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National Organic Standards Board,
c/o Robert Pooler, Agricultural Marketing Specialist,
USDA/AMS/TM/NOP, Room 2510-So., Ag Stop 0268,
P.O. Box 96456,
Washington, D.C. 20090-6456.
Phone: 202/720-3252. Fax: 202/205-7808. e-mail: nlpetition@usda.gov.

PETITION for Copper Sulfate in Crop Production
To add another annotation

Petitioner:
California Certified Organic Farmers, 1115 Mission St., Santa Cruz, Ca 95060.
(831)423-2263, www.ccof.org (contact: Brian McElroy, Director of Grower Certification)

This petition is submitted for this category of substance on the National List:
(1) Synthetic substances allowed for use in organic crop production; falls under "copper and sulfur" exemption in OFPA 6517(c)(1)(B)(i).

The petition includes, as applicable, the following information:
1. The substance's common name.
Bluestone, copper sulfate.
(chemical name: copper sulfate pentahydrate)
2. The manufacturer's name, address and telephone number.
Not applicable; there are many manufacturers.
3. The intended or current use of the substance.
Intended use: as an algicide and invertebrate control (for tadpole shrimp) in rice farming.
Current use: already approved by the NOSB for disease control.

4. A list of the crop activities for which the substance will be used. If used for crops, the substance's rate and method of application must be described.

See product label[1]. Used for control of algae[2] (many species - listed on last page of label instructions) and tadpole shrimp[2] (*triops longicaudatus*) in domestic and wild rice fields. One application is made at a rate of 10 to 15 pounds per acre after the rice field has been flooded in spring. Concentration in water should remain less than 10ppm. Usually dry copper sulfate is applied by airplanes.

5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.

"Copper sulfate is made by oxidizing copper turnings (from mining copper ore) in (special) furnaces in a current of air. The mass is then dissolved in sulfuric acid and then purified."[3] A longer description is attached in references[4, 5].

6. A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.

Material has been historically approved as a restricted material by CCOF, OCIA and QAI, although not necessarily in their written standards because "copper sulfate" was not limited to disease control in their standards. It is on the January 2001 OMRI Generic Materials List. Because it is only used on rice which is only produced in a few states, most other certifiers are silent on this use. An extensive review on the subject of copper has been prepared for the Maine Organic Farmers and Growers Association[6].

7. Information regarding EPA, and State regulatory authority registrations, including registration numbers.

EPA registration number for Phelps Dodge product: 1278-8. Other Brands may have other numbers.

8. The Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance.

CAS # for salt: 7758-98-7; for water: 7732-18-5

9. The substance's physical properties and chemical mode of action including (a) chemical interactions with other substances, especially substances used in organic production; (b) toxicity and environmental persistence; (c) environmental impacts from its use or manufacture; (d) effects on human health; and, (e) effects on soil organisms, crops, or livestock.

Physical properties: "Blue crystals, granules, or powder. Not very soluble in water. PH 8.9"[3]

Chemical mode of action: "the toxic action of copper is attributed to its ability to denature (change) the properties of cellular proteins and to deactivate enzyme systems in fungi and algae."[3]

(a) chemical interactions with other substances, especially substances used in organic production; Will produce hydrogen when mixed with magnesium. "Corrosive to metals, incompatible with strong oxidizers, reacts vigorously with reducing agents."[3]

(b) toxicity and environmental persistence; Breaks down into its components of copper, sulfur and water easily in the soil. These are then adsorbed or recombined in the soil solution. "Non-persistent as a compound, but copper itself is relatively immobile in soil and can persist and accumulate."[3] While some copper is essential for plant growth, excess can inhibit the development of microorganisms and be toxic to plants, with symptoms including disturbance in transpiration and water regulation.

(c) environmental impacts from its use or manufacture; environmental impacts of copper mining would be similar to any other mined mineral used in organic production. Copper sulfate created as a by-product from industry will have the additional concern of inadvertent inclusion of other heavy metals such as cadmium or zinc. See many of attached references in #11 below. The application rate of 10 pounds per acre would result in less copper being added to the soil than a recommended application rate for Bordeaux mix for disease control in fruit crops, a use which has already been approved for organic farming.

(d) effects on human health; "Chronic exposure- repeated ingestion of copper salts has produced hemolytic anemia, impaired immune response, liver, kidney, lung, and spleen damage and death in animals. Moderately acute toxicity to humans; LD50 (oral) 1000 mg/kg in rats. No carcinogen status, corrosive-eyes and ingestion; irritant-inhalation and skin. Acute toxicity if ingested, may cause gastroenteric pain, blue discoloration of burns and tongue, prostration, loss of consciousness, convulsions, may affect the liver kidneys, and blood. The hazards are almost entirely associated with application and not with human consumption of any residues on food."[3]

(e) effects on soil organisms, crops, or livestock - "While copper is an essential nutrient for plants in balanced amounts, excess residues in manure or organic waste materials may accumulate in soils and affect microorganisms and earthworms. Copper forms a variety of inorganic and organic complexes in the soil environment. How soluble any of the copper is (and thus potentially phytotoxic) is dependent on pH, the amount of organic matter in the soil, and other factors. Some bacteria have evolved mechanisms to regulate copper uptake and resist copper toxicity."[3] See Reference[6] for detailed review on this topic.

10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies.

MSDS is attached with Label in Reference[1].

11. Research information about the substance which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List.

For an extensive bibliography on copper in soil see the attached footnoted paper:

[6] Boyer, J. et al. 1994. Copper Fungicides and Certifiable Organic Produce. A report to the Maine Organic Farmers and Growers Association. PSE 546 Chemistry of Soils paper.

Other Research (all are attached articles except where abstract only is noted)

[8] Dunigan, E.P. & Hill, V. 1978. "Studies on the use of chemicals to control algal surface blooms in rice floodwaters." *69th Annual Progress Report of the Rice Experiment Station, Crowley, LA* p. 101-105. (Abstract attached)

[9] Gimeno-Garcia E. et al. 1996. "Heavy Metals Incidence in the Application of Inorganic Fertilizers and Pesticides to Rice Farming Soils" *Environmental Pollution* 1996 92:1 Elsevier Press.

[10] Herawati, N. et al. 1998. "Copper in Rice and in Soils according to Soil Type in Japan, Indonesia, and China: A Baseline Study". *Bulletin of Environmental Contamination Toxicology* 60:266-272 1998.

[11] Ma, Wei-Chun, 1988. "Toxicity of copper to lumbricid earthworms in sandy agricultural soils amended with Cu-enriched organic waste materials" *Ecological Bulletins* 39:53-56, Copenhagen 1988.

[12] McCurdy, S.A. et al. 1996. "Respiratory Health of California Rice Farmers". *American Journal of Respiratory Critical Care Medicine* 153:5. (Abstract attached)

[13] Miller, W.P. et al. 1987. "Short-term Transformations of Copper in Copper-amended Soils." *Journal of Environmental Quality* 16:2 1987.

[14] Payne, G.G. et al. 1988. "Form and Availability of Copper and Zinc following Long-Term Copper Sulfate and Zinc Sulfate Applications". *Journal of Environmental Quality* 17:4 1988.

[15] Payne, G.G. et al. 1988. "Availability and Form of Copper in Three Soils following Eight Annual Applications of Copper-Enriched Swine Manure." *Journal of Environmental Quality* 17:4 1988.

[16] Zalom, F.G. et al. 1979. "Toxicity of Chemicals used in California Rice Fields to Third Instar Hydrophilid Larvae." *Proceedings an Papers of the 47th Annual Conference of the CA Mosquito and Vector Control Association, Inc.* 1979. CMVCA Press. (Abstract attached)

12. A ``Petition Justification Statement" which provides justification for one of the following actions requested in the petition:

When petitioning for the inclusion of a synthetic substance on the National List, the petition should state why the synthetic substance is necessary for the production or handling of an organic product. The petition should also describe the nonsynthetic substances or alternative cultural methods that could be used in place of the petitioned synthetic substance. Additionally, the petition should summarize the beneficial effects to the environment, human health, or farm ecosystem from use of the synthetic substance that support the use of it instead of the use of a nonsynthetic substance or alternative cultural methods.

Description of Use

Copper Sulfate is used by both organic and conventional rice producers to help control two problems: algae and tadpole shrimp. These are discussed separately below.

1. Algae: Farmers use two different methods of planting in order to control aquatic (non algae) weeds: drill seeding method or the deep water and dry up method. Each method can have significant effect on the weed population. Over time each method usually favors a certain type of weed and then a farmer will need to use the other method to maintain effective weed control. Crop rotation, fallow, summer flood combined with mechanical till, will also help in long term viability of weed control methods.

The drill seeded method of organic rice farming usually does not require the use of copper sulfate, but it cannot be used frequently because of pressure from other weeds. When an organic rice farmer is using the deep water and dry up method for controlling weeds, s/he must cover the rice paddies with sufficient water to drown grass weeds. While holding the deep water a field may begin to generate unusually large amount of algae on the surface of the water. An algal mass can eliminate much of the farmers rice stand by pulling the plants down into the water, creating a scum barrier that young rice plants cannot penetrate, and blocking the transmission of sunlight to submerged leaves. A small amount of Copper Sulfate can break up the algae mass and allow rice to grow through. The impact of algae will vary depending on soil type, source of water (well water develops more algae), wind (wind can effectively break up and push around the algae), and temperature.

Copper Sulfate should only be used in conditions where plants do not have an opportunity to break through the surface of the water. These conditions can include anything that delays planting after the field has been flooded (most often windy weather or equipment breakdown). There is a very short window of opportunity (about a week to 10 days) to determine if the copper sulfate is needed and use it before crop reduction will result. The more organic matter is in the soil, the more attractive it is to algae.

2. Tadpole Shrimp: These crustaceans become a problem for organic rice farmers during a small window of time during the production cycle, otherwise the shrimp may be considered beneficial. In the water seeded (deep water) method of organic rice farming, the rice plant is very susceptible to shrimp damage during the fragile 'coleoptile' stage (before the plant turns green or has its first leaf). At this stage the plant growing can be easily broken or chewed by the shrimp. When the coleoptile is damaged by the shrimp the plant will die. During this vulnerable stage (5 - 8 days) copper sulfate may be used to control the population of shrimp so that the rice may grow through into an invulnerable stage. Many farmers have planting strategies which involve irrigating their land very quickly and having the seed ready to plant ASAP so that the rice will pass through the vulnerable stage before shrimp appear, thus making the need for copper unnecessary. When

the air and water temperatures are especially warm, it will cause the shrimp to "hatch". If the shrimp "hatch" happens while the rice is in the vulnerable stage, an organic rice farmer will need the protection of copper sulfate as a last resort to protect the crop.

As with algae, certain conditions of soil type, water source and temperature, wind and regional effects will influence the potential threat from shrimp.

Extent of Use

CCOF in the 2000 calendar year has 59 rice growers in the program. In any given year an informal estimate is that one half to two thirds of the growers use this material. It is generally used at a rate of 10 pounds per acre in one application per year. Years in which there is a very windy or very warm spring (both of which have occurred in 2001) will result in a higher need for the material because the heat promotes growth of the pests and the wind delays planting after flooding. This material is not generally needed in the first year that ground is planted in rice after pasture, because the pest populations (including weeds that necessitate deep water) have not built up enough yet.

Long term organic rice growers have developed a rotation that involves a fallow year after every one or two crop years (this is partly because no other crops can be grown on many rice soils). Therefore the copper sulfate is only used every other or every third year.

Our literature search showed only one citation from another rice producing state[8]. In southeastern rice growing states there is a whole different set of rice pests and crop management issues and so the material is not widely used or needed.

Alternatives

Since most conventional growers use this material too, there is clearly not even a very good synthetic alternative. The main alternatives for organic farmers are cultural measures as described above. These are itemized again below with discussion.

1. Drill-seeding each year will reduce the copper sulfate but is not feasible for a lot of reasons including: other weed problems which are aggravated by repeating this practice each year, soils that are two wet to use equipment on during the planting period (rice seed is normally flown onto fields), and the very large amount of acreage needing to be sown in a short planting window that is done faster by flying seed on.
2. Planting right after flooding is ideal but not always possible because of weather. Once the water warms up in the field, the algae or shrimp can outgrow the rice and without the use of a material there would be nothing to do about it.
3. Rotating into pasture is a potential idea but there are major expense issues here in bringing the fields back into rice (laser levelling and creating rice water delivery and drainage are major projects). Only about 10% of rice soils will even work as pasture because of drainage and alkalinity issues.
4. A literature search at the UC Davis library did not result in any other non-synthetic materials even being considered as alternatives. Parathion was considered as an alternative at one time, but rejected because of resistance issues[7].

Organic Regulations

The NOSB has previously reviewed copper products including copper sulfate (both as a component of Bordeaux mix and under "Coppers, fixed". They found them to be compatible with organic farming. Members of CCOF who were present for these deliberations recall that while the TAP data sheet mentioned the algae and shrimp control uses, the comments from the TAP reviewers did not specifically address rice farming but only focussed on disease control for tree crops. Therefore, the NOSB stated that they did not have enough information to include the uses in rice farming in their annotation when they voted to accept it.

