0.9 mg/m3 SA or 0.08 mg/m3 SA ± 0.46 mg/m3 fly ash	0.49, 0.54 or 2.23	22°C 50%	Body weight: Survival: Hematology/blood chemistry: Pulmonary functions: histopathology: + pollutants	No alteration No death due to exposure No alteration No alteration No alteration No interaction

Repeated dose toxicity studies by inhalation conducted with sulfuric acid aerosol (continued)

	Species (strain, sex)	Ref. (year)	Protocol	Duration, frequency	Administration	Doses	Particle size (µm)	T°(C/F) RH (%)	End-point	Value (unit)/ results
•	RABBIT (NEW ZEALAND WHITE, MALE)	165 (1992)	OTHER	4, 8, 12 MONTHS, 2H/D, 5D/WK	Inhalation, nose only	0, 125 µg/m3 (SA), ± 0.1 ppm (O3)	0.3	25°C 60 %	Tracheobronchial clearance: Lung fluids: Lung histopathology: Body and lung weight: + O3:	Altered (speed then slow) No alteration Transient alteration No change Interactions (+ and -)
•	RABBIT (NEW ZEALAND WHITE, MALE)	64 (1989)	OTHER	4, 8, 12 MONTHS, 1H/D, 5D/WK	Inhalation, nose only	0, 250 µg/m3	0.3	25°C 80 %	Tracheobronchial clearance: Pulmonary functions: Lung histopathology:	Altered (decreased) Altered Altered
•	RABBIT (NEW ZEALAND WHITE, MALE)	63 (1988)	OTHER	4, 8, 12 MONTHS, 1H/D, 5D/WK	Inhalation, nose only	0, 250 µg/m3	0.3	25°C 80 %	Tracheobronchial clearance: Lung histopathology:	Altered (decreased) Altered
•	RABBIT (NEW ZEALAND WHITE, MALE)	155 (1987)	OTHER	14 days 2h/day	Inhalation, nose only	0, 500 µg/m3 (SA) ± 0.3, 1 ppm (NO2)	0.3	25°C 60 %	Respiratory region clearance: +NO2:	Altered (decreased) Interactions (at 1 ppm)
•	RABBIT (NEW ZEALAND WHITE, MALE)	160 (1987)	OTHER	14 days 2h/day	Inhalation	0, 500 µg/m3 (SA) ± 0.3, 1 ppm (NO2)	0.3	25°C 60 %	Tracheobronchial clearance: +NO2:	Altered (decreased) Interactions (at 0.3 and 1 ppm)
•	RABBIT (NEW ZEALAND WHITE, MALE)	154 (1986)	OTHER	8 months 1h/d, 5d/wk	Inhalation, nose only	0, 250 µg/m3	0.3	25°C 80 %	Alveolar clearance:	Altered (increased)
•	RABBIT (mixed breed, male)	156 (1983)	OTHER	4 weeks, 1h/d, 5d/wk	Inhalation, nose only and oral tube (at 250 µg/m3, only)	0, 250, 500 µg/m3	0.3	27°C 80 %	Tracheobronchial clearance: Lung histopathology:	Altered (increased) Altered
•	RABBIT (NEW ZEALAND WHITE, MALE)	167 (1990)	OTHER	14 days 1, 2 or 4h/d (for 50 µg/m3) 0.5, 1 or 2 h/d (for 100µg/m3)	Inhalation, nose only	0, 50, 100 µg/m3	0.3	20°C 80 %	Respiratory region clearance:	Altered (increased) only at 50 µm/m3 for 4h/d and 100 µg/m3 for 2h/d

Repeated dose toxicity studies by inhalation conducted with sulfuric acid aerosol (continued)

	Species (strain, sex)	Ref. (year)	Protocol	Duration, frequency	Administration	Doses	Particle size (µm)	T°(C/F) RH (%)	End-point	Value (unit)/ results
•	Monkey (Macaca irus, male/female)	2 (1973)	other	18 months, 23 h/d	Inhalation, whole body	0, 0.38, 2.43, 0.48, 4.79 mg/m3	2.15, 3.60, 0.54, 0.73	22°C 50 %	Body weight: Survival: Hematology/blood chemistry: Pulmonary function: Histopathology:	No alteration No death due to SA No alteration Alteration (with high dose) Alteration in lung (with high dose)
	Monkey (Macaca irus, male/female)	3 (1975)	other	18 months, 22-23 h/d	Inhalation, whole body	0, 0.1 to 5 ppm (SO2) ± 0.5 mg/m3 (fly ash) and/or 0.1 to 1 mg/m3 (SA)	0.5 to 3.35	22° 50%	Body weight: Survival: Hematology/blood chemistry: Pulmonary functions: Histopathology: + pollutants	No alteration No death due to exposure No alteration Alteration (when 1 mg/m3 SA in mixture) Alteration in lung (when 1 mg/m3 SA in mixture) No interaction
	Mouse (Swiss webster, male)	168 (1979)	other	10 to 14 days continuously	Inhalation, whole body	0, 125, 141, 154 mg/m3	0.32, 0.45, 0.62	NS NS	Death: Histopathology: Hematology: Blood and urine chemistry: Interferon (tracheal explant and alveolar macrophages):	Yes, in each group Alteration in larynx/trachea Alteration Alteration Alteration
•	Hamster, (Syrian golden, male)	105 (1978)	other	30 days, 6h/d, 5d/wk	Inhalation whole body	0, 100 mg/m3	2.6	70% 50%	Mortality: Body weight: Clinical signs: Histopathology:	No death Transient alteration Transient alteration Alteration only in larynx and trachea
•	Dog, (Beagle, female)	110 (1978)	other	620 days, 21h/d	Inhalation whole body	0, 0.9 mg/m3 (SA) ± 13.4mg/m3 (SO2)	0.5	73-76°F 43-45 %	Bodyweight: Organ weight: Hematology: Pulmonary functions: Histopathology:	No alteration Alteration for lung, heart No alteration Alteration No alteration

NS: not specified; SA: sulfuric acid; T°: temperature; RH: relative humidity

In this study, nose-only exposure of rats for 6h/d, 5d/wk for a period of 28 days to sulfuric acid aerosols resulted in pathological changes (squamous metaplasia) and in increase in cell proliferation in the larynx only. Changes of this type are commonly seen in rats exposed to irritants. Mimimal squamous metaplasia was observed in the laryngeal epithelium following exposure to the lowest concentration used (0.3 mg/m3). This effect was fully reversible. Exposure to 1.38 mg/m3 caused more severe metaplasia accompanied by cell proliferation.

Whereas the other studies presented some deficiencies and were performed using different experimental conditions, collectively, they show consistent effects in the different animals species studied.

Among the different end points measured in rats and guinea pigs, few or no alterations were observed after repeated exposure to sulfuric acid aerosol at concentration up to 10 and 20 mg/m3 in rat and guinea pig, respectively. The main alterations observed were microscopic changes in the respiratory tract (minimal proliferation of alveolar macrophages and loss of cilia in mild trachea). Sulfuric acid aerosols had no effect on hematology, blood chemistry and body weight and/or lung weight, as far as considered biological endpoints were concerned. Taken together, these results suggest that sulfuric acid aerosols seem to have a local effect and no systemic effects in these species.

Studies performed in rabbits have investigated mainly effects of sulfuric acid aerosol on respiratory tract clearance rates of labeled particles and histologic changes. Sulfuric acid aerosol at concentration ranging from 50 to 500 µg/m3 induced alterations of both tracheobronchial and respiratory region clearance as well as microscopic changes (mainly increase in epithelial secretory cell number in pulmonary airways, which could resolve by 6 months post exposure; but no evidence of inflammation) after exposure periods from 14 days to 12 months. Note that both tracheobronchial and respiratory region clearances could be accelerated or retarded according to the study considered.

In monkeys, only the highest concentrations of sulfuric acid mist (2.43 and 4.79 mg/m3) presented deleterious effects on pulmonary structures and functions while no effect on body weight, survival or hematology and blood chemistry were observed. In hamsters exposed to high concentration of sulfuric acid mist (100 mg/m3) with large particle size (2.6 µm), microscopic alterations were seen in larynx and trachea. Exposure of dogs to 0.9 mg/m3 sulfuric acid mist have induced alterations in pulmonary functions and in organ weights (lung and heart).

Overall, these results indicate that high variability in responses to repeated inhalation with sulfuric acid aerosol is found according to animal species and endpoints studied.

Taken together, these studies have shown that toxicity was confined to changes in the structure and function of the respiratory tract, suggesting that it has a local effect and no systemic effects. The observed changes are related to the irritant properties of sulfuric acid and are most likely due to the H+ ion.

No data are available on repeated dose toxicity studies by oral, dermal or by other routes for sulfuric acid.

4.2.6 Genetic Toxicity

4.2.6.1 Genetic toxicity in vitro

Sulfuric acid has been shown to be without effect in the Ames test using various strains of *S. typhimurium* (pH4 to 9) and *E. coli* (0.002 to 0.005%), both with and without S9. It has been shown to cause chromosomal aberrations in CHO cells (pH 3.5 to 7.4,both with and without S9), and in a non-standard assay in developing sea urchin embryos (pH 5 – without S9) (Scott *et al.*, 1991).

4.2.6.2 Genetic toxicity in vivo

No studies on the in-vivo mutagenicity of sulfuric acid are available.

Conclusions:
In-vitro studies have shown an effect of sulfuric acid in chromosomal assays, but not point mutation assays.

The chromosomal effects are well known to be a consequence of reduced pH, being seen using any strong acid.

Whilst the mutagenicity of sulfuric acid has not been studied using in-vivo systems, such testing would seem inappropriate because sulfuric acid will dissociate in contact with biological systems and depending on the concentration it will buffer and lead to a lowering of pH. As such, only sulfate ions would be presented to the remote target cells of the standard assay systems, including germ cells, and would be predicted to be without effect. No standard assay systems are available to study such effects in relevant target organs (e.g. larynx). Moreover, it is likely that any long-term effects of sulfuric acid on such organs would be dominated by the anticipated irritant/necrotic effects so that such mutagenicity testing would seem to be unnecessary.

4.2.7 Carcinogenicity

Carcinogenicity studies performed with sulfuric acid solution or mist are summarized in the table below. However, all of these studies present several important deficiencies (e.g. small numbers of animals per group, only pathological report available for studies n° 54 and 55). The code **3** (not reliable) for reliability/validity has been assigned to all these studies.

Carcinogenicity studies conducted with sulfuric acid

	Test Type, Species, Strain	Ref. (year)	Protocol	Duration, Frequency	Animal /group	Dose	Result
•	CARCINOGENICITY, RAT, WISTAR	187 (1997)	Chronic gastric intubation	Life-time, 1X/WK FOR LIFE	30 M + 30 F	0.5 ML OF 0.6 % SA SOLUTION (MTD)	Local and weak carcinogen.
•	CARCINIGENICITY OR CO-CARCINOGENICITY, RAT, WISTAR	187 (1997)	Chronic intratracheal instillation	LIFE-TIME, 2x/month for 12 months	30 M + 30 F	0.3ml of 0.6 % SA solution (MTD) ± BaP	Local and weak carcinogen. Synergy with BaP
•	CARCINOGENICITY OR CO-CARCINOGENICITY, MOUSE, CBAXC57BL	187 (1997)	Chronic gastric intubation	Life-time 1x/wk for life	30 M + 22 to 27 F	0.2ml of 0.2 % SA solution (MTD) ± Urethane	Local and weak carcinogen. No synergy with Urethane
•	Initiation/Promotion or co-carcinogenicity Hamster ,	105 (1978)	Inhalation (mist)	Lifetime, 6h/d, 5d/wk	60 M	0, 100 mg/m3 (particle size: 2.6 µm) ± BaP	No evidence of carcinogenic potential. Equivocal for

	Syrian golden						promoting or co-carcinogenic effect with BaP
•	Carcinogenicity Rat, Fischer 344	55 (1978)	Inhalation (mist)	2 years	No data	0, 10 mg/cm3 (SA) ± 0.5 ppm (O3)	No carcinogenic effect
•	Carcinogenicity Guinea pig	54 (1978)	Inhalation (mist)	2 years	No data	0, 10 mg/cm3 (SA) ± 0.5 ppm (O3)	No carcinogenic effect

SA: sulfuric acid; MTD: Maximal Tolerated Dose; BaP: Benzo(a)pyrene; O3: Ozone; M: male; F: female

A local and weak carcinogenic effect was observed after treatment with sulfuric acid solution by intratracheal instillation or gastric intubation in both rats and mice. Tumors appeared the second year in those organs where sulfuric acid acted directly. Tumors observed in rats and mice after exposure to sulfuric acid by gastric intubation were mainly benign forestomach tumors (papillomas or micropapillomas): 16 tumors in the treated group and 9 in untreated control for rats, and 4 tumors in the treated group and 2 in the control group for mice. Hyperplasia of the epithelium of the forestomach, hyperkeratosis and acanthosis were also seen more frequently in animals receiving sulfuric acid alone. One malignant lung tumor (a poorly differentiated adenocarcinoma was also noticed in a rat treated with sulfuric acid by gavage. The type of lesions/tumors observed in both rats and mice treated by gavage with sulfuric acid are generally related to repeated irritation/cytotoxicity. Following intratracheal instillations of sulfuric acid solution, various tumors appeared, mainly of the respiratory tract (1chrondrosarcoma of trachea, 1 bronchial adenocarcinoma and 1 histiocytoma of lung), forestomach (6 malignant oesophagus/forestomach tumors) and lymphomas with a higher incidence than the untreated control. However, this study is compromised by several deficiencies (e.g. too few animals/group, inappropriate control groups, design of the study, analyses, and reporting of the results).

No carcinogenic effects were observed in studies performed with sulfuric acid mist although these studies also have been compromised by deficiencies.

It is noticeable that, in chronic/long term studies performed with sulfuric acid mist, no neoplastic lesions were evidenced in different animal species (see chapter *4.2.5: Repeated dose toxicity*).

4.2.8. Toxicity to reproduction and developmental toxicity/teratogenicity

4.2.8.1 Effects on Fertility

No studies were identified regarding toxicity to reproduction in animals after oral, dermal or inhalation exposure to sulfuric acid.

However, due to irritant/corrosive effects of H_2SO_4, oral and dermal routes are not appropriate for testing toxicity to reproduction. In addition, H_2SO_4 is a direct-acting toxicant. The acid as such, is not expected to be absorbed or distributed throughout the body. Therefore, it is not likely that it will reach male and female reproductive organs following exposures by any route. The anion sulfate probably does not play a specific toxicological role because it is a normal metabolite of sulfur-containing amino acids and it is excreted in the urine when in excess (144).

In long term/chronic or carcinogenicity studies no gross histological alterations were found in reproductive organs in 2 different species (rat and guinea pig) after exposure to 1-10 mg/m3 sulfuric acid aerosol, and therefore, microscopic examination was not judged necessary (54, 55, 184).

4.2.8.2 Developmental toxicity/teratogenicity

In a developmental toxicity study conducted under a method similar to OECD test Guideline 414, no significant effects on mean numbers of implants/dam, live fetuses/litter or resorptions/litter were observed in mice and rabbits exposed by inhalation to sulfuric acid aerosol at 5 and 20 mg/m3 during gestation (129).

Developmental toxicity/teratogenicity studies conducted with sulfuric acid mist								
	Species, Strain	**Ref. (year)**	**Protocole**	**Administration**	**Exposure time, frequency**	**Doses**	**Endpoint**	**Value**
•	Mouse, CF-1	129 (1979)	Similar to OECD Test guideline 414	Inhalation, whole body	Day 6 to 15 of gestation, 7h/d	0, 5, 20 mg/m3	NOAEL maternal NOEL teratogenicity	20 mg/m3 20 mg/m3
•	Rabbit, New Zealand white	129 (1979)	Similar to OECD Test guideline 414	Inhalation, whole body	Day 6 to 18 of gestation, 7h/d	0, 5, 20 mg/m3	NOAEL maternal NOEL teratogenicity.	20 mg/m3 20 mg/m3

NOAEL for maternal toxicity appear to be 20 mg/m3 for both species. No evidence of foetotoxicity or teratogenicity was seen in either species.

As demonstrated by numerous studies, sulfuric acid is a direct-acting toxicant. Because of the irritant/corrosive effect of sulfuric acid and the absence of effects observed on reproductive organs in long term/chronic studies as well as in a study related to reproduction, it may be concluded that a specific study to reproduction is not necessary.

4.2.9 Other relevant information

Among the experiments studying sulfuric acid effects that could not be integrated into the above chapters due to their special design, the most reliable or informative of them are summarized in the following table.

Other studies conducted with sulfuric acid									
Species, Strain	**Ref. (year)**	**Test Type**	**Administra-tion**	**Exposure time, frequency**	**Doses**	**Particle size (µm)**	**T° RH**	**Endpoint**	**Result**
Guinea pig NS	6 (1958)	Acute inhalation Influence of aerosol particle size on alteration of pulmonary functions	Inhalation, head only	1 hour	1.9-43.6 mg/m3	0.8, 2.5, 7	NS 38 %	Pulmonary functions:	Alterations : various degree according to particle size, the 7 µm is the less effective
Guinea pig Hartley	171 (1981)	Acute inhalation, pulmonary functions	Inhalation, head only	1 hour	0, 1.2, 14.6, 24.3, 48.3 mg/m3	1	NS, 40 or 80%	Pulmonary functions:	All-or- non response, 2 polpulations: responsive and non-responsive animals
Guinea pig Hartley	148 (1998)	Acute inhalation, pulmonary functions	Inhalation, whole body	4 hours	0, 14.1, 20.1, 43.3 mg/m3	0.95	21-22 °C, 70-80 %	Pulmonary functions: Lung histopathology: Bronchoalveolar lavage fluis:	Alteration dose- and time-dependent Alteration (high dose) Alteration (high dose)
Guinea pig NS	198 (1986)	Acute inhalation, Tracheal clearance Airway fluid	Inhalation, whole body	6 hours	0, 1, 10, 27 mg/m3	~0.9	NS 80%	Tracheal clearance: Bronchoalveolar fluids: Lung and trachea histopathology:	Transient alteration (slower at 1 mg/m3) Transient alteration in responsive animals Marked alterations in responsive animals
Guinea pig, Hartley	37 (1978)	Respiratory changes	Inhalation, whole body	2days, 6h/d	0, 25 mg/m3	1	55-60 %	Respiratory tract histopathology:	Alteration in all animals
Guinea pig, Hartley	143 (1993)	Acute/repeated inhalation, in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, nose only	3 hours or 3h/d, 5 days	0, 970 µg/m3	0.3	NS, NS	Lavage fluids: Macrophage intracellular pH :	No alteration Alteration
Guinea pig, Hartley	31 (1992)	Acute/repeated inhalation, in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, nose only	3 hours or 3h/d, 4 days	0, 300 µg/m3	0.04 or 0.3	NS, NS	Lavage fluids: Macrophage intracellular pH: Products: Phagocytic activity Effect of particle size:	Transient alteration Alteration Alteration Alteration Different effect for fine or ultrafine

Other studies conducted with sulfuric acid (continued)									
Species, Strain	**Ref. (year)**	**Test Type**	**Administra-tion**	**Exposure time, frequency**	**Doses**	**Particle size (µm)**	**T° RH**	**Endpoint**	**Result**
Guinea pig Hartley	57 (1975)	Acute inhalation, Respiratory deposition of ^{33}P-labeled bacteria aerosol	Inhalation, whole body	1 hour	0 30, 300, 3020 µg/m3	 0.25 0.6 1.8	24-26 °C, 50-55 %	Respiratory functions: Aerosol deposition in respiratory tract:	No alteration Increased in trachea and nasopharynx at 30 and 3020 µm/m3, respectively
Guinea pig, Hartley	101 (1993)	Chronic inhalation Airway responsiveness to histamine	Inhalation, whole body	3, 7, 14, 30 days, 24h/d	0, 1, 3.2 mg/m3	0.55	25 °C, 55 %	Lung wet weight Airway responsiveness:	No alteration Transient alteration at 3.2 mg/m3
Guinea pig, Hartley	61 (1992)	Chronic inhalation Lung mast cell function in culture	Inhalation, whole body	2, 4 wk 24h/d	0, 0.3, 1, 3.2 mg/m3 (SA) ± NO2	0.65 0.55 0.73	NS NS	Number of isolated cell: Mast cell histamine release: + NO2	No alteration Transient alteration at ≥ 1 mg/m3 No interaction
Rabbit, Mixed breed	30 (1983)	Acute inhalation, Tracheobronchial clearance Comparison between effects of sulfuric acid mist and other sulfites: (Fe(III)-S(IV) or Na2SO3	Oral delivery	1 hour	0, 260-2155 µg/m3 (SA) or 238–1227 µg/m3 Fe(III)-S(IV) or 270–1950 µg/m3 Na2SO3	0.3	24°C 75%	Tracheobronchial clearance	Alteration, dose-related, for SA No alteration with Fe(III)-S(IV) Alteration with Na2SO3 at ≥ 1200 µg/m3
Rabbit, New Zealand White	159 (1984)	Acute inhalation, Tracheobronchial clearance	Oral delivery	1 hour	100-1084 µg/m3	0.3	24°C 75%	Tracheobronchial clearance	Alteration, dose-related: transient acceleration at low doses / retardation at higher doses
Rabbit, New Zealand White	166 (1989)	Acute/Repeated inhalation, Tracheobronchial and alveolar clearance Comparison: effects of SA, NH4HSO4, $(NH4)_2SO4$	Oral delivery or nasal mask	2-4 hours , or 2-4h/d, 14 day 2 HOURS 2 HOURS	0.1-2 mg/m3 (SA) or 0.5, 1, 2 mg/m3 NH4HSO4 or 2 mg/m3 $(NH4)_2SO4$	0.3	24-25°C 75 or 80%	Tracheobronchial and alveolar Clearance Comparison effect of the different acids	Alteration concentration and time dependent SA is the more irritant

Other studies conducted with sulfuric acid (continued)

Species, Strain	Ref. (year)	Test Type	Administra-tion	Exposure time, frequency	Doses	Particle size (µm)	T° RH	Endpoint	Result
Rabbit, New Zealand White	163 (1992)	Acute inhalation, in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, nose only	3 hours	0, 50, 75, 125 µ/m3 (SA) ± O3	0.3	25 °C, 55%	Lavage fluids: Macrophage products: Phagocytic activity: +O3	No alteration Alteration (high dose) Alteration (high dose) Interaction
Rabbit, New Zealand White	201 (1992)	Acute inhalation , in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, nose only	2 hours	0, 50, 75, 125, 500 µg/m3	0.3	25 °C, 60%	Lavage fluids: Macrophage products:	No alteration Alteration (except 50 µg/m3)
Rabbit, New Zealand White Human	203 (1997)	Acute inhalation, in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, Rabbit: nose only, at rest. Human: whole body, exercising	3 hours	0, 1mg/m3	0.8	25 °C, 60% (rabbit) 21°C 38% (human)	Lavage fluids: Macrophage products and properties: Phagocytic activity:	Few alteration in rabbit Few alteration, mainly in rabbit Alteration in rabbit No alteration in human
Rabbit, New Zealand White	32 (1995)	Acute inhalation, in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, nose only	3 hours	0, 50, 125 µg/m3 (SA) ± O3	0.3	25 °C, 55 %	Lavage fluids: Macrophage intracellular pH : and H+ extrusion: +O3	No alteration Alteration at 125 µg/m3 Alteration Some interaction
Rabbit, New Zealand White	153 (1987)	Repeated inhalation, in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, nose only	2, 6, 13 days 2h/d	0, 500µg/m3	0.3	25 °C, 60%	Lavage fluids: Macrophage properties : Phagocytic activity:	Transient alteration Alteration (for mobility) Alteration
Rabbit, New Zealand White	202 (1994)	Repeated inhalation, in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, nose only	4 days 2h/d	0, 500, 750, 1000 µg/m3 (SA) or 1 mg/m3 (NH4HSO4)	0.3	25 °C, 60%	Lavage fluids: Macrophage products: Phagocytic activity: NH4HSO4 :	Alteration at 1 mg/m3 for some endpoints Alteration Alteration at 1 mg/m3 No alteration
Rabbit, New Zealand White	161 (1990)	Repeated inhalation, in vivo, Bronchoalveolar lavage Lung macrophage culture	Inhalation, nose only	5 days, 1h/d	0, 0.25, 0.5, 1, 2 mg/m3 (SA) or 0.5-4mg/m3 (NH4HSO4)	0.3	25 °C, 60%	Lavage fluids: Macrophage phagocytic activity:	No alteration Alteration (at smaller doses than NH4HSO4)

Other studies conducted with sulfuric acid (continued)									
Species, Strain	**Ref. (year)**	**Test Type**	**Administra-tion**	**Exposure time, frequency**	**Doses**	**Particle size (µm)**	**T° RH**	**Endpoint**	**Result**
Rabbit, New Zealand White	162 (1990)	In vivo repeated inhalation, in vivo, bronchoalveolar lavage In vitro exposure of tracheal explants : pH, osmolarity and associated anion effects	Inhalation, nose only Culture with various medium	5 days, 1 h/d 5 hours	0, 250, 500, 1000 µg/m3 Effect of HCl and Na2SO4, (in vitro)	0.3	24 °C, 60 %	PGE2,PGF2, TxB2: LTB4 : pH effect (in vitro) on eicosanoid products: associated anion:, osmolarity effect	Decreased: in vivo, No alteration: in vivo Similar than in vivo exp. No alteration No alteration
Rabbit, New Zealand White	65 (1986)	Repeated inhalation, Bronchial responsiveness to Ach	Inhalation, nose only	4, 8, 12 months 1h/d, 5d/wk	0, 250 µg/m3	0.3	25 °C, 80 %	Pulmonary functions: Bronchial responsiveness to Ach:	No alteration Hyperresponsive airways
Mice Swiss-Webster derived	56 (1975)	Acute/repeated inhalation, Respiratory tract clearance of ^{33}P-labeled bacteria	Inhalation	4 hours or 90 min/d, 4 days	1.5 mg/m3 15 mg/m3	0.6 3.2	23 °C, 70 %	Respiratory tract histopathology: Respiratory tract clearance of bacteria:	No alteration Alteration with concentrated acid only
Mice Swiss-Webster	138 (1980)	Intermittent inhalation, Enhancement of allergic lung sensitization	Inhalation	Repeated period of 3 to 7 days	0, 1 mg/m3 (SA) ± O3	0.041 (CMD)	25 °C, 50 %	Immune responsiveness to inhaled antigen: Allergic sensitization : + O3	No alteration No alteration Interaction
Donkey	157 (1978)	Acute inhalation, Pulmonary functions and tracheobronchial clearance Comparison:effects of SA and $(NH4)_2SO4$	Inhalation, nasal catheter	1 hour	71-1506 µg/m3 (SA) 290-3140 µg/m3 $(NH4)_2SO4$	0.3-0.6	28°C 45%	Pulmonary functions: Tracheobronchial clearance: $(NH4)_2SO4$:	No alteration Transient or more persitent alteration according to animal No effect on clearance
Donkey	158 (1979)	Repeated inhalation, Tracheobronchial clearance	Inhalation, nasal catheter	6MONTHS, 1h/d, 5d/wk	~104 µg/m3	0.5	28°C 45%	Tracheobronchial clearance	Alteration :variable degree among animals

Other studies conducted with sulfuric acid (continued)									
Species, Strain	**Ref (year)**	**Test Type**	**Administra-tion**	**Exposure time, frequency**	**Doses**	**Particle size (µm)**	**T° RH**	**Endpoint**	**Result**
Rat, F344	198 (1986)	Acute inhalation, Tracheal clearance Airway fluid	Inhalation, whole body	6 hours	0, 1, 10, 100 mg/m3	~0.9	NS 80%	Tracheal clearance: Bronchoalveolar fluids: Lung and trachea histopathology:	Transient alteration (faster at 10 mg/m3) No alteration Alteration in trachea
Rat , Sprague Dawley	98 (1997)	Repeated inhalation Influence of acid aerosol droplet size	Inhalation, Nose only	2days, 4h/d	0, 0.5 mg/m3 (SA) ± O3	0.06 (ultrafine) and 0.3 (fine) for labeling : 0.7	NS NS	Morphology Pulmonary functions: Pulmonary lesions: Lung labeling index in periacinar region: +O3	No alteration Change: ultrafine only Change: ultrafine only Change: ultrafine only Interaction with ultrafine
Dog, Beagle; Rat, F344; Guinea pig, Hartley	45 (1983)	Acute inhalation, Clearance via blood	Inhalation, nose only or nasal instillation	1minute (dog), 30 seconds (other)	1-20 mg/m3	0.45-1.10	NS 20 or 80%	Blood clearance half-time of an ^{35}S-labeled sulfuric acid solution	Half time of clearance from lung to blood ranges from2 to 9 min. No effect of RH
Human, Rats, Rabbits, Guinea pigs	164 (1992)	Lung macrophage culture, Sulfuric acid challenge in vitro (up to pH:4.5)						Macrophage viability: Phagocytic acivity: Sensitivity:	No alteration Decreased with decreasing pH in all species Guinea pig> rat> rabbit> human

NS: not specified; RH: relative humidity

Several experiments have examined changes in pulmonary structures and functions, in respiratory tract clearance, in bronchoalveolar lavage fluids, and in i*n vitro* pulmonary macrophage properties in different laboratory animal species, after acute or short-term repeated exposures to sulfuric acid mist. Taken together, these results indicate that there is a considerable interspecies variation in sensitivity to sulfuric acid aerosols among laboratory animals. Effects of sulfuric acid are also highly dependent on the characteristics of the aerosol, on the endpoint measured and on the experimental conditions.

In experiments studying the active component in inorganic acids on various endpoints, the observed responses seem to be due to the H+ ion while the anion appears to have no effect. The sulfur from sulfuric acid is rapidly cleared from the lungs of animals into the blood following inhalation exposure (see also Chapter *4.2.1 Mode of action of the chemical, toxicokinetics and metabolism*).

Human appears to be the less sensitive to the effects of the acid in studies investigating *in vitro* functional properties of pulmonary macrophages recovered from different species exposed *in vivo* or *in vitro* to sulfuric acid. For some authors, one of the reason of higher tolerance of human cells to the effects of sulfuric acid aerosol could be that human cells are normally exposed *in situ* to pollutants and microbes from ambient environment, while laboratory animals are raised and housed in facilities that are relatively free of ambient pollutant and microorganisms.

4.2.10 Human data

Acute inhalation exposure to sulfuric acid aerosols causes a range of effects in the respiratory system including decrease in particle clearance rates at lower concentrations (< 1.0 mg/m^3) to changes in lung function (>1.0 mg/m^3). Asthmatics and those with hyper-reactive airways appear more sensitive to the broncho-constrictive effects of the aerosol. Repeated exposure to higher concentrations of aerosol (>3.0 mg/m^3) has been reported to cause damage to the incisors.

Sixteen retrospective mortality or cancer incidence studies have been reported on populations with potential exposure to sulfuric acid aerosols or mists from a wide range of industries, including the manufacture of sulfuric acid, isopropanol, fertilisers and soaps and detergents, lead battery manufacture, metal pickling and the steel industry. In general, these studies have shown increases in lung cancer incidence or cancer of the respiratory tract and, in some cases, laryngeal cancer. Other studies in similar populations have shown no such increases. A feature of all of the studies was the potential for co-exposure to a range of different chemicals, some of which are known to be carcinogenic. Some of the studies were also inadequately controlled for known confounding factors such as smoking.

The occupational factors associated with the in occurrence of laryngeal cancer have been studied in three case-control studies, in which increased odds ratios for laryngeal cancer have been shown for those with occupational exposure to sulfuric acid mist. A fourth case-control study of laryngeal cancer cases on the Texas Gulf Coast failed to demonstrate this relationship.

A case-control study of stomach cancer showed an increased odds ratio in those with occupational exposure to sulfuric acid mists. This study could only be considered as an hypothesis-generating study.

These studies suggest that there is a moderate association between occupational exposure to acid mists containing sulfuric acid and laryngeal cancer that cannot be wholly explained by chance or by confounding by smoking or alcohol. However, given the uncertainty regarding a possible carcinogenic mechanism for sulfuric acid and the likelihood of multiple exposures to other agents in

the work environment, of which sulfuric acid mist is a part, these data are insufficient to demonstrate a causal relationship for this association. There is also little evidence to support a causal relationship between occupational exposure to sulfuric acid mist and lung cancer and there is inadequate information for drawing any meaningful conclusion about an association between occupational exposure to sulfuric acid mist and nasal and other respiratory tract cancers.

The WHO International Agency for Research on Cancer (IARC) reviewed the epidemiology studies and reported in a Monograph in 1992 that "there is sufficient evidence that occupational exposure to strong inorganic mists containing sulfuric acid is carcinogenic to humans". This conclusion has led IARC to classify "occupational exposure to strong inorganic acid mists containing sulfuric acid" as a Group 1 carcinogenic activity (88). It is stressed this classification applies to exposure to the mist (or aerosol) and not to sulfuric acid per se

However, it seems likely that sulfuric acid aerosols in sufficiently high concentrations are deposited in preferred locations in the nasopharyngeal and/or laryngeal regions, where they cause repetitive injury, inflammation and repair. The resulting increased cell proliferation, in conjunction with other carcinogenic agents, may well be responsible for the observed, rather weak association between exposure and effect. Such preferential deposition and extremely localised induced effects (squamous metaplasia and persistent proliferation) have recently been demonstrated in rodents in a 28 day inhalation study in rats (74).

CONCLUSIONS AND RECOMMENDATIONS

The chemical is a candidate for further work :

Environment: the collection of information about exposure during agricultural use should be considered

Health: the collection of information about occupational exposure to sulfuric acid mist should be considered.

REFERENCES

References not reported in the IUCLID Dossier:

- Klimish *et al.*, (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology, 25, 1-5.
- Rosner G (1994), Fraunhofer-Institut fur Toxikologie und aerosolforschung, VAL0811. doc
- ILO encyclopedia of Occupatioal health and Safety, Vol. 2, 3d ed, (1985) ISBN 92-2-103291-4
- ECETOC Technical Report n° 77: Skin and Respiratory Sensitisers: Reference Chemical Data Bank. Aug 99, ECETOC, Brussels.
- Scott *et al*., (1991). Genotoxicity under extreme culture conditions. A report from ICPEMC Task Group 9. Mutation Res. 257, 147-205.
- Food and Environment Protection Act, 1985, Part III, Control of Pesticides Regulations 1986, Evaluation on : Sulphuric acid, April 1998, Ministry of Agriculture, Fisheries and Food, Pesticides Safety Directorate, UK.

References reported in the IUCLID Dossier :

(1) Ahlborg G, Hogstedt C, Sundell M and Aman C. Laryngeal cancer and pickling house vapours. Scand. J Work. Environ. Health, 7, 239, 1981

(2) Alarie et al. (1973). Long-term continuous exposure to sulfuric acid mist in cynomolgus monkeys and guinea pigs. Arch. Environ. Health 27, 16-24.

(3) Alarie et al., (1975). Long-term exposure to sulfur dioxide, sulfuric acid mist, fly ash, and their mixtures. Arch. Environ. Health; 30, 254-262.

(4) Alderson M and Rattan N. Mortality of workers on an isopropyl alcohol plant and two MEK dewaxing plants. Br. J. Ind. Med., 37, 85, 1980.

(5) Amdur et al., Arch. Ind. Hyg. Occup. Med. 6, 305-329 (1951) zitiert in Wolff et al., J. Toxicol. Environ. Health 5, 1037-1047 (1979)

(6) Amdur M.O. (1958). The respiratory response of guinea pigs to sulfuric acid mist. Arch. Ind. Health, 18, 407-414.

(7) Amdur M.O., (1989). Health effects of air pollutants: sulfuric acid, the old and the new. Environ. Health Perspect., 81, 109-113.

(8) Amdur M.O., (1989). Sulfuric acid: the animals tried to tell us. Appl. Ind. Hyg. 4(8), 189-197.

(9) Amdur MO, Schulz RZ, Drinker P. (1952) Toxicity of sulfuric acid mist to guinea pigs. Arch. Ind. Hyg. Occup. Med. 5, 318-329.

(10) Amdur MO. (1971). Aerosols formed by oxidation of sulfur dioxide. Arch. Environ. Health, 23, 459-468.

(11) Ato-Fina data

(12) Avol et al., Toxicol. Ind. Health 4, 173-184 (1988).

(13) BASF AG data

(14) BASF AG, Werksaerztlicher Dienst, unveroeffentlichte Mitteilung, 1992.

(15) Bayer A G results

(16) Bayer AG data

(17) Beaumont JJ, Leveton J, Knox K, Bloom T et al. Lung cancer mortality in workers exposed to sulphuric acid mist and other acid mists. J. N. C. I., 79, 911, 1987.

(18) Belding D.L. (1927) Trans. Amer. Fish. Soc. 57, 100-119

(19) Bell H.L. (1970) Effects of pH on the life cycle of the midge Tanytarsus dissimilis Can. Ent. 102, 636-639.

(20) Birnbaum et al., (1983). The pathogenesis of synergistic lung damage in mice by an environmental irritant (H2SO4) and particulate antigen. Toxicology 28, 261-269.

(21) Block T, Mantanoski G, Seltser R and Mitchell T. Cancer morbidity and mortality in phosphate workers. Cancer Res., 48, 7298, 1988.

(22) Boulet, Chest 94, 476-481 (1988).

(23) Bretherick (1979) Handbook of Reactive Chemical Hazards,Butterworths, London

(24) Brown L, Mason T, Pickle L, Stewart P et al. Occupational risk factors for laryngeal cancer on the Texas Gulf coast. Cancer Res., 48, 1969, 1988.

(25) Cavender et al., (1977). Effects in rats and guinea pigs of short-term exposures to sulfuric acid mist, ozone, and their conmbination. J. Toxicol. Environ. Health. 3, 521-533.

(26) Cavender et al., (1978). Effects in rats and guinea pigs of six-month exposures to sulfuric acid mist, ozone, and their combination. J. Toxicol. Environ. Health 4, 845-852.

(27) Chaney et al., Arch. Environ. Health 35, 211-215 (1980).

(28) Checkoway H, Matthew R, Hickey J, Shy C et al. Mortalijty among workers in the Florida phosphate industry (I). Industry wide cause-specific patterns. J. Occup. Med., 27, 885, 1985.

(29) Checkoway H, Matthew R, Hickey J, Shy C et al. Mortalijty among workers in the Florida phosphate industry (II). Cause-specific mortality relationships with work areas and exposures. J. Occup. Med., 27, 893, 1985.

(30) Chen and Schlesinger, (1983). Response of the bronchial mucociliary clearance system in rabbits to inhaled sulfite and sulfuric acid aerosols. Toxicol. Appl. Pharmacol., 71, 123-131.

(31) Chen et al., (1992). Effects of fine and ultrafine sulfuric acid aerosols in guinea pigs: alterations in alveolar macrophage function and intracellular pH. Toxicol. Appl. Pharmacol., 113, 109-117.

(32) Chen et al., (1995). Alteration of pulmonary macrophage intracellular pH following inhalation exposure to sulfuric acid/ozone mixtures. Exp. Lung Res. 21, 113-128.

(33) Cipollaro M. et al., (1986). Sublethal pH decrease may cause genetic damage to eukaryotic cell: a study on sea urchins and Salmonella typhimurium. Terat. Carc. and Mutagen, 6, 275 - 287.

(34) Cocco P, Ward M, Dosemeci M. Occupational risk factors for cancer of the gastric cardia. J. Occup. Environ. Med. 40, 855 - 861, 1998.

(35) Cockrell et al. (1976), Am. Rev. Respir. Dis. 113, 91

(36) Cockrell et al., (1977). Correlation of light and electron microscopic pulmonary lesions in guinea pigs exposed to sulfuric acid mist. Lab. Invest. 36, 334 (abstract).

(37) Cockrell et al., (1978) . Respiratory tract lesion in guinea pigs exposed to sulfuric acid mist. J. Toxicol. Environ.Health, 4, 835-844.

(38) Cockrell et al., Am. Rev. Respir. Dis. 113, 91 (1976).

(39) Coggon D, Pannett B, Wield G. Upper aerodigestive cancer in battery manufacturers and steel workers exposed to mineral acid mists. Occupational and Environmental Medicine, 1996, 53, 445 - 449.

(40) Cookfair D, Wende K, Michalek A and Vena J. A case-control stiudy of laryngeal cancer among workers exposed to sulfuric acid (abstract). Am. J. Epidemiol., 122, S21, 1985.

(41) Cooper WC and Gaffey WR. Mortality of lead workers. J. Occup. Med., 17, 100, 1975.

(42) Cooper WC, Wong O and Kheifets L., Mortality among workers at lead battery plants and lead producing plants, 1947 - 1980. Scand. J. Work. Environ. Health, 11, 331, 1985.

(43) Craig G.R. and W.F. Baski (1977). The effects of depressed pH on flagfish reproduction, growth and survival. Water Research, 11, 621-626.

(44) CRC Handbook of chemistry and Physics, 71th Ed., 1990-1991, CRC Press Inc.

(45) Dahl et al., (1983). Clearance of sulfuric acid-introduced 35S from the respiratory tracts of rats, guinea pigs and dogs following inhalation or instillation. Fundam. Appl.Toxicol. 3, 293-297.

(46) Davis P. and G.W. Ozburn (1969). The pH tolerance of Daphnia pulex (Leydig, emend., Richard). Can. J. Zool., 47, 1173-1175.

(47) Demerec M. et al., (1951). A Survey of chemicals for mutagenic action on E. coli. The Amer. Naturalist, 85, 119 - 136.

(48) Denzer (1961), Merkblatt uber die Schaedigung der Fischerei durch Abwaesser, Landesanstalt fuer Fischerei NW.

(49) Elgaard E.G. and J.Y. Gilmore III (1984) J. Fish. Biol. 25 (2), 133-138

(50) Ellis (1937), Bulletin of the Bureau of Fisheries 48, 365-437

(51) Ellis M.M. (1937), Bulletin of the Bureau of Fisheries 48, 365-437

(52) Englander V, Sjoberb A, Hagmar L, Attewell R et al. Mortality and cancer morbidity in workers exposed to sulphur dioxide in a sulphuric acid plant. Int. Arch. Occup. Env. Health, 61, 157, 1988.

(53) Enterline P. Importance of sequential exposure in the production of epichlorhydrin and isopropanol. Ann. NY Acad. Sci., 381, 344, 1982.

(54) Experimental pathology Laboratories, Inc. (1978) : 2-year inhalation - Guinea pigs, EPL 119-009, Pathology Report. Project DB-009, revised in 1979.

(55) Experimental pathology Laboratories, Inc. (1978) : 2-year inhalation - Rats, EPL 119-009, Pathology Report. Project DB-009.

(56) Fairchild et al., (1975). Sulfuric acid and streptococci clearance from respiratory tracts of mice. Arc. Environ. Health, 30, 538-545.

(57) Fairchild et al., (1975). Sulfuric acid effect on the deposition of radioactive aerosol in the respiratory tract of guinea pigs. Am. Ind. Hyg. Assoc. J., pp.: 584-594.

(58) Findlay D.L. and S.E.M. Kasian (1986). Phytoplankton community responses to acidification of Lake 223, experimental lakes area, Northwestern Ontario Water, Air and Soil Pollution, 30, 719-726.

(59) Forastiere F, Valesini S, Salimei E, Magliola E et al. Respiratory cancer among soap production workers. Scand. J. Work. Environ. Health, 13, 258, 1987.

(60) France R.L. (1987). Reproductive impairment of the crayfsh Orconectes virilis in response to acidification of lake 223. Can. J. Fish. Aquat. Sci., 44, 97-106

(61) Fujimaki et al., (1992). Enhanced histamine release from lung mast cells of guinea pigs exposed to sulfuric acid aerosols. Environ. Res., 58 (1), 117-123.

(62) Fujisawa et al., Arerugi 35, 137-144 (1986) zitiert in der Datenbank TOXLINE.

(63) Gearhart and Schlesinger (1988). Response of the tracheobronchial mucociliary clearance system to repeated irritant exposure: effect of sulfuric acid mist on function and structure. Exp. Lung Res. 14, 587-605.

(64) Gearhart and schlesinger (1989). Sulfuric acid-induced changes in the physiology and structure of the tracheobronchial airways. Environ. Health Pers. 79, 127-137

(65) Gearhart and Schlesinger. (1986). Sulfuric acid-induced airway hyperresponsiveness. Fundam. Appl. Toxicol. 7, 681-689.

(66) Gomez et al., (1979). The effects on inhaled sulfuric acid aerosols on alveolar macrophage phagocytosis. Toxicol. Appl. Pharmacol. 48, A67 Abstract n¿134.

(67) Graham J.A., (1989). Review, discussion, and summary: toxicology. Environ. Health Perspect., 79, 191-194.

(68) Griffith et al. (1980) Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. Toxicology and Applied Pharmacology, 55, 501-513.

(69) Grose et al., (1978) The effect of ozone and sulfuric acid on ciliary activity of syrian hamsters. Pharmacologist 20, 211 (Abstract).

(70) Grose et al., (1982) Pulmonary host defense responses to inhalation of sulfuric acid and ozone. J. Toxicol. Environ. Health, 10, 351-362.

(71) Hagmar L, Bellander T, Anderson C, Linden K et al. Cancer morbidity in nitrate fertilizer workers. Int. Arch. Occup. Environ. Health, 63, 63, 1991.

(72) Hah et al., J. Cathol. Med. Coll. 41, 69-76 (1988) zitiert in der Datenbank TOXALL.

(73) Hermann (1959) Ind. Eng. Chem. 51 (4) 84A-87A

(74) Hext P. et al., (in preparation). Sulphuric acid: 28 day inhalation study in the rat. Zeneca Report No CTL/P/6278.

(75) Hoechst AG (1985) Unveroffentl. Unters. (Ber.-Nr. 85.0450)

(76) Hoechst AG (1985) Unveroffentl. Unters. (Ber.-Nr. 85.0487

(77) Hoechst AG (1985) Unveroffentl. Unters. (Ber.-Nr. 85.0525)

(78) Hoechst AG (1985) Unveroffentl. Unters. (Ber.-Nr. 86.1283)

(79) Hoechst AG (1985): Unveroffentl. Unters. (Ber.-Nr 85.0427)

(80) Hoechst AG (1985): Unveroffentl. Unters. (Ber.-Nr 85.0449)

(81) Hoechst AG data

(82) Hoffman and Campbell (1977) Embryotoxicity of irradiated and nonirradiated catalytic converter-treated automotive exhaust. J. Toxicol. Environ. Health 3, 705-712.

(83) Horton et al.Biological services division, Hoboken, USA, Report N DOT/MTB/ DHMO-76/2 (1976).

(84) HSDB, Hazardous Substances Data Bank, No. 1811 (28/04/1992)

(85) Hu, Diss. Abs. Int. 44, 2107-B (1984).

(86) Hurley G.V. and T.P. Foyle (1989). Differences in acid tolerance during the early life stages of three strains of Brook Trout, Salvelinus fontinalis. Water air soil pollut. 46, 387-398.

(87) IARC Monograph on the Evaluation of Carcinogenic Risks to Humans - Occupational Exposures to mists and Vapours from strong Inorganic Acids; and Other industrial Chemicals .(1992), Vol 54, p41.

(88) IARC, IARC Monographs on the Evaluation of the Carcinogenic. Risk of Chemicals to Humans, 54, 41, 1992, Sulphuric acid and other strong inorganic acids, occupational exposures to mists and vapours from.

(89) Ichinose and Sagai (1992). Combined exposure to NO2, O3 and H2SO4-aerosol and lung tumor formation in rats. Toxicology. 74, 173-184.

(90) Iguchi et al. (1986), Arerugi 35, 402-408 ; citated in Toxline databank

(91) INRS (1988) Fiche Toxicolologique No. 30 (Acide sulfurique), Cahiers de notes documentaires 130, 167-171

(92) IRCHA et MinistÞre de l'Environnement et du Cadre de Vie, Les produits chimiques dans l'environnement (1981)

(93) Izmerov NF et al. (1982). Toxicometric Parameters of Industrial Toxic Chemicals. LIP Moscow, p107.

(94) Jacobs G. A. and Martens M. A. (1989) An objective method for the evaluation of eye irritation in vivo. Fd Chem. Toxic. 27(4), 255-258.

(95) Jacobs Guido A. (1992) OECD eye irritation tests on two strong acids. J. Am. Coll. Toxicol., 11 (6) 734

(96) Jelenko, Z>M. Surg. 40, 97-104 (1974) zitiert in U.S. Department.

(97) John et al. (1979) Teratology 19, 32A

(98) Kimmel et al., (1997). Influence of Acid aerosol droplet size on structural changes in the rat lung caused by acute exposure to sulfuric acid and ozone. Toxicol. Appl. Pharmacol. 144, 348-355.

(99) KIRK-OTHMER (1978-1984), Encyclopedia of Chemical Technology, 3rd Ed., 22, 190-232, J Wiley and Sons

(100) Kleinman et al., (1989) Health effects of acid aerosols formed by atmospheric mixtures. Environ. Health Perspect. 79, 137-145.

(101) Kobayashi and Shinozaki (1993). Effects of exposure to sulfuric acid-aerosol on airway responsiveness in guinea pigs: concentration and time dependency. J. Toxicol. Environ. Health, 39 (2), 261-272.

(102) Kristensen P (1993). Inorganic acid aerosols. Arbete och Halsa, 1, 7-54.

(103) Kulle et al., Am. Rev; Respir. Dis. 126, 996-1000 (1982).

(104) Kulle et al., National Technical Information Service, Bericht Nr. PB 2-255126 (1982).

(105) Laskin S. and Sellakumar A. (1978) Comparison of pulmonary carcinogenicity of know carcinogens with and without added H2SO4 mists, airborne respirable particles, and gases. Final

Report of Progress to the Environmental Protection Agency, Project n¿ 68-02-1750.

(106) Last and Pinkerton (1997). Chronic exposure of rats to ozone and sulfuric acid aerosol: biochemical and structural responses. Toxicology 116, 133-146.

(107) Last et al. (1978) A new model for health effects of air pollutants: evidence for synergistic effects of mixture of ozone and sulfuric acid aerosols on rat lungs. J Lab. Clin. Med. 91, 328-339

(108) Leikauf et al., Am. Ind. Hyg. Assoc. J. 45, 285-292 (1984?.

(109) Lewis (1991) Hazardous Chemicals Desk Reference, 2nd Ed., Van Nostrand, Reinhold

(110) Lewis et al., (1973). Toxicity of long-term exposure to oxides of sulfur. Arch. Environ. Health; 26, 16-21.

(111) Lewkowski et al. (1979). Effects of chronic exposure of rats to automobile exhaust, H2SO4, SO2, AL2(SO4)3 and CO. Assessing Toxic Eff. Environ. Pollut. Chapter 11, pp: 187-217.

(112) Linn et al., J. Air Pollut. Control Assoc. 36, 1323-1328 (1986).

(113) Lippmann and Schlesinger (1984). Interspecies comparisons of particle deposition and mucociliary clearance in tracheobronchial airways. J. Toxicol. Environ. Health, 13(2-3), 441-469.

(114) Logue JN, Koontz MD and Hatwick MAW. A historical prospective mortality study of workers in copper and zinc refineries. J. Occup. Med., 24, 398, 1982.

(115) Lynch J, Hanis N, Bird M, Murray K et al. An association of upper respiratory cancer and exposure to diethyl sulfate. J. Occup. Med., 21, 333, 1979.

(116) Malcolm D and Barnett H. A mortality study of lead workers. 1926 - 1976. Br. J. Ind. Med., 39, 404, 1982.

(117) Malcom et al., Brit. J. Indust. Med. 18, 63-69 (1961)

(118) Malley D.F. and P.S.S. Chang (1986). Increase in the abundance of Cladocera at pH 5.1 in experimentally-acidified lake 223, Experimental Lakes Area, Ontario. Water, Air, Soil Pollut., 30, 629-638.

(119) Mazumdar S, Lerer T and Redmond C. Long term mortality study of steel workers. IX. Mortality patterns among sheet and tin mill workers. J Occup. Med., 17, 751, 1975.

(120) McKee et al. (1963), Water Quality Criteria. The Resources Agency of California, State Water Quality Control Board, Publ. No. 3-A, 279, USA.

(121) McMahon et al. (1983), Freshwater crayfish Pap. Int. Symp. 5th 71-85

(122) Menendez R. (1976). Chronic effects of reduced pH on Brook Trout (Salvelinus fontinalis). J. Fish. Res. Boar. Can., 33, 118-123.

(123) Merck Index (1989) 11th Ed.

(124) Mills K.H., S.M. Chalanchuk, L.C. Mohr and I.J. Davies (1987). Responses of Fish populations in Lake 223 to 8 years of experimental acidification. Can. J. Fish. Aquat. Sci., 44, 114-125.

(125) Moeschlin, Klinik und Therapie der Vergiftungen, Thieme-verlag, S 172-174 (1956).

(126) Morita T. et al., (1989). Effects of pH in the in vitro chromosomal aberration test." Mut Res, 225, 55 - 60.

(127) Mount D.I. (1973). Chronic effect of low pH on fathead minnow survival, growth and reproduction. Water Res., 7, 987-993

(128) Murphy et al. (1982) Ocular irritancy responses to various pHs of acids and bases Toxicology

23, 281-291.

(129) Murray FJ. (1979). Embryotoxicity of inhaled sulfuric acid aerosol in mice and rabbits. J. Environ. Sci. Health C13(3), 251-266

(130) Musk et al., Br. J. Ind. Med. 45, 381-386 (1988)

(131) National Research Council Canada (1977), Sulphur and its inorganic derivates, 266-267 NRCC No. 15015

(132) Newhouse et al., Arch. Environ. Health 33? 24-32 (1978).

(133) Niederlehner B.R. and J. Cairns Jr. (1990). Effects of increasing acidity on aquatic protozoan communities. Water, Air and Soil Pollut., 52, 183-196.

(134) Nixon et al. (1990) Evaluation of modified methods for determining skin irritation. Regul. Toxicol. Pharmacol. 12(2), 127-126.

(135) Nixon et al., (1975) Interspecies comparisons of skin irritancy. Toxicol. Appl. Pharmacol. 31, 481-490.

(136) Occupational Health Guideline for Sulfuric Acid (1978), US Department of Health and Human Services.

(137) Oehme et al., (1996). A review of the toxicology of air pollutants: toxicology of chemical mixtures. Vet. Human. Toxicol., 38 (5), 371-377.

(138) Osebold et al., (1980). Studies on the enhancement of allergic lung sensitization by inhalation of ozone and sulfuric acid aerosol. J. Environ. Pathol. Toxicol., 3, 221-234.

(139) Parent S. and R.D. Chhetham (1980). Effects of acid precipitation on Daphnia magna. Bull. Environm. Contam. Toxicol., 25, 298-304.

(140) Pierson W.R. (1987) Environ. Sci. Technol. 21(7), 679-691, cited in HSDB.

(141) Portman et al. (1971) Ministry of Agriculture, Fisheries and Food, Shellfish Information Leaflet No. 22 (142) Pough F.H. and R.E. Wilson (1977) Acid precipitation and reproductive success of Ambystoma salamanders. Water, Air and Soil Pollut., 7, 307-316.

(143) Qu et al., (1993). Alteration of pulmonary macrophage intracellular pH regulation by sulfuric acid aerosol exposures. Toxicol. Appl. Pharmacol., 121,138-143.

(144) Research Triangle Inst. (1998) Toxicological profile for sulfur trioxide and sulfuric acid. U.S. Department of Commerce, National Technical information Service.

(145) Rhodia data

(146) Rhone-Poulenc (1993) Safety Data Sheet (23/03/93). Internal unpublished results.

(147) Roth (1982), Wassergefaehrdende Stoffe

(148) Roth et al., (1998). Ventilatory responses in awake guinea pigs exposed to acid aerosols. J. Toxicol. Environ. Health, 54, 261-283.

(149) RRhodia data

(150) Runkle BK. and Hahn FF., (1976). The toxicity of H2SO4 aerosols to CD-1 mice and Fischer-344 rats. Ann. Rep. Inh. Toxi. Res. Inst. pp.: 435-439.

(151) Sathiakumar N, Delzell E, Amoeteng-Adjepong Y, Larson R and Cole P. Epidemiological evidence on the relationship between mists containing sulphuric acid and respiratory tract cancer. Critical Reviews in Toxicology, 27(3) 233-251 (1997).

(152) Sax et al. (1988) Dangerous Properties of Industrial Materials, 7th Ed., Van Nostrand,

Reinhold

(153) Schlesinger (1987). Functional assessment of rabbit alveolar macrophages following intermittent inhalation exposures to sulfuric acid mist. Fundam. Appl. Toxicol. 8, 328-334

(154) Schlesinger and Gearhart (1986). Early alveolar clearance in rabbits intermittently exposed to sulfuric acid mist. J. Toxicol. Environ. Health 17, 213-220.

(155) Schlesinger and Gearhart (1987). Intermittent exposures to mixed atmospheres of nitrogen dioxide and sulfuric acid: effect on particle clearance from the respiratory region of rabbit lungs Toxicology 44, 309-319

(156) Schlesinger et al. (1983) Physiological and histological alterations in the bronchial mucociliary clearance system of rabbits following intermittent oral or nasal inhalation of sulfuric acid mist. J. Toxicol. Environ. Health 12, 441-465.

(157) Schlesinger et al., (1978). Effects of short-term exposures to sulfuric acid and ammonium sulfate aerosols upon bronchial airway function in the donkey. Am. Ind. Hyg. Assoc. J. 39, 275-286.

(158) Schlesinger et al., (1979). Effect of chronic inhalation of sulfuric acid mist upon mucociliary clearance from the lungs of donkeys. J. Environ. Pathol. Toxicol., 2, 1351-1367.

(159) Schlesinger et al., (1984). Exposure-response relationship of bronchial mucociliary clearance in rabbits following acute inhalations of sulfuric acid mist. Toxicol. Lett., 22, 249-254.

(160) Schlesinger et al., (1987). Effect of repeated exposures to nitrogen dioxide and sulfuric acid mist alone or in combination on mucociliary clearance from the lungs of rabbits. Environ. Res. 44, 294-301.

(161) Schlesinger et al., (1990) Comparative potency of inhaled acid sulfates : speciation and the role of hydrogen ion. Environ. Res., 52, 210-224.

(162) Schlesinger et al., (1990). Modulation of pulmonary eicosanoid metabolism following exposure to sulfuric acid. Fundam. Appl. Toxicol., 15, 151-162.

(163) Schlesinger et al., (1992). Assessement of toxicologic interactions resulting from acute inhalation exposure to sulfuric acid and ozone mixtures. Toxicol. Appl. Pharmacol., 115, 183-190.