We are hoping that this petition represents enough information about rice farming for the NOSB to add to the uses in the annotation to include control of algae and tadpole shrimp. Any other limitations to the annotation about the quantity used (10 pounds per acre) or the application once a year would also be fine. The same restriction about monitoring copper accumulation in the soil is also a good idea for rice growers who use this material.

13. A Commercial Confidential Information Statement

Not applicable.

Attachments:

[1] Product labels and instructions from Phelps Dodge Refining Corporation. Includes MSDS.

[2] Integrated Pest Management for Rice, 1983. UCD Publication 3280. IPM Project- Division of Agricultural Science, University of California at Davis.

[3] TAP review database sheets for "Coppers, Fixed" and "Bordeaux Mix" from 1995 NOSB decision.

[4] Sittig, 1980, listing for Copper Sulfate (submitted by an original TAP reviewer)

[5] Kirk-Othmer Encyclopedia of Chemical Technology, 3rd. Ed., 1982. John Wiley & Sons, NY

[6] Boyer, J. et al. 1994. Copper Fungicides and Certifiable Organic Produce. A report to the Maine Organic Farmers and Growers Association. PSE 546 Chemistry of Soils paper.

[7] Grigarik, A.A. & Lynch J.H., 1985. "Controlling Tadpole Shrimp", *California Agriculture.* CA Agriculture Experiment Station Bulletin, March/April 1985.

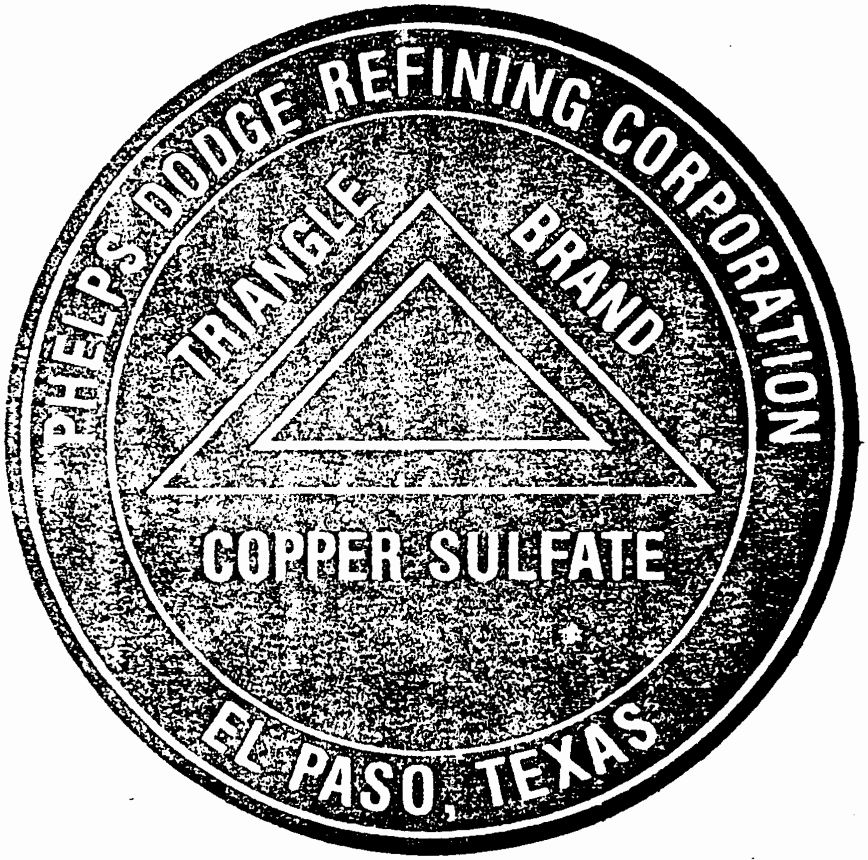
1.

phelps dodge Refining Corporation El Paso Works • P.O. Box 20001, El Paso, Texas 79998 • (915) 778-9881

ALGICIDE
-
HERBICIDE

COPPER

SULFATE PENTAHYDRATE
EPA REGISTRATION NO. 1278-8
EPA ESTABLISHMENT NO. 1278-TX-1



NATIONAL SANITATION FOUNDATION
NSF.

(Circle Containing the following)

Phelps Dodge Refining Corporation
El Paso, Texas

Triangle Brand Copper Sulfate

(Copper Sulfate)

ALGICIDE-HERBICIDE

NOT FOR MEDICINAL USE

Active ingredient : Copper sulfate pentahydrate*	99.0%
Inert ingredients:	1.0%
*Metallic Copper Equivalent	25.2%

KEEP OUT OF REACH OF CHILDREN
HARMFUL IF TAKEN INTERNALLY

DANGER/PELIGRO

PRECAUCION AL USUARIO: Si usted no lee inglés, no use este producto hasta que la etiqueta haya sido explicada ampliamente.

SEE BACK PANEL FOR ADDITIONAL PRECAUTIONARY AND PRACTICAL TREATMENT STATEMENTS.

Information for Right-to-Know States:

Copper Sulfate pentahydrate: Sulfuric acid, copper (2+) salt (1:1)/
CAS Reg No. 7758-98-7; Water / CAS Reg No. 7732-18-5

EPA REGISTRATION NO. 1278-8

EPA ESTABLISHMENT NO. 1278-TX-1

Net Weight 50 lbs. - 22.68 Kg.

MANUFACTURED BY
PHELPS DODGE REFINING CORPORATION
EL PASO, TEXAS 79998

Made in the United States of America.

DANGER/PELIGRO

PRECAUCION AI USUARIO:

Si usted no lee iglés, no use este producto hasta que la etiqueta haya sido explicada ampliamente.

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS AND DOMESTIC ANIMALS

DANGER:

Causes severe eye and skin irritation. Harmful if swallowed, inhaled, or absorbed through the skin. Avoid breathing mist or dust and contact with skin, eyes, or clothing. Causes substantial but temporary eye injury. May cause skin sensitization reactions in certain individuals. Protective clothing, including goggles, should be worn. Wash hands, arms, and face thoroughly with soap and warm water after handling. Remove contaminated clothing and wash before reuse.

STATEMENT OF PRACTICAL TREATMENT

IF SWALLOWED:

Drink promptly a large quantity of milk, egg white, gelatin solution, or if these are not available, large quantities of water. Avoid alcohol. Do not give anything by mouth to an unconscious person.

NOTE TO PHYSICIAN:

Probable mucosal damage may contraindicate the use of gastric lavage. Measures against circulatory shock respiratory depression and convulsions may be needed.

IF SPLASHED IN EYES:

Immediately flush eyes with plenty of water for at least 15 minutes, and get medical attention.

IF SPILLED ON SKIN:

Remove contaminated clothes and shoes, immediately wash skin with soap and plenty of water, and get medical attention.

IF INHALED:

Remove victim to fresh air. If not breathing, give artificial respiration, preferably mouth-to-mouth; and get medical attention.

ENVIRONMENTAL HAZARDS

This pesticide is toxic to fish and aquatic organisms. Do not apply directly to water except as directed under the specific instructions section. Drift and runoff from treated areas may be hazardous to fish and aquatic organisms in adjacent aquatic sites. Direct application of copper sulfate to water may cause a significant reduction in populations of aquatic invertebrates, plants and fish. Do not treat more than one-half of lake or pond at one time to avoid depletion of oxygen levels due to decaying vegetation. Allow one to two weeks between treatments for oxygen levels to recover.

Trout and other species of fish may be killed at application rates recommended on this label, especially in soft or acid waters. However, fish toxicity generally decreases when the hardness of water increases. Do not contaminate water by cleaning of equipment or disposal of wastes. Consult your State Fish and Game Agency before applying this product to public waters. Permits may be required before treating such water.

STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.

STORAGE:

Store unused product in orginal container only in a cool, dry area out of reach of children and animals. If container or bag is damaged, place the container or bag in a plastic bag. Shovel any spills into plastic bags and seal with tape.

PESTICIDE WASTE DISPOSAL:

Pesticide wastes are acutely hazardous. Improper disposal of pesticide, spray mixture, or rinsate is violation of Federal law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste representative at the nearest EPA Regional Office for guidance. Open dumping is prohibited.

CONTAINER DISPOSAL:

Do not reuse empty container. Completely empty liner by shaking and tapping sides and bottom to loosen clinging particles. Place the pesticide into application equipment. Then dispose of liner in a sanitary landfill or by incineration if allowed by State and local authorities. If burned, stay out of smoke.

GENERAL INSTRUCTIONS

It Is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

FEED ADDITIVE

PHELPS DODGE REFINING CORPORATION TRIANGLE BRAND COPPER SULFATE PENTAHYDRATE is a valuable dietary supplement in mixed feeds for cattle, hogs, chickens and other agricultural animals. Consult your State Agricultural Experiment Station or State Extension Service for guidance in preparing dosages and formulas.

FERTILIZER ADDITIVE

PHELPS DODGE REFINING CORPORATION TRIANGLE BRAND COPPER SULFATE PENTAHYDRATE is a valuable copper supplement in mixed fertilizers and soil conditioners. Consult your State Agricultural Experiment Station or State Extension Service for guidance in preparing dosages and formulas.

0.25 - 0.50 ppm	0.50 - 1.00 ppm	1.00 - 1.50 ppm	1.50 - 2 ppm
DIATOMACEAE ORGANISM (DIATOMS)			
Asterionella Praqilaria Navicula	Gomphonema Nitzschia Stephanodiscus Synedra Tabellaria	Achnanthes Cymbella Neidium	
PROTOZOA ORGANISM (FLAGELLATES)			
Dinobryon Synura	Ceratium Cryptomonas Euglena Glenodinium Mallomonas	Chlamydomonas Peridinium	

TO CONTROL ALGAE AND WEEDS IN FLOWING WATER

TO CONTROL POTAMOGETON PONDWEEDS, leafy and sago, in irrigation conveyance systems, use the continuous application method, selecting proper equipment to supply copper sulfate crystals at 0.25 to 0.5 pounds per hour for each cubic foot per sec. of flow for 12 hours of each 24 hours. For best control, begin copper sulfate additions when water is first turned into system to be treated and continue throughout the irrigation season. Copper sulfate becomes less effective for mature plants. Copper sulfate becomes less effective as the bicarbonate alkalinity increases and is substantially reduced above 150 ppm as $CaCO_3$. Mechanical or other means may then be required to remove excess growth.

TO CONTROL ALGAE (such as filimentous green, pigmented flagellates, diatoms) in irrigation conveyance systems begin continuous addition when water is first turned in, using suitable equipment to uniformly deliver 0.1 to 0.2 pounds of copper sulfate per hour per cubic feet per sec. of flow for 12 of each 24 hours. (Note: Triangle Copper Sulfate comes in several "free flowing" crystal sizes but should be selected to match requirements of your feeder.)

TO CONTROL ALGAE AND WEEDS IN IRRIGATION SYSTEM BY "SLUG" METHOD OF ADDITION, Add 1/2 to 2 pounds for each cubic foot per sec. flow, repeat every two weeks. A pile is required for every 5 to 30 miles of length depending on alkalinity of the water.

NOTICE TO BUYER

Seller makes no warranty, expressed or implied, concerning the use of this product other than indicated on the label. Buyer assumes all risk or use and or handling of this material when such use and/or handling is contrary to label instructions.

D.O.T. Hazard Class
RQ Environmentally hazardous solid, n.o.s.
Cupric sulfate, 9, UN 3077, PG III

COPPER SULFATE PENTAHYDRATE
CAS No. 7758-99-8

DIRECTIONS FOR USE AS A PESTICIDE

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

CONTROL OF ALGAE AND TADPOLE SHRIMP (TRIOPS LONGICAUDATUS) IN RICE FIELDS (DOMESTIC AND WILD)

Tadpole Shrimp in Rice Fields may be effectively controlled by the prompt and proper use of **COPPER SULFATE.** After the Rice Field has been flooded to a depth of 6" to 8", the **COPPER SULFATE** should be uniformly applied at a rate of 10 to 15 pounds per acre at the **FIRST SIGN OF INFESTATION.** Following these directions carefully should keep the concentration of **COPPER SULFATE** less then 10 ppm. The **"DIAMOND"** size crystals are especially graded for maximum solubility.

SEWER TREATMENT FOR ROOT AND FUNGUS CONTROL

PHELPS DODGE REFINING CORPORATION TRIANGLE BRAND COPPER SULFATE PENTAHYDRATE is effective in keeping sewer lines free of roots.

A. **FOR PARTIAL STOPPAGE:** Add 1/2 pound of **PHELPS DODGE REFINING TRIANGLE BRAND COPPER SULFATE PENTAHYDRATE** to sewer or drain and flush toward blockage with 5 gallons of water. Repeat at 6 month intervals to prevent growth of new roots.

B. **FOR COMPLETE STOPPAGE:** Physically remove the root blockage and repeat as above.

C. **FOR HOUSEHOLD SEWERS:** Use 2 to 6 lbs. **COPPER SULFATE SMALL CRYSTALS** twice yearly in spring and early fall. Apply in toilet bowl near sewer line. Flush each 1/2 lb. portion at a time. Or, remove the clean-out plug and pour entire quantity directly into sewer line and flush with water.

NOTE: DO NOT APPLY IN SINK OR TUB DRAINS.

D. **FOR COMMERCIAL, INSTITUTIONAL AND MUNICIPAL USE:**

SEWERS: Use 2 lbs. of **COPPER SULFATE SMALL CRYSTALS** each 6 to 12 months, applied to each junction or terminal manhole.

STORM DRAINS: Use 2 lbs. of **COPPER SULFATE SMALL CRYSTALS** per drain per year. Apply during period of light flow. In dry weather induce a flow with hose. If storm drains become almost plugged, repeat treatment 3 or 4 times at two week intervals.

SEWER PUMPS AND FORCE MAINS: Place 2 lbs. of **COPPER SULFATE SMALL CRYSTALS** in a cloth bag at the storage wall inlet. Repeat as needed.

CONTROLLING WEEDS, ALGAE, AND MICROSCOPIC ORGANISMS

TO CONTROL ALGAE IN IMPOUNDED WATERS, LAKES, PONDS, AND RESERVOIS

WARNING CONCERNING FISH

NOTE: The treatment of algae with **COPPER SULFATE** can result in oxygen loss in the water from decomposition of dead algae. This can cause the fish to suffocate. Therefore, to minimize this hazard, treat 1/3 to 1/2 of the water area in a single operation. Wait 7 to 14 days between treatments. Begin treatments along the shore and proceed outwards in bands to allow fish to move into untreated water.

APPLICATION BY DRAGGING BURLAP BAGS WITH COPPER SULFATE UNDER WATER

Large or Small sized **COPPER SULFATE** are placed in burlap bags and dragged through the water by means of a boat. Begin treatment along the shoreline and proceed outward until 1/3 to 1/2 of the total area has been treated. The path of the boat should insure a distribution that is even. In large lakes the boat should move in parallel lines about 60 feet apart. Continue dragging until all of the weighed **COPPER SULFATE** is dissolved.

APPLICATION BY SPRAYING COPPER SULFATE SOLUTON ON WATER SUFACE

A solution can be made with **COPPER SULFATE POWDER OR FINE CRYSTALS** which dissolves easily in water. This solution can then be sprayed on the pond or lake surface from a boat. When using this method the wind direction is important as well as the operation of the boat. **DO NOT ENDANGER PEOPLE OR ANIMALS IN THE BOAT WITH THE COPPER SULFATE SPRAY.**

APPLICATION BY SPRAYING DRY COPPER SULFATE CRYSTAL FROM AIRPLANES

Professional personnel licensed by the State Agricultural Extension Service are allowed to apply **COPPER SULFATE CRYSTALS** in some states.

If treated water is to be used as a source of potable water, the metallic copper residual must not exceed 1 ppm copper. This equals 10.64# per acre foot of water or 4 ppm of this product.

HOW TO FIND THE POUNDS OF COPPER SULFATE TO ADD TO WATER

To find Acre-Feet of water in a body of water, measure the body of water in feet. Calculate the surface area in square feet, divided by 43,560 (sq.ft. /Acre) times the average depth in feet.

1 Acre-Foot of Water = Water measuring 208.7 feet long by 208.7 wide by 1 foot deep.
1 Acre-Foot of Water = 43,560. cubic feet of water.
1 Cubic Foot of Water = 62.4 pounds
1 Acre-Foot of Water = (43,560.) (62.4) = 2,720,000. Pounds

COPPER SULFATE PENTAHYDRATE IN WATER

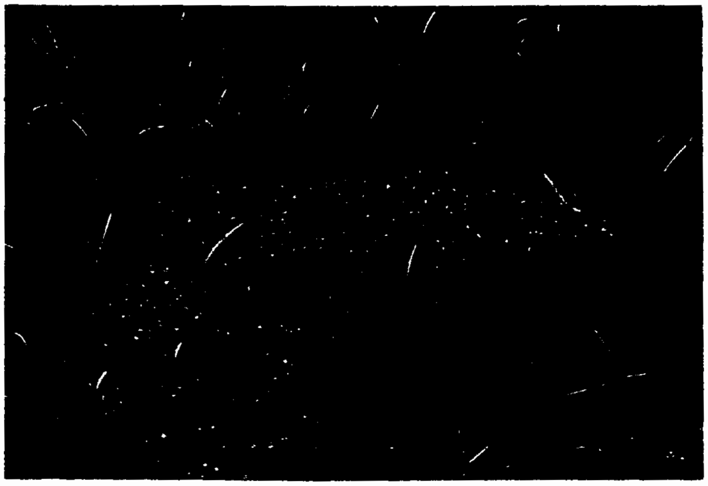
POUNDS OF COPPER SULFATE PENTAHYDRATE PER ACRE-FOOT OF WATER	=	PARTS (BY WEIGHT) COPPER SULFATE PENTAHYDRATE PER MILLION PARTS (BY WEIGHT) OF WATER	=	PARTS (BY WEIGHT) COPPER PER MILLION PARTS (BY WEIGHT) OF WATER
0.67#/Acre-Foot	=	1/4 ppm	=	0.0625 ppm
1.3 #/Acre-Foot	=	1/2 ppm	=	0.125 ppm
2.6 #/Acre-Foot	=	1 ppm	=	0.25 ppm
5.32 #/Acre-Foot	=	2 ppm	=	0.50 ppm

TREATMENT OF SOME ALGAE WITH COPPER SULFATE PENTAHYDRATE

(Dosage is in ppm of copper sulfate pentahydrate) A higher concentration is required if the water is hard. Consult with the State Fish and Game Agency before applying product in municipal waters.

0.25 - 0.50 ppm	0.50 - 1.00 ppm	1.00 - 1.50 ppm	1.50 - 2 ppm
CYANOPHYCEAE ORGANISM (BLUE GREEN)			
Anabaena Anacystis Aphanizomenon Gloeotrichia Gomphosphaeria Polycystis Rivularia	Cylindrospermum Oscillatoria Plectonema	Nostoc Phormidium	Calothrix Symploca
CHLOROPHYCEAE ORGANISM (GREEN)			
Closterium Hydrodictyon Spirogyra Ulothrix	Botryococcus Cladophora Coelastrum Draparnaldia Enteromorpha Gloeocystis Microspora Tribonema Zygnema	Chlorella Crucigenia Golenkinia Oocystis Palmella Staurastrum Tetraedron	Ankistrodesmus Scenedesmus

INTEGRATED PEST MANAGEMENT FOR RICE 12.
UCD PUBLICATION #3280



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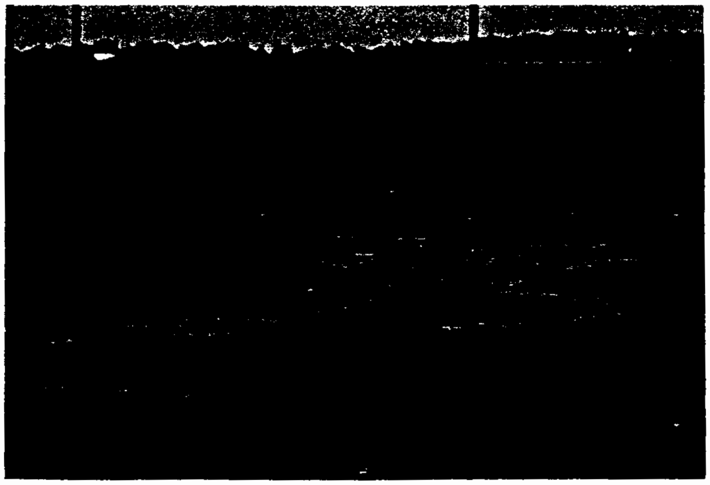

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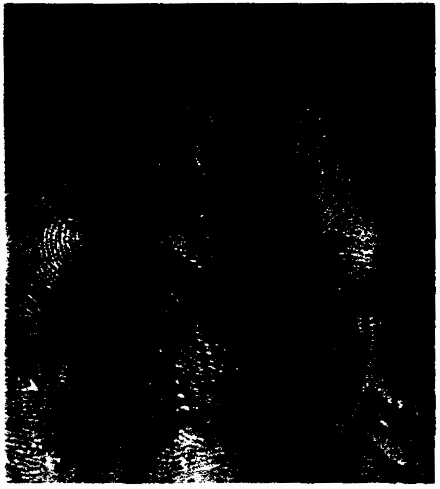


C

Algae

Algae are nonflowering plantlike organisms that reproduce by cell division or through the production of spores. The types that commonly occur in rice fields include phytoplankton, which grow suspended in water as single cells or in microscopic colonies, filamentous algae, which grow in long strands or filaments large enough to be seen with the unaided eye, and an advanced form called chara, which resembles a higher plant. The more troublesome are the filamentous algae. Although phytoplankton in large numbers may cloud the water, they rarely cause economic losses. Chara algae cause problems only in highly alkaline fields.

Filamentous algae, on the other hand, may clog waterways and impede the growth of young rice plants. Masses of filamentous algae may uproot seedlings. Algae grow best in shallow water, 3 inches (7.5 cm) deep or less, especially where poor seedbed preparation has created low areas with poor surface drainage that has resulted in inadequate decomposition of organic matter. Phosphate fertilizer left on the soil surface or applied after flooding may also stimulate algal growth. Various copper compounds are available to control filamentous algae. To be effective, these materials must be applied before algae mats float to the water surface. Check for the bubbling on the soil surface that is an indicator of a serious algal infestation.

A. Filamentous algae are most abundant in newly flooded rice fields early in the season. The algal units unite to form a solid mass of scum suspended from the water surface by gas bubbles and extending to the bottom of the flooded paddy.

B. This photo shows an algal mass that has eliminated much of a rice stand by pulling rice plants down into the water, creating a scum barrier that young rice tillers cannot penetrate, and blocking the transmission of sunlight to submerged leaves.

C. Anchored into the mud at the bottom of the paddy, *Chara* plants are branched with evenly spaced whorls of leaflike structures at the nodes. They may be mistaken for more advanced plants like naiads. When crushed, *Chara* plants emit a musky garlic or skunklike odor. Plants are usually gray green but may turn brown or chalky due to calcareous deposits or accumulated dead plants. They are usually found associated with alkaline soil of a pH 8 or higher. Chara persists through the season, holding water and preventing soil from drying at harvest. No satisfactory controls are available, although temporarily draining the field may limit the infestation.

~~e always advisable. Beginning in July and continuing rough August, make weekly observations for leaf-oppers, armyworms, and their damage. If armyworms gin to damage your crop, start sampling the field ice a week following the program outlined in the myworm section.~~

~~Keep written records of your monitoring results. his book contains sample record forms for many ajor rice pests. Well-maintained records will help you ake effective pest control decisions and predict future st management needs.~~

~~The equipment you will need for your monitoring ogram includes a hand lens, a metal or plastic cylinder closing a one square foot area (with a diameter of proximately 13.5 inches or 34 cm) (Figure 22), a simi-rly sized ring of floating plastic tubing (Figure 23), a ass jar 5 to 6 inches (12 to 15 cm) deep, a fish seine, d a pencil and sampling forms or paper for record eping. To carry out an effective monitoring program, u will have to move out into the flooded field and get et, so wear suitable clothing.~~

~~When you are sampling, be sure to sample each ddy or check within a field because different water vels will influence invertebrate populations. Addi-onally, except for the special case of the rice water eevil, you should take your samples in one or two agonal lines transecting the field (Figure 24) to be sure u get a representative sampling of field conditions. lways move against the wind when observing aquatic sts; otherwise, the movement of your feet will muddy e water ahead of you.~~

~~The pictures in this manual will help you identify any common invertebrates in the rice field. Check~~ th your farm advisor for help in identifying others.

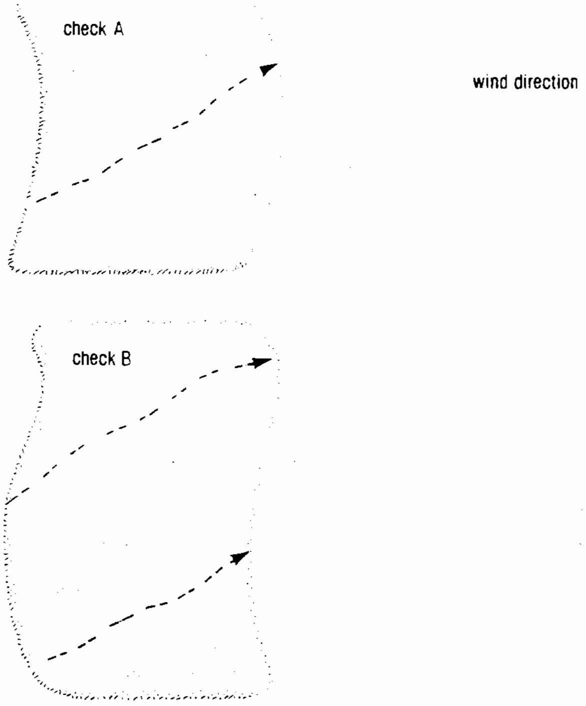


Figure 24. Sampling plan for most invertebrate pests in rice. When sampling for tadpole shrimp, seed midges, leafminers, or armyworms in rice fields, monitor each check following a path that transects the field diagonally. In a large check (check B above), take two diagonal sample sets. When sampling for underwater pests such as tadpole shrimp and midges, be sure to move against the wind so you don't muddy the water ahead of you.

adpole Shrimp
riops longicaudatus

Although they are crustaceans, tadpole shrimp re-mble tadpoles in size, shape, color and mobility. They mmonly inhabit temporary freshwater pools in low, ncultivated areas; however, when they are present in ge numbers in fields with young submerged seedlings, eir feeding and digging activities can reduce stands.

escription and Seasonal Development

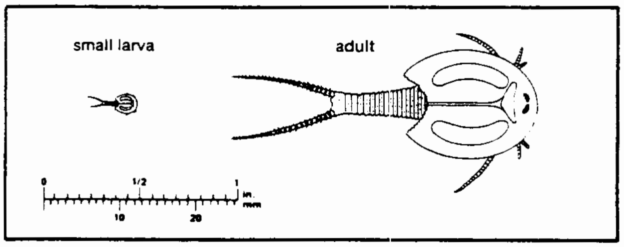
Tadpole shrimp have about 35 body segments, all t the last six or seven with pairs of leaflike, gill-bearing pendages. A thin, olive brown shield (the carapace) vers the front part, and two long tails (the cercopods) tend from the last segment. The true antennae are all and inconspicuous, but two longer, jointed pro-sses resembling antennae extend from below the well-veloped mandibles.