(164) Schlesinger et al., (1992). Interspecies differences in the phagocytic activity of pulmonary macrophages subjected to acid challenge. Fundam. Appl. Toxicol. 19, 584-589.

(165) Schlesinger et al., (1992). Long-term intermittent exposure to sulfuric acid aerosol, ozone, and their combination: altrations in tracheobronchial mucociliary clearance and epithelial secretory cell. Exp. Lung. Res., 18, 505-534.

(166) Schlesinger RB (1989) Factors affecting the response of lung clearance systems to acid aerosols: role of exposure concentration, exposure time, and relative acidity. Environ. Health Perpect., 79, 121-126 (167) Schlesinger RB (1990). Exposure-reponse pattern for sulfuric acid-induced effects on particle clearance from the respiratory region of rabbits lungs. Inhal. Toxicol., 2, 21-27.

(168) Schwartz et al. (1979). Pulmonary responses to sulfuric acid aerosols. Asses. Toxicol. Eff. Environ. Pollut., Chapter 10, pp: 173-186

(169) Serin I.F. Review and Evaluation of Recent Literature Relevant to Occupational Exposure to Sulphuric Acid. US National Institute of Environmental Health and Safety, PB87-213898, 1981

(170) Shinshima K., Ishikawa Y. (1992) Denryoku Chuo Kenkyusho Hokoku 1-25

(171) Silbaugh et al., (1981). Effects of sulfuric acid aerosols on pulmonary functions of guinea pigs. J. Toxicol. Environ. Health, 7, 339-352.

(172) Smyth et al., (1969) Range-finding toxicity data : list VII. Am. Ind. Hyg. Ass. J. 30, 470 - 476.

(173) Soskolne C, Jhangri G, Siemiatycki J, Lakhani R et al. Occupational exposure to sulphuric acid associated with laryngeal cancer, Southern Ontario, Canada. Scand. J. Work Environ Health, 18, 225, 1992.

(174) Soskolne C, Zeighami E, Hanis N, Kupper L et al. Laryngeal cancer and occupational exposure to sulfuric acid. Am. J. Epidemiol., 120, 358, 1984.

(175) Spector et al., Environ. Health Pers. 79, 167-172 (1989).

(176) Stayner L, Meinhardt T, Lemen R, Bayliss D et al. A retrospective cohort mortality study of a phosphate fertilizer production facility. Arch. Environ. Health, 40, 133, 1985.

(177) Steenland K and Beaumont J. Further follow-up and adjustment for smoking in a study of lung cancer and acid mists. Am. J. Ind. Med., 16, 347, 1989.

(178) Steenland K, Schnorr T, Beaumont J, Halperin W et al. Incidence of laryngeal cancer and exposure to acid mists. Br. J. Ind. Med., 45, 766, 1988.

(179) Swenberg and Beauchamp (1997) A review of the chronic toxicity, carcinogenicity and possible mechanisms of action of inorganic acid mists in animals. Crit. Rev. Toxicol. 27(3), 253-259.

(180) Tam W.H. and P.D. Payson (1986). Effects of chronic exposure to sublethal pH on growth, egg production and ovulation in Brook Trout, Salvelinus fontinalis. Can. J. Fish. Aquat. Sci., 43, 275-280.

(181) Teta M, Perlman G, Ott M. Mortality study of ethanol and isopropanol production workers at two facilities. Scand. J. Work Environ. Health, 18, 90, 1992.

(182) Theiss A, Oettel H and Uhl C. Occupational lung cancers. Long-term observations at BASF, Ludwigshafen am Rhein, 2nd Communication (German). Ab. Arbeitsmed Arbeitsschutz, 19, 97, 1969.

(183) Thiess, A. M., (1969); Sichere Arbeit 3/69, 11-18

(184) Thomas et al., (1958). Prolonged exposure of guinea pigs to sulfuric acid aerosol. Arch. Ind. Health 17, 70-80.

(185) Treon et al., (1950) Toxicity of sulfuric acid mist. Arch. Indust. Hyg. Occup. Med. 2, 716-734.

(186) Turner and Fairhurst (1992) Toxicology of substances in relation to major hazards - Sulphuric acid mist. Health and Safety Executive, published by HMSO Books, London, pp 1-21.

(187) Uleckiene and Griciute (1997) Carcinogenicity of sulfuric acid in rats and mice. Pathol. Oncol. Res. 3, 38-43 (1997).

(188) Utell et al., Aerosols SCi, Med. Technol. 14, 202-205 (1983).

(189) Utell et al., Am. Rev. Resp. Disp. 128, 444-450 (1983).

(190) Vernot et al. (1977). Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicol. Appl. Pharmacol., 42 (2), 417-423.

(191) Wakisaka et al., (1988). Combined effects of experimental exposures to sulfur dioxide and sulfuric acid aerosol on the respiratory response in mice. Acta Med. Univ. Kagoshima 30, 1-9.

(192) Walcek C.J., Chang T.S. (1987) Atmos. Environ. 21(5), 1107-1114, cited in HSDB.

(193) Wallen et al. (1957), Sewage and Industrial Wastes 29 (6) 695-711

(194) Warren and Last (1987). Synergistic interaction of ozone and respirable aerosols on rat lungs. Toxicol. Appl. Pharmacol. 88, 203-216.

(195) Weast R., Handbook of Chemistry and physics

(196) Weil C, Smyth H and Nale T. Quest for a suspected industrial carcinogen. Arch Ind. Hyg., 5, 535, 1952.

(197) Weiss (1980) Hazardous Chemicals Data Book, Noyes Data Corp., Park Ridge.

(198) Wolf et al., (1986). Effects of sulfuric acid mist inhalation on mucous clearance and on airway fluids of rats and guinea pigs. J. Toxicol. Environ. Health, 17(1), 129-142.

(199) Wolff R. K. (1986). Effects of airborne pollutants on mucociliary clearance. Environ. Health Perspect., 66, 223-237.

(200) Wolff RK et al., (1979) Toxicity of 0.4- and 0.8-µm sulfuric acid aerosols in the guinea pigs. J. Toxicol. Environ. Health 5, 1037-1047.

(201) Zelikoff and Schlesinger (1992). Modulation of pulmonary immune defense mechanisms by sulfuric acid: effects on macrophage-derived tumor necrosis factor and superoxide. Toxicology, 76, 271-281.

(202) Zelikoff et al., (1994). Immunotoxicity of sulfuric acid aerosol: effects on pulmonary macrophage effector and functional activities critical for maintaining host resistance against infectious diseases. Toxicology, 92, 269-286.

(203) Zelikoff et al., (1997). Effects of inhaled sulfuric acid aerosols on pulmonary immunocompetence: a comparative study in humans and animals. Inhal. Toxicol., 9, 731-752.

(204) Zmela B, Day N, Swiatnicka J and Banasik R. Larynx cancer risk factors. Neoplasma, 34, 223, 1987.

ANNEX 1

ACGIH TLV-STEL	3 MG/M3	DTLVS* TLV/BEI,1999
ACGIH TLV-TWA	1 MG/M3	DTLVS* TLV/BEI,1999
OSHA PEL (GEN INDU):8H TWA	1 MG/M3	CFRGBR 29,1910.1000,1994
OSHA PEL (CONSTRUC):8H TWA	1 MG/M3	CFRGBR 29,1926.55,1994
OSHA PEL (SHIPYARD):8H TWA	1 MG/M3	CFRGBR 29,1915.1000,1993
OSHA PEL (FED CONT):8H TWA	1 MG/M3	CFRGBR 41,50-204.50,1994
OEL-ARAB REPUBLIC OF EGYPT: TWA	1 MG/M3,	JAN1993
OEL-AUSTRALIA: TWA	1 MG/M3,	JAN1993
OEL-AUSTRIA: MAK	1 MG/M3,	JAN1999
OEL-BELGIUM: TWA	1 MG/M3,	
STEL	3 MG/M3,	JAN1993
OEL-DENMARK: TWA	1 MG/M3,	JAN1999
OEL-FINLAND: TWA	1 MG/M3,	
STEL	3 MG/M3, SKIN,	JAN1999
OEL-FRANCE: VME	1 MG/M3,	
VLE	3 MG/M3,	JAN1999
OEL-GERMANY: MAK	1 MG/M3,	JAN1999
OEL-HUNGARY: STEL	1 MG/M3,	JAN1993
OEL-JAPAN: OEL	1 MG/M3,	JAN1999
OEL-THE NETHERLANDS: MAC-TGG	1 MG/M3,	JAN1999
OEL-NORWAY: TWA	1 MG/M3,	JAN1999
OEL-POLAND: MAC(TWA)	1 MG/M3,	
MAC(STEL)	3 MG/M3,	JAN1999
OEL-RUSSIA: STEL	1 MG/M3, SKIN,	JAN1993
OEL-SWEDEN: NGV	1 MG/M3,	
TKV	3 MG/M3,	JAN1999
OEL-SWITZERLAND: MAK-W	1 MG/M3, KZG-W 2 MG/M3,	JAN1999
OEL-THAILAND: TWA	1 MG/M3,	JAN1993
OEL-TURKEY: TWA	1 MG/M3,	JAN1993
OEL-UNITED KINGDOM: TWA	1 MG/M3,	1996

OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN, KOREA CHECK ACGIH TLV;
OEL IN NEW ZEALAND, SINGAPORE, VIETNAM **CHECK ACGIH TLV**

APPENDIX III - SULFURIC ACID MSDS

Material Safety Data Sheet
Sulfuric acid MSDS
Section 1: Chemical Product and Company Identification
Product Name: Sulfuric acid
Catalog Codes: SLS2539, SLS1741, SLS3166, SLS2371, SLS3793
CAS#: 7664-93-9
RTECS: WS5600000
TSCA: TSCA 8(b) inventory: Sulfuric acid
CI#: Not applicable.
Synonym: Oil of Vitriol; Sulfuric Acid
Chemical Name: Hydrogen sulfate
Chemical Formula: H2SO4
Section 2: Composition and Information on Ingredients
Composition:
Name CAS # % by Weight
Sulfuric acid 7664-93-9 95 - 98
Toxicological Data on Ingredients: Sulfuric acid: ORAL (LD50): Acute: 2140 mg/kg [Rat.]. VAPOR (LC50): Acute: 510 mg/m 2 hours [Rat]. 320 mg/m 2 hours [Mouse].
Section 3: Hazards Identification
Potential Acute Health Effects:
Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion, of inhalation. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.
Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Classified 1 (Proven for human.) by IARC, + (Proven.) by OSHA. Classified A2 (Suspected for human.) by ACGIH. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. The substance may be toxic to kidneys, lungs, heart, cardiovascular system, upper respiratory tract, eyes, teeth. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated or prolonged

contact with spray mist may produce chronic eye irritation and severe skin irritation. Repeated or prolonged exposure to spray mist may produce respiratory tract irritation leading to frequent attacks of bronchial infection. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.
Section 4: First Aid Measures
Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

Skin Contact:
In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing
and shoes. Cover the irritated skin with an emollient. Cold water may be used.Wash clothing before reuse. Thoroughly clean
shoes before reuse. Get medical attention immediately.
Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical
attention.
Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical
attention immediately.
Serious Inhalation:
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If
breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may
be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or
corrosive. Seek immediate medical attention.
Ingestion:
Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious
person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.
Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: Non-flammable.
Auto-Ignition Temperature: Not applicable.
Flash Points: Not applicable.
Flammable Limits: Not applicable.
Products of Combustion:
Products of combustion are not available since material is non-flammable. However, products of decompostion include fumes
of oxides of sulfur. Will react with water or steam to produce toxic and corrosive fumes. Reacts with carbonates to generate
carbon dioxide gas. Reacts with cyanides and sulfides to form poisonous hydrogen cyanide and hydrogen sulfide respectively.
Fire Hazards in Presence of Various Substances: Combustible materials
Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in
presence of static discharge: Not available. Slightly explosive in presence of oxidizing materials.
Fire Fighting Media and Instructions: Not applicable.
Special Remarks on Fire Hazards:
Metal acetylides (Monocesium and Monorubidium), and carbides ignite with concentrated sulfuric acid. White Phosphorous +
boiling Sulfuric acid or its vapor ignites on contact. May ignite other combustible materials. May cause fire when sulfuric acid
is mixed with Cyclopentadiene, cyclopentanone oxime, nitroaryl amines, hexalithium disilicide, phorphorous (III) oxide, and
oxidizing agents such as chlorates, halogens, permanganates.

Special Remarks on Explosion Hazards:
Mixtures of sulfuric acid and any of the following can explode: p-nitrotoluene, pentasilver trihydroxydiaminophosphate, perchlorates, alcohols with strong hydrogen peroxide, ammonium tetraperoxychromate,

mercuric nitrite, potassium chlorate, potassium permanganate with potassium chloride, carbides, nitro compounds, nitrates,
carbides, phosphorous, iodides, picratres, fulminats, dienes, alcohols (when heated) Nitramide decomposes explosively
on contact with concentrated sulfuric acid. 1,3,5-Trinitrosohexahydro-1,3,5-triazine + sulfuric acid causes explosive
decompositon.

Section 6: Accidental Release Measures

Small Spill:

Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container. If
necessary: Neutralize the residue with a dilute solution of sodium carbonate.

Large Spill:

Corrosive liquid. Poisonous liquid. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material.
Do not get water inside container. Do not touch spilled material. Use water spray curtain to divert vapor drift. Use water spray
to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal.
Neutralize the residue with a dilute solution of sodium carbonate. Be careful that the product is not present at a concentration
level above TLV. Check TLV on the MSDS and with local authorities.

Section 7: Handling and Storage

Precautions:

Keep locked up.. Keep container dry. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Never add water to this product.
In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show
the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing
agents, combustible materials, organic materials, metals, acids, alkalis, moisture. May corrode metallic surfaces. Store in a
metallic or coated fiberboard drum using a strong polyethylene inner package.

Storage:

Hygroscopic. Reacts. violently with water. Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not
store above 23°C (73.4°F).

Section 8: Exposure Controls/Personal Protection

Engineering Controls:

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective
threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

Personal Protection:

Face shield. Full suit. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves. Boots.

Personal Protection in Case of a Large Spill:

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid
inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this
product.

Exposure Limits:

TWA: 1 STEL: 3 (mg/m3) [Australia] Inhalation TWA: 1 (mg/m3) from OSHA (PEL) [United States] Inhalation TWA: 1 STEL: 3
(mg/m3) from ACGIH (TLV) [United States] [1999] Inhalation TWA: 1 (mg/m3) from NIOSH [United States] Inhalation TWA: 1
(mg/m3) [United Kingdom (UK)]Consult local authorities for acceptable exposure limits.

Section 9: Physical and Chemical Properties

Physical state and appearance: Liquid. (Thick oily liquid.)
Odor: Odorless, but has a choking odor when hot.
Taste: Marked acid taste. (Strong.)
Molecular Weight: 98.08 g/mole
Color: Colorless.
pH (1% soln/water): Acidic.
Boiling Point:
270°C (518°F) - 340 deg. C Decomposes at 340 deg. C
Melting Point: -35°C (-31°F) to 10.36 deg. C (93% to 100% purity)
Critical Temperature: Not available.
Specific Gravity: 1.84 (Water = 1)
Vapor Pressure: Not available.
Vapor Density: 3.4 (Air = 1)
Volatility: Not available.
Odor Threshold: Not available.
Water/Oil Dist. Coeff.: Not available.
Ionicity (in Water): Not available.
Dispersion Properties: See solubility in water.
Solubility:
Easily soluble in cold water. Sulfuric is soluble in water with liberation of much heat. Soluble in ethyl alcohol.

Section 10: Stability and Reactivity Data

Stability: The product is stable.
Instability Temperature: Not available.
Conditions of Instability:
Conditions to Avoid: Incompatible materials, excess heat, combustible material materials, organic materials, exposure to moist
air or water, oxidizers, amines, bases. Always add the acid to water, never the reverse.
Incompatibility with various substances:
Reactive with oxidizing agents, reducing agents, combustible materials, organic materials, metals, acids, alkalis, moisture.
Corrosivity:
Extremely corrosive in presence of aluminum, of copper, of stainless steel(316). Highly corrosive in presence of stainless
steel(304). Non-corrosive in presence of glass.
Special Remarks on Reactivity:
Hygroscopic. Strong oxidizer. Reacts violently with water and alcohol especially when water is added to the product.
Incompatible (can react explosively or dangerously) with the following: ACETIC ACID, ACRYLIC ACID, AMMONIUM
HYDROXIDE, CRESOL, CUMENE, DICHLOROETHYL ETHER, ETHYLENE CYANOHYDRIN, ETHYLENEIMINE, NITRIC
ACID, 2-NITROPROPANE, PROPYLENE OXIDE, SULFOLANE, VINYLIDENE CHLORIDE, DIETHYLENE GLYCOL
MONOMETHYL ETHER, ETHYL ACETATE, ETHYLENE CYANOHYDRIN, ETHYLENE GLYCOL MONOETHYL ETHER
ACETATE, GLYOXAL, METHYL ETHYL KETONE, dehydrating agents, organic materials, moisture (water), Acetic anhydride,
Acetone, cyanohydrin, Acetone+nitric acid, Acetone + potassium dichromate, Acetonitrile, Acrolein, Acrylonitrile, Acrylonitrile
+water, Alcohols + hydrogen peroxide, ally compounds such as Allyl alcohol, and Allyl Chloride, 2-Aminoethanol, Ammonium
hydroxide, Ammonium triperchromate, Aniline, Bromate + metals, Bromine pentafluoride, n-Butyraldehyde, Carbides, Cesium
acetylene carbide, Chlorates, Cyclopentanone oxime, chlorinates, Chlorates + metals, Chlorine trifluoride, Chlorosulfonic
acid, 2-cyano-4-nitrobenzenediazonium hydrogen sulfate, Cuprous nitride, p-chloronitrobenzene, 1,5-Dinitronaphthlene +

sulfur, Diisobutylene, p-dimethylaminobenzaldehyde, 1,3-Diazidobenzene, Dimethylbenzylcarbinol + hydrogen peroxide,
Epichlorohydrin, Ethyl alcohol + hydrogen peroxide, Ethylene diamine, Ethylene glycol and other glycols, , Ethylenimine,
Fulminates, hydrogen peroxide, Hydrochloric acid, Hydrofluoric acid, Iodine heptafluoride, Indane + nitric acid, Iron, Isoprene,
Lithium silicide, Mercuric nitride, Mesityl oxide, Mercury nitride, Metals (powdered), Nitromethane, Nitric acid + glycerides,
p-Nitrotoluene, Pentasilver trihydroxydiaminophosphate, Perchlorates, Perchloric acid, Permanganates + benzene, 1-
Phenyl-2-methylpropyl alcohol + hydrogen peroxide, Phosphorus, Phosphorus isocyanate, Picrates, Potassium tert-butoxide,
Potassium chlorate, Potassium Permanganate and other permanganates, halogens, amines, Potassium Permanganate +
Potassium chloride, Potassium Permanganate + water, Propiolactone (beta)-, Pyridine, Rubidium aceteylene carbide, Silver
permanganate, Sodium, Sodium carbonate, sodium hydroxide, Steel, styrene monomer, toluene + nitric acid, Vinyl acetate,
Thalium (I) azidodithiocarbonate, Zinc chlorate, Zinc Iodide, azides, carbonates, cyanides, sulfides, sulfites, alkali hydrides,
carboxylic acid anhydrides, nitriles, olefinic organics, aqueous acids, cyclopentadiene, cyano-alcohols, metal acetylides,
Hydrogen gas is generated by the action of the acid on most metals (i.e. lead, copper, tin, zinc, aluminum, etc.). Concentrated
sulfuric acid oxidizes, dehydrates, or sulfonates most organic compounds.

Special Remarks on Corrosivity:

Non-corrosive to lead and mild steel, but dillute acid attacks most metals. Attacks many metals releasing hydrogen. Minor
corrosive effect on bronze. No corrosion data on brass or zinc.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Absorbed through skin. Dermal contact. Eye contact. Inhalation. Ingestion.

Toxicity to Animals:

WARNING: THE LC50 VALUES HEREUNDER ARE ESTIMATED ON THE BASIS OF A 4-HOUR EXPOSURE. Acute oral
toxicity (LD50): 2140 mg/kg [Rat.]. Acute toxicity of the vapor (LC50): 320 mg/m3 2 hours [Mouse].

Chronic Effects on Humans:

CARCINOGENIC EFFECTS: Classified 1 (Proven for human.) by IARC, + (Proven.) by OSHA. Classified A2 (Suspected
for human.) by ACGIH. May cause damage to the following organs: kidneys, lungs, heart, cardiovascular system, upper
respiratory tract, eyes, teeth.

Other Toxic Effects on Humans:

Extremely hazardous in case of inhalation (lung corrosive). Very hazardous in case of skin contact (corrosive, irritant,
permeator), of eye contact (corrosive), of ingestion, .

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:

Mutagenicity: Cytogenetic Analysis: Hamster, ovary = 4mmol/L Reproductive effects: May cause adverse reproductive effects
based on animal data. Developmental abnormalities (musculoskeletal) in rabbits at a dose of 20 mg/m3 for 7 hrs.(RTECS)
Teratogenecity: neither embryotoxic, fetoxic, nor teratogenetic in mice or rabbits at inhaled doses producing some maternal
toxicity

Special Remarks on other Toxic Effects on Humans:

Acute Potential Health Effects: Skin: Causes severe skin irritation and burns. Continued contact can cause tissue necrosis.
Eye: Causes severe eye irritation and burns. May cause irreversible eye injury. Ingestion: Harmful if swallowed. May cause

permanent damage to the digestive tract. Causes gastrointestial tract burns. May cause perforation of the stomach, GI
bleeding, edema of the glottis, necrosis and scarring, and sudden circulatory collapse(similar to acute inhalation). It may
also cause systemic toxicity with acidosis. Inhalation: May cause severe irritation of the respiratory tract and mucous
membranes with sore throat, coughing, shortness of breath, and delayed lung edema. Causes chemical burns to the repiratory
tract. Inhalation may be fatal as a result of spasm, inflammation, edema of the larynx and bronchi, chemical pneumonitis,
and pulmonary edema. Cause corrosive action on mucous membranes. May affect cardiovascular system (hypotension,
depressed cardiac output, bradycardia). Circulatory collapse with clammy skin, weak and rapid pulse, shallow respiration, and
scanty urine may follow. Circulatory shock is often the immediate cause of death. May also affect teeth(changes in teeth and
supporting structures - erosion, discoloration). Chronic Potential Health Effects: Inhalation: Prolonged or repeated inhalation
may affect behavior (muscle contraction or spasticity), urinary system (kidney damage), and cardiovascular system, heart
(ischemic heart leisons), and respiratory system/lungs(pulmonary edema, lung damage), teeth (dental discoloration, erosion).
Skin: Prolonged or repeated skin contact may cause dermatitis, an allergic skin reaction.

Section 12: Ecological Information

Ecotoxicity: Ecotoxicity in water (LC50): 49 mg/l 48 hours [bluegill/sunfish].
BOD5 and COD: Not available.
Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.
Toxicity of the Products of Biodegradation: The products of degradation are less toxic than the product itself.
Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Sulfuric acid may be placed in sealed container or absorbed in vermiculite, dry sand, earth, or a similar material. It may also
be diluted and neutralized. Be sure to consult with local or regional authorities (waste regulators) prior to any disposal. Waste
must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: Class 8: Corrosive material
Identification: : Sulfuric acid UNNA: 1830 PG: II
Special Provisions for Transport: Not available.

Section 15: Other Regulatory Information

Federal and State Regulations:
Illinois toxic substances disclosure to employee act: Sulfuric acid New York release reporting list: Sulfuric acid Rhode
Island RTK hazardous substances: Sulfuric acid Pennsylvania RTK: Sulfuric acid Minnesota: Sulfuric acid Massachusetts
RTK: Sulfuric acid New Jersey: Sulfuric acid California Director's List of Hazardous Substances (8 CCR 339): Sulfuric acid
Tennessee RTK: Sulfuric acid TSCA 8(b) inventory: Sulfuric acid SARA 302/304/311/312 extremely hazardous substances:
Sulfuric acid SARA 313 toxic chemical notification and release reporting: Sulfuric acid CERCLA: Hazardous substances.:
Sulfuric acid: 1000 lbs. (453.6 kg)
Other Regulations:

OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200). EINECS: This product is on the
European Inventory of Existing Commercial Chemical Substances.
Other Classifications:
WHMIS (Canada):
CLASS D-1A: Material causing immediate and serious toxic effects (VERY TOXIC). CLASS E: Corrosive liquid.
DSCL (EEC):
R35- Causes severe burns. S2- Keep out of the reach of children. S26- In case of contact with eyes, rinse immediately with
plenty of water and seek medical advice. S30- Never add water to this product. S45- In case of accident or if you feel unwell,
seek medical advice immediately (show the label where possible).
HMIS (U.S.A.):
Health Hazard: 3
Fire Hazard: 0
Reactivity: 2
p. 7
Personal Protection:
National Fire Protection Association (U.S.A.):
Health: 3
Flammability: 0
Reactivity: 2
Specific hazard:
Protective Equipment:
Gloves. Full suit. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator
when ventilation is inadequate. Face shield.

Section 16: Other Information

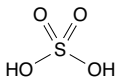
References:
-Material safety data sheet emitted by: la Commission de la Santé et de la Sécurité du Travail du Québec. -The Sigma-Aldrich
Library of Chemical Safety Data, Edition II. -Hawley, G.G.. The Condensed Chemical Dictionary, 11e ed., New York N.Y., Van
Nostrand Reinold, 1987.
Other Special Considerations: Not available.
Created: 10/09/2005 11:58 PM
Last Updated: 05/21/2013 12:00 PM

Strong Inorganic Acid Mists Containing Sulfuric Acid

CAS No. 7664-93-9 (Sulfuric acid)

Known to be human carcinogens

First listed in the *Ninth Report on Carcinogens* (2000)



Sulfuric acid

Carcinogenicity

Strong inorganic acid mists containing sulfuric acid are *known to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in humans.

Cancer Studies in Humans

Occupational exposure to strong inorganic acid mists containing sulfuric acid is specifically associated with laryngeal and lung cancer. Studies of one U.S. cohort of male workers in pickling operations in the steel industry found excesses of laryngeal cancer (approximately twofold) after adjustment for smoking and other potentially confounding variables (Steenland *et al*. 1988). A ten-year follow-up of this cohort also found a twofold excess of laryngeal cancer, consistent with the earlier findings (Steenland 1997). The same cohort showed an excess of lung cancer after adjustment for smoking and other potentially confounding variables (Steenland and Beaumont 1989). A nested case-control study of workers in a U.S. petrochemical plant found a dose-related increase in the risk of laryngeal cancer among workers exposed to sulfuric acid at moderate levels (odds ratio [OR] = 4.6; 95% confidence interval [CI] = 0.83 to 25.35) or high levels (OR = 13.4; 95% CI = 2.08 to 85.99) (Soskolne *et al*. 1984). A Canadian population-based case-control study also found a dose-related risk of laryngeal cancer for workers exposed to sulfuric acid mist, after controlling for tobacco and alcohol use and using only the most specific exposure scale (Soskolne *et al*. 1992). A similar Canadian population-based case-control study suggested an increased risk of lung cancer (oat-cell carcinoma) (Siemiatycki 1991).

Additional Information Relevant to Carcinogenicity

The manufacture of isopropyl alcohol by the strong-acid process, which uses sulfuric acid, has been classified by the International Agency for Research on Cancer as carcinogenic to humans, based on increased incidence of cancer of the paranasal sinuses in workers (IARC 1977). The carcinogenic activity of sulfuric acid is most likely related to the genotoxicity of low-pH environments, which are known to increase the rates of depurination of DNA and deamination of cytidine (IARC 1992a).

Cancer Studies in Experimental Animals

No adequate studies in experimental animals of the carcinogenicity of sulfuric acid or strong inorganic acid mists containing sulfuric acid have been reported in the literature.

Properties

Sulfuric acid is a strong acid that is a clear, colorless oily liquid at room temperature. Impure or spent sulfuric acid is a dark-brown to black liquid. Sulfuric acide is soluble in water and ethanol and is very corrosive (IARC 1992b). Physical and chemical properties of sulfuric acid are listed in the following table.

Property	Information
Molecular weight	98.1[a]
Density	1.8 g/cm^3[a]
Melting point	10.31°C[a]
Boiling point	290°C[a]
Log K_{ow}	1.92[b]
Vapor pressure	5.93×10^{-5} mm Hg at 25°C[a]
Vapor density relative to air	3.4[a]
Dissociation constant (pK_a)	1.98 at 25°C[a]

Sources: [a]HSDB 2009, [b]ATSDR 1998.

A mist is defined as a liquid aerosol formed by condensation of a vapor or by atomization of a liquid. Strong inorganic acid mists containing sulfuric acid may be generated during a process when factors such as evaporation, solution strength, temperature, and pressure combine to result in release of a mist (IARC 1992a). Sulfuric acid mists are the most extensively studied of the acid mists. Liquid sulfuric acid may exist in air as a vapor or a mist; however, it exists most often as mist, because of its low volatility and high affinity for water.

Acid strength is based on the position of equilibrium in an acid-base reaction and is measured by the negative logarithm (to the base 10) of the acid dissociation constant (pK_a). The lower the pK_a, the stronger the acid. Sulfuric acid has two pK_a values because it releases two hydrogen atoms in aqueous solution, but the first pK_a cannot be measured accurately and is reported as less than 0. Dehydration occurs because sulfuric acid has a strong affinity for water. It forms various hydrates when in contact with organic matter or water vapor. Although it is miscible with water, contact with water generates heat and may produce a violent reaction. The reaction with water releases toxic and corrosive fumes and mists. Sulfuric acid is noncombustible, but it can release flammable hydrogen gas when in contact with metals. Thermal decomposition to sulfur trioxide and water occurs at 340°C. Sulfuric acids are available in the following grades: commercial, electrolyte (high purity), textile (low organic content), and chemically pure or reagent grades (IARC 1992b, ATSDR 1998, HSDB 2009).

Sulfur trioxide is added to sulfuric acid to produce fuming sulfuric acid (also known as oleum). Oleum has a molecular weight of 178.1, may contain up to 80% free sulfur trioxide, and is a colorless to slightly colored oily liquid. Sulfur trioxide has a molecular weight of 80.1 and can exist as a gas, liquid, or solid. Liquid sulfur trioxide is colorless and fumes in air at ambient conditions. In the presence of moisture, sulfur trioxide forms solid polymers consisting of alpha and beta forms. The melting points of the alpha (62.3°C) and beta (32.5°C) forms are the temperatures at which they depolymerize back to the liquid form. The liquid form has a boiling point of 44.8°C and a density of 1.92 g/cm^3 at 20°C. Both oleum and sulfur trioxide react with water and water vapor to form sulfuric acid mists. Oleum is available in several grades with free sulfur trioxide content ranging from 20% to 99.9% and corresponding sulfuric acid equivalents ranging from 104.5% to 122.5%. Sulfur trioxide is available with a minimum purity of 99.5% as a stabilized technical grade or unstabilized liquid (IARC 1992b).

Use

Strong inorganic acid mists containing sulfuric acid are not used *per se* in industry or in commercial products but are generated from both natural and industrial sources. In particular, sulfuric acid mists may be produced during the manufacture or use of sulfuric acid, sulfur trioxide, or oleum. Sulfur trioxide is primarily used to make sulfuric acid, but it is also used as a sulfonating or oxidizing agent. Oleum is used as a sulfonating or dehydrating agent, in petroleum refining, and as a laboratory reagent. Sulfuric acid is one of the most widely used industrial chemicals; however, most of it is used as a reagent

rather than an ingredient. Therefore, most of the sulfuric acid used ends up as a spent acid or a sulfate waste. Exacting purity grades are required for use in storage batteries and for the rayon, dye, and pharmaceutical industries. Sulfuric acids used in the steel, chemical, and fertilizer industries have less exacting standards (IARC 1992b, ATSDR 1998, HSDB 2009).

Sulfuric acid is used in the following industries: fertilizer, petroleum refining, mining and metallurgy, ore processing, inorganic and organic chemicals, synthetic rubber and plastics, pulp and paper, soap and detergents, water treatment, cellulose fibers and films, and inorganic pigments and paints. Between 60% and 70% of the sulfuric acid used in the United States is used by the fertilizer industry to convert phosphate rock to phosphoric acid. All other individual uses account for less than 1% to less than 10% of the total consumption. Sulfuric acid use is declining in some industries. There is a trend in the steel industry to use hydrochloric acid instead of sulfuric acid in pickling, and hydrofluoric acid has replaced sulfuric acid for some uses in the petroleum industry. The primary consumer product that contains sulfuric acid is the lead-acid battery; however, this accounts for a small fraction of the overall use. Sulfuric acid is also used as a general-purpose food additive (IARC 1992b, ATSDR 1998).

Production

Strong inorganic acid mists containing sulfuric acid may be produced as a result of the use of mixtures of strong inorganic acids, including sulfuric acid, in industrial processes such as acid treatment of metals, phosphate fertilizer manufacture, and lead battery manufacture (IARC 1992b). The degree of vapor or mist evolution varies with the process and method. In pickling, for instance, mist may escape from acid tanks when hydrogen bubbles and steam rise from the surface of the solution.

Sulfuric acid is the largest-volume chemical produced in the United States (CEN 1996). Annual production increased from 28.3 million metric tons (62.4 billion pounds) in 1972 to 40.1 million metric tons (88.4 billion pounds) in 1980 (IARC 1992b, ATSDR 1998). Between 1981 and 2002, annual production remained fairly steady, ranging from a low of 32.6 million metric tons (71.9 billion pounds) in 1986 (IARC 1992b) to a high of 44 million metric tons (97 billion pounds) in 1998 (CEN 2003). Between 1992 and 2002, annual production declined by only 1% (CEN 2003). Many different grades and strengths of sulfuric acid are produced. The primary method of production is the contact process, which consists of the following steps: (1) oxidation of sulfur to sulfur dioxide, (2) cooling of the gases, (3) oxidation of sulfur dioxide to sulfur trioxide, (4) cooling of the sulfur trioxide gas, and (5) addition of sulfur trioxide to water to produce sulfuric acid. Oleum is produced at sulfuric acid plants by adding sulfur trioxide to sulfuric acid. In addition to primary production, large quantities of spent sulfuric acid are reprocessed (IARC 1992b, ATSDR 1998). In 2009, sulfuric acid was available from 76 U.S. suppliers, and oleum from 6 U.S. suppliers (ChemSources 2009).

The United States is a net importer of sulfuric acid and oleum. U.S. imports were 275,000 metric tons (600 million pounds) in 1975, 426,000 metric tons (940 million pounds) in 1984, and 2.3 million metric tons (5 billion pounds) in 1993, and exports were 129,000 metric tons (284 million pounds) in 1975, 119,000 metric tons (262 million pounds) in 1984, and 136,000 metric tons (300 million pounds) in 1993 (HSDB 2009). In 2009, imports were about 5 million kilograms (11 million pounds), and exports were 262,000 kg (578,000 lb) (USITC 2009).

Exposure

Human exposure to strong inorganic acid mists containing sulfuric acid may occur by inhalation, ingestion, or dermal contact. Exposure depends on many factors, including particle size, proximity to the source, and control measures such as ventilation and containment. Data on particle size distribution of acid mists are limited, and sampling methods have generally not differentiated between liquid and gaseous forms of acids. One study of sulfuric acid mists in several U.S. battery manufacturing plants found that particles had a mass median aerodynamic diameter of 5 to 6 μm, which indicates that sulfuric acid mists contain aerosol particles that can be deposited in both the upper and lower airways (IARC 1992a).

Sulfuric acid and mists and vapors containing sulfuric acid are present in the environment because of releases of sulfur compounds from both natural and anthropogenic sources. Volcanic eruptions, biogenic gas emissions, and oceans are the primary natural sources of sulfur emissions. Volcanoes release 0.75 million to 42 million metric tons (1.7 billion to 93 billion pounds) of sulfur per year, and airborne sea spray and marine organisms release between 12 million and 15 million metric tons per year (26 billion to 33 billion pounds). Coal combustion by electric plants is the major anthropogenic source of sulfur dioxide release. Sulfur dioxide emissions in the United States declined by more than 60% from the early 1970s (28 million metric tons [62 billion pounds]) to 1994 (18 million metric tons [40 billion pounds]) and decreased by another 13% from 1994 to 1995 (ATSDR 1998).

According to the U.S. Environmental Protection Agency's Toxics Release Inventory, environmental releases of sulfuric acid fluctuated from year to year, but remained in the range of 26 million to 197 million pounds from 1994 and 2007. In 2007, 840 facilities released over 138.5 million pounds of sulfuric acid, of which over 99% was released to air (TRI 2009). Ambient air may contain particulate-associated mixtures of sulfuric acid and ammonium sulfates (sulfuric acid partially or completely neutralized by atmospheric ammonia). The relative amounts of sulfuric acid and total sulfates depend on meteorological and chemical parameters. The presence of sulfuric acid and sulfates in the atmosphere is believed to be due to oxidation of sulfur dioxide in cloud water and other atmospheric media. Ambient-air concentrations of sulfuric acid are at least an order of magnitude lower than concentrations in occupational settings (IARC 1992a).

The industries in which occupational exposure to strong acid mists may occur include chemical manufacture (sulfuric acid, nitric acid, synthetic ethanol, and vinyl chloride), building and construction, manufacture of lead-acid batteries, manufacture of phosphate fertilizers, pickling and other acid treatments of metals, manufacture of petroleum and coal products, oil and gas extraction, printing and publishing, manufacture of paper and allied products, and tanneries. Most of the available occupational exposure data comes from the pickling and plating industries. In the 1970s and 1980s, average concentrations of strong inorganic acid mists containing sulfuric acid in workplace air were less than 0.01 to 7.3 mg/m^3 for pickling and acid cleaning, less than 0.07 to 0.57 mg/m^3 for phosphate fertilizer manufacture, 0.01 to 1.03 mg/m^3 for lead battery manufacture, and less than 0.005 to 0.5 mg/m^3 for other industries (IARC 1992a).

The National Occupational Hazard Survey (conducted from 1972 to 1974) estimated that 499,446 workers were exposed to sulfuric acid, 824,985 to hydrochloric acid, 132,401 to nitric acid, and 454,920 to phosphoric acid (NIOSH 1976). The National Occupational Exposure Survey (conducted from 1981 to 1983), which reported on more than 54,500 plants with potential workplace exposure to strong inorganic acids, estimated that 775,587 workers, including 173,653 women, potentially were exposed to sulfuric acid; 1,238,572 workers,

including 388,130 women, to hydrochloric acid; 297,627 workers, including 76,316 women, to nitric acid; and 1,256,907 workers, including 450,478 women, to phosphoric acid (NIOSH 1990).

Regulations

Coast Guard, Department of Homeland Security

Minimum requirements have been established for safe transport of sulfuric acid on ships and barges.

Consumer Product Safety Commission (CPSC)

Sulfuric acid and any preparation containing sulfuric acid in a concentration of 10% or more must have a label containing the word "poison."

Department of Transportation (DOT)

Sulfuric acid and numerous sulfuric acid mixtures are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Environmental Protection Agency (EPA)

Clean Air Act

New Source Performance Standards: Standards of performance have been established for sulfuric acid production units, including a limit on acid mist (expressed as H_2SO_4) emissions of 0.15 lb/ton of acid produced.

Clean Water Act

Sulfuric acid is designated a hazardous substance.

Comprehensive Environmental Response, Compensation, and Liability Act

Reportable quantity (RQ) = 1,000 lb for sulfuric acid.

Emergency Planning and Community Right-To-Know Act

Toxics Release Inventory: Aerosol forms of sulfuric acid are listed and thus subject to reporting requirements.
Threshold planning quantity (TPQ) = 1,000 lb for sulfuric acid.
Reportable quantity (RQ) = 1,000 lb for sulfuric acid.

Resource Conservation and Recovery Act

Listed Hazardous Waste: Waste codes for which the listing is based wholly or partly on the presence of sulfuric acid = U103, P115.

Occupational Safety and Health Administration (OSHA)

While this section accurately identifies OSHA's legally enforceable PELs for this substance in 2010, specific PELs may not reflect the more current studies and may not adequately protect workers.
Permissible exposure limit (PEL) = 1 mg/m^3 for sulfuric acid.

Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH)

Threshold limit value – time-weighted average (TLV-TWA) = 0.2 mg/m^3 for sulfuric acid contained in strong inorganic acid mists.

National Institute for Occupational Safety and Health (NIOSH)

Recommended exposure limit (REL) = 1 mg/m^3 for sulfuric acid.
Immediately dangerous to life and health (IDLH) limit = 15 mg/m^3 for sulfuric acid.

References

ATSDR. 1998. *Toxicological Profile for Sulfur Trioxide and Sulfuric Acid*. Atlanta, GA: Agency for Toxic Substances and Disease Registry. 189 pp.

CEN. 1996. Top 50 Chemicals: Organics outpaced inorganics as production of chemicals rose overall. *Chem Eng News* 74(26): 14 pp.

CEN. 2003. Production inches up in most countries. *Chem Eng News* 81(27): 51-61.

ChemSources. 2009. *Chem Sources - Chemical Search.* Chemical Sources International. http://www.chemsources.com/chemonline.html and search on CAS number. Last accessed: 10/22/09.

HSDB. 2009. *Hazardous Substances Data Bank*. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search on CAS number. Last accessed: 10/22/09.

IARC. 1977. Isopropyl alcohol and isopropyl oils. In *Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 15. Lyon, France: International Agency for Research on Cancer. pp. 223-243.

IARC. 1992a. Occupational exposure to mists and vapours from sulfuric acid and other strong inorganic acids. In *Occupational Exposures to Mists and Vapours from Strong Inorganic Acids and Other Industrial Chemicals*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 54. Lyon, France: International Agency for Research on Cancer. pp. 41-119.

IARC. 1992b. Annex: Chemical and physical properties and uses of sulfuric acid and sulfur trioxide. In *Occupational Exposures to Mists and Vapours from Strong Inorganic Acids and Other Industrial Chemicals*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 54. Lyon, France: International Agency for Research on Cancer. pp. 120-130.

NIOSH. 1976. *National Occupational Hazard Survey (1972-74)*. DHEW (NIOSH) Publication No. 78-114. Cincinnati, OH: National Institute for Occupational Safety and Health.

NIOSH. 1990. *National Occupational Exposure Survey (1981-83)*. National Institute for Occupational Safety and Health. Last updated: 7/1/90. http://www.cdc.gov/noes/noes1/38580sic.html, http://www.cdc.gov/noes/noes1/50742sic.html, http://www.cdc.gov/noes/noes1/58520sic.html, http://www.cdc.gov/noes/noes1/70870sic.html,

Siemiatycki J, ed. 1991. *Risk Factors for Cancer in the Workplace*. Boca Raton, FL: CRC Press.

Soskolne CL, Jhangri GS, Siemiatycki J, Lakhani R, Dewar R, Burch JD, Howe GR, Miller AB. 1992. Occupational exposure to sulfuric acid in southern Ontario, Canada, in association with laryngeal cancer. *Scand J Work Environ Health* 18(4): 225-232.

Soskolne CL, Zeighami EA, Hanis NM, Kupper LL, Herrmann N, Amsel J, Mausner JS, Stellman JM. 1984. Laryngeal cancer and occupational exposure to sulfuric acid. *Am J Epidemiol* 120(3): 358-369.

Steenland K. 1997. Laryngeal cancer incidence among workers exposed to acid mists (United States). *Cancer Causes Control* 8(1): 34-38.

Steenland K, Beaumont J. 1989. Further follow-up and adjustment for smoking in a study of lung cancer and acid mists. *Am J Ind Med* 16(4): 347-354.

Steenland K, Schnorr T, Beaumont J, Halperin W, Bloom T. 1988. Incidence of laryngeal cancer and exposure to acid mists. *Br J Ind Med* 45(11): 766-776.

TRI. 2009. *TRI Explorer Chemical Report.* U.S. Environmental Protection Agency. http://www.epa.gov/triexplorer and select Sulfuric Acid.

USITC. 2009. *USITC Interactive Tariff and Trade DataWeb.* United States International Trade Commission. http://dataweb.usitc.gov/scripts/user_set.asp and search on HTS no. 280700. Last accessed: 10/22/09.

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SHORT COMMUNICATION

Effect of Organically Chelated Micronutrients on Growth and Productivity in Okra

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ABSTRACT

Soybean and mungbean seeds were germinated and extracted after 48 hours in glass distilled water. The homogenate was subjected to protein digestion with Aspergillus protease for 12 hours. After enzymatic digestion the content was filtered and centrifuged in refrigerated centrifuge. The supernatant was used as source of amino acids for chelating the micronutrients. The micronutrient were mixed with amino acid solution and kept on shaker for 4 hours. The amino acid micronutrient chelates were confirmed with FTIR. The seeds of okra var. Phule Utkarsha were obtained from MPKV Rahuri dist. Ahmednagar and used for filed experiments. The experimental plants were sprayed with 0.4 to 2.0 % solutions of organically chelated micronutrients on 15th day and 30th day after plantation. The control plants were sprayed with distilled water. The results of present investigation revealed that all the treatments showed significant increase in growth and productivity of okra. The plants treated with 1.2, 1.6 and 2.0 % sprays of chelated micronutrient solution showed higher yields as compared to control

KEYWORDS: *Amino acids, chelated micronutrients, productivity, okra*

INTRODUCTION

Okra is one of the most popular fruit vegetables grown throughout India and even in tropical regions of the world during all the seasons. The growth, yield and quality of okra are largely influenced by the application of fertilizers as it is a short duration vegetable crop. It requires proper and sufficient N and K and micronutrients for regular fruiting and subsequent pickings. Recent developments in intensive agriculture, though contributed immensely towards surplus food, caused degradation of fertile land. Recently problem of micronutrient deficiencies have been increased markedly due to intensive cropping, introduction of high yielding varieties, irrigation, greater use of chemical fertilizers, loss of top soil by erosion, loss of micronutrients by leaching, liming of soil and decreased availability and use of farm yard manure. It is realized that productivity of crops is being adversely affected due to deficiencies of micronutrients. Deficiency of essential mineral nutrients especially micronutrients in intensive cropping system is of general occurrence. Major parts of agricultural land have been found to be deficient in one or other micronutrients. Micronutrients are usually required in minute quantities, nevertheless are vital to the growth of plant. They improve general condition of plants and are known to act as catalysts in promoting organic reactions taking place in plant. Direct application of micronutrients like copper, iron, zinc, molebdenum, magnesium, manganese cause toxic effects in the plants, hence chelated forms of micronutrients are recommended for better yields. The chelating agents of the metal ions protect the chelated ions from unfavorable chemical reactions and hence increase the availability of these ions to plants. Large numbers of metal complexing agents are available to chelate micronutrients. The strongest metal chelating agents are mostly synthetic and these are very expensive. On the other hand natural organic chelating agents such as polyflavonoids, lignosulfonates, humic and fulvic acids, amino acids, glutamic acids, and polyphosphates do help the plant in translocating the micronutrients. They are easy to produce and are inexpensive. In the present study, an attempt has been made to study the effect of foliar application of organically chelated micronutrients on growth and yield of okra.

MATERIALS AND METHODS

Production of amino acid-micronutrient chelates

Soybean and mungbean seeds were germinated for 48 hours in the dark and homogenized in blender. The homogenate was subjected to protein digestion with *Aspergillus* protease (5 U/100 ml) for 12 hours. After enzymatic digestion the content was filtered and centrifuged in refrigerated centrifuge. The supernatant was used as source of amino acids for chelating the micronutrients. The micronutrient like Zinc as zinc sulphate, iron as ferrous sulphate, and copper as copper sulphate and manganese as manganese sulphate, each of 1 g were separately dissolved in 20 ml of 0.5 % boric acid solution and then mixed with 80 ml amino acid solution. The mixtures were then kept on shaker for 4 hours to form chelates. The amino acid micronutrient chelates were confirmed with FTIR. After confirmation all the solutions were mixed together to form composite chelate solution. Molybdenum was added at the end of preparation.

Foliar application of organically chelated micronutrient

The present investigation was carried out in farmers filed at Village Pimpri Tal Sangamner Dist-Ahmednagar to find out the effect of organically chelated micronutrients on growth and yield of Okra. Total five treatments involving 0.4 %, 0.8 %, 1.2 %, 1.6 % and 2.0 % composite of chelate solution along with distilled water control was planned in Randomized Block Design with three replicates. The solutions were applied in the form of foliar sprays at two growth stages that are 15 and 30 days after seed sowing. The experimental plots were of size 4 x 3 m with ridges 60 cm spacing. The recommended doses of NPK were applied uniformly in all the plots. Intercultural operations and crop management were followed as per the schedule. The growth characters like plant height, number of leaves, leaf area, and yield per plant were recorded from 10 randomly selected plants from each treatment

RESULTS AND DISCUSSION

As evident from table the growth characters viz, plant height, number of leaves per plant, leaf area per plant were increased significantly with the application of chelated micronutrients. Among the different treatments of organically chelated micronutrients, 2.0% treatment showed better results than the other treatments through improved characters. The maximum plant height of 42.04 cm over control 20.64 cm was recorded in 2.0 % treatment. The number of leaves per plant was maximum with the spray of 2.0 % over control. Similarly maximum leaf area per plant 3380.97cm^2 was recorded in 2.0 % treatment. Yield of marketable fruits per plant was also recorded maximum 25.75 g. in 2.0 % treatment over 11.81g.in control. All the treatments of organically chelated micronutrients proved superior over control through growth and yield characters.

Boron is associated with the development of cell wall and cell differentiation and hence, helps in root elongation and shoot growth of plant. The need of boron has been emphasized earlier for normal growth of tomato plant. Boric acid is essential for better growth and development in plant. Application of micronutrients like Zn, Cu, Fe, Mo, etc is essential for increase in yield, quality and ascorbic acid content in tomato fruits. The photosynthesis enhanced in presence of zinc and boron was also explained that presence of zinc activates the synthesis of tryptophan, the precursor of IAA and it is responsible to stimulate plant growth. Iron plays an important role in promoting growth characters, being a component of ferrodoxin, an electron transport protein and is associated with chloroplast. It helps in photosynthesis might have helped in better vegetative growth. Response to applied micronutrients like zinc, copper, ferrous, molybdenum etc. for better crop growth and yield of several filed crops have been reported from almost all the part of country. All the micronutrient treatments were found significantly effective in increasing fruits per plant and fruit weight. Improvement in growth characters as a result of application of micronutrients might be due to the enhanced photosynthetic and other metabolic activity which leads to an increase in various plant metabolites responsible for cell division and elongation. Application of appreciable quantities of magnesium might have helped in chlorophyll synthesis which in turn increased the rate of photosynthesis. The results are in agreement with the findings of [1, 2]. Average fruit yield per plant were significantly influenced by different treatments with the foliar application of micronutrients. The maximum fruit yield per plant with application of 2.0 % mixture of micronutrients was recorded 25.75 g over control 11.81 g.

Table 1 Showing plant height, no.of leaves, leaf area and yield per plant

Treatments	Plant height (cm.)	No. of leaves Per plant	Leaf area per plant (cm^2)	Yield Per Plant (g)
Control	20.64	21.64	1786.05	11.81
0.4 %	27.62	18.84	2361.28	14.24

0.8 %	28.08	20.62	2759.37	17.90
1.2 %	33.36	24.02	3380.97	21.38
1.6 %	35.82	25.10	3650.40	22.10
2.0 %	42.04	28.22	4893.00	25.75

Increased yield due to micronutrients application may be attributed to enhanced photosynthesis activity and increased production and accumulation of carbohydrates and favorable effect on vegetative growth and retention of flowers and fruits, which increased number of fruits per plant. Similarly the increased dry matter production may be attributed to greater accumulation of photosynthates by vegetative parts and fruits in okra. These findings are in conformity with the observations of previous study, who obtained maximum cost benefit ratio with mixture of micronutrients.

CONCLUSION

The results revealed that among the different treatments of organically chelated micronutrients, Okra responded well to the 2.0% treatment. The growth characters as well as the yield of Okra were significantly enhanced by the application of 2.0% organically chelated micronutrients. The results of present investigation has given insight in application of chelated micronutrients in immiediate rectification of micronutrient deficency in fruit vegetable and also in organic farming.

REFERENCES

[1]. Nehra, A.S., I.S. Hooda, and K.P. Singh (2001). Effect of integrated nutrient management on growth and yieldof wheat (*Triticurn aestivum L.*) Indian J. Agron, 45: 112-17.

[2]. Sanwal, S.K., K. Lakminarayana, R.K. Yadav, N. Rai, D.S. Yadav, and B. Mousumi (2007). Effect of organic manures on soil fertility, growth, physiology, yield and quality of turmeric. Indian J. Hort., 64(4): 444-449.

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APPLICATION OF AMINO ACID CHELATED MICRONUTRIENTS FOR ENHANCING GROWTH AND PRODUCTIVITY IN CHILI (*CAPSICUM ANNUM* L.)

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Present investigation was aimed to determine the effects of foliar application of organically chelated micronutrients on growth and yield in chili (*Capsicum annum* L.). The micronutrients like iron, zink, copper and manganese were organically chelated with seed amino acids. A pot experiment was carried out to study the effect of foliar application of micronutrients, amino acids and amino acid micronutrient chelates on growth and yield of chili (*Phule Jyoti*) during 2009 and 2010 at the Yeola, District Nasik. Forty day's old seedlings of chili were transplanted in pots. The experimental plants were sprayed with three doses (0.5, 1.5 and 2.0 %) of organically chelated micronutrients along with unchelated micronutrients, amino acid solution and untreated control plants on 15th and 30th days after transplantation. The results based on two years mean revealed that out of five different treatments, the application of amino acid-micronutrient chelate at the concentration of 1.5 and 2.0% resulted in maximum plant height, number of primary branches, higher leaf area per plant, fruits per plant and more total yield per plant.

KEYWORDS : Amino acid-micronutrient chelate, foliar spray, *Capsicum annum* L., Chili, growth, yields.

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1. Introduction

Deficiency of essential mineral nutrients especially micronutrients is of general occurrence during the past few decades, due to intensive cropping, with introduction of high yielding varieties, greater use of chemical fertilizers, loss of micronutrients by leaching, and decreased use of farm yard manure. Large area of agricultural land has been found to be deficient in one or other micronutrients. It is realized that productivity of crops is being adversely affected due to deficiencies of micronutrients [1]. Micronutrients are usually required in minute quantities, nevertheless are vital to the growth of plant [2]. They improve general condition of plants and are known to act as catalysts in promoting organic reactions taking place in plant. Micronutrients like iron, copper, zinc, molybdenum, magnesium, manganese if applied directly as inorganic salts, become insoluble forms, so their absorption by the plants decreases and also cause toxic effects in the plants, hence chelated forms of micronutrients is recommended for better yields. The chelating agent protects the metal ions from undesirable chemical reactions such as precipitation and hence increases the availability of these metal ions to plants. Large numbers of metal chelating agents are available to chelate micronutrients. Well known strongest metal chelating agents like EDTA (ethylenediaminetetra acetic acid) and EDDHA (ethylenediamine hydroxyphenylacetic acid) are synthetic and these are expensive. On the other hand natural organic chelating agents such as polyflavonoids, lignosulfonates, humic and fulvic acids, amino acids, and polyphosphates do help the plant in translocating the micronutrients. These chelators are not phytotoxic to plants [3]. They are easy to produce and are inexpensive. In recent times, consumers are highly interested in organic products and demanding quality and safer food [4]. Hence there is urgent need to produce organic chelate of micronutrients for organic vegetable cultivation.

Hence, present study was carried out to test the effect of foliar application of micronutrients chelated with amino acids on growth and yield in vegetable crops, for that we selected chilli as testing crop. Because, chilli (*C. annum L*) is one of the important commercial high value spice cum vegetable crop with tremendous export potential cultivated extensively in India [5] and it is reported to respond well to fertilizer application as it is a short duration crop.

2. Materials And Methodology

Production of organic chelate of micronutrients:

Organic chelate of micronutrients was prepared in the laboratory by using seed amino acids. The micronutrient like zinc as zinc sulphate, iron as ferrous sulphate, copper as copper sulphate, and manganese as manganese sulphate, each of 1 g were separately dissolved in 20 ml of 0.5 % boric acid solution and then mixed with 80 ml of amino acids solution. The mixture was then agitated on shaker to form chelate. The amino acid micronutrient chelates were confirmed with FTIR. After confirmation all the solutions were mixed together to form composite chelate solution. Molybdenum (1.0 g) was added at the end of preparation. The micronutrient each of 1.0 g was dissolved separately in 20 ml of 0.5 % boric acid and final volume was made to 100 ml with glass distilled water. These micronutrient solutions were then mixed in 500 ml flask and Molybdenum (1.0 g) was added at the end. This was used at 2.0 % as un-chelated micronutrients (40 ppm of each micronutrient). Amino acid solution (400 ml) was diluted with 100 ml of distilled water and used at 2.0 % as amino acid treatment.

Foliar application of organically chelated micronutrients

The experiments were conducted at Yeola, District Nasik on potted plants to find out the effect of organically chelated micronutrients on growth and productivity of chilli. The seeds of chilli var. *Phule Jyoti* were obtained from MPKV Rahuri. Seedlings were raised and four weeks old chilli seedlings were transplanted in the pots (five seedlings each). Plastic pots having a diameter of 30 cm and height of 25 cm. filled with garden soil were used for pot experiments. Total five treatments involving T0 untreated control, T1- 2.0 % micronutrient solution (equivalent to 40 ppm of each micronutrient) T2- 2.0 % amino acid solution, T3-1.0 % amino acid micronutrient chelate solution (equivalent to 20 ppm of each micronutrient) T4 1.5 % amino acid micronutrient chelate solution (equivalent to 30 ppm of each micronutrient) and T5 2.0 % amino acid micronutrient chelate solution (equivalent to 40 ppm of each micronutrient) was planned in potted plants with three replicates. The concentrations of the organically chelated micronutrients were prepared prior to spray with distilled water. The solutions were applied in the form of foliar sprays at two growth stages on 15th and 30th days after transplantation. The control plants were sprayed with distilled water. The growth characters like plant height, number of branches per plant, number of leaves per plant and leaf area were recorded from each treatment at the time of flowering. Whereas, number of fruits and fruit weight was measured from each harvest and total yield per plant was determined after last harvest.