Figure 25. Digging activity of a tadpole shrimp preparing to lay eggs.

If the water is clear, you can often see tadpole shrimp swimming at the bottom of the paddy.

Tadpole shrimp adults may vary in size from 1 3/4 to nearly 3 inches.

TADPOLE SHRIMP



Tadpole shrimp may chew the tips of shoots (right, top) as they emerge from the germinating seed.

Adults are hermaphrodites (that is, they poss both male and female reproductive organs) and car masses of orange eggs in two brood pouches on t appendages of the 11th segment, just at the lower ma gin of the carapace. The adult deposits the eggs sing on soil or on plants at the bottom of the padd Although a few eggs may develop into larvae just aft laying, most require a period of drying to hatch, ar populations develop almost entirely from eggs laid previous years. The eggs are highly resistant to desicc tion, remaining viable for several years in dry soil.

Most larvae begin hatching one to three days aft spring flooding of the rice fields, but hatching ma continue for one to two weeks. Larvae develop rapidl molting and developing a carapace and tails to resemb young adults in less than 24 hours. Very small shrim (0.5 cm or ¼ inch) feed primarily on microorganism but larger shrimp (1 cm or ½ inch) may chew emergin shoots and roots of rice seedlings. Larvae shed the skins several times before reaching sexual maturity an continue to molt and increase in size after they ar adults. Floating skins may sometimes be mistaken fo dead shrimp. About nine days after hatching, tadpo shrimp begin their reproductive phase by first diggin and stirring up the silt on the bottom of the paddy pond (Figure 25) and then laying eggs in the soil or o plants. The digging and chewing activities may uproo entire seedlings or cut off leaves, causing them to rise t the surface of the water. Masses of wind-blown, cu leaves and uprooted seedlings floating along dikes ar good evidence of tadpole shrimp activity. The shrim causing the damage may occur only in localized popu lations in one or more areas of the field.

Damage

Tadpole shrimp can cause losses in seedling ric stands in two ways. First, they may chew off seedlin roots and coleoptiles or uproot seedlings with the digging, killing or injuring plants and reducing th stand. Second, their digging activities muddy the wate reducing light penetration and thereby slowing th growth of submerged seedlings. Low populations shrimp do not cause significant economic damage. Tad pole shrimp cause no injury to rice once leaves hav reached the water surface and roots are well establishe in the soil.

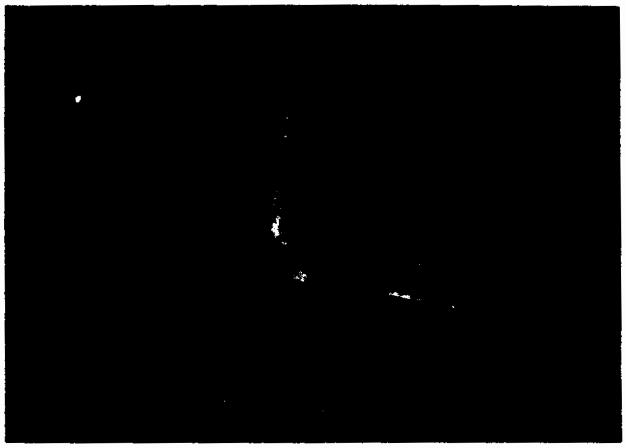
Management Guidelines

Prevention. Most tadpole shrimp eggs hatch withi two days after flood water contacts them. The longe the time between hatching of the eggs and planting, th larger the size of the shrimp and the greater the poten tial for plant injury. Flood the field as fast as possibl and seed as soon as possible after flooding has been initiated. In very large fields that take more than a few days to flood, you may want to seed parts of the field in sequence as they fill with water.

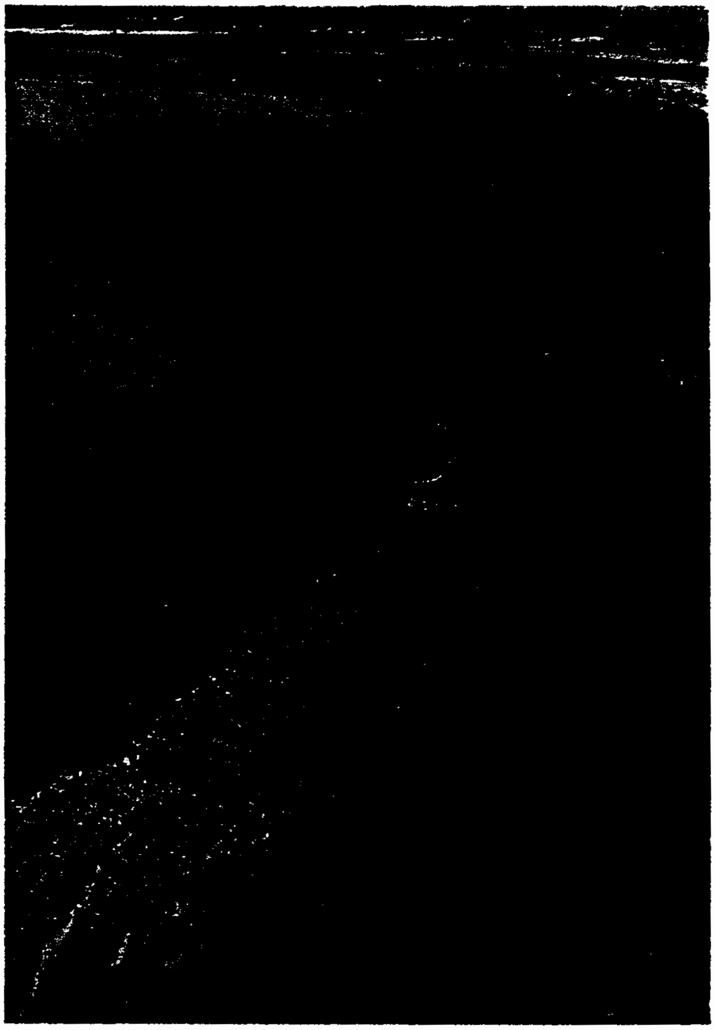
Monitoring and Control. Check all your fields, but give special attention to those that had significant populations of tadpole shrimp last year. Even though infestations may have been localized in previous years, monitor all quadrants of the field because the shrimp can occur well beyond previously observed boundaries. The presence of cut or uprooted rice seedlings and muddied water may indicate an infestation, but check for shrimp and floating shrimp skins to confirm your suspicions because seed midges and crayfish may cause similar damage. If the water is clear, you can see the shrimp swimming on the bottom. Tadpole shrimp are small, so look carefully. If it is muddy, drag a fish seine (1/8 inch mesh) across the bottom of the paddy and check the net for shrimp.

Whether any control measure is needed is determined by counting numbers of seedlings at various stages of development. These samples are generally taken twice during seedling development: at five to seven days after flooding is initiated and again three days to a week later. Use a metal or plastic cylinder enclosing one square foot (0.09 m^2) and with sides higher than the water depth (Figure 22) to count the plant stand. Place it in the water gently so the area to be observed remains clear. Wind and wave action may cause a field to become murky during the first eight days of flooding; if this occurs, you may have to wait a day or two before the water clears. An alternative to waiting is to view the seedlings through a clear glass container or ar held within the sampling ring. Hold the jar right side up and push it down into the cloudy water until you can see the bottom of the paddy and move the jar around to see the seedlings. Be careful not to fill the jar with water as you push it into the water. Take 10

adpole shrimp have chewed on the leaves of this uprooted, oating seedling. At right is the molted skin of a tadpole hrimp.



Seedlings damaged by tadpole shrimp may have all or part of their roots gnawed off.



Masses of uprooted seedlings are an indication that tadpole shrimp may be causing damage. Uprooted seedlings usually drift to the borders of the rice paddy.

samples across the check on each sampling date. If your checks are large, you may want to take 20 samples. Keep records of sampling results for shrimp and other pests of stand establishment on a form such as the one in Figure 26. The tentative guidelines below are based on a seeding rate of 150 pounds per acre.

- ***First Sample Period: 5 to 7 Days after Flooding Begins.*** Count all the germinating seeds and seedlings within the ring at each site. Examine all floating seeds and a sample of the ungerminated seeds for chewing injury. If tadpole shrimp or their molted skins are present and less than 30 healthy seedlings per square foot are found, and the damage is due to shrimp feeding, a chemical treatment is needed. If 30 or more healthy seedlings are found per square foot, but there is also evidence of shrimp activity, recheck the field in two days, using the same guidelines for treatment.

- ***Second Sample Period: 8 to 14 Days after Flooding.*** Count the rooted, healthy (nonfloating) seedlings within the sampling ring at each site. An average of 25 or more healthy seedlings per square foot at this time should provide a good stand. If less than 25 healthy seedlings are found and evidence of shrimp is present in the monitoring area, treatment is necessary. f 25 or more healthy seedlings are found, but there is also evidence of tadpole shrimp activity, repeat the ampling procedure every 2 to 3 days until rice plants emerge above the water surface. Treat if the average number of healthy seedlings drops below 25 plants. Always check for shrimp or their shed skins because midges and crayfish may cause similar injury to seedlings.

If muddy water does not allow an adequate visual inspection of the plant stand after eight days, treatment decisions must be based on the presence of shrimp and hed skins, together with observations of uprooted oating seedlings and seedlings with chewed leaf tips r roots, regardless of stand density.

Rice Seed Midges

Chironomidae

The midges are the most common group of insects n rice fields. Although about 30 species may be found, nly 3 are known to cause significant losses. These are *ricotopus sylvestris*, *Paralauterborniella subcincta*, and a pecies in the genus *Paratanytarsus*. All three can be ound in nearly every rice field every year, often in large umbers, particularly *Paratanytarsus*. Economic damage y midge larvae in rice is limited to germinating seeds nd very young seedlings.

Description and Seasonal Development

Adult midges swarm in the air over rice fields, levees, and around other bodies of water. Midges resemble small mosquitoes, but the mouthparts of midges are undeveloped, and they lack the scales on wing margins and wing veins characteristic of mosquitoes. Midges prefer to deposit their eggs on open water. Masses of eggs are laid on the water surface in strings held together by a sticky, mucuslike material that swells in contact with water to form a protective gelatinous envelope around the eggs. Eggs hatch in one to two days. The larvae use secreted silk and bits of algae or debris to build tubes on the bottom of the rice check and on submerged vegetation. The tubes serve as retreats and also act as webs to entrap algae, diatoms and detritus for food. Larvae may also feed on seedlings and leaves of rice and other plants. The larvae go through 4 instars in about 7 to 10 days in the spring when the water is warm and unshaded. The 3rd and 4th instars cause the greatest damage to rice. Larvae pupate in the silk tubes under water. Adults emerge from pupae in two to three days. Three to four generations occur each summer, but because damage is limited to seedlings, only the first two are of economic concern to rice growers.

Damage

Serious damage to rice is limited to germinating seeds and very young seedlings. Midge larvae feed on emerging leaves or root shoots or may hollow out embryos, killing plants. Once the seedling is several inches long, it can usually outgrow the feeding of midge larvae, even though irregular holes may be eaten in the leaf. Midge larvae may also feed on floating leaves, causing small holes that extend completely through the leaves. Again, injury to these older leaves is not of economic concern if other leaves are upright.

Seed and seedling injury caused by midges is most easily distinguished from damage by tadpole shrimp and other seedling pests when it is recent and the seed tissue is still firm. Midge larvae often eat the inside out of the seed, leaving it hollow; tadpole shrimp never cause this kind of damage. Sometimes damage by midges is limited to chewing away shoots and roots. Tadpole shrimp may cause similar damage, but the chewed areas on the roots, coleoptile, and young leaves will generally be larger and more irregular because of the larger size of shrimp mandibles. If the damage is caused by midges, the midge larva and tube are often still on or in the seed. If the injury is several days old, secondary organisms may invade the tissue and the actual cause of damage will be difficult to determine. Feeding damage caused by midge larvae on older leaves can be distinguished from rice leafminer damage because

NOSB Materials Database

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Identification

Common Name **Coppers, fixed** **Chemical Name**

Other Names fixed copper, copper hydroxides, basic sulfates, oxychlorides and oxides.

Code #: CAS 7758-98-7 (Copper Sulfate) **Code #: Other** 20427-59-2 (Copper Hydroxide)

N. L. Category Synthetic Allowed **MSDS** yes (Kocide)

Chemistry

Family

Composition Copper sulfate: $CuSO_4$, copper hydroxide: $Cu(OH)_2$, copper oxide: CuO.

Properties Blue crystals, granules, or powder.($CuSO_4$). Light blue powder with characteristic copper odor ($Cu(OH_2)$). Not very soluble in water. pH 8.9.

How Made

Copper Sulfate is made by oxidizing copper turnings (from mining copper ore) in (special) furnaces in a current of air. The mass is then dissolved in sulfuric acid. and then purified. Copper (II) Oxide is made the same way only without the sulfuric acid, or it can be made by ignition of copper (II) nitrate or the basic carbonate. Copper (II) Hydroxide is obtained by electrolyzing a copper anode in an electrolyte containing sodium sulfate and trisodium phophate.

Use/Action

Type of Use Crops

Use(s) Disease control ~~in tree fruit and vegetables~~. Shrimpicide in rice.
Many of the other fixed coppers are less caustic and thus safer for the applicator than copper sulfate.

Action The toxic action of copper is attributed to its abiltiy to denature (change) the properties of cellular proteins and to deactivate enzyme systems in fungi and algae.

Combinations

Status

OFPA 6517 (c) (1) (B)(i) synthetic on list as copper

N. L. Restriction Must be used in a manner that prevents accumulation of copper in the soil. Fixed coppers must be exempt from tolerance by the EPA.

EPA, FDA, etc

Safety Guidelines wear protective clothing **Directions**

Registration **State Differences**

Historical status OFPANA allowed

Internationl status IFOAM ok; CODEX ok ($CuSO_4$)

OFPA Criteria

2119(m)1: chemical interactions

Corrosive to metals, incompatible with strong oxidizers, reacts vigorously with reducing agents. Solutions of sodium hypobromite are decomposed by catalytic action of cupric ions.

2119(m)2: toxicity & persistence

--Excess copper is toxic to plants, with symptoms being disturbances in transpiration, water regulation, and cell membrane permeability. Also root stunting and reduced growth vigor.
--Breaks down into its components of calcium, sulfur, water, and copper easily in the soil. These are then adsorbed or recombined in the soil solution. Non-persistent as compounds, but copper itself is relatively immobile in soil and can persist and accumulate.

2119(m)3: manufacture & disposal consequences

All the manufacturing issues of copper mining will be relevant as well as the extraction processes.

2119(m)4: effect on human health

Chronic exposure- repeated ingestion of copper salts has produced hemolytic anemia, impaired immune response, liver, kidney, lung, and spleen damage and death in animals. Moderately acute toxicity to humans; LD50 (oral) 1000 mg/kg in rats. No carcinogen status, corrosive- eyes and ingestion; irritant- inhalation and skin. Acute toxicity if ingested, may cause gastroenteric pain, blue discoloration of bums and tongue, prostration, loss of consciousness, convulsions, may affect the liver, kidneys, and blood. The hazards are almost entirely associated with application and not with human consumption of any residues on food.

2119(m)5: agroecosystem biology

While copper is an essential nutrient for plants in balanced amounts, excess residues in manure or organic waste materials may accumulate in soils and effect microorganisms and earthworms. Copper forms a variety of inorganic and organic complexes in the soil environment. How soluble any of the copper is (and thus potentially phytotoxic) is dependent on pH, the amount of organic matter in the soil, and other factors. Some bacteria have evolved mechanisms to regulate copper uptade and resist copper toxicity.

2119(m)6: alternatives to substance

Lime sulfur, silica, kelp, baking soda, plant nutrition, resistant varieties, cultural controls(pruning & sanitation), teas, microbial fungicides. Many of above have limited effect and are scientifically untested.

2119(m)7: Is it compatible?

References

McMurtry,-M.J., “Avoidance of sublethal doses of copper and zinc by tubificid oligochaetes.” J-Great-Lakes-Res. [Toronto] : International Association for Great Lakes Research. 1984. v. 10 (3) p. 267-272.
CN: DNAL GB1627.G8J6

Miller,-W.P.; Martens,-D.C.; Zelazny,-L.W., “Short-term transformations of copper in copper-amended soils.” J-Environ-Qual. Madison, Wis. : American Society of Agronomy. Apr/June 1987. v. 16 (2) p. 176-181.
CN: DNAL QH540.J6

Boyer, J. et al. 1994. Copper Fungicides and Certifiable Organic Produce; a Report to the Maine Organic Farmers and Growers Association. PSE 546 Chemistry of Soils paper.

See also attached.

NOSB Materials Database

Identification

Common Name **Bordeaux mixes** **Chemical Name** Cupric Sulfate Pentahydrate & Calcium Hydroxide

Other Names Copper Sulfate (Bluestone) & Hydrated Lime(slaked lime)

Code #: CAS 1332-14-5 (Copper Sulfate) **Code #: Other** 1305-62-0 (Hydrated Lime)

N. L. Category Synthetic Allowed

Chemistry

Composition $CuSO4$ & $Ca(OH)2$ **Family** inorganic salt

Properties Blue crystals, granules, or powder.($CuSO4$) White powder, alkaline additive ($Ca(OH)2$)

How Made Copper Sulfate is made by oxidizing copper turnings in (special) furnaces in a current of air. The mass is then dissolved in sulfuric acid. and then purified. Hydrated lime is made by burning limestone gradually in a lime kiln and then adding the right amount of water.

Use/Action

Type of Use Crops

Use(s) Disease control on tree fruit and vegetables. Repels many insects as well. Control for potato leafhopper.

Action The toxic action of copper is attributed to its abiltiy to denature (change) the properties of cellular proteings and to deactivate enzyme systems in fungi and algae. Lime used as alkaline additive and carrier.

Combinations Combined with water at usual rate of 100/6/6

Status

OFPA 2118 (c) (1) (B)(i) synthetic on list both as copper and sulfur.

N. L. Restriction Must be used in a manner that prevents accumulation of copper in the soil.

EPA, FDA, etc

Registration

Directions

Safety Guidelines Negligible fire hazard when exposed to heat or flame

State Differences

Historical status Allowed by wide majority of certification groups.

Internationl status IFOAM , EU, Australia allowed.

BORDEAUX MIX REFERENCES

AU: Washington,-W.S.
TI: Effect of Bordeaux mixture sprays applied after flowering on fruit finish of apricot.
SO: Plant-Prot-Q. Victoria : R.G. Richardson. 1991. v. 6 (4) p. 188-189.
CN: DNAL SB950.3.A8P535

AU: Bird,-R.-M. (Robert Montgomery), 1867-
TI: Directions for making Bordeaux powder : Missouri Experiment Station formula.
SO: Columbia, Mo. : University of Missouri, Agricultural Experiment Station, 1905. [3] p.
CN: DNAL 100-M693-no.20

TI: Preparation of bordeaux mixture.
SO: New Brunswick, N.J. : New Jersey Agricultural Experiment Station, 1926. 1 sheet
CN: DNAL 100-N46S-no.190

AU: Wimalajeewa,-D.L.S.; Cahill,-R.; Hepworth,-G.; Schnieder,-H.G.; Washbourne,-J.W.
TI: Chemical control of bacterial canker (Pseudomonas syringae pv. syringae) of apricot and cherry in Victoria.
SO: Aust-J-Exp-Agric. East Melbourne : Commonwealth Scientific and Industrial Research Organization. 1991. v. 31 (5) p. 705-708.
CN: DNAL 23-AU792

AU: Menkissoglu,-O.; Lindow,-S.E.
TI: Chemical forms of copper on leaves in relation to the bactericidal activity of cupric hydroxide deposits on plants.
SO: Phytopathology. St. Paul, Minn. : American Phytopathological Society. Oct 1991. v. 81 (10) p. 1263-1270.
CN: DNAL 464.8-P56
AB: The total amount of copper, the amount of soluble but complexed copper, and the concentration of free Cu2+ ions on the surface of navel orange and bean leaves treated with different amounts of Cu(OH)2 or Bordeaux mixture were determined under field conditions. Total copper deposits decreased with time after spray application with apparent first-order kinetics with a half-life of approximately 45 and 35 days on navel orange and bean leaves, respectively.

AU: Hedrick,-U.-P.
TI: Bordeaux injury.
SO: Geneva, N.Y. : New York Agricultural Experiment Station, 1907. p. 106-189, [8] p. of plates : ill. (some col.)
CN: DNAL 100-N48-2-no.287

TI: Treatment of potato scab ; Use of Bordeaux mixture for potato blight.
SO: Geneva, N.Y. : New York Agricultural Experiment Station, 1893. 16 p. : ill.
CN: DNAL 100-N48-2-no.49

AU: Fields,-W.-S.; Elliott,-John-A. (John Asbury), 1887-1923.
TI: Making Bordeaux mixture and some other spraying problems.
SO: Fayetteville, Ark. : University of Arkansas, College of Agriculture, Agricultural Experiment Station, 1920. 12 p. : ill.
CN: DNAL 100-Ar42-no.172

AU: Teviotdale,-B.L.; Sibbett,-G.S.; Harper,-D.H.

4

Sittig 1980

COPPER OXYCHLORIDE

Function: Fungicide (1)(2)

Chemical Name: Copper chloride hydroxide

Formula: $3Cu(OH)_2 \cdot CuCl_2$

Trade Names: Recop® (Sandoz)
Cupravit® ICI
Fernacot® ICI
Pere-col® ICI

Manufacture

Copper oxychloride is made by the action of air on scrap copper in cupric chloride-sodium chloride solution.

Toxicity

The acute oral LD_{50} value for rats is 1,440 mg/kg (slightly toxic).

References

(1) Worthing, C.R., *Pesticide Manual*, 6th ed., p. 126, British Crop Protection Council (1979).
(2) Spencer, E.Y., *Guide to the Chemicals Used in Crop Protection*, 6th ed., p. 131, London, Ontario, Agriculture Canada (January 1973).

COPPER SULFATE

Function: Fungicide (1)(2)

Chemical Name: Copper sulfate

Formula: $CuSO_4 \cdot 5H_2O$

Trade Names: Blue Vitriol
Blue Copperas

Manufacture (3)

Copper shot is reacted on a batch basis with steam-heated sulfuric acid to form a supersaturated copper sulfate solution. When the solution is allowed to cool, copper sulfate crystals form. The supernatant mother liquor is decanted and either reused for the next batch of copper sulfate crystals, or used in the production of tri-basic copper sulfate or copper carbonate.

Any washwater used in cleanup is retained in the crystallization vats, and used in the copper sulfate production process. Steam to heat the solution is produced on site, from chemically softened creek water. Sludge from the softening process, plus boiler blowdown, constitute the only wastewaters associated with the copper sulfate process. These are discharged directly to a holding pond, where the sludge settles. Pond overflow is to an adjacent waterway.

Toxicity

The acute oral LD_{50} value for rats is 300 mg/kg which is moderately toxic.

Allowable Limits on Exposure and Use

Product Use: The tolerances set by the US EPA for copper sulfate in or on raw agricultural commodities are as follows:

	40 *CFR* Reference	Parts per Million
Eggs	180.102	exempt
Fish	180.102	exempt
Irrigated crops	180.102	exempt
Meat	180.102	exempt
Milk	180.102	exempt
Shellfish	180.102	exempt

References

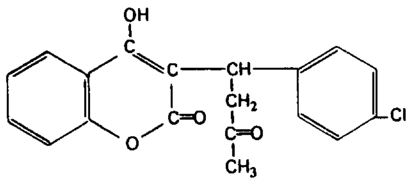
(1) Martin, H. and Worthing, C.R., *Pesticide Manual*, 5th ed., p. 127, British Crop Protection Council (January 1977).
(2) Spencer, E.Y., *Guide to the Chemicals Used in Crop Protection*, 6th ed., p. 133, London, Ontario, Agriculture Canada (January 1973).
(3) Patterson, J.W., *State of the Art of the Inorganic Chemicals Industry: Inorganic Pesticides*, Report EPA-600/2-74-0092, Wash., DC, Environmental Protection Agency (March 1975).

COUMACHLOR

Function: Rodenticide (1)(2)

Chemical Name: 3-[1-(4-chlorophenyl)-3-oxobutyl]-4-hydroxy-2H-1-benzopyran-2-one

Formula:



Trade Names: Geigy Rodenticide Exp. 332 (Ciba-Geigy)
Tomorin® (Ciba-Geigy)
Ratilan® Ciba-Geigy)

Manufacture (3)

Coumachlor is made by the condensation of 3-carbethoxy-4-hydroxycoumarin and p-chlorobenzalacetone.

Toxicity

The acute oral LD_{50} for rats is 900 mg/kg (slightly toxic).

Noyes Data Corp.

Park Ridge, NJ NDC

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Kirk-Othmer Chemical ~~Dictionary~~ Encyclopedia

an alkali salt of the acid is treated with a copper(II) sulfate solution. Copper(II) oleate [*1120-44-1*] and copper(II) stearate [*660-60-6*] are the ones most frequently mentioned in the literature but the valerate [*15432-57-2*], linoleate [*7721-15-5*], and octanoate [*20543-04-8*] have also been made. Copper(II) oleate coalesces mercury droplets and improves fuel oil combustion (55) by reducing the smoke and fumes of burning oil. It is used as a textile fungicide and in antifouling paints. Copper(II) stearate is used in coatings for xerographic plates (56) and in heat-sensitive coatings for photoduplication (see Electrophotography), as a color stabilizer in metal-containing dyes, and as a catalyst in a variety of organic reactions.