3. Results

Plant height and branches per plant

The results given in Table 1 indicated the growth characters like plant height and number of branches per plant were increased significantly with the foliar application of increased dose of organically chelated micronutrients during both the years i.e. 2009 and 2010. The pooled data indicate that chili plants received foliar application of organic micronutrient chelate at the concentration of 2 % resulted in plants of maximum height (60.13 cm), closely followed by 59.22 cm plant height observed when micronutrient chelate was foliarly applied at the concentrations 2.0%. The results further indicated that reduced concentration of 1.0% produced plants of lower height i.e. 55.60 cm. On the other hand unchelated micronutrient solution 2.0% produced plants with 50.65 cm and the amino acid solution 2.0% exhibited plants with 50.96 cm height. However, the least plant height of 45.60 cm was recorded in control. The number of branches per plant was maximum (17.58) with the spray of 2.0 % organic chelate. It was followed by 1.5 % foliar spray of organic chelate with 17.47 branches per plant. Whereas foliar spray of unchelated micronutrient solution at 2.0% produced 13.42 branches per plant and the amino acid solution at the concentration of 2.0% showed 13.58 branches per plant. However, the least number of branches per plant i.e. 11.00 was recorded in control.

The results given in Table 2 indicated the growth characters like number of leaves per plant and leaf area per plant were increased significantly with the application of increased dose of chelated micronutrients. Among the different treatments of chelated micronutrients, 1.5 and 2.0 % treatments showed better results than the other treatments through improved characters at the time of flowering. The maximum number of leaves per plant 238.73 and 239.96 were recorded in 1.5% 2.0 % treatments respectively over control (177.12). The leaf area per plant was maximum (3746.92 cm2) with the spray of 2.0 % over control.

Number of leaves and leaf area per plant

The results pertaining to number of leaves and leaf area are given in table 2. It is clear from the data number of leaves and leaf area increased significantly with the foliar application of increased dose of organically chelated micronutrients during both the years i.e. 2009 and 2010. The pooled data indicate that chili plants received foliar application of organic micronutrient chelate at the concentration of 2 % resulted in plants with maximum number of leaves (239.96), at par number of leaves per plant (238.73) was observed with 1.5 % micronutrient chelate application. The results further indicated that reduced concentration of 1.0% produced significantly less leaves i.e.232.25. On the other hand unchelated micronutrient solution at 2.0% concentration showed 212.35 leaves per plant and the amino acid solution at 2.0% dilution exhibited 214.67 leaves per plant. However, the least number of leaves per plant (177.12) was recorded in control plants.

Leaf area per plant was maximum (3746.92 cm^2) with the spray of 2.0 % organic chelate. It was followed by 1.5 % foliar spray of organic chelate with 3735.21 cm2 per plant. Whereas foliar spray of unchelated micronutrient solution at 2.0% concentration produced 2866.73 cm^2 averaged leaf area per plant and the amino acid solution at the concentration of 2.0% showed 2936.72 cm^2 averaged leaf area per plant. However, the lowest leaf area i.e. 2137.56 cm^2 per plant was recorded in control.

Fruits per plant and fruit length

Foliar application of organic micronutrient chelates at the concentration of 2.0% produced maximum number of fruits (139.93 plant-1), followed by 138.53 and 116.91 fruits plant-1 at foliar application of 1.5 % and 1.0 % micronutrient chelate respectively. The un-chelated micronutrients and amino acid solution at 2.0% concentration resulted in average of 91.90 and 93.80 fruits plant-1 respectively. However, the lowest number of fruits (80.03 $plant^{-1}$) was recorded in control pots.

Foliar application of organic micronutrient chelates at the concentration of 2.0% produced resulted significantly longer fruits (10.73 cm) followed by average fruit length of 10.65 and 10.34 cm at the concentration of 1.5 % and 1.0 % chelates respectively. The un-chelated micronutrients and amino acid solution at 2.0% concentration resulted in fruits with length of 9.15 cm and 9.20 cm respectively. However, the shorter fruits with average 7.99 cm length were recorded in control plants.

Fresh fruit weight and fruit yield per plant.

The fresh fruit weight was remarkably maximum (4.50 g fruit-1) in plants treated with foliar application of organic micronutrient chleate at the concentration of 2.0% followed by average fresh fruit weight of 4.48 and 4.14 g fruit-1 achieved from the treatments under foliar application of 1.5 % and 1.0 % organic micronutrient chealte respectively. The un-chelated micronutrients and amino acid solutions at 2.0 % foliar spray showed fresh fruit weight of 3.39 and 3.45 g fruit-1 respectively. However, the minimum average fresh fruit weight of 2.90 g fruit-1 was obtained in control plants.

Table 1 - Effect of foliar application of amino acid-micronutrient chelates on plant height and number of branches per plant in *Capsicum annum* at the flowering stage

Treatments	Plant height (cm)				Branches per plant			
	2009	2010	Pooled	PI	2009	2010	Pooled	PI
T0	45.09	46.11	45.60	--	10.87	11.13	11.00	--
T1	49.50	50.65	50.08	9.83	13.17	13.67	13.42	21.97
T2	49.80	50.96	50.38	10.49	13.37	13.80	13.58	23.48
T3	54.95	56.25	55.60	21.94	15.97	16.57	16.27	47.88
T4	59.51	60.76	60.13	31.88	17.17	17.77	17.47	58.79
T5	58.55	59.88	59.22	29.87	17.27	17.90	17.58	59.85
SEm±	0.32	0.31	0.20	--	0.27	0.25	0.08	--
CD (0.05)	1.26	1.22	1.08	--	1.06	1.02	1.04	--
CD (0.01)	2.05	1.98	1.76		1.73	1.66	1.69	

Table 2: Effect of foliar application organic-micronutrient chelates on leaves and leaf area per plant in chili.

Treatments	Leaves per plant				Leaf area per plant (cm^2)			
	2009	2010	Pooled	PI	2009	2010	Pooled	PI
T0	176.93	177.30	177.12	--	2134.19	2140.93	2137.56	--
T1	211.97	212.73	212.35	19.89	2861.55	2871.90	2866.73	34.11
T2	214.03	214.67	214.35	21.02	2932.26	2941.19	2936.72	37.39
T3	231.50	233.00	232.25	31.13	3434.15	3445.68	3439.91	60.93
T4	238.33	239.13	238.73	34.79	3722.43	3747.98	3735.21	74.74
T5	239.48	240.44	239.96	35.48	3736.14	3757.69	3746.92	75.29
SEm±	0.58	0.61	0.14	--	4.41	2.71	2.35	--
CD (0.05)	2.42	2.44	0.81	--	17.99	11.52	14.11	--
CD (0.01)	3.94	3.96	1.32		29.27	18.74	22.96	

Table 3: Effect of amino acid-micronutrient chelates on fruits per plant and fruit length in chili.

Treatments	Fruits per plant				Fruit length (cm)			
	2009	2010	Pooled	PI	2009	2010	Pooled	PI
T0	78.93	80.03	79.48	--	7.86	8.12	7.99	--
T1	90.37	91.90	91.13	14.66	8.98	9.31	9.15	14.43
T2	92.07	93.80	92.93	16.92	9.03	9.37	9.20	15.08
T3	114.80	116.91	115.86	45.76	10.17	10.51	10.34	29.34
T4	132.43	138.53	135.48	70.46	10.43	10.86	10.65	33.24
T5	136.53	139.93	138.23	73.91	10.51	10.95	10.73	34.26
SEm±	0.92	0.94	0.44	--	0.10	0.27	0.06	--
CD (0.05)	3.72	3.69	2.83	--	0.41	1.03	0.32	--
CD (0.01)	6.05	6.00	4.61		0.67	1.67	0.52	

Table 4: Effect of foliar application of Effect of amino acid-micronutrient chelates on fresh fruit weight and total yield per plant in chili.

Treatments	Single fruit weight (g)				Yield per plant (g)			
	2009	2010	Pooled	PI	2009	2010	Pooled	PI
T0	2.87	2.93	2.90	--	226.61	236.41	231.51	--
T1	3.34	3.44	3.39	17.02	302.10	316.01	309.06	33.50
T2	3.40	3.50	3.45	19.09	316.52	328.19	322.36	39.24
T3	4.09	4.18	4.14	42.73	470.19	487.25	478.72	106.78
T4	4.43	4.54	4.48	54.69	610.34	622.54	616.44	166.27
T5	4.45	4.55	4.50	55.15	613.47	633.11	623.29	169.23
SEm±	0.02	0.02	0.02	--	2.13	1.63	2.34	--
CD (0.05)	0.11	0.08	0.08	--	8.75	6.72	7.74	--
CD (0.01)	0.17	0.14	0.14		14.24	10.93	12.59	

Fresh fruit yield was remarkably maximum (623.29 g plant-1) in pots fertilized with foliar application of organic micronutrient chelates at the 2.0% concentration it was followed by average fresh fruit yield of 616.44 and 478.72 g plant-1 at 1.5 and 1.0 % solutions respectively. The 2.0 % concentrations of un-chelated micronutriens and amino acid solutions showed fresh fruit yield of 309.06 and 322.36 g plant-1, respectively. However, the minimum fresh fruit yield of 231.51 g plant-1 was recorded in control plots.

4. Discussion

Plant height, branches, number of leaves and leaf area per plant

Results given in table 1 and 2 indicate the growth characters like plant height, number of branches per plant, number of leaves per plant and leaf area were increased significantly with the application of chelated micronutrients.

The un-chelated micronutrient and amino acid treated plants showed significant increase in plant height (9.83 %, 10.49 %), number of branches (21.97 and 23.48 %) leaves per plant (19.89 and 21.02) and leaf area per plant (34.11 and 37.39%) respectively over control plants. Among the different treatments 1.5 and 2.0 % treatments of chelated micronutrients showed significantly better results than the other treatments at the time of flowering. Amino acids might have contributed in absorption of micronutrients and also served as source of nitrogen to the additional increase in growth contributing characters. Baloch *et al*. 2008 [7] reported increase in plant height number of branches per plant in chili with commercial macro and micronutrient formulation "HiGrow". Our results are in accordance with Hazra *et al*. 1987 [8]; they reported the similar effect of foliar application of micronutrients on growth and yield of okra. Malawadi, 2003 [6] reported the similar results by treating the chilli seedlings with micronutrients.

Fruits per plant and fruit length, fresh fruit weight and fruit yield per plant.

The un-chelated micronutrient and amino acid treated plants showed significant increase in number (9.83 %, 10.49 %), fruit length (21.97 and 23.48 %) fruit weight (19.89 and 21.02) and yield per plant (34.11 and 37.39%) respectively over control plants. Among the different treatments 1.5 and 2.0 % treatments of chelated micronutrients showed significantly enhanced results than the other treatments at the time of flowering. The significantly highest numbers of fruits, more fruit length, fresh fruit weight and yield per plant were recorded in the plants treated with 1.5 and 2.0% organically chelated micronutrients. These results are in line with those of Patil and Biradar, 2001[14] who applied foliar fertilizer "Polyfeed" and found significant effect on fruit number and fruit weight of chilies. Similar studies have also been conducted by Jiskani, 2005 [13] who found that foliar application of zinc 3.0 ppm, copper 1.0 ppm and boron 0.5 ppm produced the highest number of fruits per plant with increased fruit weight and more total yield per plant.

The results are also in agreement with the findings of Nehra *et al*. 2001[10] and Sanwal *et al*. 2007 [11]. The enhanced photosynthesis in presence of zinc and boron was also reported by Rawat and Mathpal, 1984 [12]. The fruit yield per plant was recorded maximum in 1.5 and 2.0 % treatments over in control. This might be due to increase in values of fresh weights of the fruits per plant. Similar results were obtained by Gupta and Gupta, 2004 [15] who reported that application of micronutrients like Zn, Cu, Fe and Mo are essential for increase in yield, quality and ascorbic acid content in tomato fruits.

All the treatments of organically chelated micronutrients proved superior over un-chelated micronutrients, amino acid solution and control plants for yield contributing parameters. The results of the present investigation are in concurrence with Radulovic, 1996 [16] who applied N, P, K, Ca, Mg and Fe, B, Zn, Mn and Cu as foliar spray and observed increase in growth and yield contributing parameters in chili.

4. Conclusions

The results revealed that un-chelated micronutrient significantly improved the growth and yield contributing characters to a 10-15 %, where as amino acid spray contributed to 15-20% increase. On the other hand amino acid–chelated micronutrients contributed overall 40-100 % increase in growth and yield contributing characters in chili. Among the treatments of organically chelated micronutrients, chilli responded well to the 1.5 % and 2.0% treatments. The growth characters as well as the yield of chilli were significantly enhanced by the application of 1.5 % organically chelated micronutrients. However, these two treatments are at par and hence we recommend 1.5 % foliar spray for better yield in chili. The results of present investigation has given insight in application of chelated micronutrients in immiediate rectification of micronutrient deficency in fruit vegetable and also in organic farming.

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6. References

[1] Bose, U.S. and Tripathi S.K. Effect of micronutrients on growth, yield and quality of tomato cv. Pusa Ruby in M.P. Cro. Res. 1996; 12:61-64.

[2] Benepal, P.S. Influence of micronutrients on growth and yield of potatoes. Ame.Pot.J. 1967; 44:363-369

[3] Ilhami Koksal A., Hatice Dumanoglu, Nurdan Tuna Günes, Mehmet Aktas**.** The Effects of Different Amino Acid Chelate Foliar Fertilizers on Yield, Fruit Quality, Shoot Growth and Fe, Zn, Cu, Mn Content of Leaves in Williams Pear Cultivar (*Pyrus communis* L.) Tr. J. of Agriculture and Forestry 1998; 23: 651-658.

[4] Ouda, B.A. and Mahadeen, A.Y. Effect of fertilizers on growth, yield, yield components, quality and nutrient contents in broccoli (*Brassica oleracea*). *Int. J. Agri. Biol*; 2008 10: 627–32

[5] Kondapa, D., Radder, B. M., Patil, P. L., Hebsur, N. S. and Alagundagi, S. C. Effect of integrated nutrient management on growth, yield and economics of chilli (Cv. Byadgi dabbi) in a vertisol. *Karnataka J. Agric. Sci. 2009;* 22 (2): 438-440.

[6] Malawadi, M. N. Effect of secondary and micronutrients on yield and quality of chilli (*Capsicum annuum* L.) *M. Sc. (Agri.) Thesis,* Univ.Agric. Sci., Dharwad (India) 2003.

[7] Baloch, Q. B., Chachar, Q. I. and Tareen, M. N. Effect of foliar application of macro and micro nutrients on production of green chilies (*Capsicum annuum* L.). Journal of Agricultural Technology 2008; 4(2): 174-184.

[8] Hazra, P., Maity, T.K. and Mandal, A.R. Effect of foliar application of micronutrients on growth and yield of okra *(Abelmoschus esculentus* L). Prog. Hort. 1987; 19: 219-222

[9] Kumbhar, V.S and Deshmukh S.S. Effect of soil application of ferrous sulphate on the uptake of nutrients, yield and quality of tomato cv. Rupali. Sou. Ind. Hort.1993; 41:144-147.

[10] Nehra, A.S., I.S. Hooda, and K.P. Singh. Effect of integrated nutrient management on growth and yield of wheat (*Triticurn aestivum L.*) Indian J. Agron. 2001; 45: 112-17.

[11] Sanwal, S.K., K. Lakminarayana, R.K. Yadav, N. Rai, D.S. Yadav, and B. Mousumi. Effect of organic manures on soil fertility, growth, physiology, yield and quality of turmeric. Indian J. Hort. 2007; 64(4): 444-449.

[12] Rawat, P.S. and Mathpal, K.N. Effect of micronutrients on yield and sugar metabolism of some of the vegetables under Kumaon hill conditions. Sci. Cult. 1984; 50:243-244.

[13] Jiskani, M.M. Foliar fertilizers-fast acting agents. Daily DAWN, the Internet Edition, Monday December 5, 2005.

[14] Patil, R. and Biradar, R. Effect of foliar application of essential nutrients on chilies. Agricultura Tecnica Santiago 2001; 51(3): 256-259.

[15] Gupta P.K. and Gupta A.K. Studies of PGR and Micronutrient mixtures on vitamin 'C' content in tomato (*Lycopersicon esculentum*, Mill) products. Indian Journal of horticulture 2004; 61(1) 102-103.

[16] Radulovic, M. Soil and vegetable nutrients supply in the region of the Zeta Montenegro. Review-of-Research-Work-at-the-Faculty-of-Agriculture,- Belgrade, 1996; 41(1): 31-40.

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Synthesis of Iron-Amino Acid Chelates and Evaluation of Their Efficacy as Iron Source and Growth Stimulator for Tomato in Nutrient Solution Culture

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Abstract Supplying a sufficient amount of available iron (Fe) for plant growth in hydroponic nutrient solutions is a great challenge. The chelators commonly used to supply Fe in nutrient solutions have several disadvantages and may negatively affect plant growth. In this research study we have synthesized certain Fe-amino acid chelates, including Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$], and evaluated their efficacy as an Fe source for two tomato cultivars (*Lycopersicon esculentum* Mill. cvs. 'Rani' and 'Sarika') grown in nutrient solution. Application of Fe-amino acid chelates significantly increased root and shoot dry matter yield of both tomato cultivars compared with Fe-EDTA. Tomato plants supplied with Fe-amino acid chelates also accumulated significantly higher levels of Fe, Zn, and N in their roots and shoots compared with those supplied with Fe-EDTA. In 'Sarika', the effect of Fe-amino acid chelates on shoot Fe content was in the order $Fe(His)_2 > Fe(Gly)_2 > Fe(Arg)_2$. In 'Rani', the addition of all synthesized Fe-amino acid chelates significantly increased activity of ascorbate peroxidase (APX) in comparison with Fe-EDTA, whereas in 'Sarika', only $Fe(His)_2$ increased shoot APX activity. The results obtained indicated that using Fe-amino acid chelates in the nutrient solution could supply a sufficient amount of Fe for plant uptake and also improve root and shoot growth of tomato plants, although this increase was cultivar-dependent. According to the results, Fe-amino acid chelates can be used as an alternative for Fe-EDTA to supply Fe in nutrient solutions.

Keywords Amino acids · Chelate · Fe availability · Fe-EDTA · Tomato

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Introduction

Iron (Fe) is an important micronutrient that plays a role in several crop physiological processes such as photosynthesis, respiration, and synthesis of heme proteins, DNA, RNA, and hormones (Curie and others 2009; Rivero and others 2003). In nutrient solution cultures, synthetic Fe chelates are widely used to maintain a desirable concentration of this element for the plant (Parker and Norvell 1999; Vadas and others 2007). The most common Fe sources used in nutrient solutions are Fe-EDTA and Fe-DTPA (Vadas and others 2007). Although these chelates maintain Fe solubility in hydroponic solutions, after Fe uptake by the plant, the concentration of free ligands is increased in the nutrient solution and, as a result, the possibility of complex formation between free ligands and other micronutrients (that is, Zn, Cu, and Mn) in the solution increases. Complexation with EDTA, EDDS, or DTPA reduces the concentrations of free metal cations and thereby decreases their availability for plant uptake (Albano and Miller 2001; Vadas and others 2007).

On the other hand, EDTA, EDDS, and DTPA are easily photodegradable compounds (Metsarinne and others 2004; Nowack and Baumann 1998). Significant photodegradation

of Fe-EDTA in natural waters (Nowack and Baumann 1998) and of Fe-EDTA, Fe-DTPA, or Fe-EDDS in Fe-containing nutrient solutions (Metsarinne and others 2001) by sunlight and particularly ultraviolet (UV) light has been reported. The half-lives of Fe-EDTA and Fe-DTPA photodegradation under high light density conditions have been reported to be 8 and 11 min, respectively (Svenson and others 1989). Photodegradation of the Fe-chelates in plant growth nutrient solutions in the presence of blue and UV lights has also been reported by Albano and Miller (2001). Some harmful compounds may be produced from photolytic degradation of Fe-chelates in nutrient solutions (Vadas and others 2007). For example, Hangarter and Stasinopoulos (1991) found that Fe-EDTA was decomposed in an agar growth solution under white fluorescent lamps and produced glyoxylic acid and formaldehyde, two compounds that inhibit plant growth. Metsarinne and others (2004) also reported photolytic degradation of DTPA into diethylenetriaminetriacetic acid and diethylenediaminetriacetic acid.

The chelators used in nutrient solutions may also be transported into the plant tissue (Vadas and others 2007) probably via an undeveloped casparian band at the root tip (Bell and others 1991). High concentrations of chelates can remove calcium (Ca^{2+}) from the cell membrane and impair root membrane integrity (Vassil and others 1998).

Supplying a sufficient amount of available Fe for plant growth in hydroponic nutrient solutions used in laboratory studies and commercial facilities is a great challenge. Considering the problem associated with the synthesized chelates currently used in nutrient solutions, finding a proper alternative of Fe compounds is necessary.

It has been shown that use of some amino acids in nutrient solutions improves Fe uptake by crops (Sánchez and others 2005). Advances in the understanding of the metabolic responses to Fe deficiency have also highlighted the key role of amino acids in both Strategy I and Strategy II plants (Zuchi and others 2009). Amino acids have the ability to coordinate metal ions (such as Fe) via their carboxyl groups (Aravind and Prasad 2005). On the other hand, amino acids are less sensitive to photodegradation and their degradation is completely biological (Jones and Hodge 1999). There are some results that indicate negligible degradation of amino acids in nutrient solutions (Jämtgård and others 2008). The degradability of metal–amino acid complexes is also less than free amino acids (Brynhildsen and Rosswall 1995; Renella and others 2004). Therefore, Fe–amino acid complexes seem to be stable in hydroponic nutrient solutions and prevent Fe precipitation.

In addition, amino acids are nitrogen sources for plant nutrition (Tida and others 2009). Most plants can directly absorb amino acids and use them in their physiological structures and processes (Jämtgård and others 2008; Näsholm and others 2009; Wu and others 2005). This may result in less accumulation of free ligand in the media and further impairment of other micronutrient balance.

Due to several disadvantages of Fe-EDTA (for example, toxic side effects on plants and impaired micronutrient balance), finding a suitable alternative for Fe-EDTA in hydroponic nutrient solutions is of great importance. There is limited information on the possibility of using Fe–amino acid complexes as a plant growth stimulator and Fe source in nutrient solution cultures. Therefore, this research was performed to synthesize three Fe–amino acid chelates and evaluate their efficacy as Fe sources for tomato plants grown in nutrient solution. Arginine (Arg), glycine (Gly), and histidine (His) were chosen as ligand amino acids. The L-enantiomers (natural forms in plants) of amino acids were used, and some factors considered in the selection of these amino acids were abundance in the plant rhizosphere, significance in plant and human nutrition, and stability of their Fe complexes in water.

Materials and Methods

Synthesis of Fe-Amino Acid Chelates

Iron chelates have been prepared using arginine (Arg), glycine (Gly), and histidine (His) amino acids as complexing agents. A solution of Arg, Gly, or His (2 mmol) in 5 ml distilled water was slowly added to a solution of $FeSO_4$ (1 mmol) in 2 ml distilled water. The mixture was heated at reflux temperature for 2 h while being stirred vigorously. Evaporation of solvent at room temperature yielded brown microcrystals of Fe-amino acid chelates. The products were washed with cold ethanol followed by diethyl ether and air-dried. All complexes were characterized by different analytical techniques.

Analyses

A PerkinElmer 2400 CHNS elemental analyzer was used to quantify nitrogen (N) and sulfur (S) in various operating modes. Atomic absorption measurements of Fe were recorded with atomic absorption spectrometry (PerkinElmer 3030; PerkinElmer, Wellesley, MA, USA). The FTIR spectra were measured with a JASCO FTIR 460 spectrophotometer (JASCO, Easton, MD, USA) over KBr pellet in the 4000–400 cm^{-1} range.

Plant Culture

Seeds of two tomato cultivars (*Lycopersicon esculentum* Mill. cvs. ‘Rani’ and ‘Sarika’), most commonly grown in Iran, were thoroughly rinsed with distilled water and

germinated on moist filter paper in an incubator at 28°C. Uniform-size seedlings were transferred to PVC lids that fit tightly over 2-L polyethylene containers in a greenhouse under controlled conditions, with an 8-h light period at intensity of 390 μmol m^{-2} s^{-1}, 25/20°C day/night temperature, and 65–75% relative humidity. The pots were wrapped with black polyethylene to prevent light from reaching the roots and solution. Two plants were planted in each pot. A basic nutrient solution was prepared in double-deionized water (electrical resistivity = 18 MΩ cm^{-1}). The nutrient solution contained 1.0 mM KNO_3, 1.0 mM $Ca(NO_3)_2$, 1.0 mM $NH_4H_2PO_4$, 1.0 mM $MgSO_4$, 50 μM KCl, 25 μM H_3BO_3, 2.0 μM $MnSO_4$, 2.0 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 1.0 μM $NiSO_4$, and 0.02 μM $H_2Mo_7O_4$ adjusted to pH 6 with NaOH or HCl as a buffer. Iron was supplied from four different sources: Fe-EDTA, $Fe(Arg)_2$, $Fe(Gly)_2$, and $Fe(His)_2$. The Fe level in the nutrient solution was 100 μM. All solutions were renewed every day.

Plants were harvested approximately 4 weeks after seeding and divided into shoot and roots. Shoot and root dry matter yields were determined for each pot.

Elemental Analyses

The plant materials were dried immediately in a forced-air oven at 70°C to a constant weight and ground to a fine powder in a Wiley mill to pass through a 20-mesh sieve. Dry samples (1 g) were placed into ceramic vessels and combusted in a muffle furnace at 550°C for 8 h. The ashed samples were removed from the muffle furnace, cooled, and then dissolved in 2 M HCl (Chapman and Pratt 1961). The final solution was diluted to meet the range requirements of the analytical procedures. Analyses of Fe and Zn were carried out with an atomic absorption spectrophotometer (model 3400, Perkin-Elmer). Shoot nitrogen concentration was measured using Autotech (model 300) according to the Kjeldahl method (Bremmer and Mulvancey 1982). The total amount of Fe and Zn was calculated via multiplying their concentrations by the weight of dry matter.

Enzyme Assay

The plant leaf samples (buffer volume:fresh weight = 3:1) were homogenized with mortar and pestle with 100 mM Tris–HCl buffer (pH 8) containing 2 mM EDTA, 5 mM DL-dithiothreitol, 10% glycerol, 100 mM sodium borate, 4% (w/v) insoluble polyvinylpyrrolidone (PVP), and 1 mM phenylmethylsulfonyl fluoride (PMSF). The homogenate was filtered through four layers of muslin cloth and centrifuged at 12,000 *g* for 40 min. The supernatant was stored in separate aliquots at –80°C prior to enzyme analyses. Total protein was determined using the Bradford method (Bradford 1976).

Catalase (CAT)

Catalase (EC 1.11.1.6) activity of the leaves was determined according to Cakmak and Marschner (1992). The reaction mixture contained 25 mM sodium phosphate buffer (pH 7.0) plus 10 mM H_2O_2 in a total volume of 3 ml. The reaction was initiated by the addition of 100 μl of leaf extracts to the reaction mixture, and the enzyme activity was determined by measuring the initial rate of disappearance of H_2O_2 at 240 nm (E = 39.4 mM^{-1} cm^{-1}) for 30 s.

Ascorbate Peroxidase (APX)

Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981). The reaction mixture, with a total volume of 3 mL, consisted of 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2, and 100 μl of the leaf extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (E = 2.8 mM^{-1} cm^{-1}).

Statistical Analysis

The experiments were set up in a completely randomized factorial design; each treatment contained three replicates. A total of 24 pots were used in this experiment. Two plants were planted in each pot. Treatment effects were analyzed by analysis of variance using the GLM procedure. Means were compared using least significant differences (LSD) at $p < 0.05$ (SAS Institute, Cary, NC, USA, 2000).

Results

Characteristics of Fe-Amino Acid Chelates

The Fe–amino acid complexes were synthesized by reaction of $FeSO_4$ and Arg, Gly, or His in a 1:2 mole ratio and the reaction yield was more than 84%. The amino acid ligands generally act as bidentate (N,O) chelates with respect to pH. The FTIR spectra of the complexes show an absorption pattern in the 4,000–400 cm^{-1} region, similar to amino acid ligands. Predominant vibrations for the complexes are associated with ν(CO), ν(C—O), $\nu(NH_2)$, $\delta(NH_2)$, and δ(CO). The observed vibrational bands for $-NH_2$ groups around 3,100–3,350 cm^{-1} are very sensitive to the effect of intermolecular interaction in the solid state and these bands sometimes appear very broad. The carboxylate ion of amino acid coordinates to the iron ion as a unidentate mode. The C = O groups of the complexes have approximately the same frequency of around 1,590–1,690 cm^{-1} (data not shown).

Root Dry Matter Weight

In both tomato cultivars, Fe-amino acid chelates significantly enhanced root dry matter yield compared to Fe-EDTA (Fig. 1). The positive effect of Fe-amino acid chelates on root growth varied dependent on the crop cultivar and amino acid type. $Fe(Arg)_2$ had no significant effect on root dry matter yield of 'Sarika' but there was significantly increased root dry matter yield of 'Rani'. In 'Sarika' no significant difference was found in root growth between the $Fe(Gly)_2$ and $Fe(His)_2$ treatments. In contrast, the positive effect of $Fe(Gly)_2$ on root dry matter yield of 'Rani' was greater than $Fe(His)_2$. With the Fe-EDTA treatment, no significant difference was found in root growth between 'Sarika' and 'Rani', whereas in the presence of the Fe-amino acid chelates, 'Rani' produced higher root dry matter yield than 'Sarika'.

Shoot Dry Matter Weight

Application of Fe-amino acid chelates as an Fe source significantly increased shoot dry matter yield of both tomato cultivars compared with Fe-EDTA (Figs. 1, 2). In 'Sarika', the effect of Fe-amino acid chelates on shoot dry matter yield was in the order $Fe(Arg)_2 < Fe(Gly)_2 < Fe(His)_2$. In contrast, in 'Rani' no significant difference was found in shoot growth of plants supplied with $Fe(Arg)_2$, $Fe(Gly)_2$, and $Fe(His)_2$ complexes. At all Fe treatments except $Fe(His)_2$, 'Rani' produced higher shoot dry matter yield than 'Sarika'.

Root Fe Content

Application of Fe-amino acid chelates resulted in a significant increase of root Fe content in comparison with Fe-EDTA (Fig. 3). The positive effect of Fe-amino acid chelates on root Fe content was dependent on the tomato cultivar and amino acid type. Addition of the $Fe(Arg)_2$ complex significantly increased Fe content in the roots of 'Rani', whereas it had no effect on root Fe content of 'Sarika'. In 'Rani,' the magnitude of increase in root Fe content was $Fe(Arg)_2 > Fe(Gly)_2 > Fe(His)_2$. In 'Sarika', the effect of $Fe(His)_2$ on root Fe content was greater than that of $Fe(Gly)_2$.

In the nutrient solutions containing Fe-EDTA and $Fe(His)_2$, 'Sarika' accumulated higher amounts of Fe in its roots than did 'Rani' (Fig. 3), whereas in the presence of $Fe(Arg)_2$, 'Rani' had higher root Fe content than 'Sarika'. No significant difference in root Fe content was found between the two tomato cultivars at the $Fe(Gly)_2$ treatment.

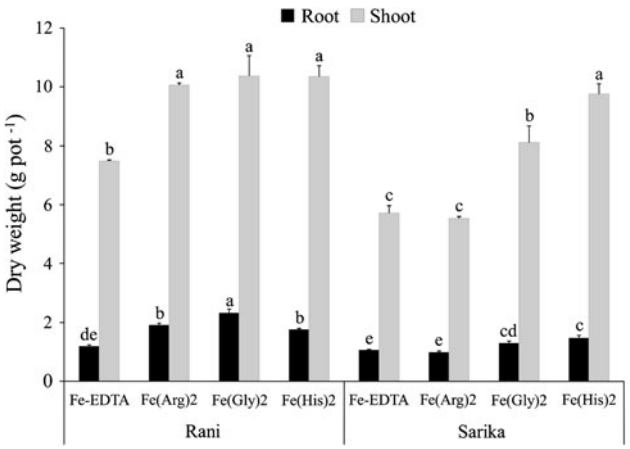

Fig. 1 Root and shoot dry matter weights of two tomato cultivars grown in nutrient solution containing Fe-EDTA, Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$]. *Error bars* represent standard error ($n = 3$). *Bars* having different letters in root or shoot are significantly different at the 5% level by LSD

Shoot Fe Content

Tomato plants supplied with Fe-amino acid chelates accumulated significantly higher Fe in their shoots compared with those supplied with Fe-EDTA (Fig. 4). The positive effect of $Fe(Gly)_2$ and $Fe(His)_2$ on shoot Fe content was higher than $Fe(Arg)_2$. In 'Sarika', addition of $Fe(Arg)_2$ had no effect on shoot Fe content. In general, 'Rani' accumulated higher Fe in its shoots than did 'Sarika'.

Root Zn Content

Addition of Fe-amino acid chelates resulted in higher root Zn content compared with Fe-EDTA (Fig. 5). The effect of $Fe(Arg)_2$ and $Fe(Gly)_2$ on root Zn content of 'Rani' was higher than that of $Fe(His)_2$, whereas in 'Sarika', $Fe(Arg)_2$ had no effect on root Zn content. In the nutrient solutions containing Fe-EDTA and $Fe(His)_2$, 'Sarika' accumulated higher Zn in its roots than did 'Rani', whereas with the $Fe(Arg)_2$ treatment, root Zn content of 'Rani' was greater than that of 'Sarika'.

Shoot Zn Content

Tomato plants supplied with Fe-amino acid chelates accumulated significantly higher Zn in their shoots compared with those supplied with Fe-EDTA (Fig. 6). In 'Rani', $Fe(Arg)_2$ and $Fe(Gly)_2$ resulted in higher shoot Zn content in comparison with $Fe(His)_2$, whereas the effect of $Fe(Arg)_2$ on shoot Zn content of 'Sarika' was less than that of $Fe(His)_2$ and $Fe(Gly)_2$. With the Fe-EDTA and $Fe(His)_2$ treatments, no significant difference was found in shoot Zn content between the two tomato cultivars, whereas with the $Fe(Arg)_2$ and $Fe(Gly)_2$ treatments, 'Rani' accumulated higher Zn in its shoots than did 'Sarika'.

Shoot N Content

In both tomato cultivars, plants supplied with Fe-amino acid chelates had higher shoot N content compared with

Fig. 2 The effect of Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$] on the shoot growth of two tomato cultivars in comparison with Fe-EDTA

those supplied with Fe-EDTA (Fig. 7). The effect of Fe-amino acid chelates on shoot N content varied significantly depending on the crop cultivar and amino acid type. Application of $Fe(Arg)_2$ had no effect on the shoot N content in 'Sarika', whereas it increased it in 'Rani'. With the Fe-EDTA treatment, no significant difference was found in the shoot N content between the two tomato cultivars, whereas with the Fe-amino acid chelate treatments, 'Rani' had higher N in its shoots than did 'Sarika'.

Activity of CAT in Shoots

In both tomato cultivars, addition of $Fe(Arg)_2$ significantly increased the shoot activity of CAT in comparison with Fe-EDTA (Fig. 8). The effect of $Fe(Gly)_2$ on shoot CAT activity was dependent on the tomato cultivar. Addition of $Fe(Gly)_2$ increased the shoot CAT activity in 'Rani', whereas it had no effect on CAT activity in 'Sarika'. In all treatments except $Fe(His)_2$, the activity of CAT was greater in 'Rani' than 'Sarika'.

Activity of APX in Shoots

The effect of Fe-amino acid chelates on the shoot activity of APX was cultivar-dependent (Fig. 9). In 'Rani', addition of all synthesized Fe-amino acid chelates significantly increased the activity of APX in comparison with Fe-EDTA (Fig. 9). In 'Sarika', the addition of $Fe(His)_2$ and $Fe(Gly)_2$ increased the shoot APX activity compared with Fe-EDTA, whereas no such effect was found for $Fe(Arg)_2$. In the presence of $Fe(Arg)_2$, the activity of APX in the shoots of 'Sarika' was greater than that in 'Rani', whereas

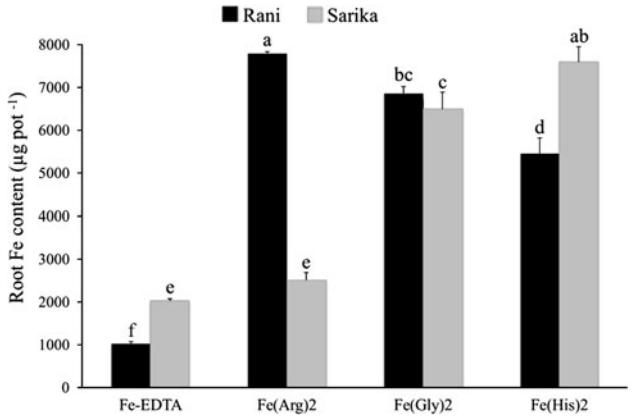

Fig. 3 Root Fe content of two tomato cultivars grown in nutrient solution containing Fe-EDTA, Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$]. *Error bars* represent standard error ($n = 3$). *Bars* having different letters are significantly different at the 5% level by LSD

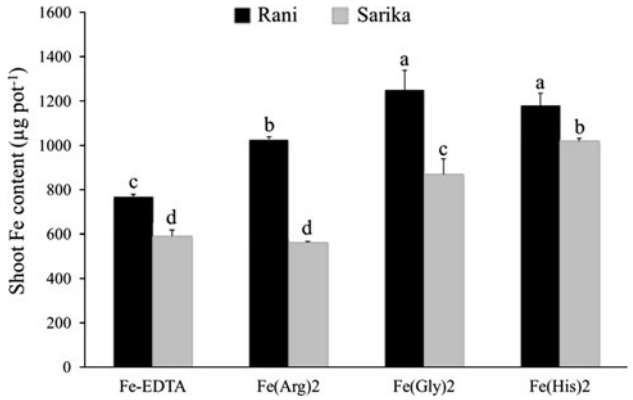

Fig. 4 Shoot Fe content of two tomato cultivars grown in nutrient solution containing Fe-EDTA, Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$]. *Error bars* represent standard error ($n = 3$). *Bars* having different letters are significantly different at the 5% level by LSD

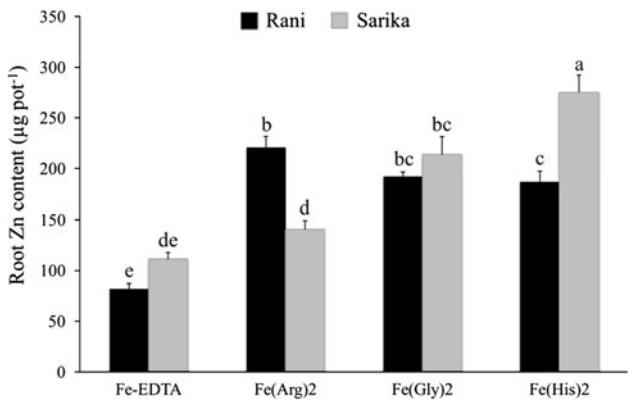

Fig. 5 Root Zn content of two tomato cultivars grown in nutrient solution containing Fe-EDTA, Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$]. *Error bars* represent standard error ($n = 3$). *Bars* having different letters are significantly different at the 5% level by LSD

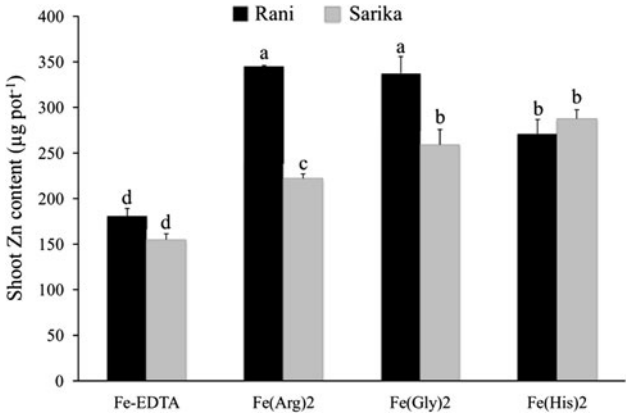

Fig. 6 Shoot Zn content of two tomato cultivars grown in nutrient solution containing Fe-EDTA, Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$]. *Error bars* represent standard error ($n = 3$). *Bars* having different letters are significantly different at the 5% level by LSD

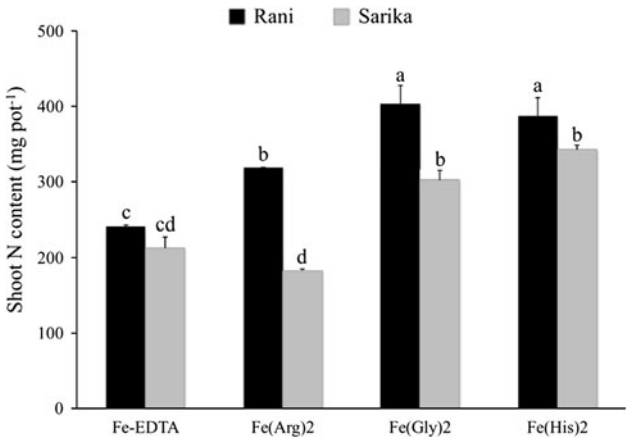

Fig. 7 Shoot N content of two tomato cultivars grown in nutrient solution containing Fe-EDTA, Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$]. *Error bars* represent standard error ($n = 3$). *Bars* having different letters are significantly different at the 5% level by LSD

with the Fe-EDTA and $Fe(His)_2$ treatments, 'Rani' had higher activity of APX in its shoots than 'Sarika'.

Discussion

The iron-amino acid chelates were synthesized using proper amounts of Arg, Gly, or His. These amino acids are abundant in the plant rhizosphere (Jones and other 2004; Rothstein 2009; Werdin-Pfisterer and others 2009) and play significant roles in plant and human nutrition (Amin and others 2011; Li and others 2007). The stability of metal complexes of these amino acids is also high in water.

The results obtained from our study indicated that using Fe-amino acid chelates in the nutrient solution could

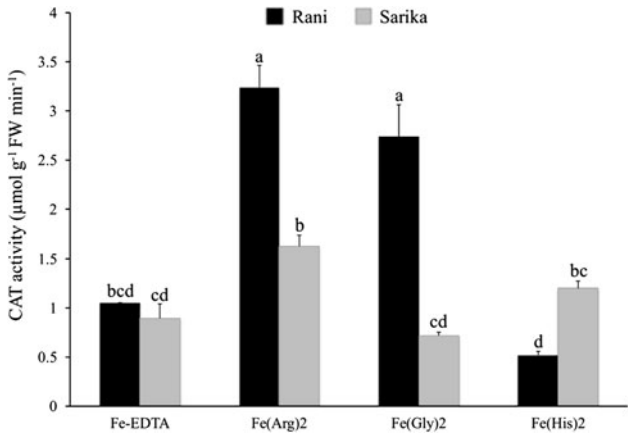

Fig. 8 Activity of CAT in the leaves of two tomato cultivars grown in nutrient solution containing Fe-EDTA, Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$]. *Error bars* represent standard error ($n = 3$). *Bars* having different letters are significantly different at the 5% level by LSD

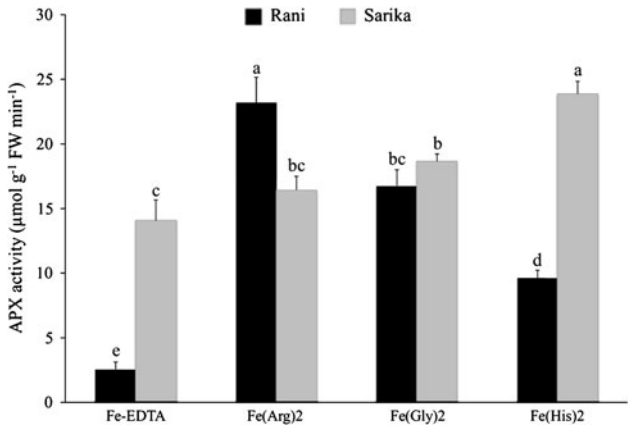

Fig. 9 Activity of APX in the leaves of two tomato cultivars grown in nutrient solution containing Fe-EDTA, Fe-arginine [$Fe(Arg)_2$], Fe-glycine [$Fe(Gly)_2$], and Fe-histidine [$Fe(His)_2$]. *Error bars* represent standard error ($n = 3$). *Bars* having different letters are significantly different at the 5% level by LSD

supply sufficient amounts of Fe for plant uptake (Figs. 3, 4) and also improve root and shoot growth of tomato plants (Figs. 1, 2). The stimulating effect of amino acids on the yield and quality of crops is due to increased mRNA transcription, activation of sugar synthesis, and increasing protein content (Keutgen and Pawelzik 2008; Nassar and others 2003; Rashad and others 2003). Amino acids induce biosynthesis of chlorophyll and thereby improve the photosynthesis rate (Amin and others 2011; Zeid 2009). Several studies (Amin and others 2011; Wang and others 2007; Zeid 2009) have reported the growth-stimulating effect of amino acids. For example, soil application of tryptophan and methionine improved plant growth via increasing auxin and ethylene production in soil and plant tissues and/or increasing the population of beneficial microorganisms (Arshad and Frankenberger 1990; Arshad and others 1995). Furthermore, some authors indicated that the level of free methionine in plants is rate limiting for ethylene production as a component of the complex regulation mechanism for the onset of the Fe-deficiency response (Zuchi and others 2009). Wang and others (2007) found that replacing $N–NO_3$ by 20% in nutrient solution with Arg resulted in a significant increase of fresh and dry matter weight of pak-choi (*Brassica chinensis* L.) shoots. Replacement of $N–NO_3$ in nutrient solution with glutamic acid and aspartic acid reduced accumulation of excess NO_3^- in plant tissues and, as a result, improved crop quality (Wang and others 2007).

Although amino acids used in the present study stimulated plant growth, it is not easy to dissect out whether the effect is due to better Fe uptake, more nitrogen supplied in the form of amino acids, or the hormonal effect of amino acids. Considering the positive and significant correlation between shoot dry matter yield and Fe and N uptake in both tomato cultivars, it seems that both enhanced Fe and N uptakes play roles in improvement of tomato growth. Without a control-free Fe treatment, it is impossible to differentiate the effects of amino acids and Fe on plant growth. Using just the amino acid ligands as the control for such a study would not be useful due to severe growth damage under free Fe conditions. Supplying Fe from Fe-EDTA or other sources was also difficult because of possible interactions between added Fe, amino acids, and other ligands. For example, Sánchez and others (2005) reported a significant interaction between free amino acids added to the nutrient solution and Fe-EDDHA in Fe uptake and plant growth. There were some restrictions to foliar spray of Fe to overcome this problem (to use just amino acid control treatment) because of the differential growth response of plants to various application methods for Fe. The possibility also exists that Fe and amino acid interactions at the root surface significantly affect plant growth as well as amino acid-N and Fe uptake by plants.

In this study, the effect of Fe-amino acid chelates on growth depended on the amino acid type and tomato cultivar (Figs. 1, 2). The growth-stimulating effect of Fe-amino acid chelates on 'Rani' was greater than that on 'Sarika'. In contrast to $Fe(Gly)_2$ and $Fe(His)_2$, addition of $Fe(Arg)_2$ had no effect on shoot and root dry matter weight of the 'Sarika' cultivar, whereas it significantly increased shoot and root growth of the 'Rani' cultivar. The different response to $Fe(Arg)_2$ application in two tomato cultivars is due to genetic diversity and/or different nutrient requirements of these cultivars. With all treatments, the shoot and root dry matter yield of 'Rani' was greater than that of 'Sarika'. The fact that there was no significant effect of $Fe(Arg)_2$ on growth and shoot Fe and N uptake in 'Sarika'

is probably because of a lower ability of this cultivar to absorb $Fe(Arg)_2$. The uptake rate of amino acids is dependent on the plant cultivar and amino acid characteristics (Falkengren-Grerup and others 2000; Okamoto and Okada 2004; Svennerstam and others 2008). Okamoto and others (2003) reported a higher ability of sorghum and rice plants to absorb organic nitrogen forms from soil solution than maize and pearl millet. Reeve and others (2009) reported that the effect of Gly addition on N uptake varied among modern and classic wheat cultivars. The greater N uptake by modern wheat cultivars seems to be due to a higher demand for N or greater root-to-shoot transport of this nutrient element in these cultivars (Reeve and others 2009). Large variations among strawberry cultivars in their amino acid uptake have been reported by Reeve and others (2008). Kielland (1994) found that Gly, aspartic acid, and glutamic acid comprised 80% and less than 10% of the total N absorbed by *Ledum palustre* and *Eriophorum angustifolium*, respectively. Variation among plant species with respect to amino acid uptake could be due to differences in the number and type of amino acid transporters. Amino acids are taken up by plants via certain transporters, for example, lysine-histidine transporter 1 (LHT1), amino acid permease 1(AAP1), and amino acid permease 5 (AAP5) (Ortiz-Lopez and others 2000; Svennerstam and others 2008). Svennerstam and others (2008) found that reduced expression of LHT1 caused a rapid decrease in Gly and His uptake, whereas it had no effect on Arg uptake. Arginine uptake is dependent on the activity of AAP5 (Svennerstam and others 2008). Therefore, lower plant uptake of $Fe(Arg)_2$ compared with $Fe(Gly)_2$ and $Fe(His)_2$ in the present study could partly be due to differences in the number and type of amino acid transporters in the root cell membrane. On the other hand, the effect of amino acids on plant growth is dependent on their characteristics. Svennerstam and others (2007) found the growth response of *Arabidopsis* sp. to glutamine application was greater than that to the other amino acids applied. Wang and others (2007) reported that certain amino acids reduced nitrate uptake and thereby inhibited plant growth. In contrast, the addition of some other amino acids improved N uptake and plant growth. In the present study, shoot growth of both tomato cultivars was significantly correlated with shoot N content (Table 2). Tomato plants supplied with $Fe(His)_2$ and $Fe(Gly)_2$ had higher N in their shoots and thus produced greater biomass compared with those plants supplied with $Fe(Arg)_2$. Another possible reason for the smaller response of tomato plants to $Fe(Arg)_2$ is its larger molecular size (Table 1) and, thus, the lower uptake of this amino acid chelate compared with the other amino chelates used. This suggestion needs to be tested in further experiments, particularly considering that the estimated diameter of Fe-amino chelate molecules is smaller than the cell wall pore diameter. Thus, there seems to be no limitation on the movement of amino chelates via these free spaces toward the cell membrane.

Table 1 Selected characteristics of Fe-amino acid chelates

Fe-amino acid chelates	Size (nm)	Fe (%)	N (%)	S (%)
$Fe(Arg)_2$	1.41	10.1	19.98	4.48
$Fe(Gly)_2$	0.48	13.25	7.39	8.04
$Fe(His)_2$	1.09	9.25	16.21	6.09

Regardless of crop cultivar, tomato plants supplied with Fe-amino acid chelates accumulated higher amounts of Fe in their shoots compared with those supplied with Fe-EDTA (Fig. 4). To better understand the effect of Fe-amino acid chelates on the nutritional status of Fe in tomato, the activity of CAT and APX was measured (Figs. 8, 9). Based on results obtained from several studies (Dasgan and others 2003; Ruiz and others 2000), biochemical indices such as activity of Fe-containing enzymes are much better indices to show the plant nutritional status of Fe than is shoot Fe concentration. The effect of Fe-amino acid chelates on the shoot activity of CAT varied with tomato cultivar and amino acid type (Fig. 8). Although $Fe(Gly)_2$ increased the activity of CAT in the shoots of 'Rani', it had no effect on the activity of this enzyme in the shoots of 'Sarika'. CAT activity was greater in both cultivars, whereas shoot Fe uptake was smaller in plants treated with $Fe(Arg)_2$ than in plants treated with $Fe(His)_2$ and $Fe(Gly)_2$. Higher activity of CAT in the presence of $Fe(Arg)_2$ is probably due to the role of Arg in the expression of genes encoding the CAT enzyme. It has been reported that Arg plays a role in several enzymatic activities within plants. Arginine and polyamines are involved in the structure and function of several proteins and antioxidant enzymes, particularly the CAT enzyme (Drolet and others 1986; Kuznetsov and Shevyakova 2007; Lovass 1991). In 'Sarika', increasing shoot Fe content was associated with elevated APX activity in the leaves. No significant difference was found in leaf activity of APX between plants fed with the $Fe(Arg)_2$ and those fed with Fe-EDTA (Fig. 9). In addition, application of $Fe(Arg)_2$ had no significant effect on shoot Fe content of the 'Sarika' cultivar. This result suggests greater dependency of APX activity on the Fe nutritional status of tomato compared with CAT. Elevated shoot Fe content and activity of CAT and APX in the presence of Fe-amino acid chelates indicates improvement of plant nutritional Fe status in comparison with Fe-EDTA treatment. In line with our results, Sánchez and others (2005) reported that addition of amino acids in the nutrient solution improved Fe uptake by tomato. Amino acids can form soluble complexes with Fe and thereby play an important role in maintaining Fe

Table 2 Correlation between root and shoot dry matter weights (DW) and Fe, Zn, and N uptake of two tomato cultivars

Plant	Parameter	Rani			Sarika		
		DW	Fe	Zn	DW	Fe	Zn
Root	Fe	0.82**			0.91***		
	Zn	0.75**	0.94***		0.94***	0.95***	
Shoot	Fe	0.94***			0.98***		
	Zn	0.85***	0.75**		0.86***	0.84***	
	N	0.87***	0.92***	0.67*	0.96***	0.96***	0.71**

* 0.05,** 0.01, and *** 0.001 significant correlations

availability for the plant (Zhou and others 2007). It has been shown that due to a larger molecular size, plant uptake of synthesized chelates (for example, Fe-EDTA and Fe-DTPA) is much lower than that of free metal cations (Marschner 1995). As mentioned before, the molecular structure of our synthesized Fe-amino acid chelates, designed using Hyperchem software, indicated that the molecular diameter of Fe-amino acid chelates is much smaller than the size of the cell wall pores (<5 nm) (Marschner 1995). Therefore, cell wall pores have no inhibitory effect on the movement of Fe-amino acid chelates into the free apoplasmic spaces, and thus Fe-amino acid chelates can pass easily through the cell wall and enter into the free spaces of the root apoplasm.

Improved Fe nutritional status of tomato plants supplied with Fe-amino acid chelates compared with Fe-EDTA-supplied plants could also be related to improved N nutritional status. Nitrogen nutritional management affects the number and activity of Fe-carrier proteins on the root cell membranes and thereby increases uptake and translocation of Fe in the plant tissues (Curie and others 2009; Murata and others 2008). Some field and pot experiments (Cakmak and others 2010; Shi and others 2010) indicated an improved concentration of Fe in wheat shoots and grain with the addition of N. An elevated Fe and Zn content in the shoots of wheat by N nutrition has also been reported by Kutman and others (2010). Although the plants absorb N in the form of $N–NO_3$ or $N–NH_4$, there is some evidence showing direct absorption of amino acids by plants (Jämtgård and others 2008; Näsholm and others 2009; Tida and others 2009). For example, Arg is an essential amino acid containing several N atoms (Abdul-Qados 2009). Glycine is easily converted to ammonium, amide, and aliphatic compounds and can be used as an N source (Schmidt and Stewart 1999).

Although the net influx and translocation of Fe and N in tomato plants were not measured in the present study, the close correlation between shoot N and Fe contents (Table 2) suggests a role of synthesized Fe-amino acid chelates in the translocation of Fe from roots to shoots. Translocation of amino acids in the plant is an important process (Ortiz-Lopez and others 2000). In contrast to assimilated carbon that is translocated and restricted to the phloem, amino acid translocation occurs in both the phloem and xylem. This translocation of amino acids in phloem and xylem helps nitrogen recycling between the roots and shoots and hastens retranslocation of nutrient elements, particularly immobile elements (for example, Fe and Zn) in the plant (Caputo and Barneix 1997; Owen and Jones 2001). In a trend similar to that of Fe, shoot Zn content was enhanced in the presence of amino acid chelates (Fig. 6).

Conclusion

Results obtained from the present study showed that in general, the Fe–amino acid complexes $Fe(Arg)_2$, $Fe(His)_2$, and $Fe(Gly)_2$ improved uptake and translocation of Fe, Zn, and N in comparison with Fe-EDTA and thus resulted in higher root and shoot growth of tomato. Elevated activity of CAT and APX confirmed the improvement of plant nutritional status of Fe. According to the results, Fe-amino acid chelates can be used as an alternative to Fe-EDTA to supply Fe in nutrient solutions. Although transport into the plant tissues is considered a potential loss of the chelator from the hydroponic solution, this problem can be resolved by frequent addition of amino acid chelates into the nutrient solution. Further research is needed to investigate the fate of these Fe-amino acid chelates in commercial hydroponic nutrient solutions where solutions are recycled for economic and environmental reasons.

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References

Abdul-Qados AMS (2009) Effect of arginine on growth, yield and chemical constituents of wheat grown under salinity condition. Acad J Plant Sci 2:267–278

Albano JP, Miller WB (2001) Photodegradation of FeDTPA in nutrient solutions. I. Effects of irradiance, wavelength and temperature. Hortscience 36:313–316

Amin AA, Gharib AEF, El-Awadia M, Rashad ESM (2011) Physiological response of onion plants to foliar application of putrescine and glutamine. Sci Hortic 129:353–360

Aravind P, Prasad MNV (2005) Cadmium-induced toxicity reversal by zinc in *Ceratophyllum demersum* L. (a free floating aquatic macrophyte) together with exogenous supplements of amino- and organic acids. Chemosphere 61:1720–1733

Arshad M, Frankenberger WTJ (1990) Response of *Zea mays* and *Lycopersicon esculentum* to the ethylene precursors, L-methionine and L-ethionine applied to soil. Plant Soil 122:219–227

Arshad M, Hussain A, Shakoor A (1995) Effect of soil applied L-tryptophan on growth and chemical composition of cotton. J Plant Nutr 18:317–329

Bell PF, Chaney RL, Angle JS (1991) Free metal activity and total metal concentrations as indices of micronutrient availability to barley (*Hordeum vulgare* L.) Klages. Plant Soil 130:51–62

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bremmer JM, Mulvancey CS (1982) Total Nitrogen. In: Page AL, Miller RH, Keeney DR (eds) Method of Soil Analysis part II. Madison, WI, ASA and SSSA, pp 599–622

Brynhildsen L, Rosswall T (1995) Effects of metals on the microbial mineralization of organic acids. Water Air Soil Poll 94:45–57

Cakmak I, Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol 98:1222–1227

Cakmak I, Kalayci M, Kaya Y, Torun AA, Aydin N, Wang Y, Arisoy Z, Erdem H, Gokmen O, Ozturk L, Horst WJ (2010) Biofortification and localization of zinc in wheat grain. J Agr Food Chem 58:9092–9102

Caputo C, Barneix AJ (1997) Export of amino acids to the phloem in relation to N supply in wheat. Physiol Plantarum 101:853–860

Chapman HD, Pratt PF (1961) Methods of analysis for soils, plants, and waters. Priced Publication 4034. Division of Agriculture Sciences, University of California, Berkeley

Curie C, Cassin G, Couch D, Divol F, Higuchi K, Jean ML, Misson J, Schikora A, Czernic P, Mari S (2009) Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters. Ann Bot 103:1–11

Dasgan HY, Römheld V, Cakmak I, Abak K (2002) Physiological root responses of iron deficiency susceptible and tolerant tomato genotypes and their reciprocal F1 hybrids. Plant Soil 241:97–104

Dasgan HY, Ozturk L, Abak K, Cakmak I (2003) Activities of iron-containing enzymes in leaves of two tomato genotypes differing in their resistance to Fe chlorosis. J Plant Nutr 26:1997–2007

Drolet G, Dumbroff EB, Legg RL, Thompson JE (1986) Radical scavenging properties of polyamines. Phytochemistry 25:367–371

Falkengren-Grerup U, Månsson KF, Olsson MO (2000) Uptake capacity of amino acids by ten grasses and forbs in relation to soil acidity and nitrogen availability. Environ Exp Bot 44:207–219

Hangarter RP, Stasinopoulos TC (1991) Effect of Fe-catalyzed photooxidation of EDTA on root-growth in plant culture media. Plant Physiol 96:843–847

Jämtgård S, Näsholm T, Huss-Danell K (2008) Characteristics of amino acid uptake in barley. Plant Soil 302:221–231

Jones DL, Hodge A (1999) Biodegradation kinetics and sorption reactions of three differently charged amino acids in soil and their effects on plant organic nitrogen availability. Soil Biol Biochem 31:1331–1342

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Keutgen A, Pawelzik E (2008) Contribution of amino acids to strawberry fruit quality and their relevance as stress indicators under NaCl salinity. Food Chem 111:642–647

Kielland K (1994) Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. Ecology 75: 2373–2383

Kutman UB, Yildiz B, Ozturk L, Cakmak I (2010) Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. Cereal Chem 87:1–9

Kuznetsov VV, Shevyakova NI (2007) Polyamines and stress tolerances of plants. Plant Stress 1:50–71

Li P, Yin YL, Li D, Kim SW, Wu G (2007) Amino acids and immune function. Br J Nutr 98:237–252

Lovass E (1991) Antioxidative effects of polyamines. J Am Oil Chem Soc 68:353–358

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, San Diego, CA

Metsarinne S, Tuhkanen T, Aksela R (2001) Photodegradation of ethylenediaminetetraacetic acid (EDTA) and ethylenediamine disuccinic acid (EDDS) within natural UV radiation range. Chemosphere 45:949–955

Metsarinne S, Rantanen P, Aksela R, Tuhkanen T (2004) Biological and photochemical degradation rates of diethylenetriaminepentaacetic acid (DTPA) in the presence and absence of Fe(III). Chemosphere 55:379–388

Murata Y, Harada E, Sugase K, Namba K, Horikawa M, Ma JF, Yamaji N, Ueno D, Nomoto K, Iwashita T, Kusumoto S (2008) Specific transporter for iron(III)-phytosiderophore complex involved in iron uptake by barley roots. Pure Appl Chem 80:2689–2697

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Näsholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. New Phytol 182:31–48

Nassar AH, El-Tarabily KA, Sivasithamparam K (2003) Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine-producing isolate of *Streptomyces griseoluteus*. Plant Growth Regul 40:97–106

Nowack B, Baumann U (1998) Biodegradation of the photolysis products of Fe(III)EDTA. Acta Hydroch Hydrob 26:104–108

Okamoto M, Okada K (2004) Differential responses of growth and nitrogen uptake to organic nitrogen in four gramineous crops. J Exp Bot 55:1577–1585

Okamoto M, Okada K, Watanabe T, Ae N (2003) Growth responses of cereal crops to organic nitrogen in the field. Soil Sci Plant Nutr 49:445–452

Ortiz-Lopez A, Chang HC, Bush DR (2000) Amino acid transporters in plants. Biochim Biophys Acta 1465:275–280

Owen AG, Jones DL (2001) Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biol Biochem 33: 651–657

Parker DR, Norvell WA (1999) Advances in solution culture methods for plant mineral nutrition research. In: Advances in Agronomy. San Diego, CA: Academic Press, pp 561–566

Rashad ESM, El-Abagg HM, Amin AA (2003) Physiological effects of some bioregulators on growth and productivity of two broad bean cultivars. Egypt J Appl Sci 18:132–149

Reeve JR, Smith JL, Carpenter-Boggs L, Reganold JP (2008) Soil-based cycling and differential uptake of amino acids by three species of strawberry (*Fragaria* spp.) plants. Soil Biol Biochem 40:2547–2552

Reeve JR, Smith JL, Carpenter-Boggs L, Reganold JP (2009) Glycine, nitrate, and ammonium uptake by classic and modern wheat varieties in a short-term microcosm study. Biol Fert Soils 45:723–732

Renella G, Landi L, Nannipieri P (2004) Degradation of low molecular weight organic acids complexed with heavy metals in soil. Geoderma 122:311–315

Rivero RM, Sánchez E, Ruiz JM, Romero L (2003) Iron metabolism in tomato and watermelon plants: influence of nitrogen source. J Plant Nutr 26:2413–2424

Rothstein DE (2009) Soil amino acid availability across a temperate-forest fertility gradient. Biochemistry 92:201–215

Ruiz JM, Baghour M, Romero L (2000) Efficiency of the different genotypes of tomato in relation to foliar content of Fe and the response of some bioindicators. J Plant Nutr 23:1777–1786

Sánchez AS, Juárez M, Sánchez-Andreu J, Jordá J, Bermúdez D (2005) Use of humic substances and amino acids to enhance iron availability for tomato plants from applications of the chelate FeEDDHA. J Plant Nutr 28:1877–1886

Schmidt S, Stewart GR (1999) Glycine metabolism by plant roots and its occurrence in Australian plant communities. Aust J Plant Physiol 26:253–264

Shi R, Zhang Y, Chen X, Sun Q, Zhang F, Römheld V, Zou C (2010) Influence of long term nitrogen fertilization on micronutrient density in grain of winter wheat (*Triticum aestivum* L.). J Cereal Sci 51:165–170

Svennerstam H, Ganeteg U, Bellini C, Näsholm T (2007) Comprehensive screening of *Arabidopsis* mutants suggests the Lysine Histidine Transporter 1 to be involved in plant uptake of amino acids. Plant Physiol 143:1853–1860

Svennerstam H, Ganeteg U, Bellini C, Näsholm T (2008) Root uptake of cationic amino acids by *Arabidopsis* depends on functional expression of amino acid permease 5. New Phytol 180:620–630

Svenson A, Kaj L, Bjorndal H (1989) Aqueous photolysis of the iron(III) complexes of NTA, EDTA and DTPA. Chemosphere 18:1805–1808

Tida G, Song S, Roberts P, Jones DL, Huang D, Iwasaki K (2009) Amino acids as a nitrogen source for tomato seedlings: The use of dual-labeled (^{13}C, ^{15}N) glycine to test for direct uptake by tomato seedlings. Environ Exp Bot 66:357–361

Vadas TM, Zhang X, Curran AM, Ahner BA (2007) Fate of DTPA, EDTA and EDDS in hydroponic media and effects on plant mineral nutrition. J Plant Nutr 30:1229–1246

Vassil AD, Kapulnik YI, Salt DE (1998) The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol 117:447–453

Wang HJ, Wu LH, Wang MY, Zhu YH, Tao QN, Zhang FS (2007) Effects of amino acids replacing nitrate on growth, nitrate accumulation, and macroelement concentrations in pak-choi (*Brassica chinensis* L.). Pedosphere 17:595–600

Werdin-Pfisterer NR, Kielland K, Boone RD (2009) Soil amino acid composition across a boreal forest successional sequence. Soil Biol Biochem 41:1210–1220

Wu L, Mo L, Fan Z, Tao Q, Zhang F (2005) Absorption of glycine by three agricultural species under sterile sand culture conditions. Pedosphere 15:286–292

Zeid IM (2009) Effect of arginine and urea on polyamines content and growth of bean under salinity stress. Acta Physiol Plant 31:65–70

Zhou Z, Zhou J, Li R, Wang H, Wang J (2007) Effect of exogenous amino acids on Cu uptake and translocation in maize seedlings. Plant Soil 292:105–117

Zuchi S, Cesco S, Varanini Z, Pinton R, Astolfi S (2009) Sulphur deprivation limits Fe-deficiency responses in tomato plants. Planta 230:85–94

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The effectiveness of foliar applications of synthesized zinc-amino acid chelates in comparison with zinc sulfate to increase yield and grain nutritional quality of wheat

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ABSTRACT

The effectiveness of foliar spray of certain Zn-amino acid chelates (ZnAAC) including Zn-arginine [$Zn(Arg)_2$], Zn-glycine [$Zn(Gly)_2$] and Zn-histidine [$Zn(His)_2$] on the yield and grain quality of two different Zn-deficiency tolerant wheat cultivars (*Triticum aestivum* cvs. 'Back Cross' and 'Kavir') was investigated under field conditions. Foliar application of Zn, regardless of the used source, significantly improved grain yield of both wheat cultivars with a mean increase of 15.2% for the first year and 19.2% for the second year. Grain Zn, iron (Fe) and protein concentrations were on average 14.3% higher in wheat plants sprayed with ZnAAC than those sprayed with $ZnSO_4$. Very significant positive correlation between grain Zn, Fe, and protein concentrations indicates that the genes affecting the grain accumulations of Zn, Fe and protein are probably closely linked. Foliar application of Zn fertilizers resulted in significant decrease (on average 17.9%) of grain phytic acid (PA) and PA:Zn molar ratio (on average 16.3%) in comparison with the control treatment although the magnitude of this reduction was greater for ZnAAC than $ZnSO_4$. According to the results obtained in this study, the ZnAAC should be considered as new Zn fertilizer sources for improving yield and total and bioavailable Zn concentrations of wheat grain.

1. Introduction

Zinc (Zn) deficiency caused by inadequate dietary intake is a global nutritional problem in human populations (Cakmak, 2008; Kutman et al., 2011). Recent estimates indicate that over two billion of the world population is affected by Zn deficiency (Cakmak et al., 2010). Wheat is one of the most widely grown cereals worldwide and plays a critical role in food security. In several developing countries, such as Iran, wheat is responsible for about half of the protein and daily calorie intake (Alloway, 2008; Cakmak, 2008). It is estimated that more than 40% of the wheat crop is cultivated on severely low Zn soils (Alloway, 2008), which produces poor yields of grain with low Zn content. An excessive and monotonous consumption of wheat-based products rapidly results in Zn malnutrition because wheat is inherently low in Zn (Cakmak et al., 2010). Increasing concentration of Zn in wheat grains and other staple foods is, therefore, an important challenge and a high-priority research task.

Although the cause of suboptimal Zn status in some cases may be inadequate dietary intake of Zn, low bioavailability of Zn is also another common causative factor. In fact, the importance of a foodstuff as a source of dietary Zn depends on both the total Zn content and the level of other constituents in the diet that affect Zn bioavailability. For example, high phytic acid (PA) intake with staple foods is one possible cause for Zn deficiency (Gargari et al., 2007). In contrast, high concentrations of protein and amino acids (AA) in cereals can contribute to higher bioavailability of micronutrients in the diet (Lonnerdal, 2000). Thus, success in alleviation of global Zn malnutrition in human diets depend upon increasing concentration of Zn and enhancers of Zn absorption such as protein combined with reduction of PA in the most commonly eaten foods.

Several approaches such as soil and foliar fertilization have been proposed and applied in developed countries to alleviate Zn deficiency problem (Khoshgoftarmanesh et al., 2010). In case of greater bioavailability of the grain Zn derived from foliar applications than from soil, agronomic biofortification would be a very attractive and useful strategy in solving Zn deficiency-related health problems globally and effectively (Cakmak, 2008; Khoshgoftarmanesh et al., 2010). Commercial $ZnSO_4$ and synthetic Zn-chelates (i.e., Zn-EDTA and Zn-DTPA) are common sources of Zn used in agricultural lands (Alloway, 2008). Most commercial inorganic Zn fertilizers contain Cd and other toxic heavy metals as impurity (Khoshgoftarmanesh et al., 2010). On the other hand, the penetration rates of synthesized chelated in leaves, due to larger molecular size, is much lower than the free metal cations (Marschner, 1995).

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In this study, the effectiveness of foliar spray of Zn-AA chelates (ZnAAC) as new Zn fertilizer sources on the grain nutritional quality of wheat was investigated. Amino acids as natural chelating agents have the ability to coordinate metal ions (such as Zn) via their carboxyl groups and thereby increase its availability for plants (Ghasemi et al., in press). In addition, several studies showed that foliar application of AA increased plant protein content. For example, Das et al. (2002) reported that foliar spray of *mulberry* plants with AA increased the protein contents as well as leaf yield as compared with the control plant. The positive increment in plant protein content due to AA application has also been recorded by Chang et al. (2005). Amino acids are also key precursors for syntheses of hormones and low-molecular weight nitrogenous substances which have the ability of forming soluble complexes with Zn and thereby increase its bioavailability for human (Lonnerdal, 2000). There is evidence for a promoter function of AA and certain chelating low-molecular-weight organic. Ekholm et al. (2003) concluded that chelating agents such as citric acid form soluble complexes with Zn and increase the availability of Zn when high dietary fiber cereal products are eaten. Graham et al. (2001) reported that AA released from proteins digestion, could bind PA and enhance Zn absorption by binding Zn and keeping it in solution.

We recently reported the growth-stimulating effects of Zn-AA chelates and their effectiveness as Zn sources for lettuce in hydroponic experiments (Ghasemi et al., in press) while a lack of information is available on how combination of Zn and AA affect the concentration and bioavailability of Zn in wheat grain. The aim of these field experiments was therefore, to examine the effects of foliar application of ZnAAC in comparison with commercial $ZnSO_4$ on the yield and nutritional quality of wheat grain.

2. Materials and methods

2.1. Synthesis and characterization of ZnAAC

The complexes of Zn with three AA, arginine (Arg), glycine (Gly) and histidine (His) as complexing agents were prepared following the procedure described by Ghasemi et al. (in press): 2 mmol of Arg, Gly or His were dissolved in distilled water and added to a solution of $Zn(OAc)_2$ (1 mmol) under stirring for several hours. Evaporation of solvent at room temperature yielded white microcrystals of ZnAAC. The microcrystals were washed with cold ethanol followed by diethyl ether and air dried.

The complexes were analyzed for their carbon (C), hydrogen (H) and nitrogen (N) using a Perkin-Elmer 2400 CHNS elemental analyzer. Atomic absorption measurements of Zn were recorded with an atomic absorption spectrometry (Model 3400, Perkin Elmer, Wellesley, MA). The FT-IR spectra were recorded with a FT-IR JASCO 460 spectrophotometer over KBr pellet in 4000-400 cm^{-1} range.

2.2. Field experiments

Field experiments were conducted in two successive years (2009–2010 and 2010–2011 growing seasons) in Rudasht Research Station (Isfahan, Iran; 32°29′N; 52°10′E). The mean temperature was 16.3 and 16.0 °C for the first and second years, respectively. The average annual rainfall was 125 mm in the first year and 1136 mm in the second year. The rainy season ranged from November to May, and the summer precipitation was negligible (Fig. 1).

For analysis of basic soil properties, soil samples were collected at 0–30 cm depth from each location before planting. The properties of the soils are shown in Table 1. Considering the critical deficiency level for the DTPA-extractable soil Zn (0.5 mg kg^{-1}) (Alloway, 2008), soils in both locations were severely deficient in available Zn.

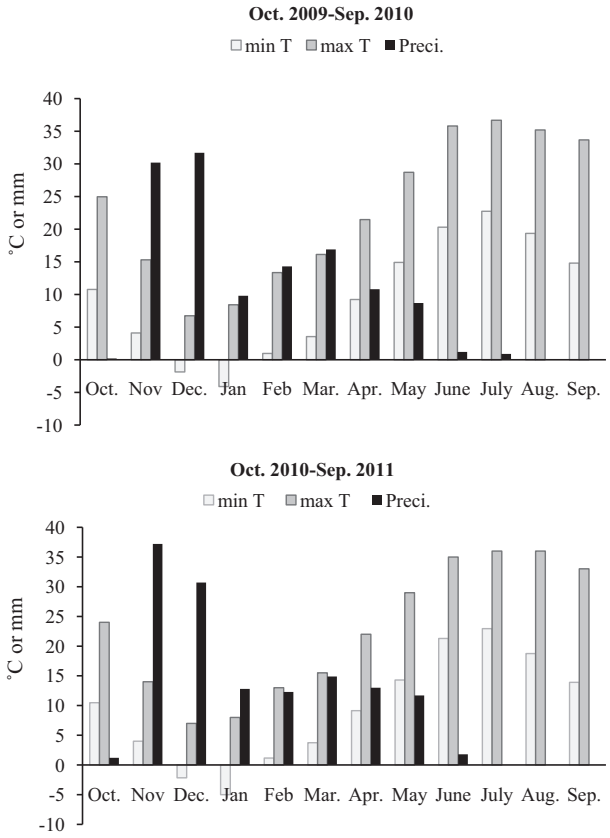

Fig. 1. The minimum (min *T*) and maximum (max *T*) temperature and mean precipitation (Preci.) of Rudasht Research Station for the growing wheat seasons in 2009–2010 and 2010–2011.

Table 1
Selected soil characteristics of the experimental locations.

Property	Year 1	Year 2
EC_e (dS m^{-1})[a]	11.5	10.7
pH (H_2O)	7.4	7.7
SAR[b]	15.7	15.4
Sand (%)	12.5	13.1
Clay (%)	41.7	40.3
$CaCO_3$ (%)	37.5	36.0
Total N (g kg^{-1} soil)	1.2	1.1
Organic C (g kg^{-1} soil)	4.8	4.6
DTPA-extractable Zn (mg kg^{-1} soil)	0.21	0.22

[a] Electrical conductivity of soil saturation extract.
[b] Sodium adsorption ratio.

Two different Zn-deficiency tolerant wheat cultivars (*Triticum aestivum* cvs. 'Back Cross' and 'Kavir'), most commonly cultivated in Iran, were used in this study. 'Back Cross' and 'Kavir' have previously been classified as Zn-deficiency tolerant and sensitive cultivars, respectively (Khoshgoftarmanesh et al., 2010). The experimental design was a split plot in randomized complete block design with three replicates. Main plot treatments consisted of control, no Zn fertilizer was applied during the whole growth stage, and foliar applications of four different Zn sources including $ZnSO_4$, Zn-arginine [$Zn(Arg)_2$], Zn-glycine [$Zn(Gly)_2$] and Zn-histidine [$Zn(His)_2$]. According to the previous findings, among various AA tested, Arg, Gly and His form stable chelates with Zn. These chelates are soluble in water and had stimulating effect on growth of lettuce (Ghasemi et al., in press). All Zn sources were sprayed at the rate of 0.2% of Zn (w/v). Subplot treatments were two wheat cultivars. Thus, there were 30 plots with each 3 m^2 (1.5 m × 1.5 m).

The agricultural practices were performed according to the local recommendations. For all treatments, about 100 kg N ha^{-1} as urea, 75 kg P ha^{-1} as superphosphate, and 100 kg K ha^{-1} as potassium sulfate were incorporated into the 0-25 cm soil layer before transplanting. At the tillering stage, on 18 March 2010 and 2011, 100 kg N ha^{-1} in the form of ammonium nitrate was top-dressed.

Foliar applied solutions contained 0.2% (w/v) Zn from each fertilizer and were applied at different growth stages as follows: (i) tillering, (ii) before emergence of main spike, and (iii) during grain filling. These stages have been recommended by Iranian Soil and Water Research Institute (Milani et al., 1998) as the best time of Zn foliar spray for improving grain quality of wheat. The solution volume of 1000 l ha^{-1} was used in all treatments. Foliar application of Zn was performed in the very late afternoon to avoid possible leaf damage caused by salts on sunny day and at high day temperature.

2.3. Wheat grain sampling

Wheat plants were sampled at maturity from the central 1 m^2 areas of each plot. The grains of wheat plants were separated from husk manually in order to avoid the risk of contamination, dried at 60 °C for 48 h, milled to pass through a 0.5 mm screen, and stored in a desiccator for nutrient analysis.

2.4. Phytic acid measurement

Phytic acid was measured according to Makower (1970) with some modification. The grain wheat sample (0.3 g) was placed into a 10 ml centrifuge tube and 3 ml trichloroacetic acid (TCA) 12% was added, stand at room temperature for 30 min, and then centrifuged at 3600 rpm for 15 min. The extraction step was repeated twice. To the combined supernatant, 2 ml cerium solution 5% and 0.8 ml concentrated H_2SO_4 was added, stand at room temperature for 30 min, and then centrifuged at 3600 rpm for 15 min. Supernatant was discarded and 3 ml concentrated H_2SO_4 was added to dissolve the residue. The resulting solution was transferred into mineralization tubes and digested at 338 °C for 3.5 h. After cooling, de-ionized water was added to the solution up to the volume of 50 ml. To 0.5 ml of the supernatant, 5 ml H_2SO_4 0.5 M was added. Finally, 1 ml of resulting solution was mixed with 1 ml 0.5 M H_2SO_4, 200 μl ammonium molybdate 1.75% and 200 μl malachite green and after 45 min absorbance was read at 610 nm. Phosphorus concentration was calculated by using phosphate standards (0.16, 0.32, 0.64, 0.96 μg ml^{-1}) curve. Phytic acid content was calculated using the following equation.

$$\text{Phytic acid}\,(\text{g}\,100\,\text{g}^{-1}) = \frac{C \times f \times 660}{m \times 1860},$$

where C = P concentration in diluted sample (μg ml^{-1}), f = dilution factor, m = sample weight (mg) (Makower, 1970).

The PA to Zn molar ratio in samples was calculated by dividing millimoles of PA with millimoles of Zn.

2.5. Grain protein

The grain protein concentration was measured using Autotech (Model 300) according to Kjeldahl method (Bremmer and Mulvancey, 1982). A factor of 5.7 was used to convert nitrogen concentration to protein concentration. Grain protein concentration was expressed on dry weight basis.

2.6. Grain Zn and Fe concentration

The samples were digested with HNO_3–H_2O_2 by a microwave accelerated reaction system (CEM, Matthews, USA), and the Zn and Fe concentrations in the digested solution were measured by atomic absorption spectrophotometer (Model 3400, Perkin Elmer, Wellesley, MA). For quality control, reagent blanks and a NIST Standard Reference Materials (NIST No. 1515 apple leaves) were included. Recovery of Zn and Fe was 87 and 92%, respectively for apple leaf standard (#1573A).

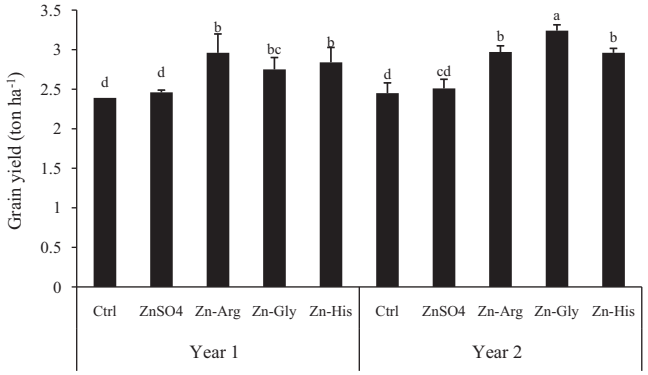

Fig. 2. The effects of foliar application of Zn-amino acid complexes on the grain yield of two wheat cultivars grown under field conditions during 2009–2010 (Year 1) and 2010-2011 (Year 2). Ctrl, $ZnSO_4$, Zn-Arg, Zn-Gly, and Zn-His are respectively no added Zn fertilizer (control) and foliar application of Zn-sulphate, $Zn(Arg)_2$, $Zn(Gly)_2$, and $Zn(His)_2$. Error bar represent standard error (n = 3). Bars having different letters are significantly different at the 5% level by LSD. Because the effect of the cultivar was not significant for grain yield, the mean grain yield of two cultivars for each year and all the Zn fertilizers are shown.

2.7. Statistical analysis

All statistical analyses were conducted using the SAS (SAS Institute, 2000). The analysis of variance (ANOVA) for the main effects (cultivar, fertilizer and year) and interactions were determined using the general linear models (GLM) procedure. Means were compared using least significant differences (LSD) at $P < 0.05$.

3. Results

3.1. Characteristics of ZnAAC

All complexes of this study were synthesized in good yield by reaction of Zn acetate with Arg, Gly, or His in refluxing water. The results of elemental analysis support the formation of ZnAAC with 2:1 ligand to metal molar ratio (Table 2). The computational results indicated that the AA ligands coordinated to Zn(II) ion via their nitrogen and oxygen atoms and support the coordination mode obtained from IR spectroscopy (data not shown).

3.2. Grain yield

Foliar application of ZnAAC significantly increased grain yield at both years, when compared to the control plots where plants were not treated with foliar Zn (Fig. 2). The positive effect of ZnAAC on the grain yield varied dependent on the AA type. The interaction of cultivation year with cultivar and fertilizer on the grain yield was also significant (Table 3). In year 1, foliar application of ZnAAC resulted in 11.8–20.3% increase in the grain yield of wheat compared to $ZnSO_4$ treatment. There was no significant difference in grain yield among ZnAAC treatments. In year 2, the grain yield of plants sprayed with ZnAAC was increased by 17.9–29.1% compared with plants sprayed with $ZnSO_4$. The $Zn(Gly)_2$ was the most effective Zn fertilizer for increase of grain yield.

Table 2
Analytical data for zinc-amino acid complexes (ZnAAC).

ZnAAC	Yield %	% Found (Calc.)[a]			
		C	H	N	Zn
$[Zn(Arg)_2]\cdot 0.5H_2O$	83.88	34.31 (34.25)	6.36 (6.47)	26.49 (26.53)	15.32 (15.54)
$[Zn(Gly)_2]$	87.11	22.54 (22.50)	3.82 (3.78)	13.06 (13.12)	30.57 (30.63)
$[Zn(His)_2]\cdot H_2O$	83.22	36.66 (36.79)	4.89 (4.63)	21.53 (21.60)	16.36 (16.70)

[a] Theoretical percentage of the elements.

Table 3
Analysis of variance of yield and grain nutritional quality of two wheat cultivars treated with five Zn fertilizers from the analysis of two growing years.

Source	df	Mean square					
		Yield	Zn	Fe	Protein	PA	PA/Zn
Year (Y)	1	0.314**	2.642***	5.32***	5.021***	0.021***	22.982*
Fertilizer (F)	4	0.921***	0.386***	2.16***	8.575***	0.007***	113.913***
Y × F	4	0.114**	0.011	0.360**	3.473***	0.005***	14.512*
Cultivar (C)	1	1.005***	0.045*	0.785**	2.00***	0.040***	200.181***
F × C	4	0.028	0.053**	0.324**	0.264**	0.000	2.473
Y × C	1	0.203**	0.005	0.066	2.784***	0.000	8.228
Y × F × C	4	0.031	0.051**	0.598***	0.178*	0.004**	10.086

* Significant at $P<0.05$.
** Significant at $P<0.01$.
*** Significant at $P<0.001$.

3.3. Grain Zn concentration

The main effect of Zn fertilizer application and its interaction with plant cultivar on the grain Zn concentration was significant (Table 3). In both years, foliar application of Zn fertilizers resulted in higher grain Zn concentration compared with the no added Zn control treatment (Fig. 3). The foliar application of ZnAAC caused a greater increase in grain Zn concentration than $ZnSO_4$. The positive effect of ZnAAC on grain Zn concentration was dependent on the wheat cultivar and type of AA. In year 1, the highest increase in grain Zn concentration of 'Back Cross' was achieved by foliar spray of $Zn(Arg)_2$ and $Zn(Gly)_2$ whereas no significant difference was found between $Zn(His)_2$ and $ZnSO_4$ treatments. In 'Kavir', foliar application of ZnAAC resulted in 8.5-25.6% increase in grain Zn concentration. Among different Zn fertilizers used as foliar spray, $Zn(Gly)_2$ and $Zn(His)_2$ resulted in the highest increase in grain Zn concentration of 'Kavir'. There was no significant difference in grain Zn concentration of 'Kavir' between $Zn(Arg)_2$ and $ZnSO_4$ treatments.

In year 2, foliar application of ZnAAC increased the grain Zn concentration by 2.9–29.2% in 'Back Cross' and by 8.2–19.9% in 'Kavir' in comparison with the $ZnSO_4$ treatment. The $Zn(Arg)_2$ and $Zn(Gly)_2$ were the most effective Zn treatments for increasing grain Zn concentration of 'Back Cross' while in 'Kavir' the highest grain Zn concentration was achieved by foliar spray of $Zn(His)_2$.

3.4. Grain Fe concentration

The effect of Zn fertilizer application and its interaction with cultivar and cultivation year significantly affected grain Fe concentration of wheat (Table 3). In both years, foliar spray of $ZnSO_4$ had no significant effect on grain Fe concentration of wheat (Fig. 4). In contrast, foliar application of ZnAAC effectively increased the grain Fe concentration of wheat plants over those of the untreated plants. In year 1, foliar application of ZnAAC increased the grain Fe concentration in 'Back Cross' by 8.8–25.8% and in 'Kavir' by 19.4–44.2%, compared with plants sprayed with $ZnSO_4$. In 'Back Cross' $Zn(Arg)_2$ and $Zn(Gly)_2$ resulted in higher grain Fe concentration in comparison with $Zn(His)_2$. The highest increase in grain Fe concentration of 'Kavir' was also related to $Zn(Gly)_2$.

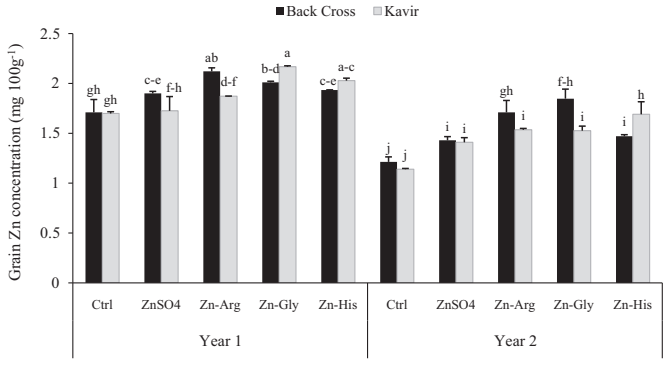

Fig. 3. The effects of foliar application of Zn-amino acid complexes on the grain Zn concentration of two wheat cultivars grown under field conditions. Ctrl, $ZnSO_4$, Zn-Arg, Zn-Gly, and Zn-His are respectively no added Zn fertilizer (control) and foliar application of Zn-sulphate, $Zn(Arg)_2$, $Zn(Gly)_2$, and $Zn(His)_2$. Error bars represent standard error ($n=3$). Bars having different letters are significantly different at the 5% level by LSD.

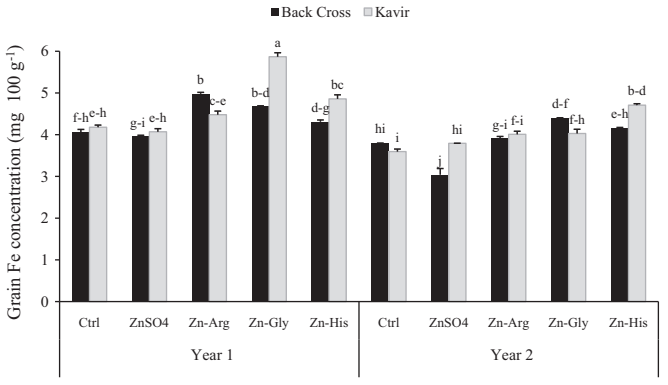

Fig. 4. The effects of foliar application of Zn-amino acid complexes on the grain Fe concentration of two wheat cultivars grown under field conditions. Ctrl, $ZnSO_4$, Zn-Arg, Zn-Gly, and Zn-His are respectively no added Zn fertilizer (control) and foliar application of Zn-sulphate, $Zn(Arg)_2$, $Zn(Gly)_2$, and $Zn(His)_2$. Error bars represent standard error ($n=3$). Bars having different letters are significantly different at the 5% level by LSD.

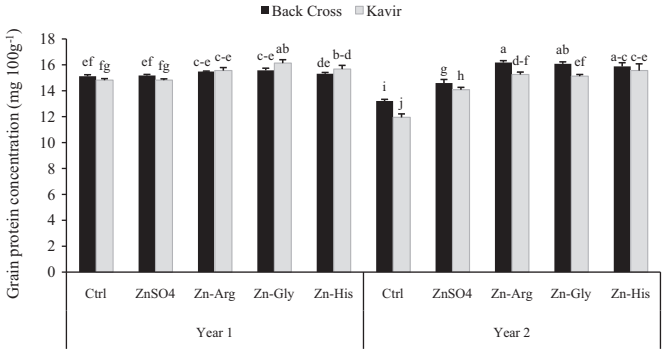

Fig. 5. The effects of foliar application of Zn-amino acid complexes on the grain protein concentration of two wheat cultivars grown under field conditions. Ctrl, $ZnSO_4$, Zn-Arg, Zn-Gly, and Zn-His are respectively no added Zn fertilizer (control) and foliar application of Zn-sulphate, $Zn(Arg)_2$, $Zn(Gly)_2$, and $Zn(His)_2$. Error bars represent standard error ($n = 3$). Bars having different letters are significantly different at the 5% level by LSD.

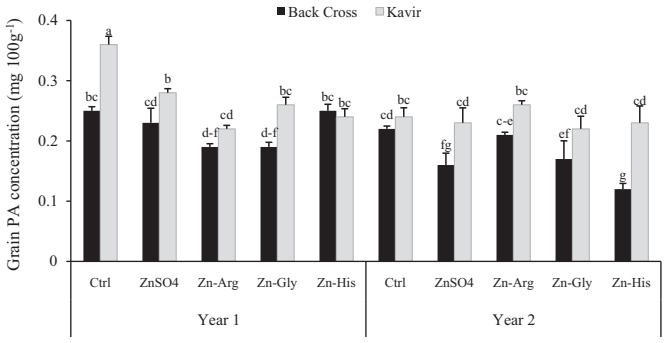

Fig. 6. The effects of foliar application of Zn-amino acid complexes on the grain phytic acid (PA) concentration of two wheat cultivars grown under field conditions. Ctrl, $ZnSO_4$, Zn-Arg, Zn-Gly, and Zn-His are respectively no added Zn fertilizer (control) and foliar application of Zn-sulphate, $Zn(Arg)_2$, $Zn(Gly)_2$, and $Zn(His)_2$. Error bars represent standard error ($n = 3$). Bars having different letters are significantly different at the 5% level by LSD.

In year 2, the highest increase in grain Fe concentration of 'Back Cross' was observed by $Zn(Gly)_2$ and $Zn(His)_2$. In 'Kavir', $Zn(His)_2$ resulted in the highest increase of grain Fe concentration.

3.5. Grain protein content

The main effect of cultivar, fertilizer, and cultivation year and their interactions on the grain protein concentration of wheat were significant (Table 3). In 'Kavir', foliar application of all ZnAAC, regardless of the year, resulted in higher grain protein concentration in comparison with the control and $ZnSO_4$ treatments (Fig. 5). In 'Back Cross', the effect of ZnAAC on the grain protein content was different between two years. In year 1, foliar application of ZnAAC increased the grain protein content in the 'Back Cross' and 'Kavir' by 0.9–2.6% and 5.0–8.9%, compared to $ZnSO_4$ treatments. The positive effect of $Zn(Gly)_2$ and $Zn(His)_2$ on the grain protein concentration of 'Kavir' was higher than $Zn(Arg)_2$ whereas no significant difference was found in grain protein concentration of 'Back Cross' among ZnAAC treatments. In year 2, the grain protein concentration of plants sprayed with ZnAAC was increased by 8.8-10.8% in 'Back Cross', and by 7.4-10.5% in 'Kavir' compared with plants sprayed with $ZnSO_4$. In spite of wheat cultivar, no significant difference was found in grain protein concentration among ZnAAC treatments.

3.6. Phytic acid

Analysis of variance showed significant effect of wheat cultivar, Zn fertilizer application, and cultivation year on grain phytic acid (PA) concentration (Table 3). In year 1, the highest grain PA concentration was found in 'Kavir' where plants were not treated with foliar Zn (Fig. 6). Foliar application of Zn fertilizers decreased grain PA concentration in both wheat cultivars. Zinc-amino acid complexes caused a greater reduction in grain PA than $ZnSO_4$ with a mean decrease of 16.0% for 'Back Cross' and 33.3% for 'Kavir', in year 1. The $Zn(Arg)_2$ and $Zn(Gly)_2$ were the most effective fertilizers for reduction of PA content in 'Back Cross'. In 'Kavir', no significant difference was found in grain PA concentration among ZnAAC treatments. In year 2, foliar application of Zn fertilizers had no effect on the grain PA concentration of 'Kavir' whereas all Zn fertilizers, except $Zn(Arg)_2$, significantly decreased the grain PA concentration of 'Back Cross' in comparison with the control treatment.

3.7. Phytic acid to Zn molar ratio

The main effect of Zn fertilizer application and its interaction with year on grain PA to Zn molar ratio was significant (Table 3). Foliar application of Zn fertilizer resulted in significant decrease of grain PA to Zn molar ratio in comparison with the no Zn added control treatments (Fig. 7). In year 1, all ZnAAC treatments except $Zn(His)_2$ significantly reduced grain PA to Zn molar ratio in comparison with $ZnSO_4$ treatment. In year 2, foliar application of ZnAAC, resulted in 25.6-45.1% reductions in PA to Zn molar ratio in comparison with the control treatment while no significant difference was found in the grain PA to Zn molar ratio between ZnAAC and $ZnSO_4$ treatments.

4. Discussion

Foliar application of Zn fertilizers is an effective agronomical practice in crop production, with substantial influence on both yield and particularly grain quality (Khoshgoftarmanesh et al., 2010). In this study, we investigated the efficacy of foliar spray of certain synthesized ZnAAC in improving yield and nutritional quality (total and bioavailable Zn content) of wheat grain. Results obtained in this experiment showed that with foliar application of both ZnAAC and $ZnSO_4$, grain concentrations of Zn and total protein increased whereas anti-nutrient PA concentration decreased. The effectiveness of ZnAAC in increasing grain concentrations of Zn and total protein and reducing grain PA content was higher than $ZnSO_4$.

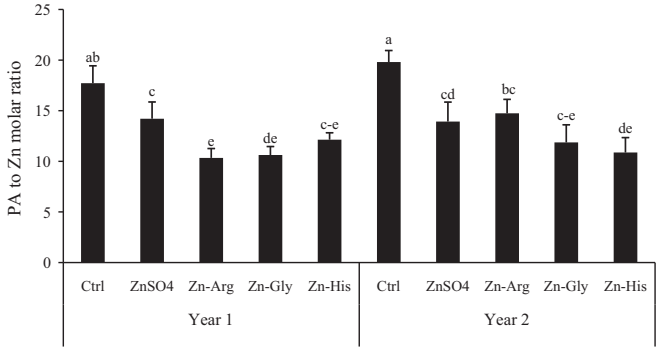

Fig. 7. The effects of foliar application of Zn-amino acid complexes on the grain phytic acid (PA) to Zn molar ratio of two wheat cultivars grown under field conditions. Ctrl, $ZnSO_4$, Zn-Arg, Zn-Gly, and Zn-His are respectively no added Zn fertilizer (control) and foliar application of Zn-sulphate, $Zn(Arg)_2$, $Zn(Gly)_2$, and $Zn(His)_2$. Error bars represent standard error ($n = 3$). Bars having different letters are significantly different at the 5% level by LSD. Because the effect of the cultivar was not significant for PA to Zn molar ratio, the mean PA to Zn molar ratio of two cultivars for each year and all the Zn fertilizers are shown.

These results suggest that ZnAAC are proper Zn sources to improve nutritional quality of wheat grain.

As presented in Fig. 2, in addition of measured quality attributes, grain yield was increased by foliar application of ZnAAC. The more positive grain yield response to ZnAAC of wheat cultivars may be due to the role of AA in different biological processes, including cell division, growth, somatic embryogenesis, and seed development (El-Bassiouny et al., 2008). The results obtained in this experiment is in accordance with findings of Gupta et al. (2003) who found that, putrescine increased grain yield, biological yield and seed weight index of wheat. Similarly, promoting effects of Arg on wheat growth were observed by Iqbal and Ashraf (2005). In $Zn(Gly)_2$ treatment, the grain yield of plants grown in year 2 was higher than that in year 1. The climatic characteristics for the growing wheat season in two years were relatively similar. Therefore, the variation in grain yield of plants treated with $Zn(Gly)_2$ among years was probably associated with different soil characteristics, in particular, soil salinity level. The soil EC in year 2 was less than that in year 1 (Table 1). Soil salinity not only promotes nutritional imbalances and deficiencies, but also increases osmotic pressure of the soil solution (Maas and Hoffman, 1977).

Elevated Zn and Fe concentration in grains of wheat plants sprayed with ZnAAC compared with those sprayed with $ZnSO_4$ might partly be associated with N nutritional status improvement. Nitrogen nutritional management affects the number and activity of Zn- and Fe-carrier proteins on the root cell membranes and thereby increases uptake and translocation of these elements in the plant tissues (Kutman et al., 2011). Irshad et al. (2002) found that higher N application improved plant growth and thereby improved uptake of nutrient elements. Amino acids are nitrogen sources for plant nutrition. Most plants can directly absorb AA and use them in their physiological structures and processes (Näsholm et al., 2009). Ohlund and Näsholm (2001) illustrated that, 100% of Arg in pine seedlings was derived from the uptake of intact AA through seedling roots and concluded that, Arg act as N sources for growth. Results from several field and greenhouse experiments (Cakmak et al., 2010; Shi et al., 2010) indicated improved concentration of Zn and Fe in wheat shoots and grain by addition of N. An elevated Fe and Zn content in the shoots of wheat by N nutrition has also been reported by Kutman et al. (2011).

In this study, significant cultivar variation in grain yield was evident in both growing seasons. Under Zn-deficient conditions (control), 'Back Cross' produced higher grain yield than 'Kavir'. In contrast, grain Zn concentrations did not show large variation between two wheat genotypes studied. No correlation was found between Zn-efficiency and grain Zn density and Zn-efficient 'Back Cross' and Zn-inefficient 'Kavir' had identical grain Zn concentration indicating that grain Zn concentration cannot be a reliable parameter for evaluating differential Zn efficiency among cultivars. These results are well agreement with the results published by Peleg et al. (2008) for wild emmer wheat cultivars and Cakmak et al. (2001) for modern wheat cultivars. In contrast to the Zn concentrations, the grain production response of cultivars to Zn fertilizer application was related to their Zn efficiency. Similar conclusions have been also reported by Cakmak et al. (2001) for durum wheat cultivars.

Increasing grain Zn concentration can increase grain protein content. Starks and Johnson (1985) showed that the majority of Zn applied to bread wheat at anthesis was incorporated into grain protein and that the greatest proportion of the ^{65}Zn applied was found in the glutenin. In the present study, foliar spray of all Zn fertilizers increased grain protein content of wheat plants over those of control plants. Both grain Zn and Fe concentrations also correlated positively and significantly with grain protein content (Table 4). As reviewed by Cakmak et al. (2010), in a number of wheat collections, grain Zn and Fe concentrations showed a very significant positive correlation with grain protein. Most probably, the genes affecting the grain accumulations of Zn, Fe and protein are closely linked as shown in *Triticum dicoccoides* (Cakmak et al., 2004).

Table 4
Correlation coefficients between grain Zn and Fe concentration with protein content of two wheat cultivars.

Cultivar	Micronutrient	Protein	
		Year 1	Year 2
Back Cross	Zn	0.62*	0.53*
	Fe	0.75**	0.79***
Kavir	Zn	0.67**	0.40ns
	Fe	0.45ns	0.74**

ns Not significant.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

In the present study, ZnAAC produced greater increases in grain protein concentration in comparison with $ZnSO_4$. This might be due to greater increase in grain Zn concentration of wheat plants by foliar spray of ZnAAC. As an exception, in 'Back Cross' plants, the effect of $Zn(His)_2$ on the grain Zn concentration was similar to $ZnSO_4$, while grain protein content in $Zn(His)_2$ treatment was significantly greater than $ZnSO_4$. Therefore, elevated grain protein content in plants sprayed with ZnAAC might also be due to the role of AA in protein synthesis. El-Bassiouny et al. (2008) demonstrated that external supply of Arg and putrescine significantly increased the seed protein content of wheat plants. This increase was attributed to the translocation of AA from shoots to seeds and the increase in protein synthesis in wheat shoot. In addition, Sood and Nagar (2003) suggested that, polyamines act as activators to RNA, protein synthesis, and/or inhibit certain proteolytic enzymes. In the present study, the most pronounced effect of ZnAAC on the protein content was observed in response to $Zn(Arg)_2$ and $Zn(Gly)_2$. Chang et al. (2005) demonstrated that Arg (rich intracellular peptide) is capable of efficiently delivering proteins into different plant tissues of both tomato and onion in a fully bioactive form. Vervaeke et al. (2005) also stated that, the involvement of Arg was probably related to protein synthesis in *Aechmea fasciata*.

In the present study, the mean concentrations of Zn and Fe in wheat grains were 1.71 and 4.24 mg $100\,g^{-1}$, respectively. These values are in the range of those reported by Malakouti et al. (1999), 0.9–2.4 mg $100\,g^{-1}$ Zn and 1.5–5.1 mg $100\,g^{-1}$ Fe in various wheat flours consumed in Iran. Gargari et al. (2007) reported 1.40 and 1.82 mg $100\,g^{-1}$ of Zn and Fe in wheat flour consumed in Tabriz city, Iran. It is well known that the total concentration of Zn in grain does not necessarily reflect the actual potential of foodstuff as the source of this element since those compounds have been known to impair mineral bioaccessibility. High PA intake with staple foods is one possible cause for Zn deficiency. Reinhold (1971) attributed Zn deficiency in rural communities of Iran to high content of PA in consumed staple foods, such as bread. Mameesh and Tomar (1993) showed 0.30 g $100\,g^{-1}$ PA in the Iranian bread. In the present study, PA concentration ranged from 0.12 to 0.36 g $100\,g^{-1}$ with average value of 0.23 g $100\,g^{-1}$. The effect of PA on the Zn absorption depends on relative levels of both Zn and PA. Hence, the PA to Zn molar ratio is considered a better indicator of Zn bioavailability than total dietary PA levels alone. According to results obtained from this study, there was a significant decrease in PA content of flour with foliar application of Zn fertilizers. In year 2, foliar spray of ZnAAC had no significant effect on grain PA content of 'Kavir' while it resulted in significant decrease of grain PA to Zn molar ratio.

In the present study, PA to Zn molar ratio among different treatments ranged from 8.1 to 21.1 with average value of 13.6. This level is close to the range (14.9–23.7) reported by Gargari et al. (2007). According to the World Health Organization (2002) in a

special food, PA to Zn molar ratio of ≥15, 5–15, and <5 is equal to Zn bioavailability as low (10–15%), moderate (30–35%), and high (50–55%), respectively. The present study showed a significant decrease in grain PA to Zn molar ratio of plants treated with Zn fertilizers. The PA to Zn molar ratios calculated for many plants treated with Zn fertilizers were 5-15 while this ratio in untreated plants was higher than 15. Our results also, indicated that foliar application of ZnAAC resulted in a considerable decrease of grain PA to Zn molar ratio compared with $ZnSO_4$.

5. Conclusions

The results obtained from this study indicated that ZnAAC were more effective Zn sources than $ZnSO_4$ to increase yield and grain Zn, Fe and protein concentrations of wheat. The effect of ZnAAC on reducing grain PA to Zn molar ratio was also, greater than $ZnSO_4$. According to the results obtained, ZnAAC not only increase the concentration of Zn in wheat grains, but makes it more bioavailable for human. In regard with high consumption of wheat bread in Iran, foliar spray of ZnAAC can be considered as an effective approach for improving human Zn nutritional status.

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References

Alloway, B.J., 2008. Zinc in Soils and Crop Nutrition. IZA Publications, Brussels.
Bremmer, J.M., Mulvancey, C.S., 1982. Total nitrogen. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Method of Soil Analysis. ASA and SSSA, Madison, pp. 599–622.
Cakmak, I., 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant and Soil 302, 1–17.
Cakmak, O., Ozturk, L., Torun, B., Ozkan, H., Kaya, Z., Cakmak, I., 2001. Tolerance of 65 durum wheat genotypes to zinc deficiency in a calcareous soil. Journal of Plant Nutrition 24, 1831–1847.
Cakmak, I., Pfeiffer, W.H., McClafferty, B., 2010. Biofortification of durum wheat with zinc and iron. Cereal Chemistry 87, 10–20.
Cakmak, I., Torun, A., Millet, E., Feldman, M., Fahima, T., Korol, A., Nevo, E., Braun, H.J., Özkan, H., 2004. *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. Soil Science and Plant Nutrition 50, 1047–1054.
Chang, M., Chou, J.C., Lee, H.J., 2005. Cellular internalization of fluorescent proteins via arginine – rich intracellular delivery peptide in plant cells. Plant and Cell Physiology 46, 482–488.
Das, C., Sengupta, T., Chattopadhyay, S., Setua, M., Das, N.K., Saratchandra, B., 2002. Involvement of kinetin and spermidine in controlling salinity stress in mulberry (*Morus alba* L. cv. S1). Acta Physiologiae Plantarum 24, 53–57.
Ekholm, P., Virkki, L., Ylinen, M., Johansson, L., 2003. The effect of phytic acid and some natural chelating agents on the solubility of mineral elements in oat bran. Food Chemistry 80, 165–170.
El-Bassiouny, H.M.S., Mostafa, H.A., El-Khawas, S.A., Hassanein, R.A., Khalil, S.I., Abd El-Monem, A.A., 2008. Physiological responses of wheat plant to foliar treatments with arginine or putrescine. Australian Journal of Basic and Applied Sciences 2, 1390–1403.
Gargari, B.P., Mahboob, S., Razavieh, S.V., 2007. Content of phytic acid and its mole ratio to zinc in flour and breads consumed in Tabriz, Iran. Food Chemistry 100, 1115–1119.
Ghasemi, S., Khoshgoftarmanesh, A.H., Hadadzadeh, H., Afyuni, M. Synthesis, characterization, and theoretical and experimental investigations of zinc(II)-amino acid complexes as eco-friendly plant growth promoters and highly bioavailable sources of zinc. Journal of Plant Growth Regulation, in press, http://dx.doi.org/10.1007/s00344-012-9300-x
Graham, R.D., Welch, R.M., Bouis, H.E., 2001. Addressing micronutrients malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Advances in Agronomy 70, 77–142.
Gupta, S., Sharma, M.L., Gupta, N.K., Kumar, A., 2003. Productivity enhancement by putrescine in wheat (*Triticum aestivum* L.). Physiology and Molecular Biology of Plants 9, 279–282.
Iqbal, M., Ashraf, M., 2005. Changes in growth, photosynthesis capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre-sowing seed treatment with polyamines. Plant Growth Regulation 46, 19–30.
Irshad, M., Yamamoto, S., Eneji, A.E., Endo, T., Honna, T., 2002. Urea and manure effect on growth and mineral contents of maize under saline conditions. Journal of Plant Nutrition 25, 189–200.
Khoshgoftarmanesh, A.H., Schulin, R., Chaney, R.L., Daneshbakhsh, B., Afyuni, M., 2010. Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. Agronomy for Sustainable Development 30, 83–107.
Kutman, U.B., Yildiz, B., Cakmak, I., 2011. Improved nitrogen status enhances zinc and iron concentrations both in the whole grain and the endosperm fraction of wheat. Journal of Cereal Science 53, 118–125.
Lonnerdal, B., 2000. Dietary factors influencing zinc absorption. Journal of Nutrition 130, 1378–1383.
Maas, E.V., Hoffman, G.J., 1977. Crop salt tolerance: current assessment. Journal of Irrigation and Drainage Engineering (ASCE) 103, 115–134.
Makower, R.U., 1970. Extraction and determination of phytic acid in beans. Cereal Chemistry 47, 288–295.
Malakouti, M.J., Sawaghebi, G., Balali, M., 1999. Effect of micronutrient supplementation on phytic acid content of wheat, bran, and flour. Soil and Water Research Journal 12, 177–186.
Mameesh, M.S., Tomar, M., 1993. Phytate content of some popular Kuwaiti foods. Cereal Chemistry 70, 502–503.
Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, San Diego, CA.
Milani, P.M., Malakouti, M.J., Khademi, Z., Balali, M.R., Mashayekhi, M., 1998. A fertilizer recommendation model for the wheat field of Iran. Soil and Water Research 19, 35–49.
Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. New Phytologist 182, 31–48.
Ohlund, J., Näsholm, T., 2001. Growth of conifer seedlings on organic and inorganic nitrogen sources. Tree Physiology 21, 1319–1326.
Peleg, Z., Saranga, Y., Yazici, A.M., Fahima, T., Ozturk, L., Cakmak, I., 2008. Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. Plant and Soil 306, 57–67.
Reinhold, J.G., 1971. High phytate content of rural Iranian bread: a possible cause of human zinc deficiency. American Journal of Clinical Nutrition 24, 1204–1206.
SAS Institute, 2000. SAS/STAT User's Guide, Release 8. SAS Institute, Cary, NC.
Shi, R., Zhang, Y., Chen, X., Sun, Q., Zhang, F., Römheld, V., Zou, C., 2010. Influence of long term nitrogen fertilization on micronutrient density in grain of winter wheat (*Triticum aestivum* L.). Journal of Cereal Science 51, 165–170.
Sood, S., Nagar, P.K., 2003. The effect of polyamines on leaf senescence in two diverse rose species. Plant Growth Regulation 392, 155–160.
Starks, T.L., Johnson, P.E., 1985. Techniques for intrinsically labelingwheat with ^{65}Zn. Journal of Agricultural and Food Chemistry 33, 691–698.
Vervaeke, I., Sitichelbout, L., Londers, E., Deroose, R., De Proft, M.P., 2005. Influence of arginine, ornithine, DFMO and polyamines on division of the generative nucleus in cultured pollen tubes of *Aechmea fasciata* (Bromeliaceae). Plant Cell, Tissue and Organ Culture 81, 77–82.
World Health Organization, 2002. Reducing Risks, Promoting Healthy Lifes, p. 284.

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Synthesis, Characterization, and Theoretical and Experimental Investigations of Zinc(II)–Amino Acid Complexes as Ecofriendly Plant Growth Promoters and Highly Bioavailable Sources of Zinc

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Abstract Amino acids (AA) as metal complexing agents have the ability to form relatively stable complexes with zinc (Zn) and thereby increase its availability for plants. In this study, the complexes of Zn(II), [Zn(L–L′)$_2$] [where L–L′ = monoanion of arginine (Arg), glycine (Gly), glutamine (Gln), histidine (His), and methionine (Met)], were synthesized and characterized by different analytical techniques. The results of elemental analysis support the formation of Zn(II)–AA complexes (ZnAAC) with a 2:1 ligand-to-metal molar ratio. The computational results indicated that the AA ligands coordinated to the Zn(II) ion via their nitrogen and oxygen atoms and support the coordination mode obtained from IR spectroscopy. For the first time, the semiempirical calculations were also performed to investigate the passive uptake of ZnAAC by root cells. The proposed transport pathway indicated that ZnAAC can pass via plant root cell wall pores without any strict hindrances. The efficacy of ZnAAC as a Zn source was evaluated for two lettuce cultivars (*Lactuca sativa* L., cvs. 'Lollo Bionda' and 'Lollo Rossa') grown in nutrient solution. The results confirmed the higher efficacy of ZnAAC in supplying Zn for lettuce in comparison with $ZnSO_4$. The synthesized ZnAAC also had a stimulating effect on root and shoot growth of both lettuce cultivars. According to the results, ZnAAC can be used as ecofriendly plant growth stimulators and sources of Zn to supply plants with readily available Zn.

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Introduction

Amino acids (AA) as natural chelating agents play a significant role in increasing solubility and availability of micronutrients, that is, zinc (Zn) in soil–plant systems (Aravind and Prasad 2005). These organic ligands change the dissolvability of metal nutrients in soils through chelation, oxidation/reduction, and acidification of the rhizosphere (Xu and others 2007; Oburger and others 2009). It has been shown that the exudation of chelating agents such as AA from roots is a possible mechanism for plant tolerance to Zn-deficiency conditions, particularly in calcareous soils (Kalaycia and others 1999; Rasouli-Sadaghiani and others 2011). Zinc is an important and essential micronutrient that plays a role in several crop physiological processes such as metabolism of carbohydrates, proteins, and hormones, membrane integrity, and reproduction (Broadley and others 2007). Zinc deficiency in soils and plants is a global micronutrient deficiency problem in most agricultural regions of the world (Alloway 2008). The easiest and most straightforward practice to correct micronutrient deficiency is to apply Zn fertilizers. Commercial $ZnSO_4$ and synthetic Zn-chelates (that is, Zn–EDTA and Zn–DTPA) are common sources of Zn used in agricultural lands (Alloway 2008). Applied soluble micronutrient fertilizers become ineffective rather rapidly as the dissolved metals react with soil minerals and organic matter (Khoshgoftarmanesh and others 2010). Most commercial inorganic Zn fertilizers contain Cd and other toxic heavy metals as impurities (Afyuni and others 2007). In comparison with inorganic fertilizers, synthetic and natural chelates have the advantage of keeping the applied nutrient in solution in a less reactive form

(Khoshgoftarmanesh and others 2010). Chelates are particularly appropriate for applications of Zn to alkaline and calcareous soils. In contrast with inorganic salts, synthetic Zn-chelates are effective for correction of Zn deficiency (Wallace and Wallace 1982) for a longer period. However, application of metal chelates may result in a potential leaching risk because the less biodegradable the carrier, the greater the risk for leaching (Gonzalez and others 2007). Zinc synthetic chelates, mainly Zn–EDTA and Zn–DTPA, are widely used to supply Zn in hydroponic nutrient solutions, but after Zn uptake by the plant, the concentration of free ligands is increased in the nutrient solution and as a result, the possibility of complex formation between free ligands and other micronutrients (that is, Cu and Mn) in the solution increases. Complexation with EDTA or DTPA reduces concentrations of free metal cations and thereby decreases their availability for plant uptake (Albano and Miller 2001; Vadas and others 2007). On the other hand, EDTA and DTPA are easily photodegradable compounds and their phytodegradation results in production of certain compounds such as glyoxylic acid, formaldehyde, diethylenetriaminetriacetic acid, and diethylenediaminetriacetic acid that are harmful for plant growth (Nowack and Baumann 1998; Hangarter and Stasinopoulos 1991; Metsarinne and others 2004).