Copper(II) Sulfate. Copper(II) sulfate [*7758-98-7*], $CuSO_4$, as the pentahydrate [*7758-99-8*], $CuSO_4.5H_2O$, is the most important copper salt in terms of the amount produced. It usually occurs in the form of blue triclinic crystals (blue vitriol). In nature it is found as the mineral chalcanthite. The pentahydrate can be dehydrated to intermediate hydrates and the anhydrous salt. The anhydrous salt, which also occurs naturally as the mineral hydrocyanite [*14567-54-5*], is white. Copper(II) sulfate solutions are usually obtained in the processing of copper ores and most of the copper metal produced is either electrorefined or recovered electrolytically from acidic copper(II) sulfate solutions. The pentahydrate is usually made by dissolving scrap copper in hot, concentrated sulfuric acid with the generation of sulfur dioxide, or by the air oxidation of scrap copper in dilute sulfuric acid. Large quantities of copper(II) sulfate are used in agriculture as a fungicide and algicide, a source of copper in animal nutrition, and as fertilizer. It is also a primary source from which other copper compounds can be derived.

Table 2. United States Copper Sulfate Production, 1967–1976

Year	Thousands of metric tons	¢/kg
1967	36.4	40.8
1969	45.9	46.1
1971	31.4	50.3
1973	39.4	55.6
1974	38.2	82.0
1975	32.3	77.6
1976	29.1	

Table 3. United States Consumption of a Selection of Copper Compounds, 1975[a]

Compound	Metric tons
copper(II) carbonate	45.4
copper(II) naphthenate	408.2
copper(II) oleate	90.7
copper(II) oxychloride sulfate	498.9
copper(I) oxide	181.4

[a] Ref. 57.

16.

COPPER FUNGICIDES AND CERTIFIABLE ORGANIC PRODUCE

A REPORT TO THE MAINE ORGANIC FARMERS AND GROWERS ASSOCIATION

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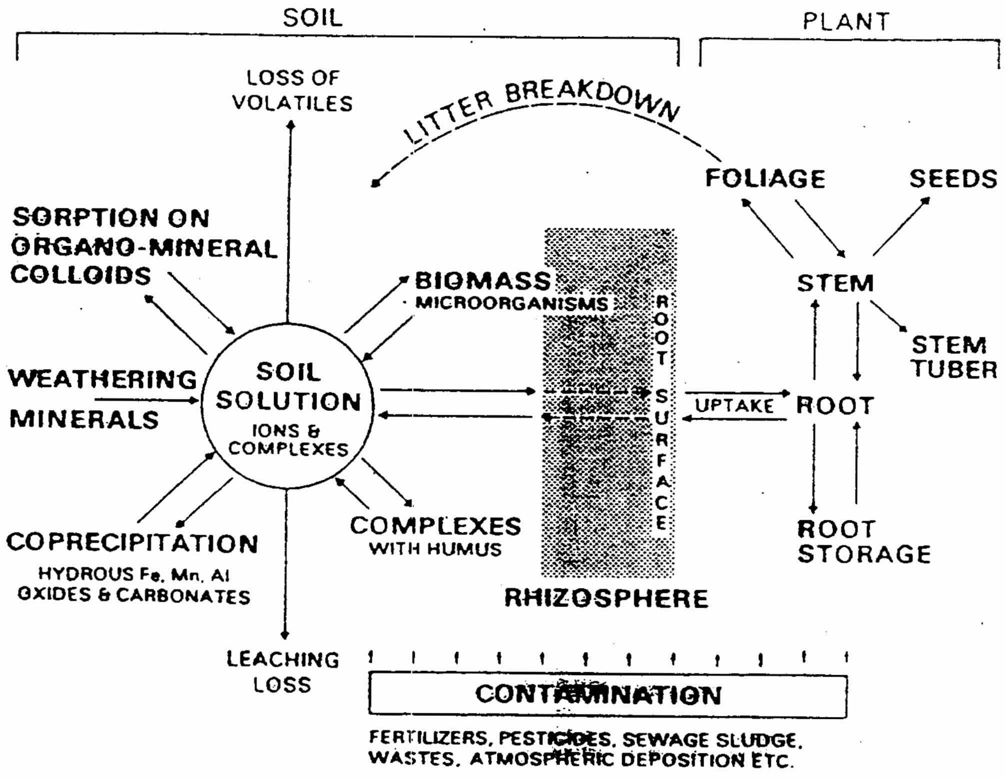

Figure 1: Copper Soil Cycling
(from Alloway 1990)

Introduction

Copper is an essential micronutrient which also occurs at toxic levels in soils and plants. If copper is to be applied regularly to agricultural land it is critical to understand its chemical interactions in the soil as well as its effects on soil organisms, plants and animals. Suitable soil test methods should be chosen which correlate soil copper levels with toxicity to plants and soil biota. This paper summarizes currently available information on copper as follows:

- -- copper interactions in the soil;
- -- soil factors which influence copper availability to plants;
- -- effects of copper on soil biota;
- -- effects of copper on plants;
- -- testing for soil copper.

Sources of copper and background levels

Copper is found in the soil solution, on exchange sites, in organic matter, and in rocks and minerals. The primary sources of copper are igneous rocks; basaltic rocks contain higher copper concentrations (60-120 parts per million [ppm]) than granitic rocks (Kabata-Pendias & Pendias 1992). In rocks, copper occurs most commonly as sulfide minerals (Barber 1984) such as chalcocite, chalcopyrite and covellite. Local soil levels are determined by geology, soil formation processes, point source contamination and atmospheric deposition. In "uncontaminated" soils, copper has been found at levels ranging from 1 to 300 ppm dry weight. Agricultural soils have average background copper levels of 20-30 ppm (Baker 1990).

Soil contamination occurs most often as a result of application of waste sludge, animal manure or copper fungicide. Together with soil additions of copper, atmospheric copper from industrial sources (such as waste incineration) is a component of many ecosystems. Tree ring analyses indicate steadily increasing concentrations of copper and other metals over the past 25 years in the eastern United States (Baes et al. 1983). Sewage sludge may contain copper concentrations as high as 1,000 ppm (Adriano 1986). Copper levels have been measured as high as 3,900 ppm on banana plantations with a history of copper fungicide use (Thrupp 1991).

Prior to the twentieth century, copper sulfate solutions were commonly applied as herbicides to cropping areas. Copper fungicides have been in use since the early 1800s (Richardson 1991) and are still applied to many crops on a regular basis, particularly fruit crops. Copper (0.1-0.2%) in water suspensions of insoluble hydroxides, carbonates, or oxides was found to be an effective fungicide. Bordeaux mixture, which was the first widely-used, foliar-applied fungicide, is still prepared by dissolving five to ten pounds of copper sulfate with an equal amount of lime or sodium carbonate in 100 gallons of water. Copper is also applied deliberately to copper-deficient agricultural soils as a fertilizer and may also be added inadvertently as a component of mineral fertilizers and animal manures. For example, when swine and poultry are raised on diets rich in copper, the high level of copper in their manure may significantly increase copper levels in soil.

Because copper is relatively immobile in most soil systems, annual applications may cause copper levels in the soil to increase to levels which are toxic to plants. Also of great concern to organic farmers are the possible effects of elevated soil copper levels on soil biota. Because copper often accumulates in soils as a result of routine management practice (Alva 1993; Committee 1977; Holmgren et al. 1993) and contamination of soil with respect to copper and some other trace elements appears to be virtually permanent (Davies 1980), copper levels should be carefully monitored if amendments are added. The level of copper required to adversely affect plant growth depends upon soil mineralogy and organic matter content, plant species, age of plant, and location.

Copper Reactions in Soils

To study the effects of copper toxicity and possible impacts of copper on soil microbial populations, it is imperative to understand the various interactions of organic and inorganic forms of copper in soils. This section is aimed at understanding the basic reaction mechanisms that control the fate of copper in soil environments. More specifically, this section looks at the following reaction mechanisms that are known to affect copper in agricultural soils:

-- solubility of copper minerals in soil;
-- hydrolysis reactions;
-- copper complexes;
-- redox effects on copper;

Soil Factors Influencing Availability to Plants

Solubility of Copper Minerals in Soils

Copper occurs in a reduced state with sulfide in primary minerals. In soils, these reduced minerals are oxidized to form oxides, hydroxides, carbonates, sulfates and phosphates. The specific minerals that govern copper solubility in soil are not known. In the absence of such information, experimental measurements performed by Norvell and Lindsay (1969 and 1972) on soils of varying pH have helped in establishing the reference solubility of

Cu^{2+} in soils (soil Cu) against which the solubilities of various oxidized copper minerals are compared. Assessing the extent to which copper is solubilized in soils is important as it affects the amount of copper present in soil solutions and defines the dominant species that will be present in soils and soil solutions, directly influencing the bioavailability and toxicity of copper in soil environments.

Important orthophosphate minerals are $Cu_3(PO_4)_2.2H_2O(c)$ and $Cu_3(PO_4)_2$. Thermodynamically, $Cu_3(PO_4)_2$ is more stable then $Cu_3(PO_4)_2.2H_2O(c)$. Both of these copper phosphates are more soluble than the soil Cu (Lindsay 1979). Thus, copper phosphates can dissolve sufficiently in soils to provide both copper and phosphorus simultaneously. This occurs to a greater extent in acidic soils. Hence, background levels of phosphorus could be an important factor while considering any potential use of copper based fungicides.

Hydrolysis

Copper participates in hydrolysis, which is a chemical transformation process resulting in liberation of the proton and inclusion of the hydroxyl group from a water molecule into the copper compound. Various hydrolysis reaction pathways for Cu^{2+} are listed in Table 1. The hydrolysis species in equilibrium with soil Cu are shown in Figure 2.

Table 1: Hydrolysis Pathways for Copper(II)
(Lindsay 1979)

LOG K	REACTIONS
-7.7	$Cu^{2+} + H_2O = CuOH^+ + H^+$
-13.78	$Cu^{2+} + 2H_2O = CuOH_2 + 2H^+$
-26.75	$Cu^{2+} + 3H_2O = CuOH_3^- + 3H^+$
-39.59	$Cu^{2+} + 4H_2O = CuOH_4^{2-} + 4H^+$
-10.68	$2Cu^{2+} + 2H_2O = Cu_2OH_2^{2+} + 2H^+$

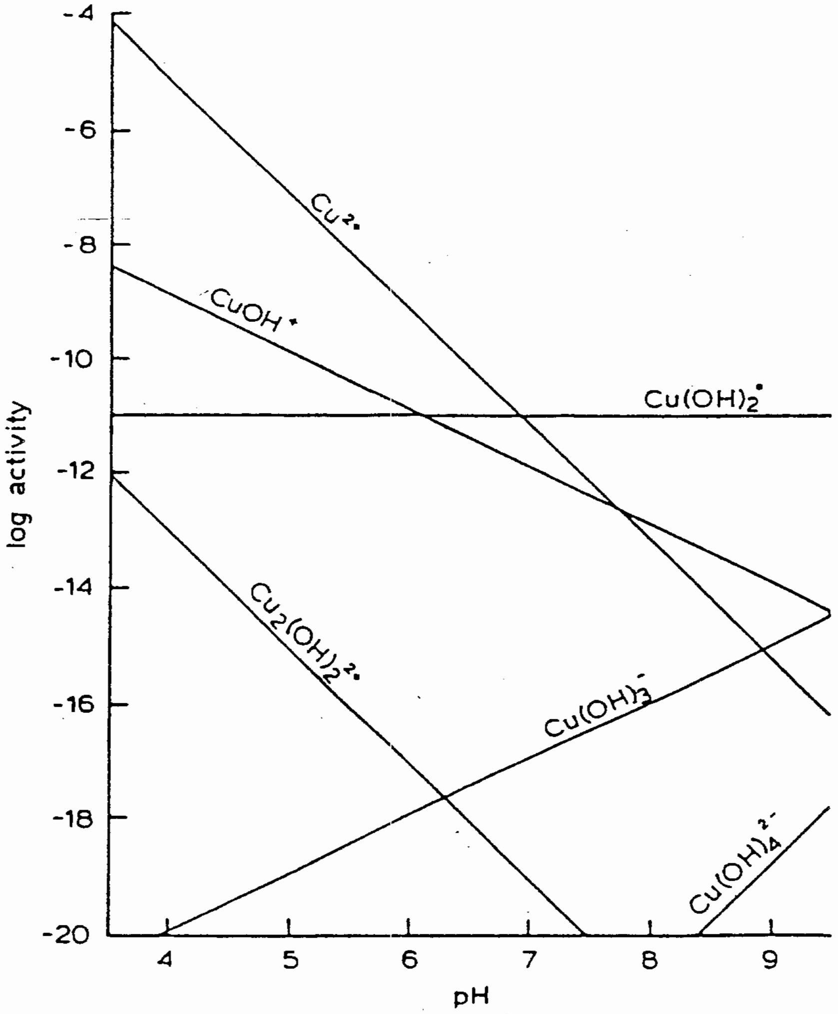

Figure 2: Hydrolysis Species of Cu^{2+} in Equilibrium with Soil Cu
(Lindsay 1979)

Hydrolysis significantly alters total copper in solution. Cu^{2+} and $Cu(OH)_2$ are the major solution species. Cu^{2+} is the dominant species below pH 6.9 and $Cu(OH)_2$ is dominant above pH 6.9. Copper complexes with ligands other than OH are more likely to form at low pHs.