Recently, we successfully synthesized Fe(II)–AA chelates and evaluated their efficiency as a Fe source for tomato plants in comparison with the Fe–EDTA chelate. Addition of Fe–AA chelates into the hydroponic nutrient solution significantly increased tissue concentrations of Fe, Zn, and N in two tomato genotypes in comparison with Fe–EDTA (Ghasemi and others 2012). The complexes of metal–AA are weaker than synthetic chelates (Alloway 2008) and their degradation in the nutrient solution is negligible (Jämtgård and others 2008). Therefore, the disadvantages of metal–AA complexes are much less than those of synthetic chelates. In addition, AA are precursors of certain plant hormones and improve plant growth via improving photosynthesis (Zeid 2009; Amin and others 2011), mRNA transcription, and sugar and protein production (Nassar and others 2003; Rashad and others 2003; Keutgen and Pawelzik 2008). The uses of AA to improve growth and yield of various crops have yielded very encouraging results with some plants. For example, exogenous application of arginine significantly increased the fresh and dry weights and concentrations of certain endogenous plant growth regulators in wheat (El-Bassiouny and others 2008), bean (Nassar and others 2003), and onion (Amin and others 2011). A significant increase in growth and yield of bean in the presence of glutamine has also been reported by Rashad and others (2003).

Considering the significant role of AA in increasing soil availability of micronutrients for plants, we hypothesized the possibility of synthesis and use of Zn(II)–AA complexes (ZnAAC) as a plant growth stimulator and Zn source in agricultural systems. The work reported in this article describes the synthesis, characterization, and theoretical investigation of $[Zn(L–L')_2]$ (where L–L′ = monoanion of arginine, glycine, glutamine, histidine, and methionine) complexes. Arginine (Arg), glycine (Gly), glutamine (Gln), histidine (His), and methionine (Met) were chosen as ligands. These AA were used because of their significance in plant nutrition and the relatively high stability of their Zn complexes in water. The efficacy of synthesized ZnAAC as growth stimulating and Zn sources was also investigated in a nutrient solution culture with lettuce. For the first time, the semiempirical PM6 calculations for ZnAAC were used to investigate the passive uptake of ZnAAC by root cells.

Materials and Methods

Synthesis of Zinc–Amino Acid Complexes (ZnAAC)

Zinc(II)–amino acid complexes have been prepared using five AA, arginine (Arg), glycine (Gly), glutamine (Gln), histidine (His), and methionine (Met) as complexing agents. All complexes were characterized by different analytical techniques.

General Methods

A solution of Arg, Gly, Glu, His, or Met (2 mmol) in 5 ml distilled water was slowly added to a solution of $Zn(OAc)_2$ (1 mmol) in 2 ml distilled water. The mixture was heated at reflux temperature for 2 h while being stirred vigorously. Evaporation of solvent at room temperature yielded white microcrystals of ZnAAC. The products were washed with cold ethanol followed by diethyl ether and air-dried.

Analyses

A PerkinElmer 2400 CHN elemental analyzer was used for quantitative determination of carbon (C), nitrogen (N), hydrogen (H), and oxygen (O) in various operating modes. Atomic absorption measurements of Zn were recorded with an atomic absorption spectrometer (PerkinElmer 3030; PerkinElmer, Wellesley, MA, USA). The FTIR spectra were measured with a FTIR JASCO 460 spectrophotometer over KBr pellets in the 4,000–400-cm^{-1} range. Electronic spectra were recorded by a JASCO-570 spectrophotometer.

Efficacy Test: Lettuce Culture in Hydroponic Nutrient Solution

Seeds of two lettuce cultivars (*Lactuca sativa* L., cvs. ‘Lollo Bionda’ and ‘Lollo Rossa’) were thoroughly rinsed with distilled water and germinated on moist filter paper in an

incubator at 28 °C. Uniform-sized seedlings were transferred to PVC lids that fit tightly over 2-L polyethylene containers in a greenhouse under controlled conditions with an 8-h light period at an intensity of 390 μmol m^{-2} s^{-1}, 25/20 °C day/night temperature, and 65–75 % relative humidity. The pots were wrapped with black polyethylene to prevent light from reaching the roots and solution. Two plants were planted in each pot. A basic nutrient solution was prepared in double-deionized water (electrical resistivity = 18 Mohm cm^{-1}). The nutrient solution contained 1.0 mM KNO_3, 1.0 mM $Ca(NO_3)_2$, 1.0 mM $NH_4H_2PO_4$, 1.0 mM $MgSO_4$, 50 μM KCl, 25 μM H_3BO_3, 2.0 μM $MnSO_4$, 2.0 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 1.0 μM $NiSO_4$, and 0.02 μM $H_2Mo_7O_4$ adjusted to pH 6 with NaOH or HCl as a buffer. Zinc was supplied from five different sources of $ZnSO_4$ (the most common Zn source used in nutrient solutions) and ZnAAC of $Zn(Arg)_2$, $Zn(Gly)_2$, $Zn(Gln)_2$, and $Zn(His)_2$. $Zn(Met)_2$ is insoluble in water so this complex was not used as a Zn source in nutrient solution culture. The Zn level in the nutrient solution was 10 μM. All solutions were renewed every day.

Plants were harvested approximately 4 weeks after seeding and divided into shoot and roots. The plant materials were dried immediately in a forced-air oven at 70 °C to a constant weight and ground to a fine powder in a Wiley mill to pass through a 20-mesh sieve. Dry samples (1 g) were placed into ceramic vessels and combusted in a muffle furnace at 550 °C for 8 h. The ashed samples were removed from the muffle furnace, cooled, and then dissolved in 2 M HCl (Chapman and Pratt 1961). The final solution was diluted to meet the range requirements of the analytical procedures. Analyses of Zn were carried out with an atomic absorption spectrophotometer (PerkinElmer model 3400).

Results and Discussion

Synthesis of ZnAAC

All $[Zn(L–L')_2]$ complexes of this study were synthesized in good yield by reaction of Zn acetate with the AA ligands in refluxing water according to the following reaction:

$$Zn(OAc)_2 + 2L - L' \text{ (e.g., Gly, } H_2NCH_2COOH) \rightarrow [Zn(L - L')_2] \text{ or } [Zn(Gly)_2] + 2HOAc$$

The acetate anion OAc^- can act as a weak base and remove a proton (H^+) from the neutral AA ligand. The $[Zn(L–L')_2]$ complexes, except $[Zn(Met)_2]$, are air-stable and soluble in water.

The spectral features (UV–Vis) of the ZnAAC did not change on keeping the aqueous solutions for 48 h, and no precipitation was observed, even after long storage at room temperature (at least 3 months after preparation), which indicates stability of the ZnAAC. Essential metal ions such as Zn in biology most frequently bind to donor ligands according to preferences dictated by the hard–soft theory of acids and bases (HSAB). The affinity of metal ions for ligands is controlled by size, charge, and electronegativity. This can be refined further by noting that for some metal ions, their chemistry is dominated by size and charge, whereas for others it is dominated by their electronegativity. According to Pearson's principle of HSAB, Zn(II) is a hard acid. This ion tends to bind to hard bases such as N-chelating, O-chelating, and N,O-chelating agents such as AA.

Elemental Analysis

The analytical data for the complexes are given in Table 1. The elemental analysis of the complexes is consistent with their formulation, $[Zn(L–L')_2]$, as are the following spectroscopic characterizations and theoretical investigations. The mole ratio of Zn(II)/L–L′ is 1:2 (Table 1).

Table 2 Selected IR bands (cm^{-1}) of zinc(II)–amino acid complexes (ZnAAC) (KBr disk)

ZnAAC	$\nu(NH_2)$	ν(C=O)	ν(C–O)	$\delta(NH_2)$	δ(C=O)
$[Zn(Gly)_2]$	3,306, 3,268	1,599	1,407	–	723
$[Zn(Glu)_2]$	3,267, 3,210	1,645	1,409	1,686	777
$[Zn(Arg)_2]\cdot 0.5H_2O$	3,139, 3,010	1,594	1,403	–	655
$[Zn(His)_2]\cdot H_2O$	3,178	1,695	1,407	–	799
$[Zn(Met)_2]$	3,249	1,606	1,409	1,601	698

Table 1 Analytical data for zinc(II)–amino acid complexes (ZnAAC)

ZnAAC	Formula weight	Yield (%)	Size (nm)	% Found (calculated)[a]			
				C	H	N	Zn
$[Zn(Arg)_2]\cdot 0.5H_2O$	420.83	83.88	1.41	34.31 (34.25)	6.36 (6.47)	26.49 (26.53)	15.32 (15.54)
$[Zn(Gly)_2]$	213.53	87.11	0.55	22.54 (22.50)	3.82 (3.78)	13.06 (13.12)	30.57 (30.63)
$[Zn(Gln)_2]$	355.69	80.69	1.10	33.94 (33.76)	4.98 (5.10)	15.47 (15.76)	18.12 (18.39)
$[Zn(His)_2]\cdot H_2O$	391.73	83.22	1.09	36.66 (36.79)	4.89 (4.63)	21.53 (21.60)	16. 36 (16.70)
$[Zn(Met)_2]$	361.81	87.73	0.99	33.63 (33.19)	5.60 (5.57)	7.93 (7.74)	18.02 (18.08)

[a] Theoretical percentage of the elements

FTIR Spectroscopy

Amino acids exist as zwitterions in the crystalline state and predominant vibrations for the free AA ligands are associated with $\nu_a(COO^-)$, $\nu_s(COO^-)$, $\delta_d(NH_3^+)$, $\delta_s(NH_3^+)$, $\nu_a(CCN)$, $\nu_s(CCN)$, and $\delta(COO^-)$. In their complexes, the AA act as bidentate ligands and bind to the metal via one oxygen and one nitrogen atom. Also, the noncoordinating C=O groups are hydrogen-bonded to the neighboring complex or lattice water, or weakly bonded to the metal of the neighboring complex. Thus, ν(COO) of AA complexes are affected by coordination as well as by intermolecular interactions (Nakamato 2009).

The effect of coordination is the major factor in determining the frequency order in AA complexes. The reported

Table 3 Calculated bond lengths (Å) and angles (°) of Zn(II)–amino acid complexes (ZnAAC)

ZnAAC	Zn–O	Zn–N	N–Zn–N	O–Zn–O	N–Zn–O	ΔH_f (kcal mol^{-1})
$[Zn(Gly)_2]$	1.899, 1.899	2.032, 2.032	131.955	129.729	88.824, 88.822, 111.356, 111.579	−193.768
$[Zn(Glu)_2]$	1.923, 1.923	2.013, 2.013	131.237	123.961	88.114, 88.074, 114.939, 114.871	−315.471
$[Zn(Arg)_2]\cdot 0.5H_2O$	1.918, 1.907	2.029, 2.010	138.465	121.280	87.201, 87.912, 103.652, 120.801	−176.504
$[Zn(His)_2]\cdot H_2O$	1.913, 1.913	2.018, 2.018	130.330	125.823	88.520, 88.515, 114.124, 114.081	−145.903
$[Zn(Met)_2]$	1.855, 1.856	1.938, 1.938	116.783	117.078	95.981, 95.600, 115.541, 117.440	−208.822

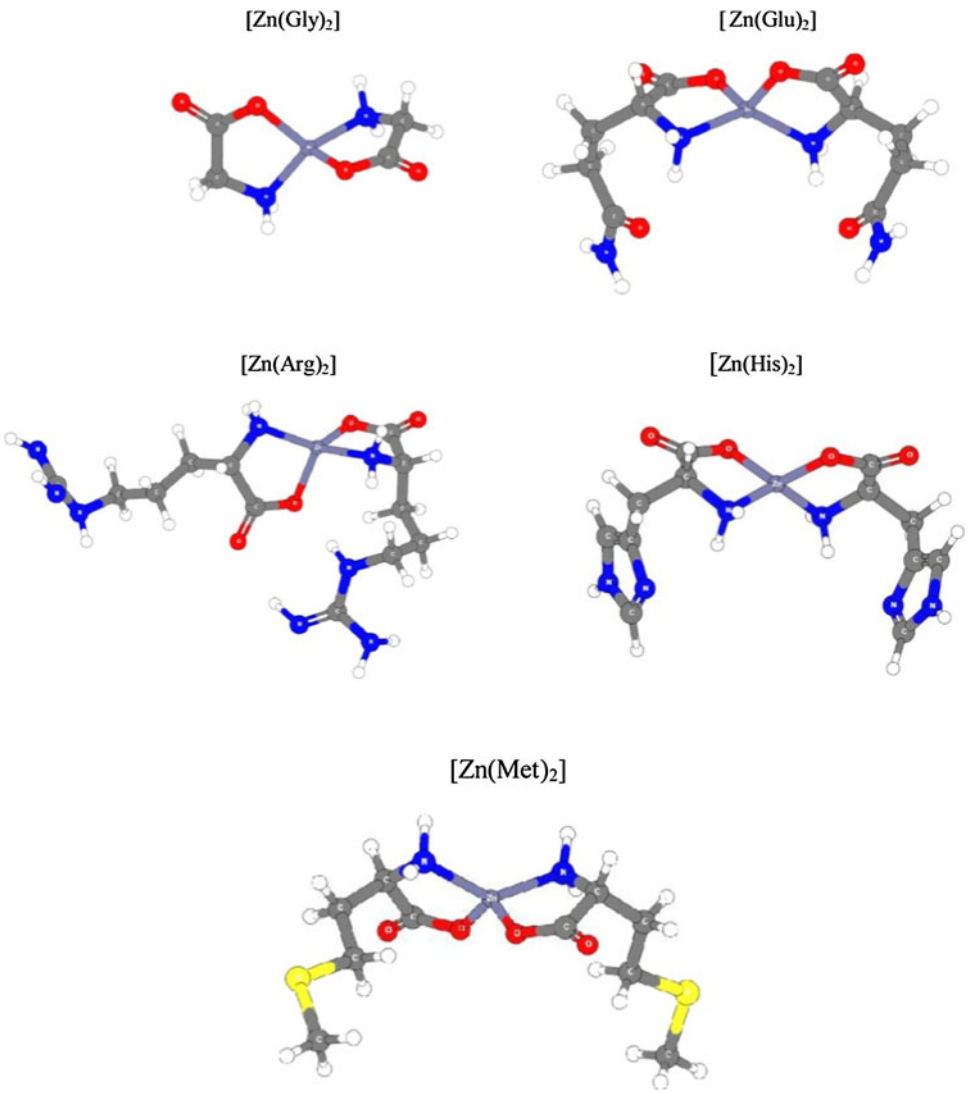

Fig. 1 Possible structure of Zn(II)–amino acid complexes (ZnAAC)

data indicate the increasing order of the metal–oxygen interaction because the COO group becomes more asymmetrical as the metal–oxygen interaction becomes stronger. The selected vibrations and assignments of ZnAAC are given in Table 2. The FTIR spectra of ZnAAC show an absorption pattern in the 4,000–400-cm^{-1} region, similar to AA. Predominant vibrations for the ZnAAC are associated with ν(CO), ν(C–O), $\nu(NH_2)$, $\delta(NH_2)$, and δ(CO). The observed vibrational bands for $-NH_2$ groups around 3,100–3,350 cm^{-1} are very sensitive to the effect of intermolecular interaction in the solid state and these bands sometimes appear very broad. Also, it is difficult to discuss the strength of the Zn(II)–NH_2– bond from the $\nu(NH_2)$. In comparison to free AA, the vibration of N–H bands appears to be shifted toward a higher frequency in the ZnAAC, proving the involvement of the amine group in the complex formation. The carboxylate ion of AA coordinates to Zn(II) as a unidentate mode. The C=O groups of ZnAAC have approximately the same frequency around 1,594–1,695 cm^{-1} and the ν(CO) is metal-sensitive (Nakamato 2009).

The electronic spectra of ZnAAC were measured in aqueous solution. Because the Zn(II) ion has a d^{10} configuration and is difficult to oxidize or reduce due to charge transfer transitions, the absorption bands in the UV region are assigned to intraligand transitions (Lever 1984).

Semiempirical Calculations

There is no example of semiempirical PM6 calculations for ZnAAC. In the present study, different possible coordination modes of the AA and donor atoms and also the variable number of AA ligands were considered. Preferences between different coordination numbers and geometries tend to be controlled by steric and electronic effects. The variation found in coordination geometries for a given coordination number is consistent with the argument that spatial requirements of a ligand and coordination restrictions of multidentate ligands are controlling factors.

The bonding parameters of ZnAAC were calculated with PM6 (Table 3). The average bond lengths between the Zn(II) and each nitrogen and oxygen of the AA ligands are 1.985 and 1.902 Å, respectively. Coordination of AA to Zn(II) leads to the formation of a chelate ring (N–Zn–O) and the average bite angle of O–Zn–N is 89.943°. The computational results indicate that the AA coordinated to the Zn(II) ion via their nitrogen and oxygen atoms, which supports the coordination mode obtained from IR spectroscopy.

The AA ligand-to = Zn(II) mole ratio of 2:1 was observed after the optimization of the complexes in the solution state. The optimized structure of the complexes in

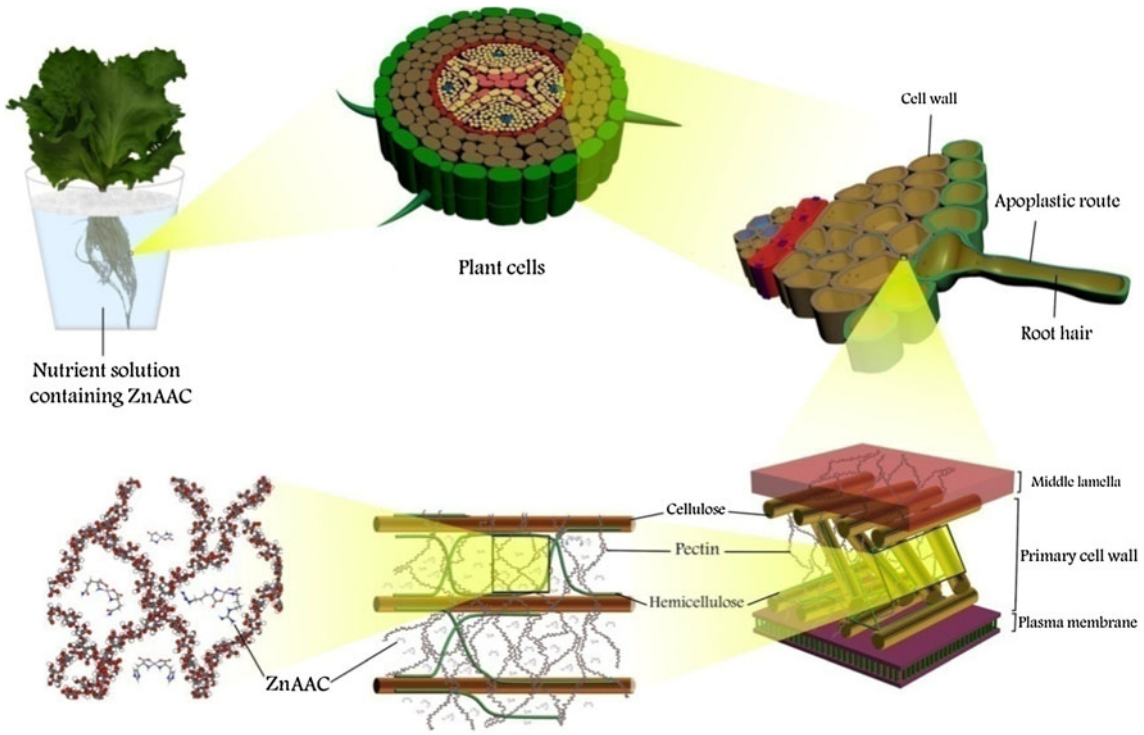

Fig. 2 A proposed transport pathway for Zn(II)–amino acid complexes (ZnAAC) uptake by lettuce roots

Fig. 3 The effect of Zn–arginine [$Zn(Arg)_2$] (*left*) on the growth of two lettuce cultivars in comparison with $ZnSO_4$ (*right*)

the solution state is shown in Fig. 1. The calculated standard enthalpies of the complex formation of ZnAAC (Table 1) show that all complexes have negative enthalpy values and are thermodynamically favored.

Efficacy of Synthesized ZnAAC in Stimulating Growth and Supplying Zn for Lettuce in Nutrient Solution Culture

A mechanistic understanding of uptake, translocation, and utilization of nutrients in plants is a prerequisite in the production of fertilizers. There are some physiochemical properties of ions and other solutes (for example, ion diameter and valence) that determine their uptake by roots. It has been indicated that due to a larger molecular size, plant uptake of the synthesized chelate (for example, Zn–EDTA, Zn–DTPA) is much lower than the free-metal cations (Marschner 1995). In this study, five ZnAAC were synthesized and characterized by different analytical techniques to determine their physiochemical properties. According to the semiempirical calculations, all ZnAAC have a molecular dimension of around 0.55–1.41 nm (Table 1). Because the pore diameter of plant cell walls is about 5 nm, the ZnAAC can pass through pores without any strict hindrances and enter into the free spaces of the root apoplasm. A proposed transport pathway for ZnAAC via the free spaces of the lettuce root cell wall from nutrient solution is shown in Fig. 2. The pathway indicates a passive uptake of ZnAAC by root cells. The mass transport of ZnAAC from the bulk of the nutrient solution to the primary cell wall occurs by diffusion and mass flow mechanisms.

The characteristics of ion uptake by roots are also affected by their interaction with charged groups in the cell wall of the apoplasm and membrane constituents. The strength of this interaction increases with increasing ion valence; conversely, the uptake rate often decreases (Marschner 1995). In the present study, the ZnAAC were synthesized with a 2:1 ligand-to-metal molar ratio. Coordination of AA to Zn(II) leads to the formation of uncharged molecular compounds. According to the results obtained from ZnAAC synthesis, we hypothesized that complexation of Zn with AA may improve Zn uptake by the plant. Therefore, we have investigated our hypothesis by comparing Zn uptake between ZnAAC and Zn^{2+} via lettuce roots in hydroponic culture.

The positive effect of ZnAAC on root and shoot growth (Figs. 3, 4) of lettuce plants was greater than that of $ZnSO_4$, although the stimulating effect of ZnAAC on lettuce growth was dependent on the plant cultivar and AA type.

In 'Lollo Bionda' $Zn(Gln)_2$ and in 'Lollo Rossa' $Zn(Arg)_2$ caused the greatest increase in root growth (Fig. 4a). Shoot dry matter weight of 'Lollo Bionda' plants supplied with $Zn(Arg)_2$ and $Zn(Gln)_2$ was higher than those supplied with $Zn(Gly)_2$ and $Zn(His)_2$ (Fig. 4b). In, 'Lollo Rossa', no significant difference was found in shoot growth between ZnAAC treatments. The stimulating effect of ZnAAC on lettuce growth could be due to the role of AA in improving the plant growth rate, cell division, and/or cell development (Abdul-Qados 2009). Nassar and others (2003) found that the positive effect of Arg on the shoot and root growth of bean was associated with the elevated level of certain plant growth regulators. A significant increase in shoot growth of pak-choi by Arg application has also been reported (Wang and others 2007). In the present study, the growth-stimulating effect of $Zn(Gln)_2$ and $Zn(Arg)_2$ was greater than that of $Zn(Gly)_2$ and $Zn(His)_2$. Differential effects of various AA on plant growth have been reported by other researchers (Wang and others 2007). For example, Svennerstam and others (2007) reported that the effect of Gln on the growth of *Arabidopsis* was greater than that of other AA studied. In another experiment, Rashad and others (2003) found a greater

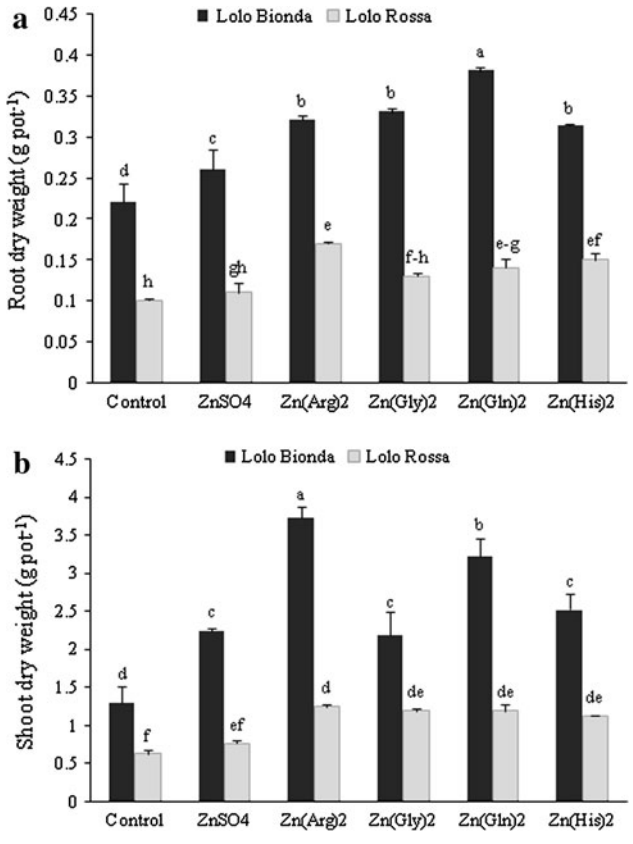

Fig. 4 Root (**a**) and shoot (**b**) dry matter weight of two lettuce cultivars grown in nutrient solution containing $ZnSO_4$, Zn–arginine [$Zn(Arg)_2$], Zn–glycine [$Zn(Gly)_2$], Zn–glutamine [$Zn(Gln)_2$], and Zn–histidine [$Zn(His)_2$]. Control presents Zn-free nutrient solution. *Error bar* represents standard error ($n = 3$). *Bars* having *different letters* are significantly different at the 5 % level by LSD

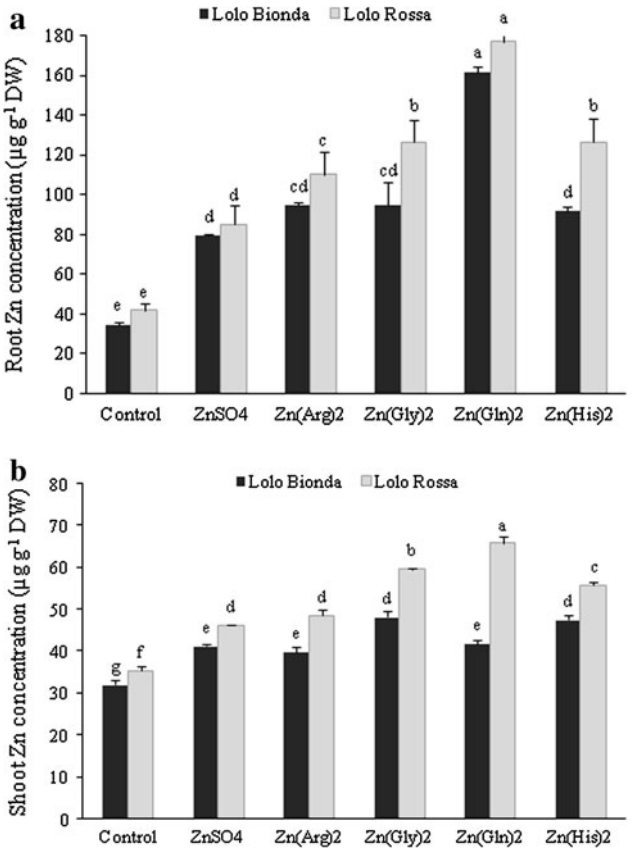

Fig. 5 Root (**a**) and shoot (**b**) Zn concentration of two lettuce cultivars grown in nutrient solution containing $ZnSO_4$, Zn–arginine [$Zn(Arg)_2$], Zn–glycine [$Zn(Gly)_2$], Zn–glutamine [$Zn(Gln)_2$], and Zn–histidine [$Zn(His)_2$]. Control presents Zn free nutrient solution. *Error bar* represent standard error ($n = 3$). *Bars* having *different letters* are significantly different at the 5 % level by LSD

effect of Gln on the growth and yield of bean plants compared with that of the other AA.

The results of the present study confirmed the greater efficacy of ZnAAC in supplying Zn to lettuce plants compared with that of $ZnSO_4$ (Fig. 5). For both lettuce cultivars, the increase in root Zn concentration was greater in plants supplied with $Zn(Gln)_2$ in comparison with those supplied with the other ZnAAC. In 'Lollo Rossa', the effect of ZnAAC on shoot Zn concentration was in the order: $Zn(Gln)_2 > Zn(Gly)_2 > Zn(His)_2 > Zn(Arg)_2$. In 'Lollo Bionda', $Zn(His)_2$ and $Zn(Gln)_2$ had similar effects on shoot Zn concentrations. In all treatments, 'Lollo Rossa' accumulated higher amounts of Zn in its roots and shoots compared with 'Lollo Bionda'. Amino acids have a great ability for forming complexes with Zn and thereby increase the bioavailability of this metal for plant uptake (Zhou and others 2007). Furthermore, stimulated plant growth by AA may result in a greater ability for Zn uptake in roots. Accordingly, Zhang and others (2009) found that addition of AA to the nutrient solutions increased uptake and root-to-shoot translocation of Zn in tomato. Eid and others (2011) reported the positive effect of AA on growth and nutrient uptake in marigold plants. According to the results obtained from the present study, the effect of ZnAAC on shoot and root Zn accumulation varied with AA type. In 'Lollo Rossa', $Zn(Gln)_2$ caused a higher accumulation of Zn in roots and shoots compared with the other ZnAAC. A possible reason for the different effects of ZnAAC on plant growth and Zn tissue content is variation in their uptake by the plant. The results obtained from the present study cannot show whether these complexes are absorbed directly with no dissociation or they dissociate at the root surface and then free amino acids and Zn pass through the cell membrane individually. It is also unclear whether the increased Zn uptake is due to plant growth improvement or increasing Zn transport through cell membrane. These hypotheses will be tested by isotopic experiments (labeled Zn and AA) in future studies. Based on the results of this study, ZnAAC are stable in nutrient solution and can improve the growth and the Zn nutritional status of lettuce plants in comparison with $ZnSO_4$. Consequently, due to

several disadvantages of synthetic chelates of Zn (for example, toxic side effects, impaired micronutrient balance), ZnAAC can be used as a suitable source of Zn in hydroponic nutrient solutions. The effectiveness of soil application of ZnAAC in plant Zn nutrition depends on the residence time of these complexes in the soil. Further research is therefore required to investigate the biodegradability of ZnAAC and to determine the role of AA in bioavailability of Zn in soil.

Conclusions

The ZnAAC in aqueous solution, $Zn(Arg)_2$, $Zn(Gly)_2$, $Zn(Gln)_2$, $Zn(His)_2$ and $Zn(Met)_2$, were synthesized and characterized by elemental analysis, atomic absorption, and FTIR spectroscopy. The results indicated the formation of 1:2 complexes of Zn(II) with all AA. Results also indicated that using ZnAAC in the nutrient solution could supply a sufficient amount of Zn for plant uptake and also improve root and shoot growth of lettuce plants. Although the size of Zn^{2+} would be increased due to complexation with AA, the results suggested that the movement of ZnAAC to the free space of root cells is not restricted by the pores. Further studies are required to investigate the mechanism of ZnAAC uptake using double ^{15}N–^{65}Zn-labeled compounds.

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References

Abdul-Qados AMS (2009) Effect of arginine on growth, yield and chemical constituents of wheat grown under salinity condition. Acad J Plant Sci 2:267–278

Afyuni M, Khoshgoftarmanesh AH, Dorostkar V, Moshiri R (2007) Zinc and cadmium content in fertilizers commonly used in Iran. International Conference of Zinc Crops, Istanbul

Albano JP, Miller WB (2001) Photodegradation of FeDTPA in nutrient solutions. I. Effects of irradiance, wavelength and temperature. HortScience 36:313–316

Alloway BJ (2008) Zinc in soils and crop nutrition, 2nd edn. IZA and IFA, Brussels/Paris

Amin AA, Gharib AEF, El-Awadia M, Rashad ESM (2011) Physiological response of onion plants to foliar application of putrescine and glutamine. Sci Hortic 129:353–360

Aravind P, Prasad MNV (2005) Cadmium-induced toxicity reversal by zinc in *Ceratophyllum demersum* L. (a free floating aquatic macrophyte) together with exogenous supplements of amino- and organic acids. Chemosphere 61:1720–1733

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677–702

Chapman HD, Pratt PF (1961) Methods of analysis for soils, plants, and waters. Priced Publication 4034. Division of Agriculture Sciences, University of California, Berkeley

Eid RA, Taha LS, Ibrahiem SMM (2011) Alleviation of adverse effects of salinity on growth, and chemical constituents of marigold plants by using glutathione and ascorbate. J Appl Sci Res 7:714–721

El-Bassiouny HMS, Mostafa HA, El-Khawas SA, Hassanein RA, Khalil SI, Abd El-Monem AA (2008) Physiological responses of wheat plant to foliar treatments with arginine or putrescine. Aust J Basic Appl Sci 2:1390–1403

Ghasemi S, Khoshgoftarmanesh AH, Hadadzadeh H, Jafari M (2012) Synthesis of iron–amino acid chelates and evaluation of their efficacy as iron source and growth stimulator for tomato in nutrient solution culture. J Plant Growth Regul. doi;10.1007/s00344-012-9259-7

Gonzalez D, Obrador A, Alvarez JM (2007) Behavior of zinc from six organic fertilizers applied to a navy bean crop grown in a calcareous soil. J Agric Food Chem 55:7084–7092

Hangarter RP, Stasinopoulos TC (1991) Effect of Fe-catalyzed photooxidation of EDTA on root-growth in plant culture media. Plant Physiol 96:843–847

Jämtgård S, Näsholm T, Huss-Danell K (2008) Characteristics of amino acid uptake in barley. Plant Soil 302:221–231

Kalaycia M, Torunb B, Ekerb S, Aydina M, Ozturkb L, Cakmak I (1999) Grain yield, zinc efficiency and zinc concentration of wheat cultivars grown in a zinc-deficient calcareous soil in field and greenhouse. Field Crop Res 63:87–98

Keutgen A, Pawelzik E (2008) Contribution of amino acids to strawberry fruit quality and their relevance as stress indicators under NaCl salinity. Food Chem 111:642–647

Khoshgoftarmanesh AH, Schulin R, Chaney RL, Daneshbakhsh B, Afyuni M (2010) Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. Agron Sustain Dev 30:83–107

Lever ABP (1984) Inorganic electronic spectroscopy. Elsevier, Amsterdam

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, San Diego

Metsarinne S, Rantanen P, Aksela R, Tuhkanen T (2004) Biological and photochemical degradation rates of diethylenetriaminepentaacetic acid (DTPA) in the presence and absence of Fe(III). Chemosphere 55:379–388

Nakamato K (2009) Infrared and Raman spectra of inorganic and coordination compounds, 6th edn. Wiley, New York

Nassar AH, El-Tarabily KA, Sivasithamparam K (2003) Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine-producing isolate of *Streptomyces griseoluteus*. Plant Growth Regul 40:97–106

Nowack B, Baumann U (1998) Biodegradation of the photolysis products of Fe(III)EDTA. Acta Hydroch Hydrob 26:104–108

Oburger E, Kirk GJD, Wenzel WW, Puschenreiter M, Jones D (2009) Interactive effects of organic acids in the rhizosphere. Soil Biol Biochem 41:449–457

Rashad ESM, El-Abagg HM, Amin AA (2003) Physiological effects of some bioregulators on growth and productivity of two broad bean cultivars. Egypt J Appl Sci 18:132–149

Rasouli-Sadaghiani MH, Sadeghzadeh B, Sepehr E, Rengel Z (2011) Root exudation and zinc uptake by barley genotypes differing in Zn efficiency. J Plant Nutr 34:1120–1132

Svennerstam H, Ganeteg U, Bellini C, Näsholm T (2007) Comprehensive screening of *Arabidopsis* mutants suggests the lysine histidine transporter 1 to be involved in plant uptake of amino acids. Plant Physiol 143:1853–1860

Vadas TM, Zhang X, Curran AM, Ahner BA (2007) Fate of DTPA, EDTA and EDDS in hydroponic media and effects on plant mineral nutrition. J Plant Nutr 30:1229–1246

Wallace GA, Wallace A (1982) Micronutrient uptake by leaves from foliar sprays of EDTA chelated metals. In: Nelson SD (ed) Iron

nutrition and interactions in plants. Marcel Dekker, Basel, pp 975–978

Wang HJ, Wu LH, Wang MY, Zhu YH, Tao QN, Zhang FS (2007) Effects of amino acids replacing nitrate on growth, nitrate accumulation, and macroelement concentrations in pak-choi (*Brassica chinensis* L.). Pedosphere 17:595–600

Xu WH, Liu H, Ma QF, Xiong ZT (2007) Root exudates, rhizosphere Zn fractions, and Zn accumulation of ryegrass at different soil Zn levels. Pedosphere 17:389–396

Zeid IM (2009) Effect of arginine and urea on polyamines content and growth of bean under salinity stress. Acta Physiol Plant 31:65–70

Zhang S, Hu F, Li H, Li X (2009) Influence of earthworm mucus and amino acids on tomato seedling growth and cadmium accumulation. Environ Pollut 157:2737–2742

Zhou Z, Zhou J, Li R, Wang H, Wang J (2007) Effect of exogenous amino acids on Cu uptake and translocation in maize seedlings. Plant Soil 292:105–117

Chapter 25

Mineral Supplementation in Plants Via Amino Acid Chelation

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Plants must obtain their vital nutrients by absorption from air, water, and/or soil. Improvements in bioavailability and assimilation can be gained through the use of amino acid chelates of the required minerals. Research presented compares metal amino acid chelates with complementary EDTA or inorganic sources. Data for corn, tomatoes, apples, potatoes and wheat indicate improvement through the supplementation of amino acid chelates of various metals. When increased bioavailability and anabolic usage are coupled with a multiple ratio assessment of all nutrients compared to each other, as determined by plant assays from crop to crop, optimal amounts and proportions of the amino acid chelates can be supplemented and significantly improved crop yields can result.

While chelation of metals is well known, physiological sequellae for mineral bioavailability in plant and animal nutrition have been less understood. A common misconception has been that all chelates of a particular metal are the same, with identical or very similar absorptive and metabolic properties. However, each type of ligand molecule has its own unique properties and, therefore, each type of chelate resulting from a particular class of ligands has its own properties. Chelation of a metal occurs when two or more sites from the ligand molecule bond into the same metal atom. This results in one or more cyclic ring structures which give the new molecule unique properties different from those that the metal would exhibit if it were in an inorganic or ionic form. More ligands than one may join the atom and form rings in addition to the first ligand molecule. The metal atom encased in the center of four or more bonds and the outward projecting backbones of the ligand rings is subsequently protected from chemical reactions or attractions which could interfere with its absorption.

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The amino acids form chelates between the terminal carboxyl and the adjacent alpha-amino group. This results in a ring structure around a metal of five members comprising bonds between the metal, carboxyl oxygen, carbonyl carbon, alpha-carbon, alpha-nitrogen and then back into the metal with a coordinate covalent bond from the nitrogen atom. The resulting chelate bonds are compatable to animal and plant metabolic systems in that they maintain their integrity prior to their metabolic usage and yet are capable of rupture, allowing the organism to benefit from the mineral and amino acid nutrition. This may be contrasted with a powerful synthetic chelate such as EDTA which forms strong bonds which are not as readily broken for metabolic usage of the chelated metal by plants and animals. Since a historical tendency in agriculture and animal husbandry has been to lump all of the chelates together, the properties of EDTA metal chelates have been presumed to represent those of other kinds of chelates. This assumption is unwarranted. The amino acid chelates have proven to be well-adapted vehicles for increasing epidermal absorption of metals through the surfaces of leaves, stems, and fruits.

High Bioavailability of Amino Acid Chelates

In a greenhouse experiment administered by Albion Laboratories(*1*), the effects of different sources of iron were combined with different levels of nitrogen supplementation. Corn plants were grown in pots in a greenhouse with four plants per pot and each treatment was repeated three times. The pH was 8.1 in the pots. The extractable iron was determined to be five parts per million in all soils, including the control soils. Nitrogen was supplemented as null plus two levels (0, 500, and 1000 ppm) by a urea and ammonium nitrate mixture at 50:50 proportions. The controls received nitrogen as above, but no iron source. Iron was applied as a foliar spray at 400 ppm for each of Iron Amino Acid Chelate, ferrous sulfate, and Iron-EDTA. The first spraying was done when the corn plants had acheived growth of ten to twelve inches and a second spraying at the same rates followed one week later. The plants were harvested three weeks after the second spraying. Following harvest, the foliage was washed in 1% hydrochloric acid, followed by a distilled deonized water rinsing, then dried for 24 hours at 75°C. Dry matter yield was determined, thereafter. This was followed by dry ashing, extraction, and measurement of the iron and manganese by DCP (direct current plasma spectrophotometry). The results are shown in Table I.

The highest dry matter yields and, therefore, the greatest vegetative growths were obtained from the Iron Amino Acid Chelate foliar treatments. Duncan's multiple range test (DMRT) was applied to determine significance of the various data within the table. Data which are similar ($P < 0.05$) are followed by a common letter. Administration of nitrogen at 1000 ppm generally yielded less beneficial results than the 500 ppm rates. In terms of dry matter, the Iron Amino Acid Chelate groupings stand unique with a lower end exception of inorganic iron sulfate at 500 ppm nitrogen. The Iron-EDTA was inconsequential in its production of dry matter, regardless of nitrogen levels.

Table I. Comparison of Above-Ground Corn Yields From Three Iron Sources with Concomitant Nitrogen Treatments

Treatment Fe-source	*N (ppm)*	*Dry Matter (g/pot)*	*Fe (ppm)*	*Mn (ppm)*
None	0	14.32 cd[1]	28 e	35 c
None	500	15.02 bcd	31 de	37c
None	1000	13.86 cd	35 cde	39 c
Fe-AACH[2]	0	17.62 a	102 a	71 b
Fe-AACH	500	16.73 ab	114 a	90 a
Fe-AACH	1000	16.85 ab	119 a	72 b
$FeSO_4$	0	13.23 de	48 bcde	32 c
$FeSO_4$	500	15.73 abc	53 bcd	42 c
$FeSO_4$	1000	14.82 bcd	63 b	40 c
Fe-EDTA	0	14.12 cd	47 bcde	38 c
Fe-EDTA	500	15.08 bcd	56 bc	37 c
Fe-EDTA	1000	11.24 e	69 b	31 c

SOURCE: Adapted from ref. 1.
[1]Numbers in a column not followed by a common letter differ significantly at $P < 0.05$, as determined by DMRT.
[2]Fe-Amino Acid Chelate.

All of the iron foliar spray sources promoted greater iron uptakes, but results from the amino acid chelate were highest with close to four times those of the control. The EDTA source of iron did not differ significantly from the inorganic iron, although both were higher than the control amounts. Other elements can be affected by the ready absorption of a particular ion. Manganese was absorbed in greater levels in conjunction with the Iron Amino Acid Chelate foliar spray. By contrast, the manganese absorption from the Iron-EDTA source was not appreciably different from the control values.

Table II shows data collected from a field crop of corn which had no apparent deficiencies*(2)*. Iron, zinc and manganese were administered singularly as Amino Acid Chelate foliar sprays upon the visually healthy crop. Bushels per acre yields increased in all cases. Also of significance was the increase in absorption of other metals. Zinc Amino Acid Chelate generated a notable increase of both iron and manganese, as well as of zinc.

Table II. Effects of Foliar Application of Iron, Zinc, and Manganese Amino Acid Chelates on the Yield and Nutrient Content of Corn

Treatment	*Yield (Bu/A)*[1]	*N*	*P*	*K*	*Fe*	*Zn*	*Mn*
			%			ppm	
Control	126.5	2.83	0.38	3.31	218	31	62
Fe-AACH[2]	132.2	2.92	0.34	3.84	257	35	67
Zn-AACH[2]	134.6	2.84	0.37	3.24	277	76	71
Mn-AACH[2]	132.9	2.85	0.37	3.42	207	30	82

SOURCE: Adapted from ref. 2.
[1]Yield in bushels per acre.
[2]Metal-Amino Acid Chelate.

Isotope Studies of Absorption and Translocation. In order to more fully understand the absorption of the Iron Amino Acid Chelates, radioactive ^{59}iron was applied to tomato leaves as a foliar spray of either Iron Amino Acid Chelate, Iron-EDTA or iron sulfate(2). All of the iron sources were applied at the same molar concentrations of iron. The data which are shown in Table III indicate that significantly more iron was absorbed through the epidermis of the tomato leaf if in the Iron Amino Acid Chelate form.

Table III. Replicate Absorption Measurements of ^{59}Fe, Applied as a Foliar Spray to Selected Attached Tomato Leaves

	Fe-Amino Acid Chelate	*Fe-EDTA*	*$FeSO_4$*
Same Leaf	43.1[1]	26.6	29.4
Adjacent Leaf	0.20	0.10	0.20
Same Stem	0.30	0.03	0.14
Same Leaf	37.58	23.93	21.08
Adjacent Leaf	0.37	0.13	0.14
Same Stem	0.07	0.04	0.14

SOURCE: Adapted from ref. 2.
[1]Units are corrected counts per minute per milligram.

In further experiments on greenhouse corn plants, nitrogen was administered twice a week from a nutritive solution(2). The results are shown in Table IV. Sprays containing the same amounts of radioactive iron were

given to plants which were 60 centimeters tall. Analyses were made five days after spraying. Each of the three treatments was replicated three times and the values shown are the resulting means.

Table IV. Absorption and Distribution of ^{59}Fe Applied as a Foliar Spray to Selected Attached Corn Leaves

Plant Part	*Iron Amino Acid Chelate*	*Iron Sulfate*
Point of Application	227[1]	68
Point of Application + 1 cm	0.54	0.17
Opposite Leaf	0.20	0.13
Root	0.13	0.03

SOURCE: Adapted from ref. 2.
[1]Units are corrected counts per minute per milligram.

In each case of either Iron Amino Acid Chelate or iron sulfate, the highest corrected counts per minute of absorbed radioactive iron were obtained at the point of application. Points of application could be considered to be sources or reservoirs of iron that could be drawn from on an as needed basis. Within the same timeframe, nearly four times as much iron had been translocated to the root from the Iron Amino Acid Chelate as compared to the inorganic control source.

Effects of Foliar Applications of Amino Acid Chelates on Flowering Fruits. The data in Table V were generated from experimentation in San Salvador on tomato plants(*3*). In this case, a foliar spray containing several minerals was administered to field tomatoes of the Santa Cruz Kada variety. Nitrogen and phosphorus fertilizers as well as standard herbicides and pesticides were applied to the soil at the same rate in all treatments including the control. Each of the three rates of spraying of Multimineral Amino Acid Chelate were done ten days before first flowering. The study was a complete randomized block design with four treatments and five replicates. Each plot was 10 meters by 1 meter, as these were row crops.

The number of buds increased progressively according to the rate of foliar application of Multimineral Amino Acid Chelate. The most dramatic increase occurred in the number of fruits obtained from the highest application of the Multimineral. This is especially significant in that the first spraying achieved an average number of fruits that were 2.5% above controls, the second yielded 6% above the first, however, the third yielded 26% above the second, and 33% above the first. The amounts of increases in foliar applications were 15% greater from the first spraying to the second and, 13% greater from the second to the third comprising a 30% increase from the first to the third sprayings.

Table V. Effects of Foliar Applications of Multimineral Amino Acid Chelate on Tomato (*Licopersicum esculentum* L.) Yield

Treatment[1] *(ml/ha)*[2]	*Number of Buds (average)*	*Number of Fruits (average)*
0	28.2	19.8
464.28	30.5	20.3
535.71	31.5	21.5
607.14	33.2	27.0

SOURCE: Adapted from ref. 3.
[1]Multimineral Amino Acid Chelate
[2]Units are milliliters per hectare.

Effects of Mineral Amino Acid Chelate Application Directly on the Surface of Fruits. It has been found that there is a direct correlation between bitter pit and the amount of calcium in apple fruits. Calcium is important in cell wall development in plants with the integrity of cells and cell walls completely dependent on the availability of sufficient calcium. Results in Table VI were obtained when calcium was administered as a post harvest fruit dip (Hymas, T., unpublished research report).

Table VI. Calcium Concentrations in Granny Smith Apples Receiving Various Postharvest Dip Treatments

Treatment	*Dilution*	*Ca in Peel (ppm)*
Control	----	241
$CaCl_2$	1:74	219
Ca-AACH[1]	1:120	708
Ca-AACH	1:60	723
Ca-AACH	1:30	670
$CaCl_2$ + Ca-AACH	1:148 + 1:60	568

[1]Calcium Amino Acid Chelate

The calcium from Albion Laboratories was Calcium Amino Acid Chelate at 5% calcium. The commercial dip was calcium chloride at 12% calcium. Granny Smith apples were used and there were eight apples per replication in each treatment. It had previously been determined that better results could be

obtained by additional treatments with DPA (diphenylamine) administered to decrease incidence of scalding, and administration of a sucrose ester that had been shown to slow down maturation of fruits. These two products were administered to all apples with the exception of the controls. Prior to calcium measurement, all of the apples were washed in deionized distilled water. The skins were separated from the rest of the flesh for analysis. These were dried and analyzed by atomic absorption spectrophotometry.

The data indicate the amount of calcium in the peel. The control received no dips. In all cases of Amino Acid Chelate administration, the amounts of calcium in the peel were high above the control amounts. A combination of calcium chloride and Calcium Amino Acid Chelate was not as effective, being at 21% less than the highest chelate alone, and 15% less than the lowest chelate alone. The difference between the calcium chloride and highest Calcium Amino Acid Chelate represented a 330% in increase in calcium absorption from the Amino Acid Chelate source, even though the chelated source contained only 42% of the calcium present in the inorganic dip.

Improvements of Yield Through TEAM Evaluation of Albion Amino Acid Chelates

In recognizing that they had suceeded in creating a series of mineral chelates which were highly bioavailable, researchers at Albion Laboratories, Inc. decided to further expand the utilization of these products by determining a way to properly balance minerals within particular crop plants as a way to maximize yields under any prevalent environmental or edaphic conditions. This work, which incorporated several years of experimentation, resulted in what has been termed the TEAM report (Technical Evaluation of Albion's Minerals). It utilizes the mass action proportional relationship of every element pitted against every other element of eleven of the elemental nutrients that are necessary for plant growth. Critical levels were also involved in the calculation parameters. The report is based on the nutritive balances of minerals supplied by amino acid chelates in foliar sprays which will yield the highest amounts of any particular crop. The calculations and subsequent recommendations for kinds and amounts of Albion Laboratories Amino Acid Chelates to be administered by foliar spraying are handed through computer software. The result has been the ability to prescribe the nutritive needs of a particular crop on a particular field for maximized yields. Since nutritive requirements differ from plant-type to plant-type, the evaluation takes these differences into account by selecting the crop-type at the time of data entry of the assayed mineral levels of the plant tissues.

Computer Assisted Recommendations for Amino Acid Chelate Applications for Increased Potato Crop Yields. To illustrate the advantages of TEAM evaluation, some data are shown in Table VII. The eleven nutrients analyzed by the TEAM report are shown in the left column. Actual nutrient

concentrations are indicated next and these are followed by nutrient indices which are assigned by the TEAM program. In addition to these data, the critical levels obtained from other agronomic sources and publications are shown in the rightmost column(*4*).

Table VII. Nutrient Analyses for a Potato Crop Showing Actual Concentrations, TEAM Report Balanced Nutrient Indicies, and Critical Level Values

Nutrient	*Nutrient Concentration*	*Nutrient Indices*	*Critical Level*
Ca	0.85 %	-17	0.49 %
S	0.26 %	-16	0.18 %
Mg	0.24 %	-15	0.24 %
P	0.36 %	- 5	0.36 %
Fe	203 ppm	- 1	75 ppm
K	4.32 %	2	3.50 %
Cu	10 ppm	2	6 ppm
B	29 ppm	5	9 ppm
N	5.36 %	8	3.1 %
Mn	105 ppm	11	7 ppm
Zn	36 ppm	26	19 ppm

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The nutrient indices should be considered to show mineral balance proportions, either as a decriment or adequacy for any particular nutrient that was analyzed from the plant tissues that were submitted for analysis. The most needful nutrients (or the ones most severely out of balance) are listed first, with decreasing needfulness as the series moves downward towards the nutrient measured in the highest abundance and present in sufficient quantities for growth and yield.

The nutrient which is most out of balance and is listed first in this example is calcium. By referring to the assayed nutrient concentration and comparing it with its critical level, calcium would appear to be present in sufficient quantities. Similar arguements could be made for sulfur and iron which are additionally listed as having some degree of imbalance for highest yields of potato crops. In order to assess the results of following the customary farm management practice of determining mineral supplementation needs by comparing tissue analyses with known critical levels, the professional recommendations of an experienced and independent farm manager were used. Both recommendations were followed on separate portions of the field. When assessing the nutrient concentrations indicated in Table VII, the recommendation of the farm manager was to add phosphorus and let the other

minerals go untouched, since they appeared to be in sufficient concentrations as compared to critical levels. The TEAM recommendation, however, was to repair an imbalance seen through its evaluation as indicated by the differences of nutrient indices.

Results of following the two divergent recommendations are shown in Table VIII. The control was considered to be the hundred percent yielding value. The recommendation of the farm manager was considered as the critical treatment and the TEAM report results are also indicated. The yield in hundred-weight bags per acre of potatoes was increased by 23% by following the TEAM evaluation recommendations and supplying the correct mineral balances by foliar spraying the appropriate Amino Acid Chelates on the crops. The critical evaluation suggested by the farm manager was able to effect some improvements in yields resulting in a 10% increase over the control, but this was less than half of the increase that was obtainable through following the TEAM recommendation.

Table VIII. Yield of Potato Crops Given Nutrient Supplements Based on TEAM Analysis Versus Standard Farm Management (Critical Levels)

Treatment	*Yield (CWT/A)*[1]	% Difference
Control	323	100
Critical	354	110
TEAM	398	123

[1]Yield in hundred-weight bags per acre.

Computer Assisted Recommendations for Amino Acid Chelate Application for Increased Wheat Yields. Improvement in wheat yield through use of the TEAM evaluation is shown in Tables IX and X(*4*). These data are particularly depictive of the capabilities of the TEAM program. Zinc was listed as the most out of balance mineral, and yet had a nutrient concentration within the plant leaves of nearly twice the suggested critical level. Manganese, which was also shown as being deficient or, at least, out of balance, was seventeen times greater than the critical level. A comparison of Tables IX and X, show that the TEAM program is not just a blanket measure and ranking of nutrient concentrations. The proper balance of nutrients form a crucial part of the analysis. Mineral needs vary from crop to crop and what is in balance for one crop may be out of balance for another.

When the farm manager was allowed to assess the nutrient concentrations in Table IX, his choice was to do nothing. All of the balances seemed to be appropriate and were all sufficiently above the critical levels. Again, the recommendation of the TEAM report was to repair a measured empirical imbalance for that particular wheat crop on that particular field.

Table IX. Nutrient Analyses for a Wheat Crop Showing Actual Concentrations, Critical Level Values and TEAM Report Balanced Nutrient Indices

Nutrient	*Nutrient Concentration*	*Nutrient Indices*	*Critical Level*
Zn	28 ppm	- 9	15 ppm[1]
K	4.36 %	- 7	1.25 %
Ca	0.30 %	- 7	0.20 %[1]
P	0.50 %	- 5	0.15 %
Mn	85 ppm	- 3	5 ppm
S	0.43 %	- 2	0.15 %[1]
Cu	10 ppm	2	5 ppm
Mg	0.29 %	3	0.15 %[1]
N	5.42 %	4	1.5 %
Fe	215 ppm	5	50 ppm
B	31 ppm	19	5 ppm

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[1]Sufficiency level.

The results of these prescribed treatments are shown in Table X. In this case, the critical level treatment was the same as the control treatment, since the farm manager opted to do nothing. There was a slight decriment in bushels per acre of yield from the critical treatment over the control, but this would be considered a random difference. The TEAM recommendation, however, was able to increase the yield of bushels per acre of wheat by 20% over the control, which represented a substantial increase.

Table X. Yield of Wheat Crops Given Nutrient Supplements Based on TEAM Analysis Versus Standard Farm Management (Critical Levels)

Treatment	*Yield (Bu/A)*[1]	% Difference
Control	103	100
Critical	101	98
TEAM	124	120

[1]Yield in bushels per acre.

Effects of TEAM Recommendations of Amino Acid Chelates on Plants Exibiting Chronic Pathogenicity. In addition to helping increase yields in crops that are either deficient in mineral nutrients or out of balance in nutrients,

Amino Acid Chelates manufactured by Albion Laboratories can also benefit plants that have routine pathological problems. A field of potatoes that was commonly infected with early blight disease was given foliar spraying of Albion Amino Acid Chelates according to the TEAM recommendation(*5*). Early blight characteristics included wilt and death with symptoms commensing in mid-season. The plant tissue analyses indicated that three minerals, zinc, iron, and manganese, were low. The TEAM computer evaluation included three additional elements, sulfur, boron, and copper as being out of balance sufficiently with the first three mentioned to justify taking further corrective action. Most of the field was sprayed with the recommended chelates, but a portion along the fence was set aside as the control portion. The soil had been examined through different parts of the field and was found to have no differences between the field in general and the control area.

At mid-season, plants in the control area showed the effects of early blight disease with its necrosis and wilt. Some deaths were apparent. Plants in the treated area showed improved appearances over previous years. The treated area was able to yield 332 hundred-weight bags per acre while the control area yielded 294 hundred-weight bags per acre. This represented a 13% increase for the plants that were sprayed with Amino Acid Chelates according to the TEAM computer evaluation in comparison to the controls. This was a significant improvement in plants that were normally prone to early blight infection.

Value of TEAM Computer Assisted Recommendations of Amino Acid Chelates. It can readily be seen how crucial a proper balance of available minerals is to high-yielding crops (Tables VII, VIII, IX, and X). Even though most all elements were present within the plant tissue in sufficient quantities according to critical levels, there were important characteristics of balance which were not in alignment and required correction for yield improvement. Often, classical agronomic practice cannot assess the effects of trace mineral insufficiencies until the end of the season, and only then, if classical symptoms of mineral deficiencies become apparent. Plans may then be made to remedy the situation before the next season. However, the crop yields for that particular season are lost as to what they could have been, had the imbalance or deficiency been known in time for corrective actions to be taken. The advantage of TEAM is to allow making that evaluation early in the season. The plant sampling materials can be collected soon after they arise from the ground or emerge from trees or shrubs. The TEAM program can then assess mineral analyses and evaluate the balance on a crop-by-crop basis. This results in a recommendation for administering Albion Laboratories Amino Acid Chelated Minerals at particular concentrations to promote an appropriate balance of minerals in the plant and maximize yields for that particular crop in the same season of growth.

Conclusions

Economic uses of plants can include fruits, stocks, vegetables, fibers, wood, and chemicals. With the exceptions of some chemicals that require stress on normal plant physiology for maximal production, most of the uses of plant crops benefit from maximal health and growth. Requirements for optimal growth include sufficient carbon dioxide, water, light, temperature, and minerals. Carbon dioxide is relatively constant in most all arable lands. Presuming that the crop is grown in an appropriate climate with sufficient water, light and temperature, the only remaining hurdles to surpass in maximizing growth and yields become the issues of mineral balances and adequacies. The use of Albion Laboratories Amino Acid Chelated Minerals represents a highly bioavailable source of minerals that can be utilized by plants for both vegetative growth and fruit production by taking advantage of the rapid acquisition possible through foliar or fruit surface application. This allows the needs of a particular crop to be assessed, prescribed and fulfilled within the same season. The data that have been presented have shown how seemingly healthy plants may also benefit from foliar application of these chelated minerals. While the plants may appear to be healthy, they may not be maximized as to the balance of minerals required for optimal performance and growth. By using the highly bioavailable Amino Acid Chelates, and also taking advantage of the computer mass action rationing capability of the TEAM evaluation report, optimal crop-by-crop performance can be maximized through applying optimal mineral balances for the particular crop.

Literature Cited

1. Hsu, H. H.; Ashmead, H. D. In *Foliar Feeding of Plants with Amino Acid Chelates*; Ashmead, H. D.; Ashmead, H. H.; Miller, G. W.; Hsu, H. H., Eds.; Noyes Publications: Park Ridge, N.J. 1986; pp 273-280.
2. Hsu, H. H.; Graff, D. In *A New Era in Plant Nutrition*; Ashmead, D., Ed.; Albion Laboratories, Inc.: Clearfield, Utah, 1982, pp 37-51.
3. Reyes, N.; Escobar, C. In *A New Era in Plant Nutrition*; Ashmead, D., Ed.; Albion Laboratories, Inc.: Clearfield, Utah, 1982, pp 84-90.
4. Hsu, H. H. In *Foliar Feeding of Plants with Amino Acid Chelates*; Ashmead, H. D.; Ashmead, H. H.; Miller, G. W., Hsu, H. H., Eds.; Noyes Publications: Park Ridge, N.J. 1986; pp 183-200.
5. Moss, E. In *A New Era in Plant Nutrition*; Ashmead, D., Ed.; Albion Laboratories, Inc.: Clearfield, Utah, 1982, pp 109-119.

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The Effects of Different Amino Acid Chelate Foliar Fertilizers on Yield, Fruit Quality, Shoot Growth and Fe, Zn, Cu, Mn Content of Leaves in Williams Pear Cultivar (*Pyrus communis* L.)

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Abstract: In this study, utility opportunities of three different amino acid chelate foliar fertilizers in Williams pear trees (*Pyrus communis* L.) on seedling for reduction in yield, fruit quality and growth resulted from direct irregularities such as yellowing, browning and falling of leaves in early season were investigated. By this aim, the effects of fertilizers applied three times at 15 days of intervals on total yield, yield per trunk cross section unit area, fruits size, firmness, total soluble solids and titretable acidity, shoot length and Fe, Zn, Cu, Mn content of leaves were determined. Especially amino acid chelated–Fe increased total yield by 64% for the third year and 47% as mean, yield per trunk cross section unit area by 64% for the third year and 45%, extra fruit ratio by 75% for the third year and 11%, shoot length by 70% for the third year and 30%, Fe content of leaves by 112% for the third year and 120%, Zn content by 11% for the third year, Cu content by 22% as mean, but decreased Cu content by 4% for the third year, Mn content by 20% for the third year and 22% as mean when compared with control. Thus it was seemed that this fertilizer prevented yellowing, browning and falling of leaves. In the consideration means of three years, the highest Fe (325.5 ppm), Zn (82.9 ppm), Cu (28.4 ppm) and Mn (66.5 ppm) content of leaves was reached by amino acid chelated–Fe, Zn and multi mineral and control, respectively.

Amino Asit Kleyti Farklı Yaprak Gübrelerinin Williams Armudunda (*Pyrus communis* L.) Verim, Meyve Kalitesi, Sürgün Gelişimi ve Yaprakların Fe, Zn, Cu, Mn Kapsamı Üzerine Etkileri

Özet: Bu çalışmada, çöğür anaçlar üzerine aşılı Williams armudunda (*Pyrus communis* L.) erken dönemde yapraklarda sararma, kahverengileşme ve dökülme gibi rahatsızlıkların neden olduğu verim, meyve kalitesi ve gelişmede ortaya çıkan gerilemeye karşı amino asit kleyti üç farklı yaprak gübresinin kullanım olanakları araştırılmıştır. Bu amaçla, 15 gün aralıklarla üç kez uygulanan gübrelerin toplam verim, birim gövde kesit alanına düşen verim, meyve iriliği, meyve eti sertliği, suda eriyebilir toplam kuru madde, titre edilebilir asitlik, sürgün uzunluğu ve yaprakların Fe, Zn, Cu, Mn kapsamı üzerine etkileri belirlenmiştir. Özellikle amino asit kleyti–Fe, kontrol ile karşılaştırıldığında toplam verimi üçüncü yılda %64 ve ortalama %47, birim gövde kesit alanına düşen verimi üçüncü yılda %64 ve ortalama %45, ekstra meyve oranını üçüncü yılda %75 ve ortalama %11, sürgün uzunluğunu üçüncü yılda %70 ve ortalama %30, yaprakların Fe kapsamını üçüncü yılda %112 ve ortalama %120, Zn kapsamını üçüncü yılda %11, Cu kapsamını ortalama %22 artırmış, fakat Cu kapsamını üçüncü yılda %4, Mn kapsamını üçüncü yılda %20 ve ortalama %22 azaltmıştır. Ayrıca bu gübrenin yaprakların sararmasını, kahverengileşmesini ve dökülmesini önlediği gözlenmiştir. Üç yılın ortalaması dikkate alındığında, yapraklarda en yüksek Fe (325.5 ppm), Zn (82.9 ppm), Cu (28.4 ppm) ve Mn (66.5 ppm) kapsamına sırasıyla amino asit kleyti–Fe, –Zn, –multi mineral ve kontrolde ulaşılmıştır.

Introduction

In Williams pear trees (*Pyrus communis* L.), first becoming yellowish in early season, later brownish and falling of leaves on some or all shoots in summer mid have occured due to mineral nutrient deficiency realized by absorption and translocation affairs in tree. Westwood (1) reported that minerals absorption of roots could be prevented by high pH, high calcerous and anaerobic growing conditions and translocation in tree by graft incompatibility and discontinuities in vascular tissue. As a result, healty leaf area on the trees is not enough for photosynthesis, so trees have become partially weaker at

the begining, later completely died. For this reason, supplying of the plant with mineral nutrients effectively is the most important factor.

Micro elements are generally offered the plants by adding to medium or application to leaves. When they are applied as inorganic salts to the growing medium, above pH 6, Fe, and above pH 7 Mn, B, Cu and Zn have become insoluble forms, so their absorption by the plants has decrease. However chelates are obtained by the reaction of metalic salts with their synthetic or natural organic complexes has saved the metal cations from undesirable reactions such as precipitation. For this reason synthetic precursors which have the ability of making strong chelate is almost used in plant growing medium. EDTA (ethylene diaminetetra acetic acid) and EDDHA (ethylene diamin o–hydroxyphenylacetic acid) are well known as synthetic precursors. However because of the disadvantages mentioned above it has been suggested that micro elements as inorganic or organic complexes should be applied to the leaves instead of adding them to the growing medium in order to solve micro element requirements of the plants. The leaf fertilizers which an inorganic mineral structure hardly diffuse from the leaf surface into the plant because of high weight molecular structure. In order to eliminate these negative effects leaf fertilizers with organic structure as synthetic chelates were developed. But some difficulties such as releasing of metals from the chelating precursors and introducing into the plant cell has prevented absorption of micro elements from the plants. On the other hand, foliar fertilizers as chelate should be easily absorbed by the plants, rapidly transported and should be easily release their ions to affect the plant. Natural chelators as mid molecular weight compounds (like humic and fulvic acid, amino acids, polyflavanoids that have long organic chains) and low molecular weight compounds (like citric acid, ascorbic acid, tartaric acid that have short organic chains) diffuse easily to cell cytoplasm according to their chemical structure. These chelators are not phytotoxic to plants. They make complexes especially with heavy metals and prevent them to uptake by plants in higher ratio (1–5).

The aim of this research is to determine the effects of amino acid chelated–Fe, –Zn and –multi mineral foliar fertilizers on Fe, Zn, Cu and Mn content of leaves, shoot length, yield and fruit quality of Williams pear trees which have irregularities such as yellowing, browning and falling of leaves in early season.

Materials and Methods

This research was carried out between 1992–1994 on Williams pear trees (*Pyrus communis* L.) on seedling which are approximately 40 years old grown in Ankara conditions. Three different foliar fertilizers (Table 1), amino acid chelated–Fe, –Zn and – multi mineral (Kemito Inc.) were sprayed three times at 15 days intervals, first application was carried out a month after bud burst, during three year.

Table 1. Mineral content of amino acid chelated–Fe, –Zn and –multi mineral foliar fertilizers.

	Amino acid chelate foliar fertilizers and contents (g/kg)		
Micro Elements	Chelated–Fe	Chelated–Zn	Chelated–Multi Mineral
Fe	42.0	–	10.0
Zn	–	42.0	9.0
Mn	–	–	6.5
Cu	–	–	4.5
S	–	–	3.0
B	–	–	0.2
Co	–	–	0.05
Mo	–	–	0.01
Ni	–	–	0.005
Se	–	–	0.0005
Macro Elements			
N	80.0	80.0	80.0
Mg	–	–	7.5
Ca	–	–	1.0

In the first year while 0.2% concentration for the first and second applications, 0.4% concentration for the third application were used, 0.4% concentration was applied in the other years. Fertilizer solutions were sprayed as 10 liter per tree.

In this research Fe, Zn, Cu, Mn levels of leaves (ppm), total yield (kg), yield per trunk cross section unit area (kg/cm^2), distribution of fruit into the size classes (%), fruit firmness (lb), total soluble solids (%), titratable acidity (g/l) and shoot length (cm) parameters were investigated.

Leaves collected just before the first, second and the third applications from the trees were wet ashed with HNO_3±$HClO_4$ solution and micro element compositions were determined by atomic absorption spectrofotometer (6). Total yield was determined by weighting all fruits of each tree. Trunk cross section unit area was calculated by measuring of trunk circumference of tree at 15 cm above of grafting point and yield per trunk cross section unit area was determined by dividing of yield to trunk cross section area. All harvested fruits were sized based on their diameters into four classes such as extra (>6.0 cm), class I. (5.5–6.0 cm), class II. (5.4–5.0 cm) and discard (<5.0 cm) and calculated in total fruit amount and percentage of each class. Fruit firmness were measured by pressure tester had a plunger with 7.8 mm in diameter on ten fruit sample for three replicate. Total soluble solids were determined with hand refractometer as three times for each replicate and ten milliliters of fruit juice was titrated with 0.1 N NaOH to a malic acid endpoint of pH 8.2 for titratable acidity measurements. The lengths of ten shoots of each replication were measured and mean shoot length was calculated as arithmetical.

In this research, a randomized plots experiment design was used with five replications. 'Treatment x year' interaction was controlled by analysis of variance by means SAS and Minitab and mean comparisons were performed by Duncan's multiple range test at P<0.05 where appropriate.

Results and Discussion

Yield and Fruit Quality

Total yield was found higher in amino acid chelated–Fe and in other applications as compared to the control in all years (Table 2). But differences were not found statistically significant. In the first year, total yield was found as 136.4 kg in amino acid chelated–Fe and as 136.3 kg in amino acid chelated–multi mineral foliar fertilizer. These values are 35% higher than control. The highest total yield as 79.0 kg was also obtained amino acid chelated–Fe in the second year. This value is 45% higher than control. Total yield was determined as 60.3 and 62.2 kg in amino acid chelated–Zn and –multi mineral, respectively. In the third year, amino acid chelated–Fe, –multi mineral and –Zn being 128.0, 105.0 and 83.8 kg increased total yield at 64, 34 and 7%, respectively, as compared to the control (Table 2).

Table 2. The effect of amino acid chelate foliar fertilizers on the yield.

	Total Yield (kg/tree)							
Treatments	1992	%	1993	%	1994	%	Mean	%
Control	100.7	100	54.4	100	78.1	100	77.7	100
Chelated–Fe	136.4	135	79.0	145	128.0	164	114.5	147
Chelated–Zn	115.2	114	60.3	111	83.8	107	86.4	111
Chelated–Multi Mineral	136.3	135	62.2	114	105.0	134	101.1	130
Mean	122.1a	121	64.0c	118	98.7b	126		
LSD (P<0.05)	15.9							
	Yield per Trunk Cross Section Unit Area (kg/cm^2)							
Control	0.25	100	0.14	100	0.19	100	0.19	100
Chelated–Fe	0.33	134	0.19	141	0.31	164	0.28	145
Chelated–Zn	0.35	140	0.18	131	0.25	135	0.26	136
Chelated–Multi Mineral	0.34	136	0.15	113	0.25	134	0.25	130
Mean	0.32a	128	0.16c	114	0.23b	121		
LSD (P<0.05)	0.04							

Pehlivan (7) reported that 0.4% amino acid chelated–multi mineral foliar fertilizer increased the yield 39% in Starkspur Golden Delicious apple. But increase was not found statistically significant. Shazly (8) reported that Rakbeh et al. found amino acid chelated–multi mineral and Zn metalosote increased the yield 54% more than control in orange and mandarins. Shazly (8) determined that Zn metalosote and multimineral metalosote increased the yields 79 and 18%, respectively. According to Table 2, in consideration of mean values of three years, amino acid chelated–Fe resulted 47%, –multi mineral 30% and –Zn 11% higher yields than control, being 114.5, 101.1 and 86.4 kg, respectively. But statistically significant differences were not found among these means. Statistically important differences realized among means of years. Total yield in the first year was higher than others as 122.1 kg (Table 2).

Differences in yield per trunk cross section unit area for all treatments were not statistically significant. Furthermore, yield was higher in all treatments than control. In the first year, amino acid chelated–Zn gave better result as 0.35 kg/cm^2 than –multi mineral (0.34 kg/cm^2), –Fe (0.33 kg/cm^2) and control (0.25 kg/cm^2).

Table 3. The effect of amino acid chelate foliar fertilizers on fruit ratio in size classes.

		Fruit Ratio (%)			
Treatments	Years	Extra	Class I.	Class II.	Discard
Control	1992	44.9a* ab**	33.9	14.2b ab	7.0a
	1993	52.5a a	36.9	7.9a b	2.7a b
	1994	29.6bc b	36.8	22.4ab a	11.2a a
	Mean	42.3	35.9	14.8	7.0
Chelated–Fe	1992	32.5ab b	37.2	20.7ab a	9.6a a
	1993	57.0a a	36.4	5.9a b	0.7a b
	1994	51.8a ab	34.2	11.9b ab	2.1b b
	Mean	47.1	35.9	12.8	4.1
Chelated–Zn	1992	24.2ab b	45.6	22.5ab a	7.7a a
	1993	50.7a a	41.6	5.9a b	1.8a b
	1994	44.6ab ab	34.8	12.7b ab	7.9a a
	Mean	39.8	40.7	13.7	5.8
Chelated–Multi Mineral	1992	19.4b b	39.0	30.9a a	10.7a a
	1993	61.5a a	32.4	5.5a b	0.6a b
	1994	20.3c b	39.1	27.9a a	12.7a a
	Mean	33.7	36.8	21.4	8.0
LSD (P<0.05)		12.8	NS	12.8	12.8

* Differences in treatments for each year.

** Differences in years for each treatment.

Otherwise, amino acid chelated–Fe gave higher results than others as 0.19 and 0.31 kg/cm^2, in the second and third year, respectively. When the means of three years were compared, yield per trunk cross section unit area were 45, 36 and 30% higher in amino acid chelated–Fe, –Zn and –multi mineral, respectively, than the control. The mean as 0.32 kg/cm^2 in the first year was statistically differ than that of other years (Table 2).

Differences in extra fruit rates were statistically significant. In the first year, the highest extra fruit rate was obtained in control (44.9%), amino acid chelated–Fe (32.5%) and –Zn (24.2%). Amino acid chelated–multi mineral provided the lowest extra fruit ratio as 19.4% (Table 3).

On the other hand Pehlivan (7) found that amino acid chelated–multi mineral treated in two times at 0.2% concentration without basal fertilizer not significantly increased the extra fruits as 74.5%, and single treatment

Table 4. The effect of amino acid chelate foliar fertilizers on the fruit firmness, total soluble solids and titratable acidity.

Treatments	Firmness (lb)			
	1992	1993	1994	Mean
Control	15.9	14.0	14.9	14.9
Chelated–Fe	16.0	15.5	15.0	15.5
Chelated–Zn	15.7	14.8	14.6	15.0
Chelated–Multi Mineral	16.3	14.9	15.1	15.4
Mean	16.0a*	14.8b	14.9b	
LSD (P<0.05)		0.7		
	Total Soluble Solids (%)			
Control	11.5	12.2	11.8	11.8
Chelated–Fe	10.8	12.4	11.6	11.6
Chelated–Zn	12.1	12.0	12.0	12.0
Chelated–Multi Mineral	11.2	12.0	11.8	11.7
Mean	11.4b	12.1a	11.8ab	
LSD (P<0.05)		0.5		
	Titratable Acidity (g/l)			
Control	3.6	2.7	3.2	3.2
Chelated–Fe	3.5	3.0	3.2	3.2
Chelated–Zn	3.8	2.9	3.5	3.4
Chelated–Multi Mineral	4.0	3.0	3.3	3.4
Mean	3.7a	2.9c	3.3b	
LSD (P<0.05)		0.2		

* Differences among the years.

at 0.4% concentration caused 68.3% increases when compared with control in Strakspur Golden Delicious apple at the result of one year treatment. In current research, in the second year extra fruit ratio was high in all treatments. However in the third year, effects of amino acid chelated–Fe (51.8%) and –Zn (44.6%) on the extra fruit ratio were statistically important (Table 3).

Extra fruit ratios in amino acid chelated–Fe and –Zn were significantly higher in the last two years than the first. Differences among treatments were not statistically significant in class I. Amino acid chelated–Fe and –Zn caused decreasing in fruit ratio in the class II in the third year. Discard fruit ratio was decreased by especially amino acid chelated–Fe in the last year (Table 3).

Differences in fruit firmness were not statistically significant among treatments for each year. Differences among means of years were significant (Table 4).

The mean in 1992 as 16.0 lb was significantly higher than in 1993 as 14.8 lb and 1994 as 14.9 lb. In the total soluble solid, statistical differences were occurred only among the years. It was higher in 1993 as 12.1% and 1994 as 11.8% than 1992. Similarly differences in titratable acidity were significant only among years and titratable acidity was higher in the first year than others (Table 4).

Shoot Length

Amino acid chelated–Fe significantly increased mean shoot length as 32.71 cm for means of three years. The mean shoot length at 30% higher than control (25.15 cm) and amino acid chelated–Zn 25.09 cm and 27% higher than amino acid chelated–multi mineral (Table 5).

Pehlivan (7) reported that amino acid chelated–multi mineral at 0.2% concentration without the basal fertilizer did not increased shoot length with respect to control. In current research, shoot length was significantly lowest in the third year (Table 5).

Fe, Zn, Cu, Mn Content of Leaves

Differences in Fe content were statistically significant among treatments for each year and among years for each treatment. Fe content was significantly higher in amino acid chelated–Fe in the first as 301.8 ppm and the second year as 335.8 ppm. In the third year, foliar fertilizers significantly increased Fe content in leaves (Table 6).

According to means of years, the highest Fe content as 325.5 ppm was provided by amino acid chelated–Fe. Differences among years for each treatment were not statistically significant with the exception of amino acid chelated–Zn. Amino acid chelated–Zn significantly increased Zn content of leaves in all years. Differences among years was found statistically significant with the exception of control and the highest values were reached by the third year (Table 6).

Differences among treatments were not statistically significant. Cu content was significantly higher in the third year with 35.5 ppm than other years (Table 7).

Table 5. The effect of amino acid chelate foliar fertilizers on the shoot length.

	Shoot Length (cm)							
Treatments	1992	%	1993	%	1994	%	Mean	%
Control	29.74	100	33.28	100	12.42	100	25.15b*	100
Chelated–Fe	39.02	133	37.96	114	21.16	170	32.71a	130
Chelated–Zn	28.46	96	28.34	85	18.46	149	25.09b	100
Chelated–Multi Mineral	25.60	87	34.52	104	17.22	139	25.78b	103
Mean	30.70a**	103	33.53a	101	17.32b	139		
LSD (P<0.05)	6.20**							
LSD (P<0.05)	6.13**							

* Differences among the treatments based on means in years.

** Differences among the years based on means in treatments.

In the first year, Mn content of leaves was between 41.4 and 61.8 ppm, but differences in means were not found significantly. In the second year, Mn content was significantly higher in amino acid chelated–multi mineral as 66.4 ppm, in control as 48.6 ppm and amino acid chelated–Zn as 47.0 ppm than amino acid chelated–Fe as 42.4 ppm.

In the third year, Mn content of all treatments increased and control as 89.0 ppm, amino acid chelated–Fe as 71.6 ppm and amino acid chelated–multi mineral as 69.2 ppm were significantly higher than amino acid chelated–Zn as 62.2 ppm. Differences among the years were statistically significant with the exception of amino acid chelated–Zn and Mn content highly increased in control, amino acid chelated–Fe in the last year (Table 7).

As a result of this research, firstly Fe content of leaves and shoot length followed by yield and fruit quality were improved in amino acid chelated–Fe and so irregularities such as yellowing, browning and falling of leaves in early season were seemed to highly correct. The use of amino acid chelated–Fe is worthy of further consideration because of its beneficial effect on especially Fe nutrition.

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Table 6. The effect of amino acid chelate foliar fertilizers on Fe and Zn content of leaves before the third application.

	Fe (ppm)							
Treatments	1992	%	1993	%	1994	%	Mean	%
Control	132.0b* a**	100	152.0b a	100	160.0b a	100	148.0	100
Chelated–Fe	301.8a a	229	335.8a a	221	338.8a a	212	325.5	220
Chelated–Zn	125.4b b	95	136.8b b	90	346.6a a	217	202.9	137
Chelated–Multi Mineral	167.0b a	126	187.6b a	123	248.4ab a	155	201.0	136
Mean	181.5	137	203.0	133	273.4	171		
LSD (P<0.05)	95.7							
	Zn (ppm)							
Control	31.8b* a**	100	32.4b a	100	43.2c a	100	35.8	100
Chelated–Fe	32.6b b	102	27.0b b	83	48.0bc a	111	35.9	100
Chelated–Zn	61.2a c	192	78.2a b	241	109.2a a	253	82.9	232
Chelated–Multi Mineral	42.8b b	135	40.4b b	125	59.6b a	138	47.6	133
Mean	42.1	132	44.5	137	65.0	150		
LSD (P<0.05)	15.3							

* Differences among treatments for each year.

** Differences among years for each treatment.

Table 7. The effect of amino acid chelate foliar fertilizers on the Cu and Mn content of leaves before the third application.

	Cu (ppm)							
Treatments	1992	%	1993	%	1994	%	Mean	%
Control	14.8	100	14.8	100	35.6	100	21.7	100
Chelated–Fe	20.4	138	24.8	168	34.2	96	26.5	122
Chelated–Zn	12.0	81	14.0	95	28.4	80	18.1	83
Chelated–Multi Mineral	22.0	149	19.4	131	43.8	123	28.4	131
Mean	17.3b	117	18.2b	123	35.5a	100		
LSD (P<0.05)				13.3				
	Mn (ppm)							
Control	61.8a* b**	100	48.6ab c	100	89.0a a	100	66.5	100
Chelated–Fe	41.4a b	67	42.4b b	87	71.6ab a	80	51.8	78
Chelated–Zn	52.4a a	85	47.0ab a	97	62.2b a	70	53.9	81
Chelated–Multi Mineral	47.4a b	77	66.4a ab	137	69.2ab a	78	61.0	92
Mean	50.7	82	51.1	105	73.0	82		
LSD (P<0.05)				19.1				

* Differences among treatments for each year.

** Differences among years for each treatment.

References

1. Westwood, M.N., Temperate–Zone Pomology Physiology and Culture. Third Edition. Timber Press Portland, Oregon, p. 523, 1993.
2. Ashmead, H., World Nutritional Crisis in Agriculture, Foliar Feeding of Plants with Amino Acid Chelates. Albion Laboratories Inc., Clearfield, Utah, p. 1–9, 1986.
3. Graff, D.J., Radioactive Isotope Studies in Plants. Weber College, 1986.
4. Hsu, H.H., Nutrient Balance and Crop Yield, Foliar Feeding of Plants with Amino Acid Chelates. Albion Laboratories Inc., Clearfield, Utah, p. 183–198, 1986a.
5. Hsu, H.H., Chelates in Plant Nutrition, Foliar Feeding of Plants with Amino Acid Chelates. Albion Laboratories Inc., Clearfield, Utah, p. 209–217, 1986b.
6. Chapman, H.D. and Praff, P.F., Methods of Analyses for Soils, Plants and Waters. Univ. of California, Division of Agricultural Series, California, 1961.
7. Pehlivan, G., Yaprak Gübresi Uygulamalarının Elmada (*Malus domestica* Borch. cv. Starkspur Golden Delicious) Meyve Verim ve Kalitesi ile Sürgün Gelişimi Üzerine Etkileri. Ankara Üniversitesi, Fen Bilimleri Enstitüsü Yüksek Lisans Tezi (Basılmamış), Ankara, p. 53, 1994.
8. Shalazy, S.A., The Effects of Amino Acid Chelated Mineral Deficiencies and Increasing Fruit Production in Trees in Egypt, Foliar Feeding of Plants with Amino Acid Chelates. Albion Laboratories Inc., Clearfield, Utah, p. 289–299, 1986.

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ORIGINAL ARTICLE

Effect of Magnesium Fertilizer Sources and Rates on Yield and Fruit Quality of Date Palm cv. Hayany under Ras-Sudr Conditions

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ABSTRACT

A two years study was carried out during two successive seasons of 2012 and 2013 in a private orchard of "Hayany" date palm grown in sandy soil under drip irrigation system from a well at Ras-Sudr city, South Sinai Governorate, Egypt. Three levels (20, 30 and 40 g Mg / palm) of two magnesium fertilizer forms namely magnesium chelate (Mg EDTA, 12.5% Mg) and magnesium sulphate (9.9% Mg) were added at three equal doses as soil application, three times a year i.e. February 1st , May 1st and July 1st. Briefly, all tested treatments enhanced leaf total chlorophyll content, fruit set percentage, retained fruit percentage, yield, fruit physical and chemical properties and leaf minerals content. Magnesium chelate form at 40 g Mg/palm showed superiority than magnesium sulphate form in this respect.

Key words: **D**ate palm -Fruit quality – Hayany cv. -Magnesium fertilizer - Yield.

Introduction

Date palm (*Phoenix dactylifera*, L.) is the most important crop in Egypt. Date palm plays an important role in the economical and social life of the people in Egypt. It considered a symbol of life in desert. It can grow and produce under different types of soil from light sandy to heavy clay soil. Also, it has high adaptability to stress conditions as it tolerates high levels of salinity, drought and harsh weather (Diallo, 2005). It is more salt tolerate than any other fruit crops (FAO, 1982 and Lunde, 1978). "Hayany" cultivar is one of the most economically important cultivar of soft dates in Egypt.

In Egypt, fertilization program for almost crops does not included magnesium as a major element. In addition, there are very little attentions have been paid towards magnesium nutrient element for date palm nutrition and recommendations which lead to enhance vegetative growth and productivity, especially for those grown in sandy soil under drip irrigation system.

Magnesium is an essential element for chlorophyll molecule structure that regulates photosynthesis process. Also, it acts an activator of many enzyme systems involved in carbohydrate metabolism and synthesis of nucleic acids. Furthermore, it plays an essential role in the biological activity of ATP (Westwood, 1978 and Jones *et al.,* 1991). Concerning the action effect of the tested treatments on date palm are somewhat rare than upon the review are supported with other species rather than date palm in this respect.

In addition, new reclaimed soils are poor in their nutrient content including magnesium element. Many investigators have been started to study magnesium nutrition and determination of magnesium needs of economically important crops in Egypt (FAO 2000; Salem 2007 and El-Fouly *et al.*, 2012). Generally, the influence of magnesium on yield and fruit quality was reported by El-Safty and Rabii, 1998 on Washington navel orange tree and Abou Aziz *et al.,* 2000, on banana plants. They mentioned that magnesium fertilization improved yield and fruit quality of the abovementioned fruit species. Moreover, Mostafa *et al.,* 2007, indicated that fertilizing "Grand Naine" banana with 100 g/plant magnesium chelate as soil application plus foliar spray of 2% magnesium sulphate improved growth parameters, yield and fruit quality. In this respect, Fawzi *et al.,* 2010 stated that fertilizing Le Conte pear trees with compost 45k/ tree plus biofertlizers 20 g/ tree plus 1.5% magnesium sulphate gave the best results regarding yield and fruit quality. Also, Hanafy Ahmed *et al.,* 2012, found that foliar application of Mg (137.5 ppm), Cu (97 ppm) and growth regulators (20 ppm 2, 4-D, 30 ppm GA3 or 10 ppm BA) improved growth characters and yield of Washington Navel orange trees.

The aim of this study is to evaluate the effect of magnesium soil applications sources and rates on leaf total chlorophyll content, fruit set %, retained fruit percentage, yield, fruit quality and leaf mineral content of "Hayany" date palm grown in sandy soil under drip irrigation system at Ras- Sudr conditions.

Material and Methods

This study was carried out during two successive seasons of 2012 and 2013 in a private orchard, at Ras-Sudr city, South Sinai Governorate, Egypt. "Hayany" date palm trees of eight years old grown in sandy soil, and spaced 7x7m apart under drip irrigation system from a well were devoted for this study. Physical and chemical

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analyses of the experimental soil shown in Table 1. Meanwhile, the chemical analysis of the used water for irrigation is recorded in Table 2.

Table 1: Analysis of the experimental soil at Ras-Sudr, South Sinai Governorate, Egypt.

Soil depth (cm)	Texture class	pH soil past	E.Ce (dSm^{-1})	Organic matter %	Soluble cations (mequiv./l)				Soluble anions (mequiv./l)			
					Ca^{++}	K^{+}	Na^{+}	Mg^{++}	Cl^{-}	$So_4^{=}$	HCo_3^{-}	$Co_3^{=}$
0-30	Sand	7.28	9.1	0.53	16.2	1.3	50.4	23.1	54.5	33.9	2.5	--
30-60	Sand	7.16	8.6	0.55	15.3	1.23	47.7	21.9	51.5	32.1	2.4	--

Table 2: Chemical analysis of water used for irrigation at the experimental orchard, at Ras-Sudr, South Sinai Governorate, Egypt.

pH	EC (dSm-1)	Soluble cations (me/l)				soluble anions (me/l)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$Co_3^{=}$	HCo_3^{-}	Cl^{-}	$So_4^{=}$
7.43	8.1	14.4	20.6	44.9	1.16	--	2.3	48.5	30.2

Forty two female palms trees of healthy, nearly uniform in shape, size and productivity, received the same horticulture practices were treated with three levels of magnesium as soil application in two forms namely; magnesium chelate (Mg EDTA 12.5% Mg) and magnesium sulphate ($MgSO_4$ 9.9% Mg). Hayany date palm was subjected to seven treatments as follows:

1- Control without Mg fertilization.
2- 20 g Mg /palm (160 g magnesium chelate 12.5% Mg).
3- 30 g Mg /palm (240 g magnesium chelate 12.5% Mg).
4- 40 g Mg/palm (320 g magnesium chelate 12.5% Mg).
5- 20 g Mg /palm (202 g magnesium sulphate 9.9% Mg).
6- 30 g Mg /palm (303 g magnesium sulphate 9.9% Mg).
7- 40 g Mg/palm (404 g magnesium sulphate 9.9% Mg).

The experiment was designed as randomized complete block design with three replicates for each treatment and each replicate was represented by two palms.

Soil application of magnesium fertilizer rates was divided into equal three doses applied three times a year i.e. February, 1st, May, 1st and July, 1st in each season.

The ordinary fertilization program was 25 kg/palm of sheep manure added in December, 1.5 kg/palm of triple calcium super phosphate (45% P_2O_5) broadcasted on the soil surface through the whole area during December and 5 kg ammonium sulphate/palm (20.5% N) divided into equal three doses applied three times a year i.e. march, May, and July.

Response of "Hayany" date palms trees to the tested three levels of magnesium EDTA and magnesium sulphate fertilizers were evaluated through the following determinations.

Leaf total chlorophyll content:

Leaf total chlorophyll content was determined by Minolta chlorophyll meter SPAD-502.

Fruit set percentage:

Number of nodes and set fruits in twenty five strands per palm were recorded after 4 weeks of pollination. The percentage of fruit set was calculated using the following formula:

$$\text{The percentage of fruit set} = \frac{\text{Total number of set fruit per strand}}{\text{Total number of nods per strand}} \times 100$$

Retained fruit percentage:

The retained fruit percentage was calculated at the harvest time September 1st according to Soliman and El Kosary (2002) formula as follows:

$$\text{The retained fruit percentage} = \frac{\text{Total number of retained fruits per bunch}}{\text{Total number of the nodes per bunch}} \times 100$$

Yield (kg/palm tree):

In both seasons, dates were harvested at September 1st when fruits reached Khalal stage and the average fruit yield and bunch weight was recorded in Kilograms.

Fruit physical and chemical properties:

Forty fruits were taken at harvest from each treated palm tree at Khalal stage (full mature, crunchy and red in color) from each bunch per palm to determine the following physical and chemical properties i. e. fruit weight (g), fruit volume (cm^3), fruit length (cm), fruit diameter (cm), pulp dry matter (%), seed weight (g), total soluble solids (T.S.S.) was determined by Hand refractometer. Percentage of total acidity as g citric acid / 100 g f.wt., T.S.S./Acid ratio and total sugars (%) were determined according to A.O.A.C. (1995).

Leaf mineral content:

To determine leaf mineral content (N, P, K, Ca and Mg), leaf samples were taken during November and washed with tap water then with distilled water to remove the dust. After washing, they were dried in an electric oven at 70ºc for 72 hours. The dried leaves were ground, digested and prepared for analysis using the method described by Parkinson and Allen (1975). Total nitrogen was determined by the semi-micro kjeldahl methods Bremner (1965). Phosphorus was estimated by the method Chapman and Pratt (1961). Potassium was determined by the flame-photometer according to Jackson (1958). Calcium and magnesium were determined by titration against versente solution (Na EDTA) according to (Chapman and Pratt, 1961).

Statistical analysis:

The obtained data in 2012 and 2013 seasons were subjected to analysis of variance according to Clarke and Kempson (1997). Means were differentiated using multiple Range test at the 0.05 level (Duncan, 1955).

Results and Discussion

Leaf total chlorophyll content:

All tested treatments succeeded in increasing leaf total chlorophyll content as compared with the control treatment in both seasons of study, (Table 3). However, magnesium chelate form gave higher positive effect than magnesium sulphate form. Generally, 40 and 30g Mg/palm in the form of magnesium chelate took nearly the same trend and induced the highest leaf total chlorophyll content as compared with the control treatment and other tested treatment in both seasons.

The enhancement effect of magnesium on leaf total chlorophyll content may be attributed to the fact that magnesium is an essential element for chlorophyll molecule structure that regulates photosynthesis (Jones *et al.*, 1991; Purohit, 2007 and Spiegel – Ray and Goldschmidt, 2007). Also, the increase in the amount of magnesium application leads to an increase in leaf total chlorophyll content and hence photosynthesis level was increased (Bybordi and Shabanov, 2010).

The enhancement effect of magnesium chelate than magnesium sulphate on leaf total chlorophyll content may be due to that magnesium chelate remains in a soluble form and easy for plant uptake. However, the chelate form was more pronounced than sulphate form of "Grand Naine" banana (Mostafa *et al.*, 2007).

The obtained results of magnesium fertilizer regarding their positive effect on leaf total chlorophyll content are in harmony with the findings of Abou El-Khashab (2002) on olive seedling; Mostafa *et al.* (2007) on banana; Bybordi and Shabanov (2010) on grape; Fawzi *et al.* (2010) on Le Conte pear; Hanafy Ahmed *et al.* (2012) on Washington navel orange. They mentioned that magnesium fertilizer improved leaf chlorophyll content of the aforementioned fruit species.

Fruit set (%):

Table 3, indicates that fertilizing "Hayany" date palm with magnesium chelate and magnesium sulphate produced higher positive effect on fruit set percentage as compared with the control treatment in both seasons of this study. Moreover, chelate form of magnesium fertilization gave higher positive effect than sulphate. Generally, 40 and 30g Mg/palm in the form of magnesium chelate gave similar and high positive effect on fruit set percentage as compared with the control treatment in both seasons of this study.

Retained fruit (%):

Fertilizing "Hayany" date palm with magnesium chelate and magnesium sulphate succeeded in enhancing retained fruit percentage as compared with the control treatment in both seasons of study, (Table 3). However, magnesium chelate treatments surpassed the corresponding ones of magnesium sulphate treatments in enhancing the retained fruit percentage "Hayany" date palm trees in both seasons. Moreover, 40g Mg/palm and 30g

Mg/palm in the form of magnesium chelate took nearly the same trend and proved to be the superior treatment in this respect.

Yield (kg):

Table 3, illustrates that magnesium chelate and magnesium sulphate treatments succeeded in improving yield (kg) / palm as compared with the control treatment in both seasons. Moreover, chelate form of magnesium fertilization gave higher positive effect than sulphate form. Generally, 40g Mg/palm and 30g Mg/palm in the form of magnesium chelate induced the highest productive effect without significant differences between them in 2012 and 2013 seasons.

Table 3: Effect of magnesium chelate and magnesium sulphate rates as soil application on leaf total chlorophyll content, fruit set (%), retained fruit (%) and yield of "Hayany" date palms (2012 &2013 seasons).

Treatments	Leaf total chlorophyll content		Fruit set (%)		Retained fruit (%)		Yield (kg/palm)	
	2012	2013	2012	2013	2012	2013	2012	2013
Control " untreated"	56.26 e	57.86 e	79.1 d	79.7 e	30.4 d	31.3 c	52.1 d	51.6 d
20g Mg/palm (as Mg EDTA)	73.53 b	74.43 bc	81.8 ab	81.6 b	32.7 bc	33.0 b	56.7 b	58.1 c
30g Mg/palm (as Mg EDTA)	80.13 a	88.36 a	82.2 a	82.3 a	34.6 a	34.8 a	63.6 a	67.1 a
40g Mg/palm (as Mg EDTA)	82.10 a	91.00 a	82.8 a	82.7 a	34.7 a	34.9 a	65.6 a	68.4 a
20g Mg/palm (as $MgSO_4$)	63.83 d	64.76 d	80.3 c	80.4 d	31.1 cd	32.6 b	54.3 cd	53.9 d
30g Mg/palm (as $MgSO_4$)	68.53 c	71.43 c	80.8 bc	81.0 cd	32.3 bc	33.5 b	58.2 b	59.6 bc
40g Mg/palm (as $MgSO_4$)	72.20 bc	76.23 b	81.8 ab	81.5 bc	33.1 ab	34.8 a	59.4 b	62.3 b

Means within each column followed by the same letter (s) are not significantly different at 5% level.

Bunch weight (kg):

It is clear from Table 4, that magnesium chelate and magnesium sulphate treatments produced higher bunch weight than the control treatment in both seasons of study. Anyhow, 40g Mg/palm and 30g Mg/palm in the form of magnesium chelate showed superiority in this respect.

The enhancement effect of magnesium fertilizer on fruit set percentage, retained fruit percentage, yield and bunch weight may be due to the important role of magnesium on chlorophyll molecule structure that regulates photosynthesis (Jones *et al.*, 1991; Purohit, 2007; Spiegel – Ray and Goldschmidt, 2007). Also, the increase in the amount of magnesium application leads to an increase in leaf total chlorophyll content and consequently photosynthesis level was increased (Bybordi and Shabanov, 2010). So that, the enhancement effect on chlorophyll was reflected in more carbohydrates production through photosynthesis process and increasing vegetative growth and consequently improved fruit set percentage, retained fruit percentage, yield and bunch weight.

The enhancement effect of magnesium chelate than magnesium sulphate fruit set percentage, retained fruit percentage, yield and bunch weight may be due to that magnesium chelate remains in a soluble form and easy for plant uptake. However, the chelate form was more pronounced than sulphate form of "Grand Naine" banana (Mostafa *et al.*, 2007).

The obtained results regarding the effect of magnesium fertilizer on fruit set percentage, retained fruit percentage, yield and bunch weight in line with the findings of Abou Aziz *et al.* (2000) on banana; Abd El-Moniem *et al.* (2002) on Washington navel orange; El-Seginy *et al.* (2003) on Anna apple; Elham Dawood and Shahin (2006) on Canino apricot; Mostafa *et al.* (2007) on banana; Bybordi and Shabanov (2010) on grape; Fawzi *et al.* (2010) on Le Conte pear; Hanafy Ahmed *et al.* (2012) on Washington navel orange.

Fruit physical and chemical properties:

Fruit weight (g):

Table 4, demonstrates that magnesium chelate and magnesium sulphate treatments succeeded in improving fruit weight in both seasons as compared with the control treatment. Moreover, chelate form of magnesium fertilization induced higher positive effect than sulphate form magnesium fertilization. Generally, 40g Mg/palm magnesium chelate treatment gave the highest fruit weight (13.5 and 14.5 g) against (9.8 and 9.2 g) for the control treatment in both seasons, respectively.

Fruit volume (cm^3):

Table 4, shows that magnesium chelate and magnesium sulphate forms produced similar and higher positive effect on fruit volume of "Hayany" date palm as compared with the control treatment in both seasons of

study. Generally, magnesium chelate treatments induced high positive effect on fruit volume, especially at 40g Mg/palm as compared with the control treatment in both seasons of study.

Fruit length (cm):

Table 4, indicates that magnesium chelate and magnesium sulphate treatments produced higher positive effect on fruit length as compared with the control treatment of "Hayany" date palm in both seasons of study. Generally, 40 and 30g Mg/palm in the form of magnesium chelate took nearly the same trend and induced the highest fruit length values as compared with the control treatment in both seasons.

Table 4: Effect of magnesium chelate and magnesium sulphate rates as soil application on bunch weight and some fruit physical properties of "Hayany" date palms (2012 &2013 seasons).

Treatments	bunch weight (kg)		Fruit weight (g)		Fruit volume(cm^3)		Fruit length (cm)	
	2012	2013	2012	2013	2012	2013	2012	2013
Control " untreated"	17.4 d	17.2 d	9.8 d	9.2 d	10.2 e	10.2 d	3.4 b	3.4 e
20g Mg/palm (as Mg EDTA)	18.9 bc	19.4 b	10.9 bc	10.9 c	11.2 cde	11.3 c	3.7 ab	3.8 cd
30g Mg/palm (as Mg EDTA)	21.2 a	22.4 a	12.9 a	14.2 a	13.3 ab	14.7 a	3.9 a	4.2 a
40g Mg/palm (as Mg EDTA)	21.9 a	22.8 a	13.5 a	14.5 a	13.9 a	14.8 a	3.9 a	4.2 a
20g Mg/palm (as $MgSO_4$)	18.1 cd	18.0 d	10.5 cd	9.8 d	11.0 de	10.2 d	3.6 b	3.6 de
30g Mg/palm (as $MgSO_4$)	19.4 b	19.9 bc	11.5 b	12.0 bc	12.0 cd	12.5 b	3.6 b	3.9 bc
40g Mg/palm (as $MgSO_4$)	19.8 b	20.8 b	11.7 b	12.6 b	12.3 bc	13.0 b	3.9 ab	4.1 ab

Means within each column followed by the same letter (s) are not significantly different at 5% level.

Fruit diameter (cm):

Table 5, demonstrates the magnesium chelate and magnesium sulphate treatments succeeded in improving fruit diameter (excepted 20g Mg/palm in the form of magnesium sulphate) as compared with the control treatment in both seasons of study. Generally, 40g Mg/palm in magnesium chelate form proved to be the superior treatment in this respect.

Pulp weight (g):

Table 5, indicates that fertilizing "Hayany" date palm with magnesium chelate and magnesium sulphate produced a similar and higher positive effect on pulp weight as compared with the control treatment in both seasons of this study. Generally, 40g and 30g Mg/palm in the form of magnesium chelate gave a high positive effect on pulp weight as compared with the control treatment in both seasons of this study.

Pulp dry matter (%):

Table 5, demonstrates that the tested treatments produced similar and higher positive effect on pulp dry matter percentage as compares with the control treatment. Moreover, chelate form of magnesium fertilization gave higher positive effect than sulphate form. Generally, 40g and 30g Mg/palm in the form of magnesium chelate gave higher positive effect on pulp dry matter percentage, especially 40 g Mg/palm magnesium chelate treatment as compared with the control treatment in both seasons of study.

Seed weight (g):

Table 5, shows that the two forms of magnesium fertilizers i.e. chelate and sulphate produced similar and higher positive effect on seed weight of "Hayany" date palm as compared with the control treatment in both seasons of study. Generally, the three rates of magnesium chelate form induced higher positive effect on seed weight, especially 40 g Mg/palm gave a high positive effect on seed weight in both seasons of this study.

Table 5: Effect of magnesium chelate and magnesium sulphate rates as soil application on some fruit chemical properties of "Hayany" date palms (2012 &2013 seasons).

Treatments	Fruit diameter (cm)		Pulp weight (g)		Pulp dry matter (%)		Seed weight (g)	
	2012	2013	2012	2013	2012	2013	2012	2013
Control " untreated"	2.1 c	2.2 c	8.4 c	7.8 e	37.1 c	36.9 b	1.46 b	1.40 c
20g Mg/palm (as Mg EDTA)	2.2 bc	2.3 bc	9.3 bc	9.3 cd	37.6 bc	37.2 b	1.53 ab	1.68 abc
30g Mg/palm (as Mg EDTA)	2.3 ab	2.5 a	11.2 a	12.5 a	39.1 a	38.5 ab	1.71 ab	1.79 ab
40g Mg/palm (as Mg EDTA)	2.4 a	2.5 a	11.6 a	12.6 a	40.4 a	40.1 a	1.97 a	1.92 a
20g Mg/palm (as $MgSO_4$)	2.1 c	2.2 c	9.1 bc	8.2 de	37.0 bc	36.8 b	1.36 b	1.53 bc
30g Mg/palm (as $MgSO_4$)	2.2 bc	2.3 bc	9.8 b	10.1 bc	37.8 bc	37.4 b	1.70 ab	1.83 ab
40g Mg/palm (as $MgSO_4$)	2.2 bc	2.4 ab	10.2 b	11.0 b	38.0 bc	37.9 ab	1.53 ab	1.56 abc

Means within each column followed by the same letter (s) are not significantly different at 5% level.

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Fruit T.S.S. (%):

Table 6, illustrates that the magnesium chelate and magnesium sulphate treatments exerted high positive effect on fruit T.S.S. content than the control treatment in both seasons of study. Moreover, magnesium fertilization in chelate form gave higher positive effect than sulphate form magnesium fertilization. Generally, 40g Mg/palm in the form of magnesium chelate treatment proved to be the most efficient treatments in this concern. Other treatments showed an intermediate values in this respect.

Fruit Total acidity content (%):

Table 6, indicates that the two tested form magnesium fertilizers induced a pronounced reductive effect on fruit total acidity content as compared with the control. Briefly, 40g Mg/palm in the form of magnesium chelate treatment proved to be the most efficient treatment in reducing fruit total acidity content in both seasons of study.

Fruit T.S.S. / Acid ratio:

Statistical analysis indicates that magnesium chelate and magnesium sulphate treatments scored significantly higher values of fruit T.S.S. / Acid ratio as compared with the control treatment in both seasons of study. Generally, 40g Mg/palm in the form of magnesium chelate treatment scored the highest values (214.3 &190.1) against (92.1 & 101.7) for the control treatment in the first and second seasons, respectively (Table 6).

Fruit total sugar content:

Table 6, reveals that 40 and 30g Mg/palm in the form of magnesium chelate and 40g Mg/palm in the form of magnesium sulphate produced similar and higher positive effect on fruit total sugar content of “Hayany” date palm fruits as compared with control treatment in both seasons of study. Moreover, other treatments showed an intermediate values in this respect.

The enhancement effect of magnesium fertilizer on fruit physical and chemical properties may be due to the important role of magnesium on chlorophyll molecule structure, carbohydrate metabolism, many enzyme involved in carbohydrate metabolism and protein synthesis (Jones *et. al.*, 1991; Purohit, 2007; Spiegel-Ray and Goldschmidt, 2007; Sliva and Uchida, 2000 and Cakmak and Yazici, 2010). So that, the enhancement effect on chlorophyll was reflected in improving vegetative growth which leads to more carbohydrates production through photosynthesis process and consequently improved total soluble solids, total sugar content and finally fruit physical and chemical properties

The enhancement effect of magnesium chelate than magnesium sulphate fruit physical and chemical properties may be due to that magnesium chelate remains in a soluble form and easy for plant uptake. However, the chelate form was more pronounced than sulphate form of "Grand Naine" banana (Mostafa *et al.*, 2007).

The obtained results of magnesium fertilizer regarding their positive effect on fruit physical and chemical properties are harmony with the finding of Abou Aziz et al. (2000) on banana; Ahmed and Morsy (2001) on Canino apricot; Abd El-Moniem *et al.* (2002) on Washington navel orange; El-Seginy *et al.* (2003) on Anna apple; Elham Dawood and Shahin (2006) on Canino apricot; Mostafa *et al.* (2007) on banana; Bybordi and Shabanov (2010) on grape; Fawzi *et al.* (2010) on Le Conte pear; Hanafy Ahmed *et al.* (2012) on Washington navel orange. They mentioned that magnesium fertilizer improved fruit physical and chemical properties of the aforementioned fruit species.

Table 6: Effect of magnesium chelate and magnesium sulphate rates as soil application on leaf minerals contents of "Hayany" date palms (2012 &2013 seasons).

Treatments	T.S.S (%)		Total acidity (%)		T.S.S./acid ratio		Total sugars (%)	
	2012	2013	2012	2013	2012	2013	2012	2013
Control " untreated"	29.4 c	29.2 d	0.32 a	0.29 a	91.9 d	100.7 e	24.9 d	24.9 d
20g Mg/palm (as Mg EDTA)	30.1 bc	30.2 c	0.29 abc	0.23 bc	103.8 cd	131.3 cde	27.8 ab	27.8 ab
30g Mg/palm (as Mg EDTA)	32.0 a	31.1 b	0.25 c	0.19 cd	128.0 b	163.7 b	28.3 a	28.3 a
40g Mg/palm (as Mg EDTA)	32.0 a	32.2 a	0.15 d	0.17 d	213.3 a	189.4 a	28.7 a	28.7 a
20g Mg/palm (as $MgSO_4$)	30.1 bc	29.7 cd	0.31 ab	0.27 ab	97.1 cd	110.0 de	26.3 c	26.3 c
30g Mg/palm (as $MgSO_4$)	31.0 abc	30.3 bc	0.28 abc	0.22 c	110.7 bcd	137.7 bcd	26.9 bc	26.9 bc
40g Mg/palm (as $MgSO_4$)	31.5 ab	31.1 b	0.27 bc	0.22 c	116.7 bc	141.4 bc	28.4 a	28.4 a

Means within each column followed by the same letter (s) are not significantly different at 5% level.

Leaf mineral content:

Nitrogen:

Table 7, illustrates that magnesium fertilizers in the form of chelate and sulphate induced high positive effect on leaf nitrogen content than the control treatment in both seasons of study. Moreover, chelate form gave

higher positive effect than sulphate form. Generally, 40g Mg/palm in the form of magnesium chelate proved to be the most efficient treatments in this concern. Other treatments showed an intermediate values in this respect.

Phosphorus:

Table 7, indicates that the two tested forms of magnesium fertilizers (chelate and sulphate) produced similar and higher positive effect on leaf phosphorus content of "Hayany" date palm as compared with the control treatment in both seasons of study. Generally, magnesium chelate treatments induced higher positive effect on leaf phosphorus content, especially at 40g Mg/palm as compared with the control treatment in both seasons of this study.

Potassium:

Table 7, shows that fertilizing "Hayany" date palm with magnesium chelate and magnesium sulphate produced higher positive effect on leaf potassium content as compared with the control treatment in both seasons of study. Generally, magnesium chelate treatments induced high positive effect on leaf potassium content, especially at 40 g Mg/palm as compared with the control treatment in both seasons.

Calcium:

Table 7, demonstrates that magnesium chelate and magnesium sulphate treatments induced similar and higher positive effect on leaf calcium content than the control treatment in both seasons of study. Generally, magnesium chelate and magnesium sulphate treatment took the same trend in this concern in both seasons without any significant difference in this respect.

Magnesium:

Table 7, reveals that the two tested form of magnesium fertilizers (chelate and sulphate) produced higher positive effect on leaf magnesium content of "Hayany" date palm as compared with control treatment in both seasons of study. Generally, 40 g Mg/palm in the form of magnesium chelate gave higher positive effect on leaf magnesium content as compared with the control treatment in both seasons of study. Moreover, other treatments showed an intermediate values in this respect.

The obtained results regarding the effect of magnesium fertilizer on leaf mineral content go in line with the finding of Ahmed and Morsy (2001) on Canino apricot; Mostafa *et al.* (2007) on banana; Fawzi *et al.* (2010) on Le Conte pear; Hanafy Ahmed *et al.* (2012) on Washington navel orange. They mentioned that magnesium fertilizer improved leaf mineral content of the aforementioned fruit species. Also, existence of magnesium element is an adequate level the photosynthesis process and forming chlorophyll perfectly which produce carbohydrates in leaf and leads to perfect fruits and good yield.

Thereupon, from the obtained results and under similar conditions it is preferable to add magnesium chelate fertilizer especially, at 320 g / palm (40 g Mg/palm) as soil application in three times a year i.e. February, 1st, May, 1st and July, 1st to enhance leaf chlorophyll content, fruit set percentage, retained fruit percentage, yield, fruit physical and chemical properties as well as leaf minerals content of "Hayany" date palm.

Table 7: Effect of magnesium chelate and magnesium sulphate rates as soil application on leaf minerals contents of "Hayany" date palms (2012 &2013 seasons).

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Calcium (%)		Magnesium (%)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Control " untreated"	1.30 e	1.31 e	0.16d	0.16 c	1.54 g	1.60 e	0.49 b	0.62 b	0.24 d	0.28 c
20g Mg/palm (as Mg EDTA)	1.46 c	1.57 c	0.21 a	0.20 b	1.70 c	1.67 b	0.53 a	0.65 a	0.29 cd	0.32 bc
30g Mg/palm (as Mg EDTA)	1.56 b	1.64 b	0.21 a	0.22 ab	1.77 b	1.80 a	0.53 a	0.65 a	0.37 ab	0.37 ab
40g Mg/palm (as Mg EDTA)	1.72 a	1.74 a	0.21 a	0.25 a	1.80 a	1.81 a	0.53 a	0.63 a	0.44 a	0.41 a
20g Mg/palm (as $MgSO_4$)	1.38 d	1.52 d	0.17 c	0.19 b	1.56 f	1.62 d	0.52 a	0.64 a	0.28 cd	0.30 bc
30g Mg/palm (as $MgSO_4$)	1.44 c	1.54 d	0.17 c	0.20 b	1.64 e	1.65 c	0.52 a	0.65 a	0.30 cd	0.32 bc
40g Mg/palm (as $MgSO_4$)	1.46 c	1.57 c	0.18 b	0.21 b	1.67 d	1.66 bc	0.53 a	0.65 a	0.32 bc	0.33 abc

Means within each column followed by the same letter (s) are not significantly different at 5% level.

References

A.O.A.C., 1995. Association of Official Agricultural Chemists, Official Methods of Analysis, 15th ed. A.O.A.C., Washington, DC.

Abd El-Moniem, A., A.A. El-Helaly and H.M. El-Kader, 2002. Response of Washington navel orange trees to soil foliar application of magnesium sulphate. J. Adv. Agric. Res., 7: 605-612.

Abou Aziz, A.B., M.F. Mostafa, N.R. Samara and A.M. El-Tanahy, 2000. Nutritional studies on banana plants. J. Agric. Sci. Mansoura Univ., 25(1): 433-439.

Abou El-khashab, A.M., 2002. Growth and chemical constituents of some olive cultivars as affected by biofertilizers and different water regime. Egypt. J. Agric., NRC. 1(2): 243-265.

Ahmed, F.F. and M.H. Morsy, 2001. Response of "Canino" apricot trees grown in the new reclaimed land to application of some nutrients and ascorbic acid. The fifth Arabian Hort. Conf. Ismailia, Egypt, pp: 27-34.

Bremner, J.M., 1965. Total nitrogen. In: Methods of Soil Analysis (Part 2). Black, C.A. (Ed), pp: 1149-78. American Society of Agronomy, Madison, USA.

Bybordi, A. and J.A. Shabanov, 2010. Effects of the foliar application of magnesium and zinc on the Yield and quality of three Grape cultivars grown in the calcareous soils of Iran. Notulae Scientia Biologicae, 2(1): 81-86.

Cakmak, I. and A.M. Yazici, 2010. Magnesium: A forgotten element in crop production. Better Crops., 94(2): 23-25.

Chapman, H.D. and P.F. Pratt, 1961. Methods of Analysis for Soils Plants and Water. University of California Division of Agricultural Sciences.

Clarke, G.M. and R.E. Kempson, 1997. Introduction to the design and analysis of experiments. Arnold, a Member of the Holder Headline Group, 1st Edt. London, UK.

Diallo, H., 2005. The role of date palm in combat desertification. In: The Date Palm: From Traditional Resource to Green Wealth. pp. 13-19. UAE Center of studies and Strategy Researches. Abu Dhabi, UAE.

Duncan, D.B., 1955. Multiple range and multiple F Test. Biometrics, 11: 1-42.

El-Fouly, M.M., A.I. Rezk, O.A. Nofal and E.A.A. Abou El-Nour, 2012. Depletion of magnesium in Egyptian soils, its content in crops and estimated needs. J. Agric. Res., 1(1): 1-8.