Complexes of Copper

Copper is known to form a variety of inorganic and organic complexes (Huheey 1983). Inorganic copper complexes of carbonates, phosphates, sulfides and silicates are relatively unavailable to plants, as are hydrous copper oxides of iron, aluminum and manganese (Dosskey and Adriano 1992). Copper can form soluble complexes with organic chelating agents in the soil solution. Dissolved organic carbon (DOC) governs the concentration of ionic species of copper in soils with high organic content (Meador 1991); higher DOC levels result in higher levels of organic copper complexes in solution. The degree of decomposition of the organic matter also influences complexation. Complexation capacity increases with increasing decomposition. Although higher decomposition rates result in release of copper to the soil solution, this does not mean that higher soil solution copper levels are maintained. Maximum complexation (>98%) occurs between pH 5-6 (Kervin et al. 1984). Formation of these organic complexes affects the mobility of copper in the soil. Copper can be held tightly by the soil in the form of insoluble complexes formed by humic substances or as solid-phase coatings on clay surfaces. However, substantial differences exist among these complexes in reducing the toxicity from Cu^{2+} ions (Menkissoglu and Lindow 1991). Soil Cu often becomes phytotoxic when its level exceeds 5% of the soil's cation exchange capacity (Mathur and Levesque 1983). Thus, levels of organic carbon in the soil need to be tested and monitored before any assessment of potential toxicity from copper fungicides can be made.

Redox Effects

The ability of copper to exist in multiple valence states necessitates the study of redox effects on copper. As discussed earlier, copper normally exhibits a 2+ valence in the soil; however, copper is also known to exist as Cu^{+} and Cu^{0}. The most pertinent redox reactions are:

$$Cu^{2+} + e = Cu^{+} \; (\log K = 2.62)$$
$$Cu^{+} + e = Cu^{0} \; (\log K = 8.89)$$
$$Cu^{2+} + 2e = Cu^{0} \; (\log K = 11.89)$$

The Cu^{2+} and Cu^{+} activities are equal at pe = 2.62, and their ratio changes tenfold for each unit change of pe. The effect of redox, the solubility of Cu^{+} and the stability of copper minerals are schematically shown in Figure 3.

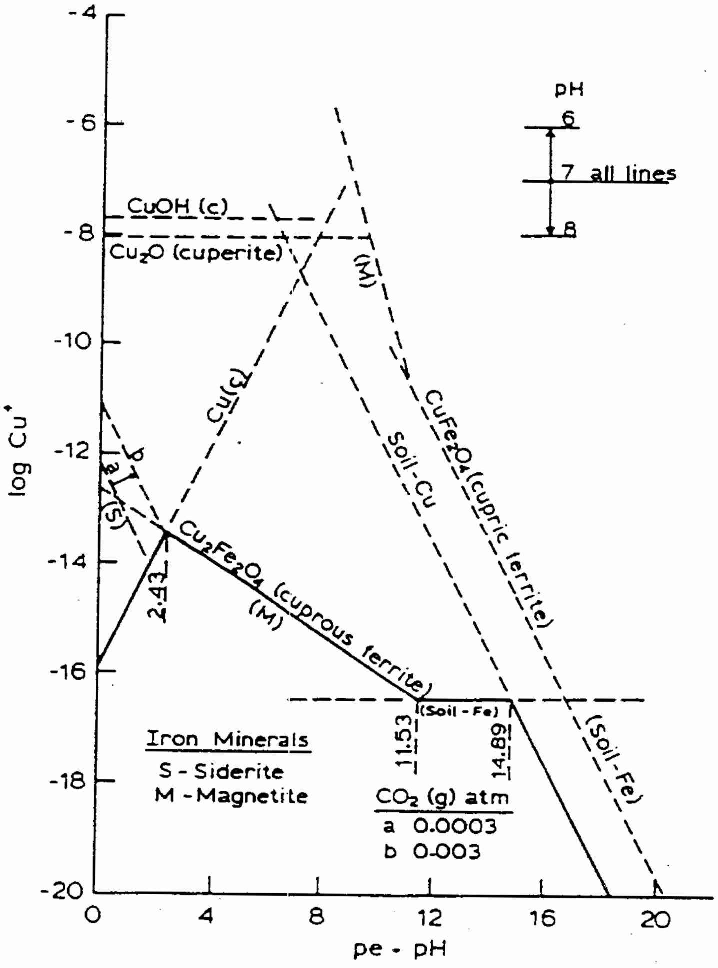

Figure 3: Stability of Cu Minerals
(Lindsay 1979)

The dashed lines in Figure 3 depict metastable equilibrium. The solubility of copper in mineral soils is expected to follow the solid lines shown in Figure 3. Shuman (1988) states that the effects of redox are not very prominent in organic soils which also contain other metals, especially Fe and Mn. However, bacterial oxidation and reduction of sulfur compounds in organic soils may facilitate redox reactions of copper sulfide (Mathur and Levesque 1989).

Copper influences on soil biota

By the early 1900s, researchers had noted that applications of the Bordeaux mixture sometimes had stimulating effects on crop vigor and yield which could not be associated with fungal control alone (Reuther and Labanauskas 1966). It was later found that copper played an essential role in plant and animal metabolism. Along with the realization that copper was a micronutrient came the first evidence of copper deficiency, observed by Bartels in 1927 in bread mold (*Aspergillus niger*). He found that varying amounts of copper changed both the color and growth status of the bread mold spores (Reuther and Labanauskas 1966). Other workers soon confirmed that copper (in various amounts) is an essential element to fungal and yeast growth.

Bacteria, actinomycetes and fungi require the same micronutrients as higher plants and will compete for those micronutrients when concentrations fall below optimum levels (Stevenson 1986). Immobilization and mineralization reactions occur for copper just as they do for macronutrients and other micronutrients. The rate of release will depend on microbial environmental conditions and will be highest in warm, moist, well-aerated soils where general decomposition rates are at their highest levels.

Organic complexing agents produced during microbial decomposition may affect the availability of trace elements, such as copper, by forming relatively unavailable soluble and insoluble complexes. Toxic copper concentrations may therefore be reduced to non-toxic levels through these complexation processes. The biochemicals responsible for forming soluble complexes with metal cations such as copper include amino acids and aliphatic acids (Stevenson 1986). These biochemicals are continually produced by microbial activity; while they tend to be very transient in nature, the net effect of all microbial species combined may be appreciable (Stevenson 1986). These complexing agents are more prevalent in soils with high levels of organic matter and may have something to do with the microbial tolerance for high levels of copper in histosols (Mathur 1983).

The effects of copper on microbial populations have been well documented. These microbial studies indicate that a variety of effects, ranging from stimulation to toxicity, can be caused by additions of copper to the soil. A study was conducted in the apple-growing regions of Aomori Prefecture, Japan, to determine the effects of copper on decomposition and microbial activity (Aoyama et al. 1993). Copper was added in concentrations of up to 2000 ppm to mineral soils amended with pulverized orchard grass and to non-amended soils. Microbial activity, which was estimated by measuring CO_2 evolution, decreased in both soils which received copper applications. Microbial biomass carbon was also significantly reduced in both copper-treated soils and actually decreased to a greater extent than the decrease in CO_2 evolution would have predicted. In summary, the copper treatments considerably reduced the size of the microbial biomass, while only slightly suppressing decomposition rates. The question arises as to whether these results indicate a decrease in species diversity.

Zibilske and Wagner (1982) added sludge-borne materials with three different levels of copper (556, 78 and 11 ppm) to a silt loam agricultural soil. Microbial populations were studied with respect to bacterial numbers, ATP content, and fungal genera distribution. Bacterial growth was severely restricted with 556 ppm copper additions for the duration of the study, but was initially stimulated in the 78 and 11 ppm copper treatments. After fifteen days, population counts in the two lower concentration treatments fell to below those of the control samples. Total soil biomass was estimated using ATP data and indicated that biomass increased initially, followed by declines for all treatments. Copper additions selectively reduced fungal spore germination, resulting in a change in fungal genera population distributions. Overall, the authors suggest that copper additions could result in reductions in energy transfer efficiency and nutrient cycling, as well as microbial population modifications.

Copper alone and in combination with other metals was investigated on sandy loam soils in the United Kingdom to determine the effects on microbial biomass (Chandler and Brookes 1993). Again, decreasing microbial biomass was noted with increasing concentrations of copper. When zinc was added with copper, the decrease in microbial biomass was much greater than with copper alone at the same concentrations, indicating an additive effect of both metals present.

Soil dehydrogenase activity assays are used as measures of microbial enzyme production for substrate decomposition and are another indicator of overall microbial activity. Rogers and Li (1985) used dehydrogenase activity for assessing potential toxicity of copper to microbial populations. A large decrease in dehydrogenase activity was noted when soil copper concentrations were from 30-300 ppm. Similar trends in dehydrogenase activity were noted in a study by Maliszewska et al. (1985) in sandy and alluvial soils. The numbers of nitrifiers present in the soil were also showed similar decreases with increasing copper concentrations, although low concentrations of copper in the sandy soils seemed to stimulate nitrifying populations above the control levels. Bacteria that fix free nitrogen and nitrifying bacteria appear to be more susceptible to metal toxicity than other bacteria (Kabata-Pendias & Pendias 1992). Chandler and Brookes (1991) urge caution when interpreting dehydrogenase activity studies, due to potential interactions between copper and triphenylformazan (TPF) used in the assay. They observed that dehydrogenase activity levels may appear lower than they actually are.

Some bacteria have evolved mechanisms to regulate copper uptake and resist copper toxicity. A copper-resistant strain of *Pseudomonas fluorescens* (09906) was investigated by Yang et al. (1993) for potential use as a biological control agent in citrus groves contaminated from years of copper fungicide use. Data suggests that copper-resistant genes play an important role in competitive fitness, even in soils with lower copper levels. Various compounds have been shown to induce transient copper resistance in plant-pathogenic bacteria (Gotto et al. 1993). Glutamate added to a $CuSO_4$ solution increased the viability of bacteria present. Induced resistance was noted for other amino acid and amide additions, as well as for phosphate buffer, plant extracts, and several antibiotics. Enhanced copper resistance seemed to be more pronounced in naturally copper-resistant bacteria than in copper-sensitive species.

On a different scale, several studies have been conducted on the effects of copper on macrofaunal populations. In one species of cladoceran (water flea) populations responded positively to low concentrations of copper in food-limited situations, while growth rates decreased for well-fed animals at the same copper concentrations (Postma et al. 1994). This suggests that the amount of food limitation is important to the interactive effects. Trials were conducted on solanaceous crops by Hare (1984) to determine if a copper-based fungicide (formulated with $Cu(OH_2)$) would have suppressive effects on the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Beetle populations were reduced between 44-100% when using the copper-based fungicide, compared to a more conventional fungicide (mancozeb). This study does not report copper concentrations in beetles; however, other studies do indicate an accumulation of copper in earthworms, centipedes and other soil macrobiota (Morgan and Morgan 1990; Weigmann 1991).

Copper Toxicity in Plants

"Average" levels of copper in plant tissue

Copper accumulates at a faster rate than cadmium, zinc or lead (Kastori et al. 1992) in plant tissue. Although copper tends to accumulate in the roots of plants (Kastori et al. 1992; Hardiman et al. 1984), the highest concentrations of copper reported were found in cereal grain embryos (Kabata-Pendias & Pendias 1992). "Normal" copper concentration ranges in plants are considered to be from 5-20 ppm. Copper is an essential nutrient and so plays an important role in many plant processes: photosynthesis, respiration, carbohydrate distribution, N reduction and fixation, protein and cell wall metabolism, enzyme mechanisms, water relations, pollen viability and disease resistance (Adriano 1986).

Toxic levels of copper in plant tissue

From 10-30 ppm dry weight has been suggested as a critical concentration (above which toxicity effects are likely) in plant tissue for copper (Kabata-Pendias & Pendias 1992). Alloway (1990) suggests higher critical levels, ranging from 20-100 ppm. Of course, age, species and site differences must be taken into account. Ten percent depression in yield has been observed at concentrations from 5-64 ppm (Alloway 1990). Tissue concentration of the metal is not always a conclusive indicator of toxicity to the plant (Rhoads et al. 1989). Toxic effects were observed at tissue concentrations of 18 ppm in barley, 11 ppm in wheat, 5 ppm in corn and 8 ppm in lettuce (Macnicol and Beckett 1985).

Uptake of copper by plants

Although most copper uptake by plant roots is probably an active process (Barber 1984), both active and passive uptake of copper occur (Kabata-Pendias & Pendias 1992). Copper uptake by oats was found to be greater in sandy soil than in organic soil (Bjerre and Schierupp 1985). The origin of copper can influence uptake, with anthropogenic copper taken up at greater concentrations than lithogenic copper, as illustrated in Figure 4.