Elham Dawood, Z.A. and M.F.M. Shahin, 2006. Effect of spraying magnesium, boron, ascorbic acid and vitamin B complex on yield and fruit quality of "Canino" apricot. Arab. Univ., J. Agric. Sci., 14(1): 337-347.

El-Safty, M.A. and R.S. Rabii, 1998. Effect of foliar and soil application of magnesium sulfate on mineral composition, yield and fruit quality of Washington Navel orange trees. J. Agric. Sci. Mansoura Univ., 23(6): 2635-2641.

El-Seginy, Amal M., S.M. Malaka Naiema and W.M. Abd El-Messeih, 2003. Response of Anna apple trees grown in newly reclaimed calcareous soil to magnesium sulfate application in different quantities and doses. Alex. J. Agric. Res., 48: 69-74.

FAO, 1982. Plant Production and Protection paper. Date Production and Protection. Food and Agriculture Organization of the United Nation. Rome, Italy.

FAO, 2000. Fertilizers and Their Use. Rome, pp: 21-23.

Fawzi, M. I. F., F.M. Shahin, A. Elham Daood and E.A. Kandil, 2010. Effect of organic, biofertilizers and magnesium sulfate on growth, yield, chemical composition and fruit quality of "Le Conte" pear trees. Nature and Science, 8(12): 273-280.

Hanafy Ahmed, A.H., M.K. Khalil, A.M. Abd El-Rahman and A.M. Nadia Hamed, 2012. Effect of magnesium, copper and growth regulators on growth, yield and chemical composition of Washington Navel orange trees. J. Applied Sci. Res., 8(2): 1271-1288.

Jackson, M.L., 1958. Soil Chemical Analysis. P. 498. Constable Ltd. Co., London.

Jones, I.B., B. Wolf and H.A. Milles, 1991. Plant analysis handbook. Micro-Macro Publishing Inc., pp: 213

Lunde P., 1978. A History of Dates. Saudi Aramco World, 29(2): 176-179.

Mostafa, E.A.M., M.M.S. Saleh and M.M.M. El-Migeed, 2007. Response of banana plants to soil and foliar application of magnesium. American Eurasian J. Agric. & Environ. Sci., 2(2): 141-146.

Parkinson, J.A. and S.E. Allen, 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun. Soil Sci. and Plant Analysis, 6(1): 1-11.

Purohit, S.S., 2007. Plant Physiology. Sarswati Purohit Student Edition, India Hinglas offset, Jodhpur. pp: 133-377.

Salem, S.E., 2007. Study the interaction effect of potassium and magnesium on yield and quality of grape vine in calcareous soils. M.Sc. Thesis Fac. Agric., Alex. Univ. Egypt., pp: 172.

Sliva, J.A. and R. Uchida, 2000. Essential nutrients for plant growth: Nutrient functions and deficiency symptoms. Plant Nutrient Mangagement in Hawaii,s Soils, Approaches for Tropical and Subtropical Agriculture, Hawaii Manoa, Chapter 3, pp: 31-51.

Soliman, S.S. and S. El Kosary, 2002. Effect of different hand pollination methods on fruit retained, yield and fruit quality of some Egyptian date palm cultivars. Egypt. J. Hort., 29(2): 281-300.

Spiegel-Roy, P. and E. Goldschmidt, 2008. Biology of Citrus. Cambridge University Press, pp: 140-184.

Westwood, M.N., 1978. Temperate Zone Pomology. W.H. Freeman Company, San Francisco, USA. pp: 428.

EFFECTS OF FOLIAR CALCIUM APPLICATION ON PEACH FRUIT QUALITY, SHELF-LIFE, AND FRUIT ROT

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Abstract

This is an intermediate report of the impacts of several calcium formulations applied throughout the peach fruit development and growth period. Calcium nitrate, calcium chloride, or a calcium amino acid chelate (Metalosate® Calcium), were assessed for their effect on the quality and shelf life of peach fruit. All caused improvements in fruit firmness, peel growth cracking, and reduced post harvest fruit rots. Metalosate® Calcium caused increased fruit size.

Introduction

Calcium is an essential component for plant cell function, and plant tissue integrity (Conway, 1982; Conway and Sams, 1987; Elad and Kirshner, 1992). Calcium's physiological activity as a second messenger in cellular biochemistry and its requirement in cell wall structure make it important to fruit growth and development, as well as general fruit quality (Kadir, 2004; Kazuhiro et al, 2004), the rate of fruit senescence (Ferguson, 1984; Gerasopoulos and Drogoudi, 2005), disease resistance (Elmer, 2006; Lanauskas and Kvikliene, 2006; Tobias et al, 1992; Volpin and Elad, 1991), and other stresses (Yuen, 1993). While not all impacts of calcium on fruit quality, shelf-life, and fruit rot appear positive (Crisosto et al, 1997; Ellis et al, 1996; Lester and Grusak, 2004), it is clear that calcium formulation, rate, and timing impact the efficacy of calcium on several parameters (Crisosto et al, 1997; Elmer et al, 2006; Kazuhiro et al, 2004). This is an intermediate report of the impacts of several calcium formulations applied throughout the peach fruit development and growth period.

Materials and Methods

During the 2005-2007 seasons, peach trees in Byron, GA, USA were either untreated or treated with calcium nitrate, calcium chloride, or a calcium amino acid chelate (Metalosate® Calcium) at two week intervals from shuck split (just before peach flower petal fall, late March) until shortly before harvest (late July). Sixteen trees for each treatment were designated in the orchard in a completely randomized design and treated as single tree replicates in the analysis. Data were analyzed by analysis of variance and means were separated by Fisher's protected least squared difference test.

In 2005 the 'Sunprince' peach trees were four years old. They were planted with a spacing of 5.5 m (18.0 ft.) between trees in the row, and 6.1 m (20 ft.) between rows. Trees were irrigated using microsprinklers, replacing evapo-transpirational loss completely on a weekly basis from bloom through harvest. Trees were maintained with standard cultural and pest management regimes (Horton and Johnson, 2006; Horton et al. 2005-2007), including a late summer $Ca(NO_3)_2$ side dressed application of 23 kg/acre (51 lbs./acre or 57 kg/Ha). Trees were pruned and thinned according to industry standard, with fruit spaced about 6 inches (15 cm) along the fruit bearing shoots, resulting in approximately 250 fruit per tree in 2005 and 300 fruit per tree in 2006. In 2007, the peach crop was severely diminished in central Georgia by a freeze event on April 9. Therefore, trees were only evaluated for impact of calcium chloride on brown rot disease incidence, as a part of a brown rot management program. Calcium chloride was chosen as the formulation based on the work or Elmer et al (2006). It was compared to other, more standard brown rot management schemes. These fruit were harvested on 18 Jul 2007, inoculated with *Monilinia fructicola*, placed at room temperature and observed for brown rot after 4 and 7 days.

Calcium applications were made, as outlined in Table 1, during seasons 2005-2007. All applications were made at a volume of 3.5 L (118.3 fl. oz.) per tree (to run off) with hand-gun application. Fruit were sampled for nutrient analysis and fruit were harvested, as outlined in Table 1. Fresh fruit samples were washed in 0.01N nitric acid with 0.01% detergent and rinsed in deionized water prior to drying at 65°C (149°F). Sampled fruit were peeled and sub samples of peel and flesh were dried as above for tissue analysis. The coded 50.0-gram (1.8-oz) samples were analyzed by Albion Laboratories, Inc. for calcium and other plant nutrients. When fruit were harvested in 2005 and 2006, they were weighed in the field and evaluated for size by passing the fruit over a grading table. Fruit in each size category were counted. A 16-fruit set was prepared by randomly selecting fruit from the total fruit from each tree for each harvest date. This sub sample was weighed, evaluated subjectively for % red overblush color, and for background color using Clemson Chips 1-6. The fruit were also assessed for firmness using a Magness-Taylor penetrometer fitted with a 8-mm (0.3-in) probe, and for total soluble solids with an Atago brix refractometer.

After evaluation of fruit on 27 Jul 2006, a set of samples of 30 fruit was taken from each treatment for post harvest evaluation of rhizopus and brown rot. After 11 and 16 days in storage at 2-4°C (36-39°F), fruit was evaluated.

Results and Discussion.

When these studies were initiated, they were designed to assess the effect of calcium nitrate on fruit firmness and shelf life. At that time, the decision was made to include two other calcium formulations at the same level of elemental calcium for comparison. This was the basis of our decision for the level of each material used that year. It is apparent in the data that follows that use of Metalosate® Calcium at that level had a negative impact on fruit quality. Assessment of the level of calcium in the fruit suggested that the Metalosate® Calcium rate should be significantly reduced. The label recommended rate of this material was then included in the 2006

experiment along with the higher rate. As stated earlier, in 2007, due to the freeze that spring, only calcium chloride was assessed for impact on brown rot suppression as part of a larger pathology study.

Table 1
Treatment Application Concentrations and Dates, Tissue Sampling Dates, Harvest Dates, and Dates of Post Harvest Evaluation of Peach Fruit

Treatment	2005	2006	2007
Calcium Nitrate 12.0 ml/L (15.4 fl. oz./100 gal.)	5 Apr, 19 Apr, 3 May, 18 May, 31 May, 15 Jun, 28 Jun, 12 Jul	27 Mar, 30 Apr, 12 May, 28 May, 11 Jun, 25 Jun, 7 Jul	
Calcium Chloride 27.0 mg/L (0.4 oz./100 gal.)	5 Apr, 19 Apr, 3 May, 18 May, 31 May, 15 Jun, 28 Jun, 12 Jul	27 Mar, 30 Apr, 12 May, 28 May, 11 Jun, 25 Jun, 7 Jul	19 Apr, 2 May, 24 May, 4 Jun, 14 Jun, 29 Jun, 11 Jul
Metalosate® Calcium 2.5 ml/L (32.0 fl. oz./100 gal.) 16.0 ml/L (204.7 fl. oz./100 gal.)	---- 5 Apr, 19 Apr, 3 May, 18 May, 31 May, 15 Jun, 28 Jun, 12 Jul	27 Mar, 30 Apr, 12 May, 28 May, 11 Jun, 25 Jun, 7 Jul 27 Mar, 30 Apr, 12 May, 28 May, 11 Jun, 25 Jun, 7 Jul	
Tissue Sampling	2 Aug	10 May, 12 Aug	3 Jun, 18 Jul
Harvest Dates	26 Jul, 29 Jul, 1 Aug, 5 Aug	13 Jul, 17 Jul, 20 Jul, 24 Jul, 27 Jul, 31 Jul, 4 Aug	18 Jul
Post Harvest Evaluation		27 Jul, 7 Aug, 13 Aug	23 Jul

Table 2
Peel and Flesh Calcium Concentrations for the 2005 through 2007 Trials

Treatment	Flesh Ca (ppm)	Peel Ca (ppm)	Flesh Ca (% of UTC)	Peel Ca (% of UTC)
2005	(Late)	(Late)	Late)	(Late)
Control	274.0b	606.4	100.00	100.00
$Ca(NO_3)_2$	285.4b	677.5	104.15	111.92
$CaCl_2$	246.7b	563.3	90.02	92.89
Metalosate® Calcium (16)	379.6a	695.0	138.7	114.68
2006	(Early/Late)	(Early/Late)	(Early/Late)	(Early/Late)
Control	239d/197d	633c/560c	100/100	100/100
$Ca(NO_3)_2$	318c/283c	705c/660c	133/143	111/118
$CaCl_2$	293c/296c	674/618	123/150	106/110
Metalosate® Calcium (16)	972a/877a	989a/802a	407/445	156/143
Metalosate® Calcium (2.5)	445b/438b	719bc/720b	186/222	114/129
2007	(Early/Late)	(Early/Late)	(Early/Late)	(Early/Late)
Control	284/248	628/585	100/100	100/100
$CaCl_2$	334/279	658/617	118/113	105/105

Values within the same sampling time (early or late) year and column were compared for differences. Means followed by the same letters are not significantly different according to Fisher's protected LSD test.

Although the calcium nitrate and calcium chloride treatments numerically improved calcium levels in fruit peel and flesh, generally the calcium chelated with amino acids was present in peel and flesh at significantly higher levels (Table 2). Calcium treatments had lower impacts on fruit size in 2005 than 2006. One explanation may be that the period from April 1 to July 31 had twice as much rainfall in 2005 as 2006. It is likely that some of the calcium was washed from the surfaces of leaves and fruit with rain events.

Assessment of fruit yield and quality in 2005 (Figure 1) demonstrated that the untreated control and Metalosate® Calcium treatments produced greater total field weight per tree than the other treatments, but individual fruit size, ground color, % red overblush, firmness, or percent total soluble solids were not changed. The proportion of fruit that was in the size category of 2.75 inches (6.99 cm) and larger was not different among calcium nitrate, $CaCl_2$, and Metalosate® Calcium treatments and was less than the untreated control. Not apparent in this data was our observation that the quality of Metalosate® Calcium fruit was visually poor. The poor appearance of this fruit was supported by the apparent advancement of its ripening when one considers the numerically redder, and less firm values demonstrated in the study. Because of the apparent poor quality of the fruit we decided to look at lowering the rate of Metalosate® Calcium to the labeled rate of 2.5 ml/L (32.0 fl. oz./100 gal) in the 2006 trial.

In 2006 a number of differences were apparent. Although the overall yield was similar among the treatments, there were differences among treatments in the

distribution of harvested fruit among the size classes (Figure 2). Both Metalosate® Calcium treatments had more fruit shifting to larger size categories, with fewer fruit in the smaller size categories. While a greater proportion of the 16.0 ml/L (204.7 fl. oz../100 gal.) treatment fruit was 3.00 inches (7.62 cm) or larger, the fruit again were of poorer quality than any other treatment. Not only was firmness lower, post harvest quality was compromised with a level of fruit rot similar to the untreated control after storage (Figure 3). It was apparent that the lower concentration of Metalosate® Calcium gave both good fruit quality, and increased fruit size, with a greater proportion of the fruit falling in the 2.75 inches (6.99 cm) and larger size group. Calcium nitrate also favored a shift to larger sizes without compromising quality. However, the size increase was not as great as Metalosate® Calcium. Again, there were no differences among the treatments in 2006 with regard to fruit color, or total soluble solids. There was a numerical trend toward increased firmness in the $CaCl_2$, calcium nitrate, and Metalosate® Calcium treatments that would be expected with the observed decrease in fruit rots after storage (Figure 2).

Tobias et al (1993) demonstrated that calcium treatment delayed degradation of cell wall structure in apples, limiting the incidence and spread of *Botrytis cinerea*. This phenomenon likely explains our finding that all the calcium treatments had a reduction in growth cracks of the peel relative to the control.

Differences among treatments in 2006 were more apparent than the previous year. With regard to fruit size, this was probably due to two factors. The first being the fact that when moisture is in excess, which was the case several times during the fruit ripening period, fruit will tend to have greater increases in size without further interventions.

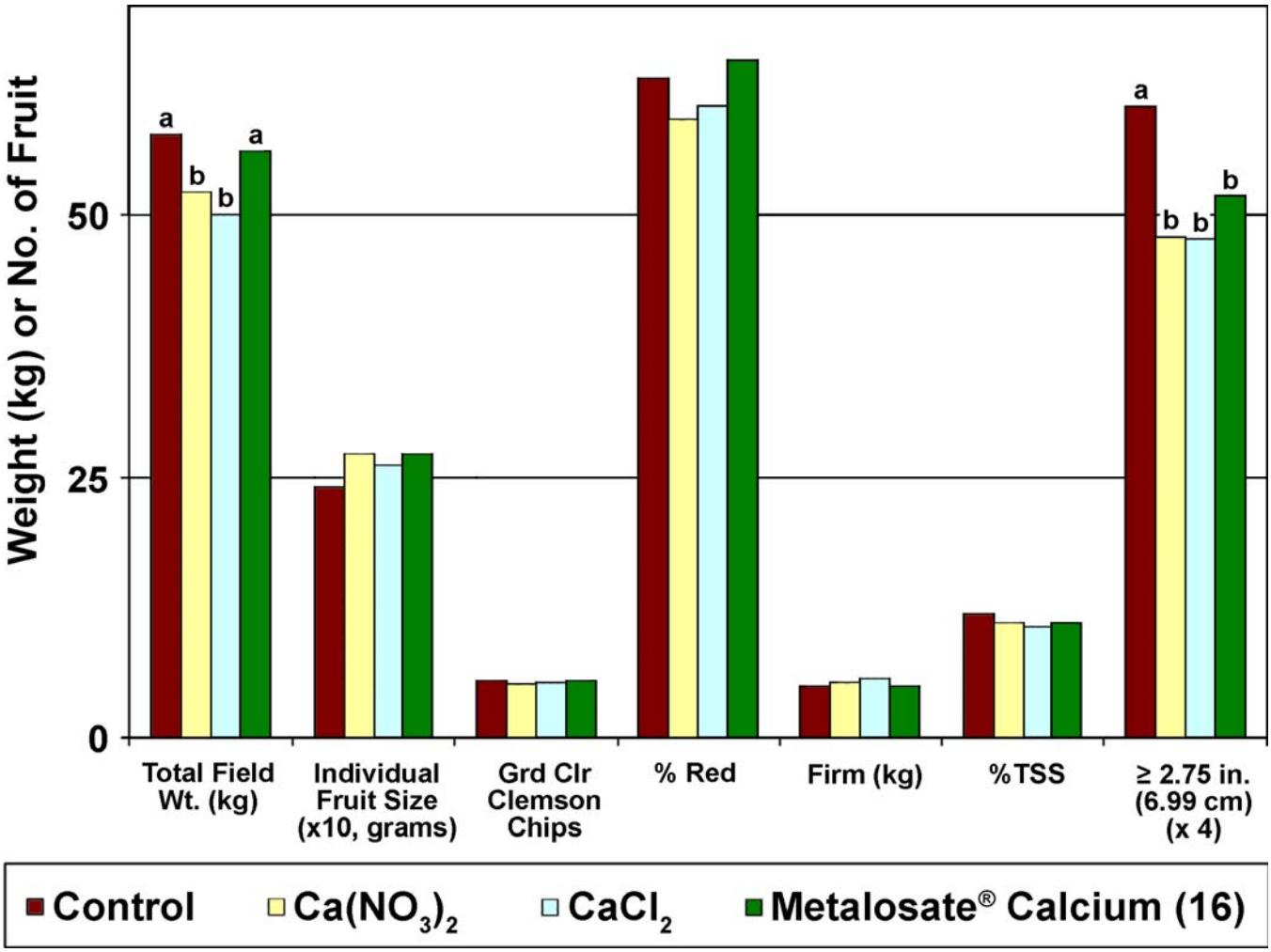

Figure 1. Calcium Effect on Fruit Yield, Size, and Quality in 2005

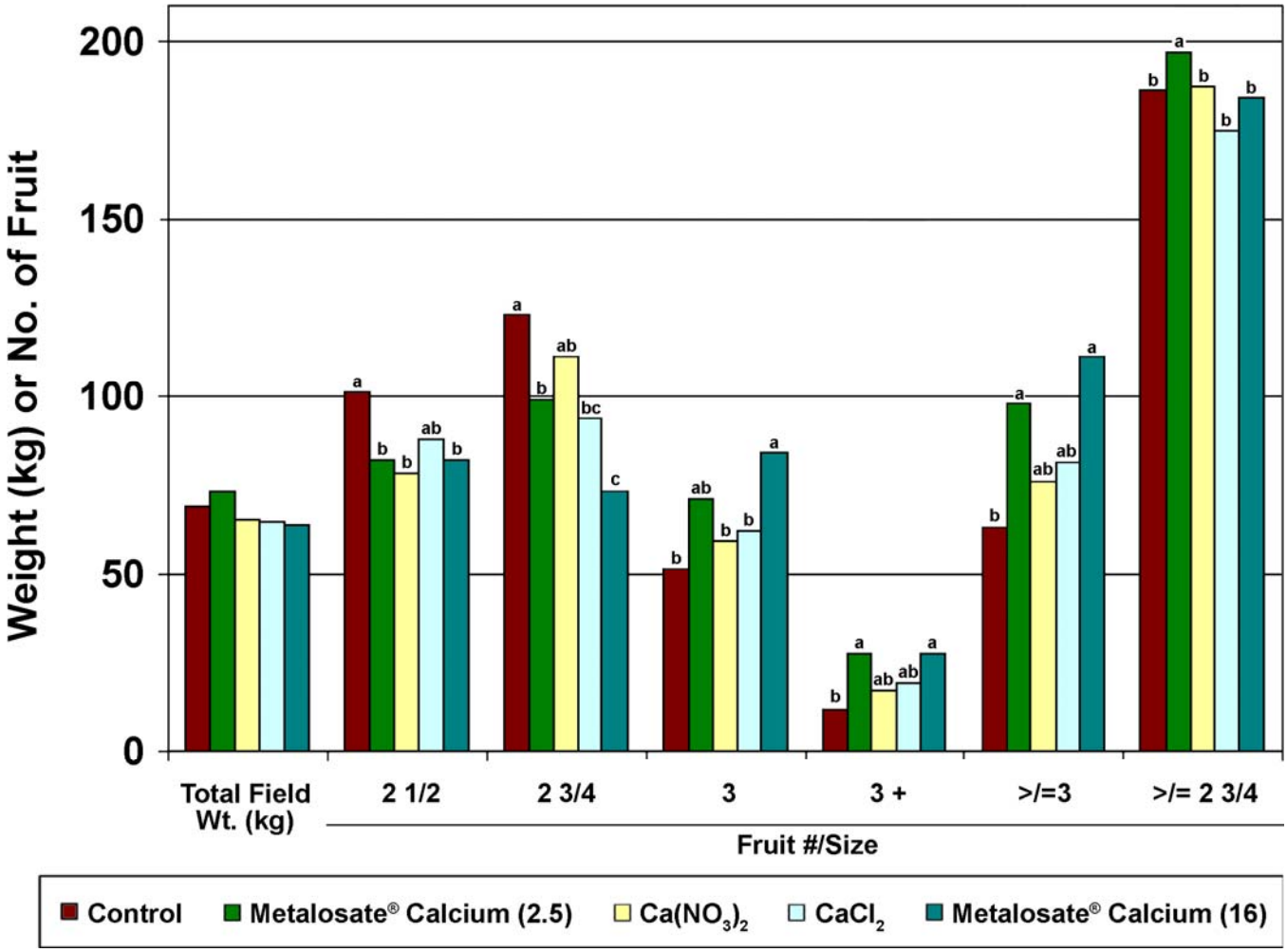

Figure 2. Calcium effect on fruit size distribution and yield in 2006

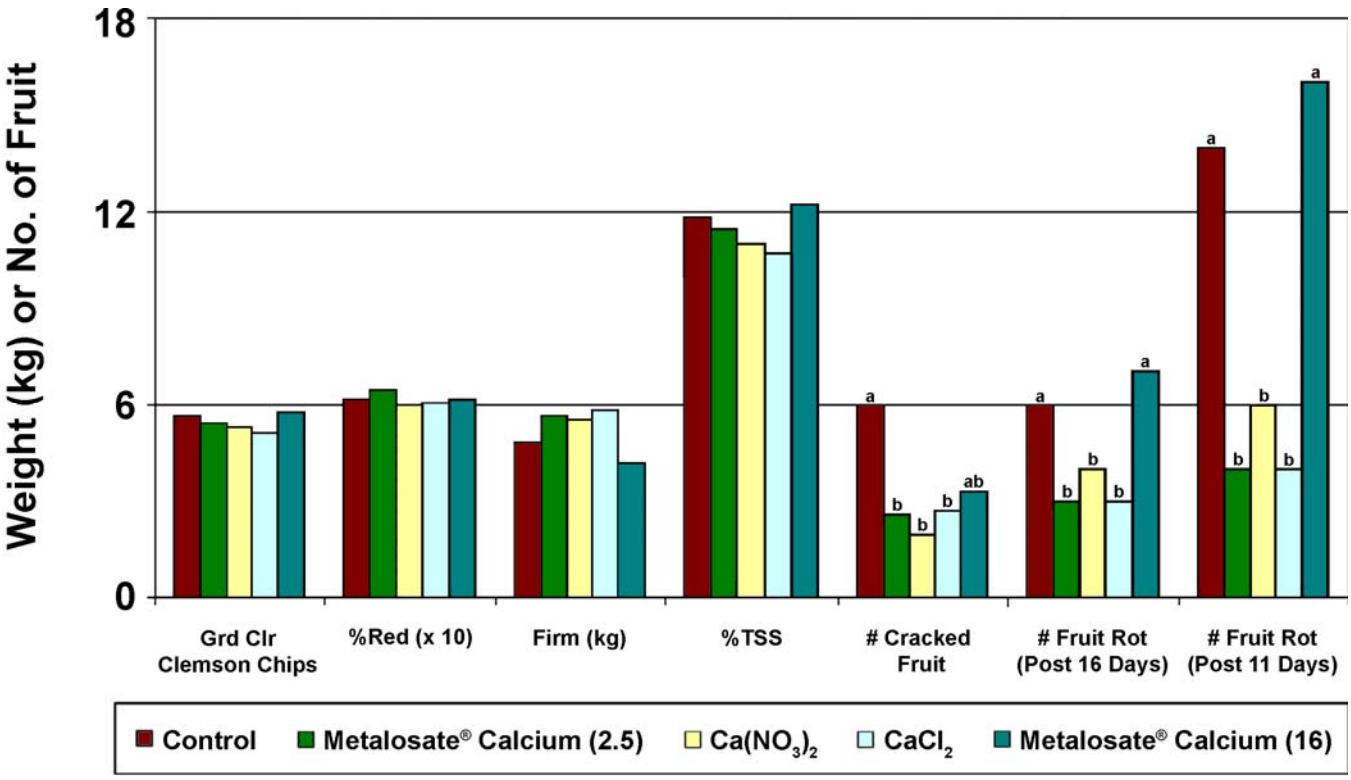

Figure 3. Calcium effect on fruit quality and post harvest rots in 2006.

Table 3
Effect of $CaCl_2$ as part of a brown rot management program

Treatment and Rate/Acre	Brown Rot Incidence* (% Infected Fruit)	
	4 Days After Harvest	7 Days After Harvest
Untreated Control	25.0 a	76.3 a
Propimax 3.6EC (119 ml)	26.9 a	78.2 a
Propimax 3.6EC (119 ml) + $CaCl_2$ (10.1g)	14.7 ab	57.7 b
Propimax 3.6EC (119 ml) + Sulfur 90EP (4.1 kg)	21.8 ab	69.9 ab
Propimax 3.6EC (119 ml) + Captan 90EP (3.6 kg)	12.8 ab	62.2 ab
LSD (P=0.05)	14.8	16.0

**Brown rot incidence was recorded on fruit stored at ambient temperature. Means followed by the same letters are not significantly different according to Fisher's protected LSD test.*

Thus, even if the calcium treatments may be responsible for some increases in fruit size and therefore yield, it is not likely to be apparent in a rainy peach season. Secondly, the excessive rain likely served to reduce foliar calcium levels and dampen the impact of the element in 2005.

In our assessment of $CaCl_2$ as a part of a pest management program, we determined that in the 2007 season, addition of $CaCl_2$ reduced the incidence of brown rot occurring in storage at room temperature (Table 2). Again, it is likely that this is attributable to maintenance of cell wall integrity by delaying the degradation of the cell wall's pectic polymers, to which calcium is essential (Tobias et al, 1992). Clearly pre-harvest application of calcium has positive impacts on fruit quality and shelf life. We still have much to do to verify these preliminary findings, but with this intermediate report of our progress, the possibility of using calcium supplementation to improve fruit quality and shelf-life seems very likely.

Additional study will be undertaken in 2008 to replicate the 2006 study, and expand the 2007 study to include the other calcium formulations.

LITERATURE CITED

Conway, W. 1987. Effects of preharvest and postharvest calcium treatments of peaches on decay caused by *Monilinia fructicola*, *Hort. Sci.* 32:820–823.

Conway, W.S. 1982. Effect of postharvest calcium treatment on decay of delicious apples, *Plant Dis.* 66:402–403.

Conway, W.S. and Sams, C.E. 1987. The effects of postharvest infiltration of calcium, magnesium, or strontium on decay, firmness, respiration, and ethylene production in apples, *J. Amer. Soc. Hort. Sci.* 112:300–303.

Crisosto, C.H., Johnson, R.S., DeJong, T. and Day, K.R. 1997. Orchard factors affecting postharvest stone fruit quality, *Hort. Sci.* 32:820–823.

Elad, Y. 1997. Responses of plants to infection by *Botrytis cinerea* and novel means involved in reducing their susceptibility to infection, *Biolog. Rev. Cambridge Philo. Soc.* 72:381–422.

Elad, Y. and Kirshner, B. 1992. Calcium reduces *Botrytis cinerea* damage to plants of *Ruscus hypoglossum*, *Phytoparasitic* 20**:**285–291.

Ellis, M., Madden, L.V., Erincik, O. 1996. Evaluation of calcium chloride for control of Botrytis Fruit Rot on Strawberry. *Ohio Pest Management.* http://ipm.osu.edu/mini/96m-5.html

Elmer, P.A.G**.,** Spiers, T.M., Wood, P.N. 2006. Effects of pre-harvest foliar calcium sprays on fruit calcium levels and brown rot of peaches. *Crop Protection.*26:11-18

Ferguson, 1984 I.B. Ferguson, Calcium in plant senescence and fruit ripening, *Plant, Cell and Environ* 7:477–489.

Horton D.L. and D, Johnson**,** Eds. 2006. *Southeastern Peach Growers' Handbook*.

Horton D.L., Brannen, P., Bellinger, R., Ritchie, D. 2005-2007. *Southeastern Peach, Nectarine and Plum Pest Management and Culture Guide.*

Kadir, S.A. 2004. Fruit quality at harvest of 'Jonathan' apple treated with foliarly-applied calcium chloride. *Journal of Plant Nutrition*. 27:1991-2006.

Kazuhiro, I., Masashi, M., Hiroyuki, F. 2004. The effect of spraying of calcium to the fruit quality, the quality keeping period and the tree vigor of 'kousui' in the green house. *Bulletin of the Saga Prefectural Fruit Tree Experiment Station*. 15:8-14.

Lanaouskas, J, Kvikliene, N. 2006. Effect of calcium foliar application on some fruit quality characteristics of 'Sinap Orlovskij' apple. *Agronomy Research.* 4:31-36.

Lester, G.E., Grusak, M.A. 2004. Field application of chelated calcium: postharvest effects on cantaloupe and honeydew fruit quality. *HortTechnology*. 14:29-38.

Tobias, R.B., Conway W.S., Sams, C.E., Gross, K.C., Whitaker,B.D. 1992. Cell wall composition of calcium-treated apples inoculated with Botrytis cinerea. *Phytochemistry*. 32:35-39.

Volpin, H. and Elad, Y. 1991. Influence of calcium nutrition on susceptibility of rose flowers to *Botrytis* blight, *Phytopathology* 81:1390–1394.

Wojcik, P. 2001. Prune fruit quality as influenced by Calcium spraying, *J. Plant Nutr.* 24:1229–1241.

Yuen, M. C., 1993. Postharvest handling of tropical fruits. In: Proceedings of the International Conference on Postharvest Handling of Tropical Fruits, 19–21 July, Chaing Mai, Thailand.

Absorption and mobility of foliar-applied boron in soybean as affected by plant boron status and application as a polyol complex

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Abstract In the present study (i) the impact of plant Boron (B) status on foliar B absorption and (ii) the effect of B complexation with polyols (sorbitol or mannitol) on B absorption and translocation was investigated. Soybean (*Glycine max* (L.) Meer.) plants grown in nutrient solution containing 0 μ*M*, 10 μ*M*, 30 μ*M* or 100 μ*M* ^{11}B labelled boric acid (BA) were treated with 50 m*M* ^{10}B labelled BA applied to the basal parts of two leaflets of one leaf, either pure or in combination with 500 m*M* sorbitol or mannitol. After one week, ^{10}B concentrations in different plant parts were determined. In B deficient leaves (0 μ*M* ^{11}B), ^{10}B absorption was significantly lower than in all other treatments (9.7% of the applied dose vs. 26%–32%). The application of BA in combination with polyols increased absorption by 18–25% as compared to pure BA. The absolute amount of applied ^{10}B moving out of the application zone was lowest in plants with 0 μ*M* ^{11}B supply (1.1% of the applied dose) and highest in those grown in 100 μ*M* ^{11}B (2.8%). The presence of sorbitol significantly decreased the share of mobile ^{10}B in relation to the amount absorbed. The results suggest that ^{11}B deficiency reduces the permeability of the leaf surface for BA. The addition of polyols may increase ^{10}B absorption, but did not improve ^{10}B distribution within the plant, which was even hindered when applied a sorbitol complex.

Keywords B deficiency · B toxicity · Foliar absorption · Mannitol · Sorbitol · Soybean · Water potential

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Introduction

Warrington (1923) proved boron (B) to be an essential micronutrient for higher plants. Even though the demand for B on a molar basis is higher than for any other micronutrient in dicotyledons, knowledge of its physiological role is still limited. Boron

deficiency appears worldwide in crop production and is reported in over 80 countries on 132 crops. The occurrence of B deficiency depends on multiple factors, such as e.g. weather conditions (drought, high precipitation, etc.), soil conditions (low pH soils B leaching, calcareous soils B fixation) and the cultivated crop species (Shorrocks 1997). Physiological responses of plants to B deficiency include the loss of membrane integrity and cell wall stability, which result in the development of structural damage in crop plants like for instance, cracked stem in celery, stalk rot in cauliflower, heart rot and internal black spot in beet, top rot in tobacco and internal cork in apple (Blevins and Lukaszewski 1998). Several studies showed that B deficiency induces leaf structural changes, including abnormal stomata and distorted guard cells in cauliflower (Sharma and Sharma 1987) and coffee (Rosolem and Leite 2007) or decreased stomatal conductance and transpiration rates in navel orange and cotton (Oosterhuis and Zhao 2001; Sheng et al. 2009). Many other effects associated with B imbalances have been described, but the direct role of B in metabolism is still little understood.

In commercial plant production, providing a sufficient B supply is particularly important for yield formation (pollination) (Khayyat et al. 2007; Wojcik et al. 1999), fruit quality and crop storability (Wojcik et al. 1999), and stress tolerance (Cakmak and Römheld 1997). In addition to B deficiency, B toxicity can also considerably limit plant production (Miwa et al. 2006). Natural B toxicity occurs in soils in arid and semi-arid environments or may derive from mining deposits, fertilizers or irrigation water. Information available on B toxicity is fragmentary (Nable et al. 1997). Brown and Hu (1996) described symptoms of toxicity such as the death of cambial tissues and stem die back, causing fruit disorders (gummy nuts, internal necrosis) and bark necrosis. A loss in membrane integrity in association with B toxicity was reported by Alpaslan and Gunes (2001).

In most plant species, B is phloem immobile and distribution of B within a plant mainly follows the transpiration stream. The first visual effects of B deficiency can be observed in young leaves and meristematic tissues, whereas B toxicity symptoms are mainly visible in older leaves especially in the leaf tips where the transpiration stream ends (Poss et al. 1999).

Within the cell wall and cytoplasm, B quickly forms stable complexes (mainly mono— and diesters) and contributes to the water insoluble fraction. Thus, re-translocation from source to sink organs is not easily accomplished in the plant. In a wide range of plants, sugar alcohols (also called polyhydric alcohols or polyols) are present in the phloem sap. Most common are the straight-chained hexiols such as mannitol and sorbitol (Bieleski 2005). They contain cis-diol groups which can form stable complexes with B. These compounds facilitate the re-translocation from old leaves to "sink" organs such as young developing leaves, roots, fruits and meristematic tissues (Brown and Hu 1996; Brown et al. 1999; Delgado et al. 1994; Shelp et al. 1998). Boron mobility was evidenced in plants mainly belonging to the Rosaceae family (e.g., apple, cherry, peach) having large quantities of the sugar-alcohol sorbitol in the phloem sap, and also in those rich in mannitol largely corresponding to the families of Apiaceae (carrots and celery), Brassicaceae (broccoli, cauliflower), Fabaceae (pea, common bean) and Oleaceae (olive) (Bieleski 1982; Brown and Shelp 1997). Blevins and Lukaszewski (1998) found a large quantity of the sugar alcohol pinitol in the phloem sap of soybean, but the possibility of complex formation with B and re-translocation remains unclear (Bieleski 2005). Lehto et al. (2004) suggested a possible role of B complex formation with inositol or pinitol in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) H. Karst), but stable complexes could not be directly demonstrated.

In the present study the impact of plant B status on B foliar absorption and the effect of B complex application on improving absorption and translocation was investigated in soybean. Some experiments carried out with soybean cultivar "Oak Erin" suggested that pinitol (i.e. the polyol detected in soybean) does not significantly contribute to B mobility. In contrast to sorbitol and mannitol, pinitol is a cyclic polyol. Since the process of complex formation between B and pinitol remains unclear, it was not investigated as a candidate for foliar B application trials.

Thereby, to assess the effect of B complex application, sorbitol and mannitol were selected since stable B complexes with these compounds have been previously reported (Hu and Brown, 1997). In preliminary trials (data not shown) a 1:10 B:sorbitol ratio was found to increase the rate of foliar B

absorption and translocation in soybean plants. Hence, the following two hypotheses were tested, namely: (i) plant B status may affect the absorption and the within-plant mobility of foliar-applied B and (ii) foliar application of B-sorbitol and B-mannitol complexes can increase absorption and the within-plant mobility of B.

Material and methods

Pre-treatment

Soybean seeds (*Glycine max* (L.) Meer., cv. "Oak Erin") were soaked for 1 h in 10 m*M* $CaSO_4$ solution and then transferred to filter paper moistened with 2.5 m*M* $CaSO_4$ until radicles emerged. Seedlings were planted into 3 l plastic pots (4 plants per pot) containing continuously aerated nutrient solution (pH 5.5) of the following composition: 0.88 m*M* K_2SO_4, 0.1 m*M* KCl, 2 m*M* $Ca(NO_3)_2$, 1 m*M* $MgSO_4$, 0.25 m*M* KH_2PO_4, 10 μ*M* $^{11}B(OH)_3$ (enrichment 99.8%), 0.5 μ*M* $MnSO_4$, 0.2 μ*M* $CuSO_4$, 0.02 μ*M* $(NH_4)_6Mo_7O_{24}$, 1 μ*M* $ZnSO_4$ and 100 μ*M* Fe(III)EDTA. The nutrient solution was prepared with de-ionized water and changed on a weekly basis. Plants were cultivated in a climate chamber (Universität Hohenheim, Germany) with a radiation of approximately 1000 μmol $m^{-2}s^{-1}$. Day (14 h) and night (10 h) temperatures were kept at 24 and 20°C respectively, with a relative humidity (RH) of 60%. After cultivation in full-strength nutrient solution for 2 weeks, the 16 pots were divided into 4 groups which were consequently supplied for 2 weeks with a nutrient solution containing 0 μ*M*, 10 μ*M*, 30 μ*M* or 100 μ*M* ^{11}B -labelled BA,

Foliar formulations

Foliar treatment solutions were prepared with a basic de-ionized water solution containing 50 m*M* ^{10}B labelled boric acid (BA) plus 0.5% (v/v) surfactant (Plantacare, Cognis, Düsseldorf). Sorbitol and mannitol were used at a concentration of 500 m*M*, because concentrations of B and sorbitol in ratio 1:10 facilitates the formation of 1:2 B-polyol complexes (Hu and Brown 1997). The basic solution (BaSol) was used as the control in order to compare whether the polyols contribute to the quantitative absorption and/or affect the within-plant mobility of absorbed B. Treatments of the experiment were as follows:

1. Boron (B): Basic solution (BaSol)
2. Mannitol (BM): BaSol with B:mannitol ratio 1:10 (w/w)
3. Sorbitol (BS): BaSol with B:sorbitol ratio 1:10 (w/w)

All chemicals were of analytical grade (Merck, Darmstadt, Germany).

Data collection and sampling design

Data were collected from 16 pots containing 4 plants per pot, in total 64 plants. The pots were set using a split plot design with 4 replicates. The main plot factor pre-treatment has 4 levels: nutrient solutions with 0, 10, 30, and 100 μ*M* ^{11}B concentration, the sub plot factor foliar formulation has three levels: basic solution, basic solution with sorbitol and basic solution with mannitol. The fourth plant within each pot was used to measure the water potential.

Application

Foliar treatment solutions were applied on leaflets of the last fully-expanded leaves. Treatments were supplied via application of 16* 2.5 μl drops (40 μl in total) of the formulation on two adjacent leaflets per leaf. Soybean leaves consist of three leaflets, two are paired and one is the upper leaflet. Drops were applied to the lower half of the paired leaflets (Fig. 1). Leaves were harvested after 1 week and separated into segments. Distribution of

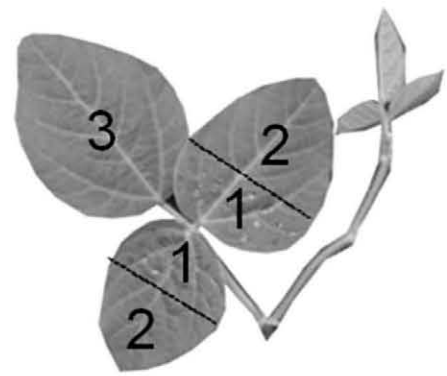


Fig. 1 Schematic presentation of soybean leaf segments used for the analysis of B absorption and translocation. 1: application zone (lower part of a leaflet), 2: leaf tip, 3: non-treated leaflet of a treated leaf

the applied ^{10}B was separately determined for the different segments as indicated in Fig. 1.: Segment 1: fraction remaining in the application zone, segment 2: fraction of ^{10}B in the leaf tip indicating acropetal translocation via transpiration stream and segment 3: fraction in non-treated leaflets of the treated leaf indicating short distance basipetal translocation via phloem. All samples from segment 1 were carefully rubbed under de-ionized water between gloved thumb and forefinger for 20 seconds to remove trapped material (Eichert and Goldbach 2008).

Analytical methods

Harvested leaves were dried in the oven at 65°C for 2 days. Ground dry leaf samples (0.05–0.1 g) were weighed in quartz crucibles. The samples were ashed in the oven with increasing temperatures (200°C, 300°C, 400°C and 500°C for 1, 1, 1 and 2 hours, respectively) then samples were cooled down overnight. Next day, samples were rewetted with some drops of 3% H_2O_2-solution and after drying, ashed again in the oven for 3 hours at 500°C. The ash was dissolved in 5 ml mixed acid solution [3.3% v/v HNO_3+10 ppb Beryllium (Be)] and centrifuged for 2 minutes at 4000×g (Hettich Universal 30 F). Boron isotopes (^{10}B, ^{11}B) were determined by inductively coupled plasma mass spectroscopy (ICP-MS, ELAN 6000, Perkin-Elmer, Überlingen, Germany), using Be as an internal standard. B concentrations and contents in each segment were calculated.

Water potential

Leaf water potentials were measured using the Scholander pressure chamber method (Scholander et al. 1965). From each pot one plant was randomly selected (n=4), the last fully-developed leaves of soybean plants were harvested and immediately fixed into the Scholander pressure chamber. For standardization of the moment when the xylem fluid appeared, tissue paper was held carefully on the top of the leaf stem. As soon as a liquid drop was visually observed, the pressure was recorded. This method was implemented to facilitate the visual detection of the sap appearance, since in former experiences with drought stressed plants, only small and disperse drops similar to foam could be seen to come out of the soybean leaf petiole.

Scanning electron microscopy (SEM) examination

Leaf surfaces were examined under a scanning electron microscope (S- 3400N, Hitachi, Tokyo, Japan; acceleration potential 15 kV, working distance 10–11 mm). Leaves from the different treatments were dried at room temperature, making sure that the surface remained flat. For observation of either the upper or lower leaf side, approximately 1 cm^2 sections were excised, and sputtered with gold. Different areas of the leaf sections were subsequently directly observed under the microscope. The abaxial and adaxial surface of five leaves was examined for each treatment. The length and width of stomatal pores (n=100) was assessed by the programme Image-Pro Plus 6 (Bethesda, USA)

Statistics

A mixed model approach was used for statistical analysis. For fixed effects general least square means were estimated and presented with their standard error in the results. An univariate analysis was performed for the ^{10}B concentration of each segment, the sum of segments 2 and 3, the propotion of ^{10}B in segment 2 or 3 compared to all segments, for the water potential and the ^{11}B concentration. A multivariate analysis was used for a combined analysis of ^{10}B over all segments. In addition, the water potential und the ^{11}B concentration were used as covariates for ^{10}B, but were dropped from the model as they had no significant influence.

To reach homogeneous residual variation for univariate and multivariate analysis, the data were logarithmically transformed for the traits ^{10}B and ^{11}B. For analysing the proportions of segment 2 or 3 the ^{10}B data were transformed using the logit as link function. In both cases estimated means were back transformed for presentation. The shown standard errors of these means were back transformed using the delta method.

The model for the univariate analysis is given by:

$$y_{ijklm} = \mu + r_k + \alpha_i + \beta_j + (\alpha\beta)_{ij} + p_{kl} + e_{ijklm}, \quad (1)$$

where r_k is the effect for the k^{th} replicate, p_{kl} is the main plot error effect of the l^{th} pot in the k^{th} replicate, α_i is the i^{th} pre-treatment effect and β_j is the j^{th} nutrient solution effect. $(\alpha\beta)_{ij}$ denotes the interaction

effect of the i^{th} pre-treatment and the j^{th} nutrient solution. e_{ijklm} denotes the subplot error or residual error effect of the i^{th} pre-treatment, j^{th} nutrient solution of the m^{th} plant in the l^{th} pot in the k^{th} replicate. All factors and interactions were taken as fixed. The main and sub plot error were taken as random. The replicate effect was treated as fixed ignoring all inter block information.

For the multivariate analyses the model is given by:

$$y_{ijklmn} = \mu + r_{kn} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + (\alpha\gamma)_{in} + (\beta\gamma)_{jn} + (\alpha\beta\gamma)_{ijn} + p_{kln} + e_{ijklmn}, \quad (2)$$

where γ_n denotes the n^{th} segment, and interactions and all other effects are denotes as in equation (1). For the pot effects p_{kln} of the three segments and for the residual error effects e_{ijklmn} of the three segments an unstructered variance-covariance matrix was assumed a priori. Because of small or fixed main plot variance component estimates the variance-covariance structure for the analysis of ^{10}B was simplified by dropping the covariances between main plot effects of one plant. Thus the optimal variance-covariance structure included heterogeneous variances for segments but no covariances. An Akaike Information Criterion (AIC) (Akaike, 1974) based model selection approach was used to find this model.

Results

Plant B status

Growth of soybean plants under various isotopically-labelled BA concentrations in the nutrient solution resulted in plants with different ^{11}B tissue concentrations. Plants grown in full-strength nutrient solution with 0, 10, 30 or 100 μM ^{11}B had average ^{11}B tissue concentrations of 2.1 (±0.2), 50.9 (±5.2), 86.7 (±8.9) and 103.7 (±10.7) μg ^{11}B g^{-1} DW, respectively.

Visual symptoms

Symptoms were observed in plants with 0 μM ^{11}B and 100 μM ^{11}B supply. Plants grown without ^{11}B showed deficiency symptoms such as diminished root and shoot growth. Root development was significantly decreased in plants without ^{11}B supply in comparison to plants treated with 10, 30 or 100 μM ^{11}B (1.4±0.1, 4.6±0.9, 5.3±0.5, 4.8±0.4 g dry weight, respectively). Roots were brownish in colour and shoot development was decelerated, due to the dying off of apical meristems. Leaves became very hairy, rigid, dark green, small and interveinal necrosis appeared. The inclination of the leaves was abnormal. They grew vertical and leaf tips pointed downwards. Moreover, alterations in the surface morphology of leaves in plants with 0 μM ^{11}B were observed. Stomata appeared closed, collapsed and sunken underneath the epidermis (Fig. 2). Whereas the pore lengths of B deficient leaves did not differ from leaves grown under adequate (10 μM ^{11}B) B supply (−B: 8.4±1.3 μm, +B: 8.5±1.3 μm, n=100), the pore widths differed significantly. In B deficient leaves average pore widths were 0.1±0.3 μm, while with adequate B supply widths were 3.2±1.3 μm (n=100).

Abnormal leaf inclinations were also observed in association with 100 μM ^{11}B supply. Furthermore, older leaves were also rigid and showed veinal browning on the lower leaf surface, with black spots on the upper leaf surface. In all treatments, the dry matters of the treated leaves varied between 300 to 400 mg and no clear trend could be detected in association with the different B regimes. Development and phenology of roots and shoots of plants with 10 μM ^{11}B and 30 μM ^{11}B supply was in accordance to normal growth of the species.

Necrotic spots appeared beneath the applied droplets in some of the treatments. The degree of damage depended on the composition of the formulations and the plant ^{11}B status. Regardless of the foliar formulations applied to the B-deficient plants (0 μM ^{11}B) necrotic spots never developed on the treated leaf areas. Increased phytotoxicity symptoms were observed in plants cultivated in 30 and 100 μM ^{11}B. The degree of damage was most severe after the application of formulations containing sorbitol.

Water status

Water potential (ψ_w) measurements showed highest values of −0.59±0.05 MPa and -0,61±0.06 MPa in plants with 0 μM ^{11}B and 100 μM ^{11}B supply, respectively. In plants with 10 μM ^{11}B and 30 μM ^{11}B supply, ψ_w was lower with values of −0.78± 0.13 MPa and −0.74±0.12 MPa, respectively.

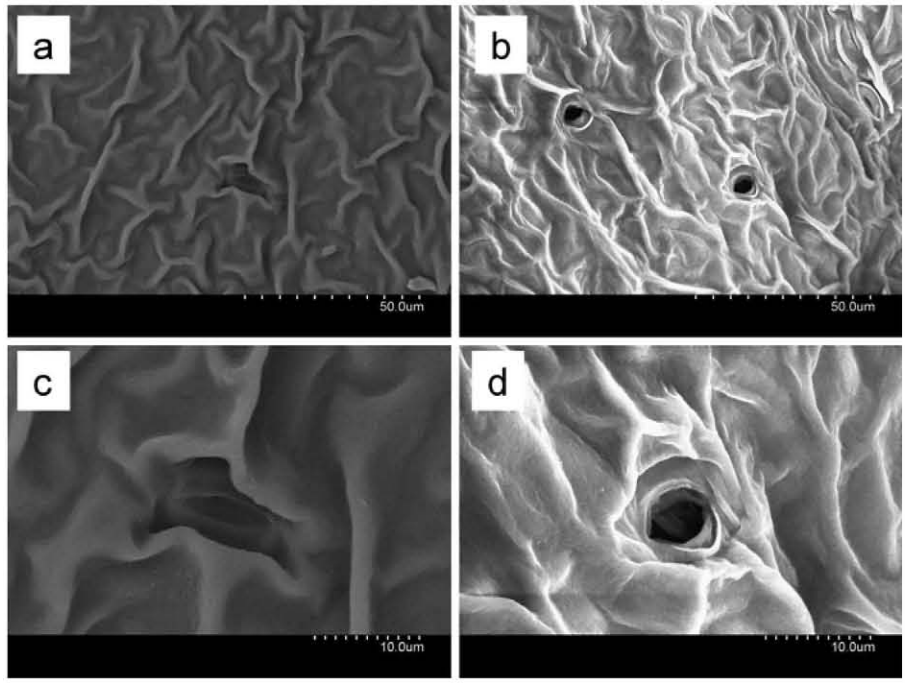


Fig. 2 SEM micrographs of the abaxial leaf surface of soybean leaves. Stomata appeared closed, collapsed and sunken underneath the epidermis on plants grown without ^{11}B (**a**, **c**) and developed regularly on plants treated with 10 μM ^{11}B in the nutrient solution (**b**, **d**)

Absorption and mobility

Both B absorption and B translocation were significantly affected by plant ^{11}B status and the addition of polyols, whereas interactions between these 2 factors were not significant (Table 1). When applied as pure BA 18.2% of the foliar-applied ^{10}B was absorbed by the leaves, while with the addition of sorbitol or mannitol the proportion of absorbed ^{10}B increased to 22.9% and 25.4%, respectively (Fig. 3a). Plants with 0 μM ^{11}B supply showed the lowest ^{10}B contents representing only 9.7% of the applied dose, whereas in the other treatments 26.5% to 32.3% of the applied ^{10}B penetrated the leaf surfaces (Fig. 3b).

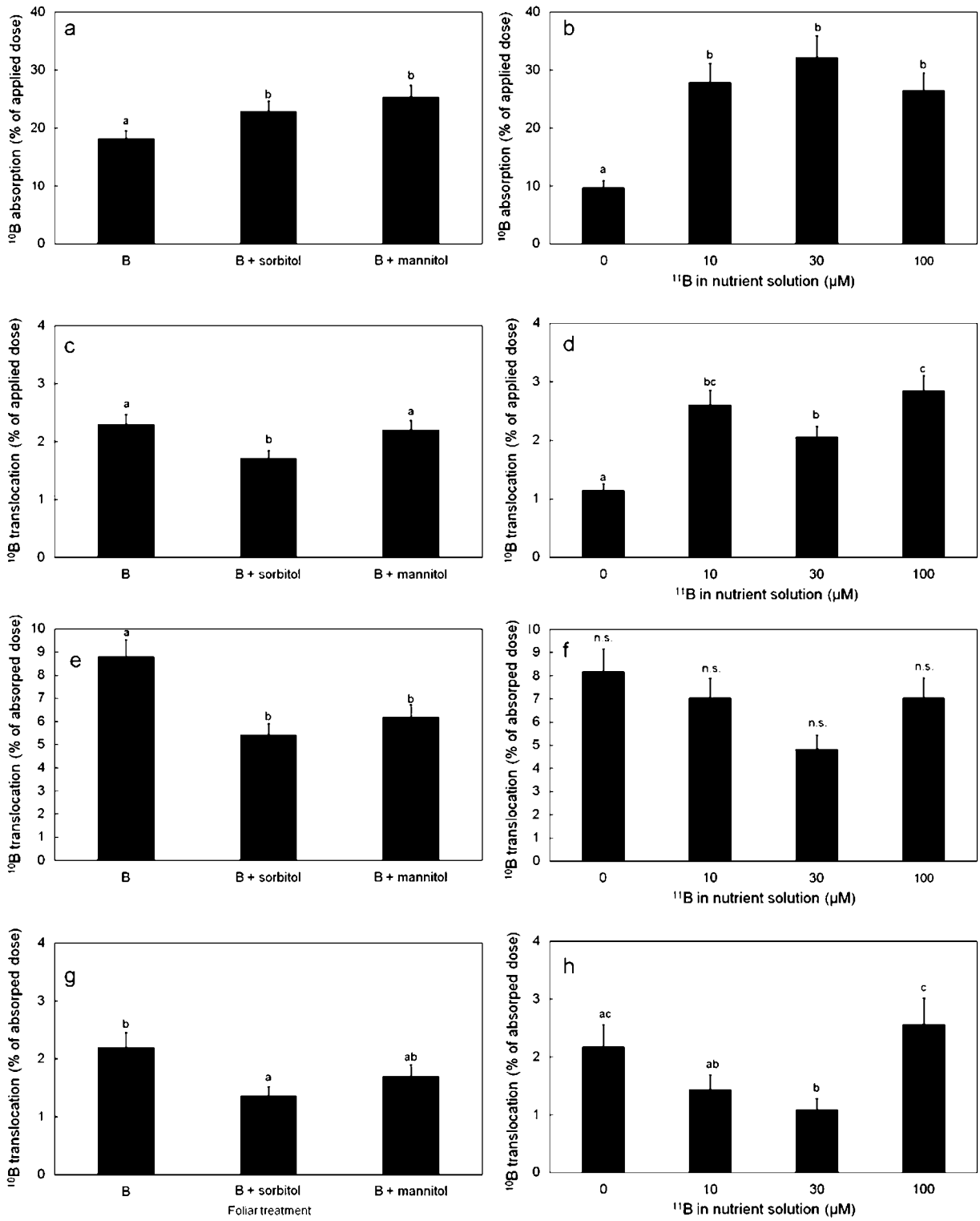
Table 1 Results of statistical analysis of ^{10}B contents in segments 1 (application zone), 2 (upper part of treated leaf), and 3 (neighbouring leaflet) as affected by the ^{11}B supply during pre-culture ("B status") and the foliar application as pure boric acid or boric acid in combination with polyols ("foliar treatment")

Source of variation	Segment 1	Segment 2	Segment 3
B status	<0.001	<0.001	0.0005
Foliar treatment	0.0038	0.0045	0.0253
Interaction	0.4901	0.3671	0.4829

The share of mobile ^{10}B which moved out of the application zone towards the tips of the treated leaves and the adjacent leaflet ranged from 1.1% to 2.8% of the applied dose (Fig. 3d). It was lowest in plants with 0 μM ^{11}B supply and overall highest in the plants grown under 100 μM ^{11}B supply. The addition of mannitol did not significantly affect the share of applied ^{10}B found in other plant parts, whereas sorbitol overall lead to a significant reduction in mobile foliar-applied ^{10}B (Fig. 3c).

While the share of mobile ^{10}B expressed as % of the applied ^{10}B is important in practical terms, the significance of this parameter for the analysis of within-plant ^{10}B mobility is rather limited, because it depends on both, the absorption process and the subsequent translocation in the plant. Therefore, we expressed the amounts of ^{10}B detected in other plant

Fig. 3 Effect of polyols (**a**, **c**, **e**, **g**) and different ^{11}B supply during growth (**b**, **d**, **f**, **h**) on foliar absorption of ^{10}B (**a**, **b**), total ^{10}B translocation to the tips of the treated leaflets and neighbouring leaflets as percentage of the applied dose (**c**, **d**), and ^{10}B translocation as percentage of absorbed B to tips of the treated leaflets (**e**, **f**) and neighbouring leaflets (**g**, **h**). Plants were treated either with 50 mM ^{10}B-labelled boric (B), 50 mM labelled boric acid and 500 mM sorbitol (B+sorbitol) or 50 mM labelled boric acid and 500 mM mannitol (B+mannitol). Error bars represent the standard errors of the means. Values marked by the same letter are not significantly different


a
b
c
d
e
f
g
h
^{10}B absorption (% of applied dose)
^{10}B translocation (% of applied dose)
^{10}B translocation (% of absorped dose)
B
B + sorbitol
B + mannitol
Foliar treatment
^{11}B in nutrient solution (μM)
0
10
30
100
n.s.

parts as % of the total amount that penetrated the leaves. The share of penetrated ^{10}B that moved to the tips of the treated leaflets (segment 2, Fig. 1) was higher in plants with 0 μM ^{11}B supply. In plants grown under 30 μM ^{11}B the share of penetrated ^{10}B was the lowest (Fig. 3f). The addition of both polyols significantly decreased ^{10}B movement into the tips of the treated leaflets (Fig. 3e).

Similar effects were found in the neighbouring leaflet (segment 3). The highest shares of penetrated ^{10}B were detected in plants grown in 0 μM ^{11}B or 100 μM ^{11}B (Fig. 3h), and polyols decreased relative mobility (Fig. 3g).

B concentrations in other plant parts were below the detection limit of 1 $\mu g\ g^{-1}$ DW. This assumption derives from former experiments where ^{10}B measured in other plant parts were below the detection limit.

Discussion

Symptoms of different B root supply

Two weeks after the onset of the ^{11}B treatments, plants showed different visual symptoms. The greatest effect was observed in plants grown without ^{11}B, indicating that they suffered from B deficiency. Shoots and roots showed a significant reduction in growth and development. Moreover, leaf surface morphology alterations were found in plants grown under ^{11}B shortage. Stomata of B-deficient leaves were closed, collapsed and sunken underneath the epidermis (Fig. 2). Several studies showed that B deficiency induced leaf structural changes including abnormal stomatal morphology and altered functionality (Oosterhuis and Zhao 2001; Rosolem and Leite 2007; Sharma and Sharma 1987; Sheng et al. 2009) but the underlying mechanisms of this physiological response to B deficiency remain speculative.

Boron deficiency and B toxicity affect membrane permeability (Alpaslan and Gunes 2001; Cakmak et al. 1995), resulting in membrane leakage and as a consequence K-efflux. Potassium is particularly important for the osmotic regulation of stomatal aperture. Due to the possible K membrane leakage in B-deficient or B -intoxicated plants this regulation could be dysfunctional, which may also explain the higher leaf water potentials at the lowest or highest B supply observed in this study. Another possible explanation for stomatal closure in B deficient plants could be the involvement of B in the structure of the cell walls and microfibrilles of the guard cells enabling stomatal opening.

The cultivation of plants under 100 μM ^{11}B in the nutrient solution induced toxicity symptoms affecting shoot but not root growth as observed visually and by measurement of the root dry mass. The shoots of plants treated with 100 μM ^{11}B supply did not differ in size and development as compared to plants with 10 μM ^{11}B and 30 μM ^{11}B concentrations in the nutrient solution, but the oldest leaves showed toxicity symptoms such as black necrotic spots. Nable et al. (1997) reported that under toxic B supply roots had adequate B concentrations in comparison to the toxic B concentrations in the shoots.

Effects on absorption of foliar-applied B

Plants with no ^{11}B supply experienced a significant reduction of foliar ^{10}B absorption as compared to plants grown under 10 μM ^{11}B, 30 μM ^{11}B and 100 μM ^{11}B (Fig. 3b). The absorption of ^{10}B was about thrice higher in all treatments in comparison to the 0 μM ^{11}B treatment. This strong decrease in foliar ^{10}B absorption under B-deficiency is rather unexpected and deserves further attention. Foliar absorption is driven by a concentration gradient across the leaf surface and modulated by the permeability of the leaf surface. In theory, a higher B concentration gradient after foliar B application could be expected in B-deficient versus B-sufficient leaves. However, lower ^{10}B penetration rates were determined in B deficient plants. The limited rate of ^{10}B absorption by B-deficient leaves must be most likely caused by a reduced permeability of the leaf surface. In leaves of plants grown without ^{11}B supply, stomata were shrunken and closed, which was earlier reported to reduce absorption of foliar-applied solutes via the stomatal pathway (Eichert and Burkhardt 2001; Eichert and Goldbach 2008). Additionally, with closed stomata, less transpiration water was released which otherwise may have re-condensated on the leaf surface (Burkhardt et al. 1999) and kept foliar-applied solutes partly dissolved and mobile even though the surrounding bulk atmosphere was dry (see below). Possibly, B deficiency also induced alterations in cuticular

structure, as was recently reported for Fe deficiency in peach and pear trees (Fernández et al. 2008). The alteration in leaf structure due to nutrient deficiencies may limit the efficiency of foliar fertilization.

The addition of polyols increased the absorption of foliar-applied B in all treatments. Generally, polyols could enhance B absorption by lowering the deliquescence humidity (DRH) of the deposited substances. This would extend the period of time during which foliar-applied B is mobile and can be absorbed, if RH of the air is above the DRH of the mixture of components (Fernandez and Eichert 2009). The RH during the experiment was 60%, which is well below the DRHs of the components, and accordingly this humectant effect should not have affected absorption. However, it has to be taken into account that leaf surfaces are surrounded by a laminar layer in which RH is higher than ambient RH (Burkhardt and Eiden 1994). As already mentioned above, water transpired by the leaves may substantially contribute to an increase in humidity, and therefore the humectant effect of polyols could have increased B absorption despite the low ambient RH. This argument may also explain why polyols did not affect B absorption in plants without B supply because B deficiency induced stomatal closure which probably reduced the amount of water released by the leaves.

Effects on B mobility

The absolute percentage of foliar-applied ^{10}B that moved out of the treated leaf parts ranged from 1.1 to 2.8% of the applied dose, and the effect of ^{11}B preculture on absolute ^{10}B mobility was similar to that on ^{10}B absorption, i.e. the lowest amount of ^{10}B moving out of the treated leaf parts was found in plants pretreated without ^{11}B supply. Polyols also significantly affected ^{10}B mobility, and overall lowest translocation was observed after the addition of sorbitol (Fig. 3c), even though the absolute absorption rate in this treatment was significantly higher than with pure BA (Fig. 3a). This might be due to the occurrence of many leaf necrotic spots in this treatment, which could have fixed ^{10}B in the dead tissues, thus preventing its translocation.

To gain further mechanistic insight into the effects of the ^{11}B status of plants and added polyols on ^{10}B mobility, we calculated the shares of translocated ^{10}B in relation to the amount absorbed by the leaf. Highest relative translocation rates were observed in plants pre-cultured in 0 μM ^{11}B or 100 μM ^{11}B (Fig. 3f, h). While high translocation rates in plants with high ^{11}B contents can be explained by the saturation of possible B binding sites in the cell wall leaving more free B for translocation, the reason for the relatively high shares of translocated ^{10}B in plants without ^{11}B supply is less obvious.

We found evidence that in this treatment stomata were disturbed and, like in plants growing under 100 μM ^{11}B, leaves sustained higher water potentials than plants cultivated under 10 μM ^{11}B or 30 μM ^{11}B indicating that both under B deficiency and high B supply the average transpiration rates were probably lower than under adequate supply, as it was reported by Eichert et al. (2010) for Fe deficient peach leaves. According to results obtained with *Ricinus communis* L., low transpiration rates may enhance phloem mobility of foliar-applied B (Eichert and Goldbach 2010), and possibly this was also the case in the present study with soybean.

Both polyols reduced the relative B mobility as compared to the application of BA alone. This may be due to the conversion of small uncharged BA molecules into relatively large, negatively charged B-polyol complexes. While BA is moderately plasmalemma-permeable and may thus easily diffuse into the phloem, the large ionic complexes are probably rather excluded from passive transmembrane transport reducing phloem mobility. This is in contrast to the situation in plants with natural polyol-assisted B mobility, where complexation takes place not until BA has entered the phloem.

Conclusion

The results of this study indicate that B deficiency symptoms may reduce B absorption through the leaf surface. From an agronomic point of view this negative feedback loop may limit the chance to alleviate B deficiency by foliar fertilization, and it can be concluded that B should therefore be applied before severe deficiency symptoms may occur. The application of B as B-sorbitol complex proved to increase absorption but reduced within-plant B mobility. Therefore, humectants that may have the same positive effect on B absorption as sorbitol, but that may not hinder B mobility should be selected in future research attempts.

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References

Akaike H (1974) New look at the statistical model identification. IEEE Trans Automat Contr AC 19:716–723

Alpaslan M, Gunes A (2001) Interactive effects of boron and salinity stress on the growth, membrane permeability and mineral composition of tomato and cucumber plants. Plant Soil 236:123–128

Bieleski RL (2005) Taxonomic patterns in the distribution of polyols within the proteaceae. Aust J Bot 53:205–217

Bieleski RL (1982) Sugar alcohols. In: Loewus A, Tanner W (eds) Encyclopedia of plant physiology NS Vol. 13A. Plant Carbohydrates. I. Intracellular carbohydrates. Springer, Berlin, pp 158–192

Blevins DG, Lukaszewski KM (1998) Boron in plant structure and function. Annu Rev Plant Physiol Plant Mol Biol 49:481–500

Brown PH, Hu H (1996) Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol-rich species. Ann Bot 77:497–506

Brown PH, Shelp B (1997) Boron mobility in plants. Plant Soil 193:85–101

Brown PH, Bellaloui N, Hu HN, Dandekar A (1999) Transgenically enhanced sorbitol synthesis facilitates phloem boron transport and increases tolerance of tobacco to boron deficiency. Plant Physiol 119:17–20

Burkhardt J, Eiden R (1994) Thin water films on coniferous needles. Atmos Environ 28:2001–2011

Burkhardt J, Kaiser H, Goldbach H, Kappen L (1999) Measurements of electrical leaf surface conductance reveal re-condensation of transpired water vapour on leaf surfaces. Plant Cell Environ 22:189–196

Cakmak I, Römheld V (1997) Boron deficiency-induced impairments of cellular functions in plants. Plant Soil 193:71–83

Cakmak I, Kurz H, Marschner H (1995) Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. Physiol Plant 95:11–18

Delgado A, Benlloch M, Fernandez Escobar R (1994) Mobilization of boron in olive trees during flowering and fruit development. Hortic Sci 29:616–618

Dell B, Huang L (1997) Physiological response of plants to low boron. Plant Soil 193:103–120

Eichert T, Burkhardt J (2001) Quantification of stomatal uptake of ionic solutes using a new model system. J Exp Bot 52:771–781

Eichert T, Goldbach HE (2008) Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces— further evidence for a stomatal pathway. Physiol Plant 132:491–502

Eichert T, Goldbach HE (2010) Transpiration rate affects the mobility of foliar-applied boron in *Ricinus communis* L cv. Impala. Plant Soil 328:165–174

Eichert T, Peguero-Pina JJ, Gil-Pelegrin E, Heredia A, Fernandez V (2010) Effects of iron chlorosis and iron resupply on leaf xylem architecture, water relations, gas exchange and stomatal performance of field-grown peach (*Prunus persica*). Physiol Plant 138:48–59

Fernandez V, Eichert T (2009) Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. Crit Rev Plant Sci 28:36–68

Fernández V, Eichert T, Del Rio V, Lopez-Casado G, Heredia-Guerrero JA, Abadia A, Heredia A, Abadia J (2008) Leaf structural changes associated with iron deficiency chlorosis in field-grown pear and peach: physiological implications. Plant Soil 311:161–172

Hu H, Brown PH (1997) Absorption of boron by plant roots. Plant Soil 193:49–58

Khayyat M, Tafazoli E, Eshghi S, Rajaee S (2007) Effect of nitrogen, boron, potassium and zinc sprays on yield and fruit quality of date palm. Am Eurasian J Agric Environ Sci 2:289–296

Lehto T, Räisänen M, Lavola A, Julkunen-Tiitto R, Aphalo PJ (2004) Boron mobility in deciduous forest trees in relation to their polyols. New Phytol 163:333–339

Marentes E, Shelp BJ, Vanderpool RA, Spiers GA (1997) Retranslocation of boron in broccoli and lupin during early reproductive growth. Physiol Plant 100:389–399

Marschner H (1995) Mineral Nutrition of Higher Plants. Academic, London

Miwa K, Takano J, Fujiwara T (2006) Improvement of seed yields under boron-limiting conditions through overexpression of BOR1, a boron transporter for xylem loading, in *Arabidopsis thaliana*. Plant J 46:1084–1091

Nable RO, Banuelos GS, Paull JG (1997) Boron toxicity. Plant Soil 193:181–198

Oosterhuis DM, Zhao D (2001) Effect of boron deficiency on the growth and carbohydrate metabolism of cotton. Plant Nutr 92(Symposium 2):166–167

Papadakis IE, Dimassi KN, Bosabalidis AM, Therios IN, Patakas A, Giannakoula A (2004) Boron toxicity in 'Clementine' mandarin plants grafted on two rootstocks. Plant Sci 166:539–547

Pfeffer H, Dannel F, Römheld V (2001) Boron compartmentation in roots of sunflower plants of different boron status: A study using the stable isotopes 10B and 11B adoping two independant approaches. Physiol Plantarum 133:346–351

Poss JA, Grattan SR, Grieve CM, Shannon MC (1999) Characterization of leaf boron injury in salt-stressed Eucalyptus by image analysis. Plant Soil 206:237–245

Rosolem CA, Leite VM (2007) Coffee leaf and stem anatomy under boron deficiency. R Bras Ci Solo 31:477–483

Roygrong S (2009) Role of Boron and Zinc in Growth and Flowering of Lychee (*Litchi chinensis* Sonn.). Dissertation, University of Hohenheim

Schlegel TK, Schönherr J, Schreiber L (2005) Size selectivity of aqueous pores in stomatous cuticles of *Vicia faba* leaves. Planta 221:648–655

Scholander PF, Hammel HT, Bradstre ED, Hemmings (1965) Sap pressure in vascular plants. negative hydrostatic pressure can be measured in plants. Science 148:339–346

Schon M, Blevins D (1990) Foliar boron applications increase the final number of branches and pods on branches of field-grown soybeans. Plant Physiol 92:602–607

Sharma CP, Sharma PN (1987) Mineral nutrient deficiencies affect plant water relations. J Plant Nutr 10:1637–1643

Shelp BJ, Marentes E, Kitheka AM, Vivekanandan P (1995) Boron mobility in plants. Physiol Plant 94:356–361

Shelp BJ, Kitheka AM, Vanderpool RA, Van Cauwenberghe OR, Spiers GA (1998) Xylem-to-phloem transfer of boron in broccoli and lupin during early reproductive growth. Physiol Plant 104:533–540

Sheng O, Song SW, Peng S, Deng XX (2009) The effects of low boron on growth, gas exchange, boron concentration and distribution of 'Newhall' navel orange (*Citrus sinensis* Osb.) plants grafted on two rootstocks. Sci Hort 121:278–283

Shorrocks VM (1997) The occurrence and correction of boron deficiency. Plant Soil 193:121–148

Sotiropoulos TE, Therios IN, Dimassi KN, Bosabalidis A, Kofidis G (2002) Nutritional status, growth, CO_2 assimilation, and leaf anatomical responses in two kiwifruit species under boron toxicity. J Plant Nutr 25:1249–1261

Warrington K (1923) The effect of boric acid and borax on the broad bean and certain other plants. Ann Bot 27:629–673

Wojcik P, Cieslinski G, Mika A (1999) Apple yield and fruit quality as influenced by boron applications. J Plant Nutr 22(9):1365–1377

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Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality

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Abstract

BACKGROUND: Foliar sprays of iron (Fe) and zinc (Zn) fertilisers are known to be an effective way to improve Fe and Zn concentrations in rice grain. However, results can differ significantly among different rice cultivars and/or types of foliar fertiliser. In this study, several Fe-rich rice cultivars were used to identify an effective foliar fertiliser for optimal Fe and Zn enrichment of rice grain.

RESULTS: Foliar Fe amino acid (Fe-AA) fertiliser significantly improved the Fe concentration in brown rice of most cultivars. Compared with the control, the average Fe concentration in all tested cultivars was increased by 14.5%. The average Fe concentration was increased by 32.5% when 1% (w/v) nicotianamine (NA) was added to Fe-AA, while the average Zn concentration was increased by 42.4% when 0.5% (w/v) $ZnSO_4 \cdot 7H_2O$ was added to Fe-AA.

CONCLUSION: The results suggested that NA at a suitable concentration added to Fe-AA fertiliser could accelerate Fe accumulation in rice grain. A relatively low concentration of $ZnSO_4 \cdot 7H_2O$ added to Fe-AA significantly increased Fe and Zn accumulation in rice grain. The study identified some useful foliar fertilisers for enhancing the levels of Fe and Zn in selected Fe-rich rice cultivars.

Keywords: rice (*Oryza sativa* L.); iron; zinc; foliar iron amino acid fertiliser; nicotianamine

INTRODUCTION

Rice (*Oryza sativa* L.) is indispensible in the diet of most of the world's population.[1] However, it is a poor source of many mineral nutrients, especially Fe and Zn, essential for humans. The International Rice Research Institute (IRRI) reported that polished rice contains an average of only 2 mg kg^{-1} Fe and 12 mg kg^{-1} Zn.[2] The biofortification target Fe concentration in polished rice, i.e. the amount needed to have a measurable effect on the Fe status of at-risk people, has been reported to be 14 mg kg^{-1}, which is seven times higher than that in wild-type rice.[3] According to a national nutritional survey, approximately 24% of all Chinese children suffer from a serious deficiency of Fe (i.e. anaemia); while over 50% show a subclinical level of Zn deficiency. Thus increasing the ability of plants to accumulate higher levels of grain Fe and Zn could have a dramatic impact on improving human health.[4]

There are several potential approaches to increasing the concentration of Fe and Zn in staple food crops, including fortification and supplementation programmes[5] as well as conventional breeding and genetic engineering.[6] Although rice fortification has proven to be effective for certain nutrients, it is costly, so many people in poor countries or areas cannot afford fortified rice.[7] Biotechnological approaches and plant-breeding programmes to increase Fe and Zn levels in rice grain are longer-term strategies.[8] More rapid ways need to be developed along with longer-term strategies that will address the present severe problem of Fe and/or Zn deficiency in humans globally. Agriculture is the primary source of all nutrients required for crops and humans, and fertilisation is the key strategy of nutrient integrated management in agronomic approaches to enhance crop quality and yield. Foliar fertiliser sprays have proved to be a sustainable, effective and low-cost strategy to improve Fe and Zn levels in edible portions of staple food crops.[9]

It has been reported that foliar Fe amino acid (Fe-AA) sprays can significantly improve the grain Fe levels in some rice cultivars.[10] However, little is known about the effects of foliar Fe-AA sprays on Fe-rich rice cultivars. Since the Fe concentration in Fe-rich rice is higher than that in common rice, spraying foliar fertilisers on Fe-rich cultivars makes it easier to reach the Fe biofortification target more quickly. It is well known that nicotianamine (NA) is indispensible for appropriate Fe translocation in plants, while a shortage of NA causes disorders in internal Fe transport. Positive responses to Zn fertilisation have been reported for a number of crops, including rice and wheat.[11] We postulated that the addition

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of NA at a suitable concentration to Fe-AA sprays could improve Fe accumulation in brown rice. We also investigated the effects of $ZnSO_4 \cdot 7H_2O$ addition to Fe-AA on grain Fe and Zn accumulation. The results of these studies were used to design experiments to identify some useful foliar fertilisers for enhancing the levels of Fe and Zn in selected Fe-rich rice cultivars.

MATERIALS AND METHODS

Rice varieties and components of amino acid fertiliser

Thirteen rice varieties were selected as follows: indica rices Zheguangxiang1 (IR1) and Zhenong40 (IR2); japonica rices T125 (JR1), R7 (JR2) and Zhenong37 (JR3); japonica hybrid rices Xiuyou-5 (JHR1), Nipponbare (JHR2), Nipponbare mutant (JHR3), HB075 (JHR4), T124 (JHR5) and Xiushui 110 (JHR6); japonica transgenic rices Xiushui110-1 (JTR1) and Xiushui110-2 (JTR2). The average Fe concentration in brown rice of these 13 cultivars was 13.65 mg kg^{-1}. The main components (w/v) of the Fe-AA fertiliser were 0.1% $FeSO_4 \cdot 7H_2O$, 0.4% amino acid (N 18.61%) and 0.2% urea (N 46%).[12]

Experimental sites and design

Field experiments, located at Bengbu County in Shaoxing City (Zhejiang Province, China), were conducted in the summer seasons of 2008 and 2009. Both experiments were in a split plot design with three replicates. Some properties of the experimental soil are presented in Table 1.

In 2008 the main treatments were the sprays with water (A_0) and Fe-AA (A_1) and the subtreatments were the 13 rice cultivars. All rice plants were sprayed on 1 June and harvested on 14 November. In 2009 we selected four rice cultivars (IR2, JR1, JHR1, JHR2) from the above 13 cultivars. The main treatments were the four rice cultivars and the subtreatments were the following Fe-containing solutions: control (CK); Fe-AA + 1% (w/v) NA (FeNA1); Fe-AA + 0.1% (w/v) NA (FeNA2); Fe-AA; Fe-AA + 0.5% (w/v) $ZnSO_4 \cdot 7H_2O$ (FeZn1); Fe-AA + 0.3% (w/v) $ZnSO_4 \cdot 7H_2O$ (FeZn2). Rice plants were sprayed in the field directly on 20 June and harvested on 26 November. The plot size of the two experiments was 10.6 m^2 (2.2 m × 4.8 m) and each plot was separated by a ridge covered with plastic film. N (as urea) at 145 kg ha^{-1}, P_2O_5 (as calcium superphosphate) at 31.5 kg ha^{-1} and K (as potassium chloride) at 72 kg ha^{-1} were applied as basal fertiliser 1–2 days prior to transplanting. Another 25.7 kg ha^{-1} of urea was applied at the tillering and booting stages separately. The foliar sprays were applied three times, once every 5 days, after anthesis. A volume of 700 mL of the appropriate solution was sprayed per plot during each application.

Table 1. Basic physicochemical properties of soil used

Characteristic	Value
pH (H_2O, 20 °C)	5.7
Organic matter (g kg^{-1})	38.9
Total Fe (mg kg^{-1})	8988
Alkali-hydrolysable N (mg kg^{-1})	54.1
Olsen P (mg kg^{-1})	23.5
NH_4OAc-extractable K (mg kg^{-1})	98.9
DTPA/$CaCl_2$/TEA-extractable Fe (mg kg^{-1})	208.8
DTPA/$CaCl_2$/TEA-extractable Zn (mg kg^{-1})	5.2

Plant sampling and analysis

Mature rice in the whole plot was harvested for grain yield analysis. Grain weight data were adjusted to 140 g kg^{-1} moisture level. Grain samples for rice quality analysis were taken randomly from panicles of mature rice plants within the harvest area. All rice grains were air dried. Blighted grains were removed before the rice seeds were dehusked in an electrical dehusker (model JLGJ-45, China). Part of the brown rice was polished with a sample polisher (model JB-20, China) before grinding.

All samples for micronutrient analysis were ground in a sample grinder (model MM301, Retsch, Germany) and digested in 2 mL of HNO_3 guaranteed reagent (GR) and 0.5 mL of H_2O_2. The digestion solutions were allowed to cool to room temperature (25 °C) and adjusted to a final volume of 25 mL with doubly deionised water. Fe and Zn concentrations were determined using an inductively coupled plasma mass spectrometer (model 7500a, Agilent, USA). Reference material (powder of the polished rice, GBW (E) 080684) and blanks were included in each digestion and Fe and Zn determination.

Samples for polished rice quality analysis were ground in a cyclone grinder (model 3010-99, Ugy, Fort Collins, CO, USA) to pass through a 0.15 mm sieve. Both protein and total amino acids of rice were detected by near-infrared reflectance spectroscopy. Rice powder samples (3 g) were placed in a small ring cup of 36 mm inner diameter and scanned using a monochromator (model 5000, NIR Systems, Silver Spring, MD, USA).

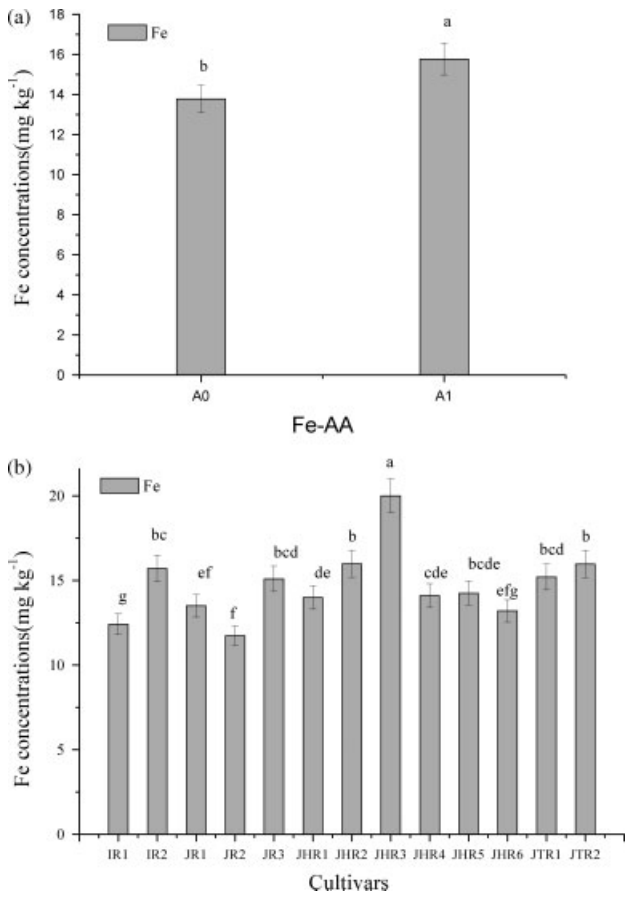

Figure 1. Effects of cultivar and foliar Fe-AA spray on average Fe concentration in brown rice. Different letters on bars indicate that values are significantly different at $P < 0.05$ according to Tukey's HSD test. Error bars denote standard error.

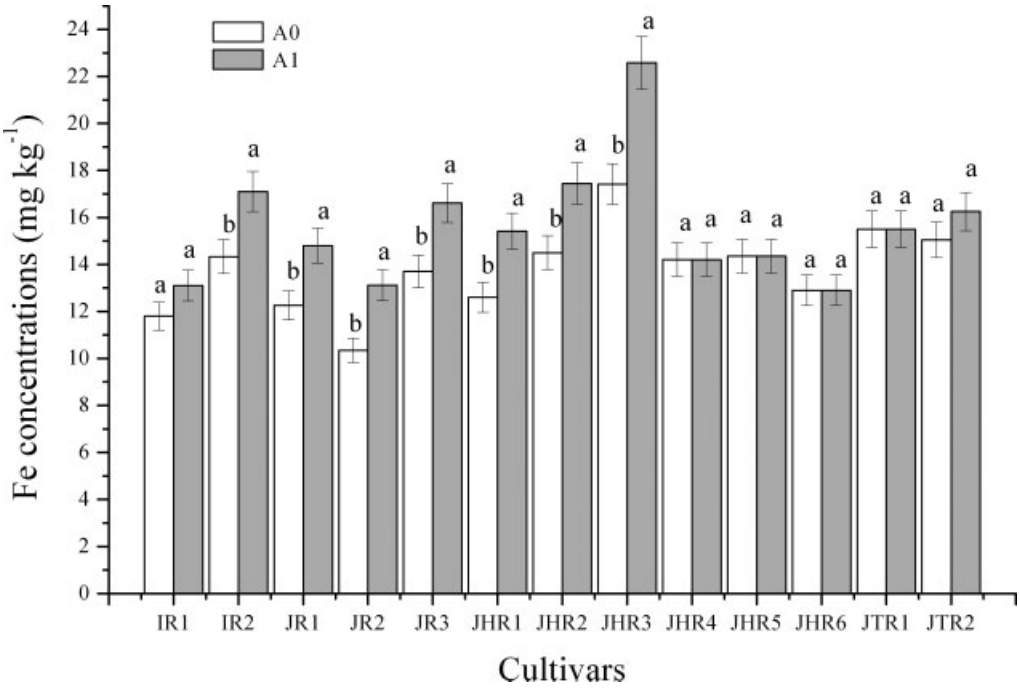

Figure 2. Effects of foliar Fe-AA spray on brown grain Fe concentration in different rice cultivars. Different letters on bars indicate that values are significantly different at $P < 0.05$ according to Tukey's HSD test. Error bars denote standard error.

Statistical analysis

All statistical analyses were performed using STATISTICA Version 5.5 (StatSoft, Tulsa, OK, USA). Each value represented the average of three replicates. Data were subjected to analysis of variance (ANOVA), and significant differences in mean values were determined using Tukey's multiple range test ($P < 0.05$).

RESULTS

Fe concentration in brown rice from 13 rice cultivars

Two conventional indica rices (IR1, IR2), three japonica rices (JR1, JR2, JR3), six japonica hybrid rices (JHR1, JHR2, JHR3, JHR4, JHR5, JHR6) and two japonica transgenic rices (JTR1, JTR2) were used in the 2008 experiment. The effects of Fe-AA spray and cultivar on the average Fe concentration in brown rice were highly significant (Fig. 1). With foliar Fe-AA spray the average Fe concentration in the 13 rice cultivars reached 15.8 mg kg^{-1}, which was a 14.5% increase compared with the control (A_0). The 13 rice cultivars differed significantly in Fe concentration.

We compared the effects of water (A_0, control) and Fe-AA (A_1) sprays on the Fe concentration in different rice cultivars (Fig. 2). Of the two indica rice cultivars tested, the Fe concentration in IR2 was 17.1 mg kg^{-1}, which was 19.3% higher than in the control. Of the 11 japonica rice cultivars tested, only JR1, JR2, JR3, JHR1, JHR2 and JHR3 showed a significant increase after foliar Fe-AA spray, with Fe concentrations of 16.6, 13.1, 14.8, 15.4, 17.4 and 22.6 mg kg^{-1} respectively. Compared with the control, the Fe concentrations increased by 21.2, 26.9, 20.7, 22.3, 20.0 and 29.6% respectively. The results suggested that spraying foliar Fe-AA fertiliser had a positive effect on improving grain Fe accumulation in the various rice cultivars tested.

Effects of NA and $ZnSO_4 \cdot 7H_2O$ added to Fe-AA on grain Fe and Zn accumulation in four rice cultivars

Foliar Fe-AA spray showed significantly positive effects on Fe accumulation in the grain of IR2, JR1, JHR1 and JHR2 in 2008 (Fig. 2). We added NA and $ZnSO_4 \cdot 7H_2O$ to the foliar Fe-AA spray to test whether these two additions could accelerate Fe accumulation and improve the Zn concentration in brown rice of these four cultivars in 2009.

Grain Fe accumulation was significantly affected by cultivar, foliar solution and their interaction, while grain Zn accumulation

Table 2. Two-way ANOVA of foliar solutions and rice cultivars as well as their interaction on reported traits of rice: degrees of freedom (DF), *F* value probabilities (*F* Pr.) and Tukey's protected $HSD_{0.05}$ test scores

		Fe		Zn		PC		TAAC		Grain yield	
Source of variation	DF	*F* Pr.	$HSD_{0.05}$	*F* Pr.	$HSD_{0.05}$	*F* Pr.	$HSD_{0.05}$	*F* Pr.	$HSD_{0.05}$	*F* Pr.	$HSD_{0.05}$
Cultivar (A)	3	0.01	3.8	0.07	NS	<0.001	0.6	0.007	0.3	<0.001	189.7
Solution (B)	5	<0.001	1.3	<0.001	2.5	<0.001	0.7	<0.001	0.1	0.03	20.6
A × B	15	<0.001	2.1	0.08	NS	<0.001	0.1	1.0	NS	0.04	17.4

PC, protein concentration; TAAC, total amino acid concentration; NS, not significant.

Table 3. Effects of different cultivars on average Fe and Zn concentrations in brown rice and average protein (PC) and total amino acid (TAAC) concentrations in polished rice

	Accumulation value (mg kg^{-1} dry weight)			
Cultivar	Fe	Zn	PC	TAAC
IR2	16.0a	24.0a	9.2a	9.7a
JR1	11.2b	19.3a	8.2b	8.0c
JHR1	12.0b	19.8ab	8.4b	9.3b
JHR2	16.1a	23.9a	9.1a	9.7a

Values in the same column followed by different letters are significantly different at $P < 0.05$ according to Tukey's HSD test.

Table 4. Effects of different Fe- and Zn-containing solution sprays on average Fe and Zn concentrations in brown rice and average protein (PC) and total amino acid (TAAC) concentrations in polished rice

	Accumulation value (mg kg^{-1} dry weight)			
Solution	Fe	Zn	PC	TAAC
CK	13.2c	16.9c	7.8d	8.5d
FeNA1	18.1a	19.9b	8.5c	9.2a
FeNA2	14.5c	19.4b	8.6bc	8.8c
Fe-AA	14.4c	20.0b	8.8bc	8.8c
FeZn1	18.8a	28.3a	9.6a	9.1a
FeZn2	15.9b	25.8a	9.2ab	9.0b

Values in the same column followed by different letters are significantly different at $P < 0.05$ according to Tukey's HSD test.

was significantly affected by foliar solution only (Table 2). The average Fe and Zn concentrations in the four cultivars suggested that grain Fe and Zn concentrations in different rice cultivars obviously differ (Table 3). Cultivars IR2 and JHR2 had higher Fe and Zn concentrations than cultivars JR1 and JHR1.

The average Fe and Zn concentrations in the four cultivars were significantly affected by different foliar solutions (Table 4). Compared with treatment Fe-AA, the Fe concentration was increased by 25.7% under treatment FeNA1. The Zn concentration was increased by 42.5 and 29.0% under treatments FeZn1 and FeZn2 respectively; at the same time a positive effect on Fe accumulation was observed.

NA (1%) added to Fe-AA significantly increased the Fe concentration in brown rice of the four cultivars (Fig. 3). Compared with treatment Fe-AA, grain Fe in IR2, JR1, JHR1 and JHR2 was increased by 51.4, 12.7, 11.9 and 27.3% respectively, while grain Zn in JHR2 was increased by 43.8%. All four cultivars had higher Fe and Zn concentrations when $ZnSO_4 \cdot 7H_2O$ (0.5%) was added to Fe-AA. Grain Fe in IR2, JR1, JHR1 and JHR2 was increased by 45.1, 18.4,

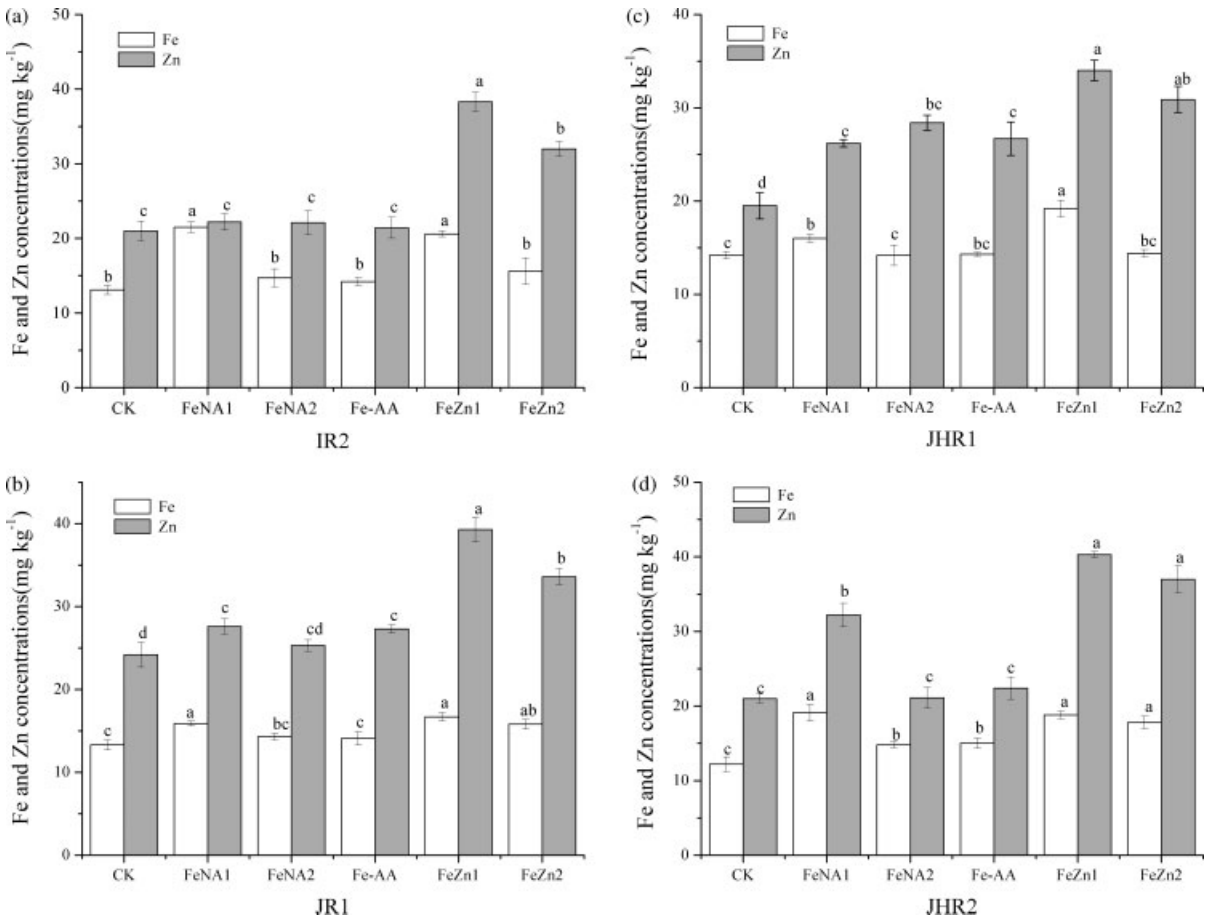

Figure 3. Effects of spraying different Fe and Zn solutions on rice grain Fe and Zn accumulation in four tested rice cultivars. Different letters on bars indicate that values are significantly different at $P < 0.05$ according to Tukey's HSD test. Error bars denote standard error.

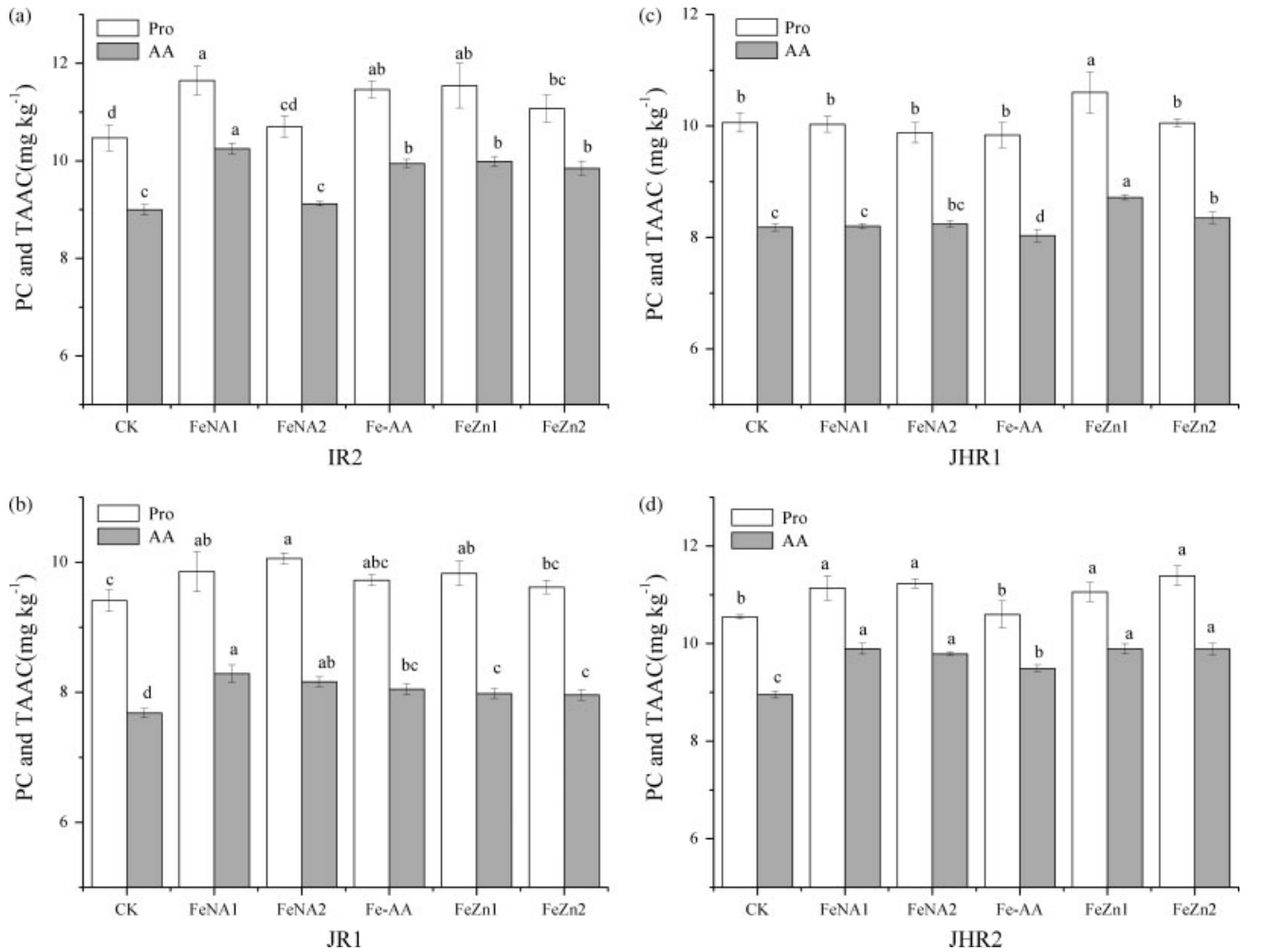

Figure 4. Effects of different foliar Fe and Zn solutions on protein (PC) and total amino acid (TAAC) concentrations in polished rice of four tested rice cultivars. Different letters on bars indicate that values are significantly different at $P < 0.05$ according to Tukey's HSD test. Error bars denote standard error.

34.3 and 27.0% respectively, while grain Zn was increased by 78.9, 44.0, 27.3 and 79.0% respectively. Apparently, a low concentration of $ZnSO_4 \cdot 7H_2O$ added to foliar Fe-AA spray fertiliser significantly improved Zn accumulation and also accelerated Fe accumulation in brown rice.

Rice nutritional quality

Rice nutritional quality was tested by determining protein content (PC) and total amino acid content (TAAC). Our study proved that foliar Fe and Zn solutions had different effects on rice nutritional quality for different genotypes. Rice PC was significantly affected by cultivar, foliar solution and their interaction, while rice TAAC was significantly affected by cultivar and foliar solution (Table 2). Our study provides evidence that JR1 and JHR2 had relatively higher average PC and TAAC in polished rice compared with IR2 and JHR1 (Table 3).

The average protein and total amino acid concentrations in the four rice cultivars differed when different foliar Fe and Zn solutions were sprayed (Table 4). NA (1%) added to Fe-AA (FeNA1) spray significantly increased grain PC and TAAC in polished rice of all four cultivars, with values 9.0 and 8.2% higher than those of treatment Fe-AA. $ZnSO_4 \cdot 7H_2O$ (0.5%) added to Fe-AA (FeZn1) led to the highest PC and TAAC. Compared with treatment Fe-AA, PC of treatment FeZn1 was increased by 9.1%. Thus NA and $ZnSO_4 \cdot 7H_2O$ added to foliar Fe-AA fertiliser had a tendency to increase PC and TAAC in polished rice.

The effects of foliar Fe and Zn solutions on rice protein and total amino acid concentrations of each cultivar are shown in Fig. 4. PC in JHR1 and JHR2 was increased by 8.2 and 4.7% when $ZnSO_4 \cdot 7H_2O$ (0.5%) was added to Fe-AA.

Grain yield as affected by cultivar and Fe-AA fertiliser

Grain yield was significantly affected by all treatments and their interaction (Table 2). Different cultivars (Fig. 5(a)) and foliar sprays (Fig. 5(b)) gave different grain yields. Compared with the control (CK), treatment FeNA1 gave a slightly higher grain yield, while grain yields under treatments FeNA2, Fe-AA and FeZn1 were lower. The results suggested that foliar solutions had a slightly negative effect on rice grain yield. Compared with treatment Fe-AA, grain yield was increased by 13.3 and 6.6% under treatments FeNA1 and FeZn1 respectively. It seemed that NA and $ZnSO_4 \cdot 7H_2O$ added to foliar Fe-AA fertiliser had a tendency to increase rice grain yield. The effects of foliar Fe and Zn solutions on the rice grain yield of each cultivar were totally different (Fig. 6). Compared with the control, grain yields of JR1 and JHR1 were significantly improved under treatments FeNA1 and FeZn1.

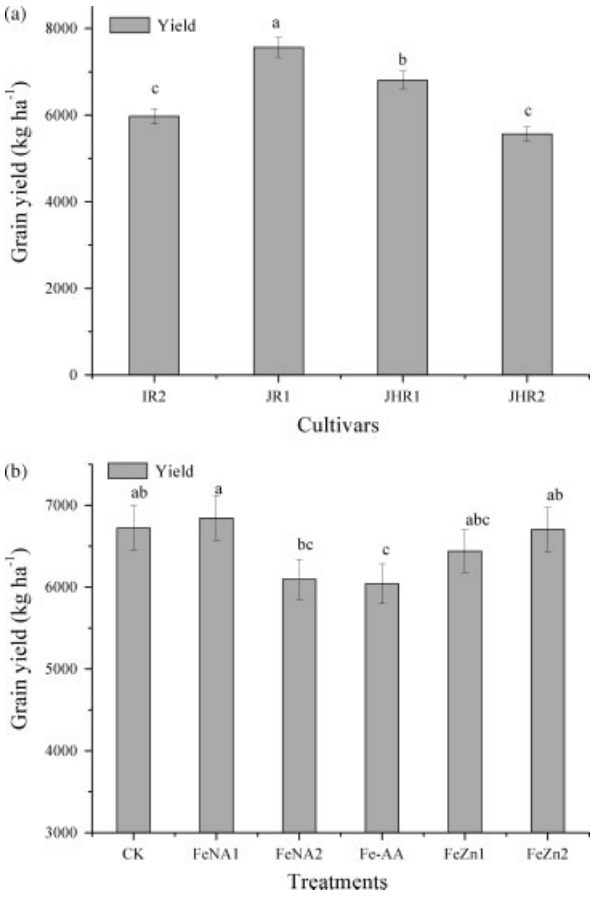

Figure 5. Effects of cultivar and foliar Fe and Zn solutions on average grain yield of four tested rice cultivars. Different letters on bars indicate that values are significantly different at $P < 0.05$ according to Tukey's HSD test. Error bars indicate standard error.

DISCUSSION

Micronutrient malnutrition, particularly Zn and Fe deficiency, afflicts over three billion people worldwide.[13] Producing micronutrient-enriched cereals via biofortification, either agronomically or genetically, and improving Fe and Zn bioavailability are considered promising and cost-effective approaches for diminishing malnutrition.[14]

The results reported here demonstrated that foliar Fe-AA could significantly increase the Fe concentration in brown rice of different cultivars. A similar observation was reported previously by Hsu and Ashmead.[15] Shenker and Chen[16] noted that one major problem impairing the success of foliar applications of Fe is the slow penetration of Fe through the leaf. High solubility and low molecular weight are key factors governing foliar uptake.[17] As the amino acid molecular ion radius is small, less resistance to the penetration of Fe-AA allows the foliar fertilisers to easily enter the plant.[18–20] In the present study, after being taken up by the leaves, low-molecular-weight amino acids might be chelated with Fe in the plant, which would increase the mobility of Fe and enhance its translocation to the sink during the development of rice grains. On the other hand, the leaf apoplasmic pH might be decreased by foliar Fe-AA application and thereby the leaf symplastic Fe uptake would be advanced. Brinch-Pedersen *et al.*[21] demonstrated that part of the mineral supply to the developing cereal grain originated from the remobilisation of minerals stored in leaves as they senesced during grain filling. Using Fe and Zn foliar applications at the phenological stage where rice was just in anthesis in the experiment provided the leaves with sufficient Fe and Zn during the grain-filling period.

Foliar Fe-AA fertiliser treatments showed different effects on grain Fe and Zn concentrations among different rice cultivars. The average concentrations of Fe and Zn in brown rice of IR2 and JHR2 were higher than those in brown rice of JR1 and JHR1. This can be explained by the genetic variability between varieties. Zimmermann and Hurrell[22] reported that variety not only influences seed morphology but may also affect the level of minerals. This would suggest that there is scope for improvement of mineral levels in seed and grain by selecting optimal varieties for specific regions and environments.

Adding NA (1%) to Fe-AA significantly improved Fe accumulation in brown rice in this experiment. Previous studies have shown that NA appears to be associated with Fe homeostasis and is easily transported within the plant; it has also been proved that NA is a low-molecular-weight compound that is ubiquitous in plants and has the capacity to bind Fe and Zn and other metals,[23,24] In our experiment, NA probably served as a mobile binding partner for Fe and Zn translocation from cell to cell after being absorbed by the leaves. NA (1%) addition to Fe-AA (FeNA1) spray significantly increased grain PC and TAAC in polished rice of the four cultivars, so the enhanced protein status may be another reason for grain Fe improvement. Our study provided evidence that adding an appropriate amount of NA to foliar spray could accelerate Fe accumulation in rice grain.

The micronutrient Zn is essential for all organisms. The use of Zn fertilisers is a conventional approach to overcome Zn deficiency. In our study, $ZnSO_4 \cdot 7H_2O$ added to foliar Fe-AA spray significantly improved rice grain Zn accumulation. Indeed, many previous studies have also reported a positive correlation between grain Zn and Fe concentrations in cereals.[25,26] Grain Fe and Zn may share similar protein-dependent mechanisms for translocation to or storage in the grain.[27] Since Zn and PC are closely associated in biological systems, in experiment 2, adding $ZnSO_4 \cdot 7H_2O$ (0.5%) to Fe-AA significantly improved grain Fe concentration, which seemed to be attributable to grain Zn and PC improvement. Our study suggests that it is possible to improve Fe and Zn accumulation together in rice grain.

The nutritional quality of rice is mostly evaluated in terms of micronutrients (e.g. Fe, Zn), PC and TAAC.[28] According to the results obtained in the present study, the foliar application of Fe- and Zn-containing solutions could improve Fe and Zn concentrations significantly, while a positive effect was also observed on rice PC and TAAC.

CONCLUSION

Under field conditions, Fe-AA could significantly improve the Fe concentration in brown rice of various cultivars/genotypes. It could be widely used for indica rice, japonica rice and some hybird rice cultivation. Foliar Fe-AA supplied with an optimal amount of NA could accelerate Fe accumulation in brown rice. Appropriate $ZnSO_4 \cdot 7H_2O$ addition to Fe-AA could improve Fe and Zn concentrations simultaneously.

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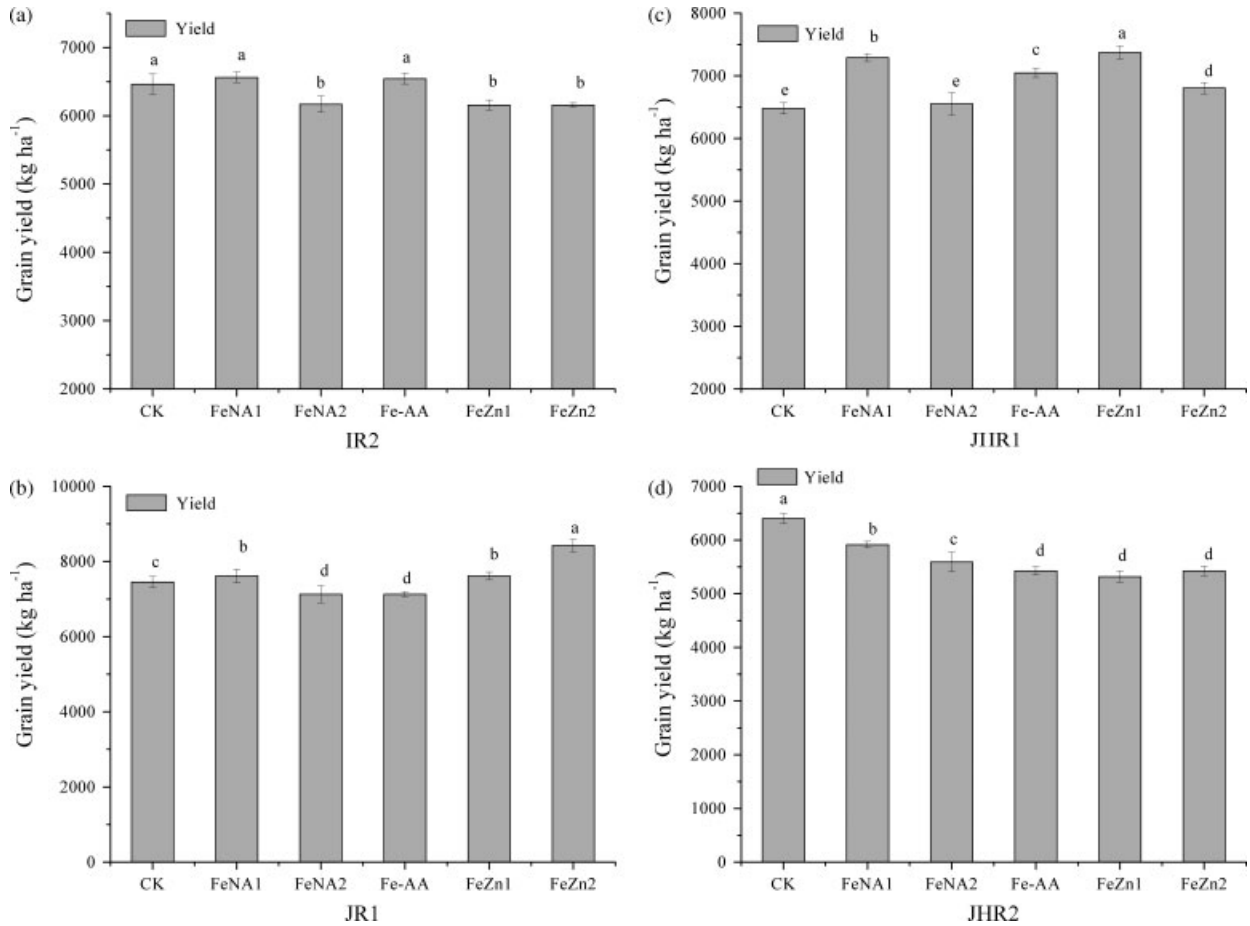

Figure 6. Effects of different foliar Fe and Zn solutions on rice grain yield of four tested rice cultivars. Different letters on bars indicate that values are significantly different at $P < 0.05$ according to Tukey's HSD test. Error bars indicate standard error.

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REFERENCES

1 Ishimaru Y, Masuda H, Bashir K, Inoue H, Tsukamoto T, Takahashi M, *et al*, Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. *Plant J* **62**:379–390 (2010).

2 International Rice Research Institute (IRRI), High-iron and -zinc rice. [Online]. (2006). Available: http://www.knowledgebank.irri.orgfactsheets/OtherResources/Health-and-Nutrition/fs-FeAndZn.

3 Pfeiffer WH and McClafferty B, HarvestPlus. Breeding crops for better nutrition. *Crop Sci* **47**:88–105 (2007).

4 Clemans S, Palmgrey MG and Kramer U, A long way ahead: understanding and engineering plant metal accumulation. *Trends Plant Sci* **7**:309–315 (2002).

5 Davidsson L, Approaches to improve iron availability from complementary foods. *J Nutr* **133**:1560–1562 (2003).

6 Holm PB, Kristiansen KN and Pedersen HB, Transgenic approaches in commonly consumed cereals to improve iron and zinc content and bioavailability. *J Nutr* **132**:514–516 (2002).

7 Poletti S, Gruissem W and Sautter C, The nutritional fortification of cereals. *Curr Opin Biotechnol* **15**:162–165 (2004).

8 Schachtman DP and Barker SJ, Molecular approaches for increasing the micronutrient density in edible portions of food crops. *Field Crops Res* **60**:81–92 (1999).

9 Rengel Z, Batten GD and Crowley DE, Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Res* **60**:27–40 (1999).

10 Mu J, Raza W, Xu YC and Shen QR, Preparation and optimization of amino acid chelated micronutrient fertilizer by hydrolyzation of chicken waste feathers and the effects on growth of rice. *J Plant Nutr* **31**:571–582 (2008).

11 Prasad R, Zinc in soils and in plant, human and animal nutrition. *Indian J Fertilisers* **2**:103–109 (2006).

12 Zhang J, Wang MY and Wu LH, Impacts of combination of foliar iron and boron application on iron biofortification and nutrition quality of rice grain. *J Plant Nutr* **58**:267–272 (2008).

13 Bouis H, The potential of genetically modified food crops to improve human nutrition in developing countries. *J Dev Stud* **43**:79–96 (2007).

14 Diftelfeld A, Cakmak I and Peleg Z, Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. *Physiol Plant* **129**:635–643 (2007).

15 Hsu HH and Ashmead HD, Effect of urea and ammonium nitrate on the uptake of iron through leaves. *Plant Nutr* **7**:291–299 (1984).

16 Shenker M and Chen Y, Increasing iron availability to crops: fertilizers, organic-fertilizers, and biological approaches. *Soil Sci Plant Nutr* **51**:1–17 (2005).

17 Schonherr J, Cuticular penetration of calcium salts: effects of humidity, anions and adjuvant. *J Plant Nutr Soil Sci* **164**:225–231 (2001).

18 Mu SH and Bai YJ, Uptake and transport of foliar applied iron by plants. *J Hebei Agrotech Teachers College* **8**:60–64 (1994). (in Chinese).

19 Frank W, Mechanisms of foliar penetration of solutions. *Annu Rev Plant Physiol* **18**:281–300 (1968).

20 Brown JC, Mechanism of iron uptake by plants. *Plant Cell Environ* **1**:249–257 (1978).

21 Brinch-Pedersen H, Borg S, Tauris B and Holm PB, Molecular genetics approaches to increasing mineral availability and vitamin content of cereals. *J Cereal Sci* **46**:308–326 (2007).

22 Zimmermann MB and Hurrell RF, Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Curr Opin Biotechnol* **13**:142–145 (2002).
23 Von Wiren N, Klair S, Bansal S, Briat JF, Khodr H, Shioiri T, *et al*, Nicotianamine chelates both Fe^{III} and Fe^{II}. Implications for metal transport in plants. *Plant Physiol* **119**:1107–1114 (1999).
24 Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T and Wiren N, ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. *J Biol Chem* **279**:9091–9096 (2004).
25 Cakmak I, Torun A, Millet E, Fahima T, Korol A, Nevo E, *et al*, *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Sci Plant Nutr* **50**:1047–1054 (2004).
26 Morgounov A, Gomez-Becerra HF and Abugalieva A, Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica* **155**:193–203 (2007).
27 Kutman UB, Yildiz B, Ozturk L and Cakmak I, Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. *Cereal Chem* **87**:1–9 (2010).
28 Lu XH, Wu LH, Pang LJ, Li YS, Wu JG, Shi CH, *et al*, Effects of plastic film mulching cultivation under non-flooded condition on rice quality. *J Sci Food Agric* **87**:334–339 (2007).