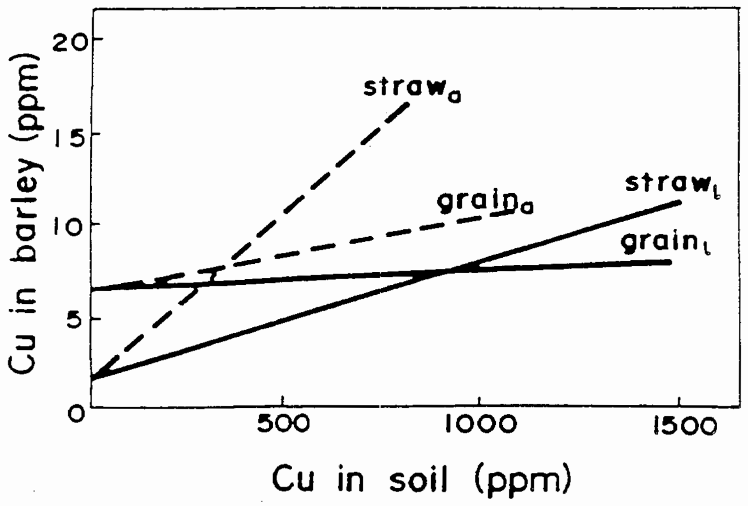

FIGURE 4. Source of Cu: a: anthropogenic; L: lithogenic (Grupe & Kuntze 1988, as in Kabata-Pendias & Pendias 1992)

Uptake of ions in soil solution is determined both by the solution concentrations of the ions and the extent of the root structure. Mycorrhizae can be instrumental in root uptake of adsorbed ions (Alloway 1990). Copper uptake varies with ion activity, which varies with pH (Barber 1984; Rhoads et al. 1989). Copper is most available to plants below pH 6. At higher pH levels Cu^{2+} may be unavailable, despite high soil levels of copper. Below pH 5.5, Cu^{2+} may be present at toxic levels, despite low levels of total soil copper (Baker 1990). Cation exchange probably plays an important role in metal uptake through the roots, although the process is not entirely understood.

In addition to root uptake, copper is also taken up in significant amounts through leaf tissue (Kabata-Pendias & Pendias 1992), probably in two phases. The primary mechanism is believed to be nonmetabolic cuticular penetration. Transport across the plasma membrane into the protoplast also occurs. Once in the leaf, copper can be transported throughout the plant. Copper is more mobile than other metals which are absorbed by foliage (Kabata-Pendias & Pendias 1992).

Copper toxicity disturbs transpiration, water regulation (Kastori et al. 1992) and cell membrane permeability (Kabata-Pendias & Pendias 1992), causes root stunting and deformation, and reduced growth vigor (Adriano 1986). Copper and iron have interactions in the plant and an excess of copper can result in a deficiency in iron (Alva 1993), resulting in chlorosis. Copper has been reported to interact antagonistically with zinc (Adriano 1986).

Critical soil levels of copper

Critical levels of copper in the soil for plants depend on soil solution Cu^{2+} concentrations, since this is the predominant species taken up by plants. Soil solution levels of Cu^{2+} are dependent on total soil copper as well as soil factors such as texture, pH and organic content. Soils containing high levels of organic matter often have low solution Cu^{2+} levels regardless of total soil copper, because of the complexation of copper by organic material discussed above. Acidic soils are also often copper deficient because of increased solubility and leaching of Cu^{2+} at low pH. However, if copper is added to an acidic soil, phytotoxicity or reduced plant growth will occur at lower total soil copper levels than would occur if the soil was neutral or alkaline (Rhoads et al. 1989).

As mentioned previously, background copper levels in agricultural soils average 20 to 30 ppm on a dry weight basis. Soil solution copper levels typically range from 0.64 to 38 parts per billion (ppb). On two different mineral soils, depressed plant growth due to excessive solution Cu^{2+} was reported at solution concentrations of 635 ppb (Barber 1984) and at total soil concentrations of 100 ppm (Kabata-Pendias & Pendias 1992). Deformed roots in snapbean were observed when copper salts were added to soil at a rate of 300 ppm (Minnich et al. 1987). Kabata-Pendias and Pendias (1992) report that suggested limits for copper levels in mineral soils range from 23-140 ppm dry weight.

In organic soils, copper levels may be much higher before plant growth is affected. Mathur and Levesque (1983) found that plant growth was not affected on three different organic soils until total soil copper concentrations were 900 ppm, 1137 ppm, and 1172 ppm. Mathur et al. (1983) report that copper concentrations of 1186 ppm on organic soils did not negatively influence onion yields. It is actually recommended that copper concentrations be maintained at 100 ppm in undecomposed peat soils and 400 ppm in well-humified muck soils. These levels are required for plant nutrition and for decreasing the rate of subsidence on organic soils.

A study by Mathur and Levesque (1983) used three organic soils, three mineral soils, and their 1|1 mixtures to investigate soil copper and its effects on plants. Plants were grown on these soils and phytotoxic effects of various levels of copper noted. They found that phytotoxic levels of copper in the soil were reached when total copper concentration in the soil was greater than 5% of the soil's cation exchange capacity.

Copper excesses often occur in soils which have been sprayed with Bordeaux mixture (or other copper-based fungicides) for disease control over a period of many years (Reuther and Labanauskas 1966). For example, many fruit orchards which received extensive copper applications over a period of years now have phytotoxic levels of copper. In these orchards growers find it difficult, if not impossible, to start young fruit trees, which are more susceptible to copper toxicity than older trees (Adriano 1986; Alva 1993). Silt, loam or peat soils may not show the adverse effects of copper accumulation for fifty years or more because of their higher organic content and their ability to adsorb Cu^{2+}; however, sandy soils may accumulate critical copper levels in as little as five years (Committee 1977). Because of differences among soils, even a single application of copper can produce strong residual effects which may last for years.

Soil Testing for Copper

It is important to monitor levels of soil copper, since regular applications of copper fungicides can cause toxic accumulations. Since Cu^{2+} is the form taken up by plants, a soil test should theoretically be able to measure Cu^{2+} activity in the soil solution and relate that to plant health and growth. This is often not possible because Cu^{2+} concentrations in soil solutions are usually too low to measure with copper probes and because tests which measure the sum of all water-extractable copper species are not accurate predictors of copper effects on plants. Measuring total soil copper on a dry weight basis is also not an effective method of predicting plant uptake because of the variability of soil factors among soils which influence soil solution Cu^{2+}.

Soil mineralogy, pH and organic matter content determine how much copper will be readily available (soil solution copper plus exchangeable copper) and how much will be fixed, or specifically adsorbed, on soil minerals and organic compounds. Total soil copper can be partitioned into six major pools: soluble species in solution, exchangeable Cu^{2+}, Cu-organic complexes, copper adsorbed by hydrous Mn, Fe, and Al oxides, copper adsorbed on colloidal clays, and residual copper. Also, high pH soils may contain large copper hydroxide and carbonate pools, and organic soils may contain a large copper sulfide pool (Levesque and Mathur 1986). Sequential extractions are used to determine the amount of copper in each pool. These extractions begin by removing water soluble and exchangeable copper with calcium chloride, magnesium chloride, potassium nitrate, or an ammonium salt. Pools of copper which are progressively more difficult to extract are then removed with increasingly stronger extractants until the organic fraction is left. This organic fraction is then extracted with sodium hydroxide, pyrophosphate or some combination of peroxide and nitric acid. What is left is the residual soil copper which is then determined by dissolving the soil with hydrofluoric acid, nitric acid, hypochloric acid or some combination thereof. Finally, an unextracted sample of soil is digested with hydrofluoric acid to determine total soil copper. This concentration is compared to the sum of the sequentially extracted pools (Yong et al. 1993; Liang et al. 1991).

Sequential extractions help to identify partitioning of copper in soils. Copper soil pools can then be correlated to plant effects and simple soil test methods. Such correlations help to determine which pools most influence plant available copper and which methodology might be the most effective for predicting plant availability of Cu^{2+} in a given soil. Liang et al. (1991) used a sequential extraction scheme to extract mineral prairie soils and then correlated copper uptake in snap beans with the soil pools they identified, a simple soil test extractant, and various soil factors. They found that the clay-adsorbed copper pool correlated best with bean uptake of copper and that a DTPA extractant was the most effective at predicting plant availability of copper. In this case sequential extraction aided in finding an effective soil test method for copper.

There are many soil extractants in use today, the most effective of which measure the readily available copper plus some part of the specifically absorbed copper. The most common test methods use an acid extractant, such as HCl, on the principle of H^+ mass action. The organic chelating extractants, EDTA and DTPA, are also commonly used. The acid extractant, Mehlich 1 (.05M HCL + .025M H_2SO_4), is a very common test method (Baker and Amacher 1982) but is not effective on alkaline soils due to excessive dissolution of copper hydroxides and carbonates. The concentrations of copper thus extracted are higher than actual plant available Cu^{2+}. The Mehlich 3 test (.2M acetic acid + .25M NH_4NO_3 + .015 NH_4F + .013M HNO_3 + .001M EDTA) was developed for use over a wide pH range (Zhu and Alva 1993). Acid extractants are widely used on mineral soils but may not be effective on soils with a high organic content. This is because Cu^{2+} ions exchanged or dissolved by the extractant are likely to resorb in organic compounds (Levesque and Mathur 1988). Thus a soil with most of the copper organically bound requires an extraction method which effectively decomplexes a higher percentage of the copper from organic compounds and then prevents recomplexation.

Levesque and Mathur (1988) compared copper extracted with eight different extractants to sequentially extracted copper. Their work was done on fifty-five organic soils under cultivation. They found that 57% of the copper was not extractable (residual pool), 28% was organically complexed, and 9% was sulfide bound (Levesque and Mathur 1986). In this case the capacity of the soil to replenish the readily available copper pool is dependent on the organic and sulfide copper pools. The acid extractants and cation exchange extractants they tested did not remove enough copper to adequately correlate with these pools. They also tested DTPA and EDTA and found that EDTA-extracted copper adequately reflected plant available copper but that DTPA over-extracted the organic soils used. The most effective extractants were .5M HCL + .05M $AlCl_3$ and .1M NH_4HCO_3 + .005M DTPA which reflected most accurately the available pool and a significant portion of the reserve pool. Further tests with oats, carrots, onions and lettuce revealed that the HCL + $AlCl_3$ extractant correlated best with plant uptake of copper (Mathur and Levesque 1989). This extractant worked well because resorption of Cu^{2+} displaced from organic complexes into solution was prevented by Al^{3+}, which is strongly held by organic chelates.

The percentage of total soil copper removed by a given extractant will change with soil texture and organic content. A given acid extractant will remove a larger percentage of the total soil copper from a sandy soil than from a fine textured soil. The following table lists a number of mineral soils with different textures which are extracted with two common soil test extractants. The first five rows represent soils from citrus orchards which were contaminated with copper fungicide applications over a period of years. The levels of copper extracted from these soils represent phytoxicity since many plants may be adversely affected by acid-extracted copper levels of 15 ppm (Adriano 1986). The last two rows represent averages of four fine-textured and four coarse-textured soils. They contain levels of copper which are in the range of background levels.

Table 2: Copper extracted from soils of different textures
(Zhu and Alva 1993; Shuman 1988)

TEXTURE	CEC	TOTAL Cu (PPM)	MEHLICH 3 EXTRACTED Cu (PPM)	DTPA EXTRACTED Cu (PPM)
FINE LOAM	6.6	223.3	119.6	128.5
FINE LOAM	19.3	123.3	57.2	64.5
FINE LOAM	14.4	269.5	141.3	163.3
SAND	7.9	121.6	80.0	73.2
SAND	2.5	83.2	66.3	42.6
LOAM	10.0	23.5	1.2	1.15
SAND	4.0	5.2	0.75	0.57

Organic soils, which are not represented in the table, may have levels of total copper approaching 1000 ppm because of the great adsorptive capacity of organic matter. Ultimately, the best criteria for the efficiency of a test method is how it correlates with plant uptake and, more importantly, with plant growth and health. An extractant should be chosen which will effectively extract a wide range of soils.

Summary

The question whether to apply copper fungicides poses a worrisome problem, because any blanket application of copper fungicide will affect more than just the target fungus. It is not well understood how copper accumulations in microbial communities and soil macrofauna transfer upward through the food chain to affect the entire ecosystem. Although it may take years to accumulate phytotoxic copper levels in soils, microbial toxicity may be reached much sooner. Large decreases in microbial biomass and activity have been noted in several studies when copper additions exceed thirty ppm on a dry weight basis. Both short-term and long-term effects of copper must be studied to determine phytotoxicity, chemical parameters and critical soil levels. The fact that there is no universally accepted critical copper level, either for soil or for plants, makes it impossible to recommend absolute limits or target areas of potential copper toxicity on a general basis. The questions arise as to whether some agricultural soils are now at critical levels, and to what extent residual effects of copper applications will affect background soil levels.

We are unable to recommend specific ppm values of copper which could be safely added to soils, due to the variability in ranges of copper resulting in toxicity and the site specificity of crop uptake of copper. However, a tentative rule of thumb is that the copper concentration of the soil should not exceed 5% of the cation exchange capacity, with the cation exchange capacity determined by ammonium acetate. Our recommendation is to consider each case on an individual basis, taking into account current copper levels in the soil, soil type and crop. For example, corn grown on a sandy, acidic soil will show phytotoxic effects much sooner than barley grown on an organic soil (especially if pH > 6.0 on the organic soil). Toxic levels on mineral soils could be reached anywhere from 25-140 ppm depending on the soil texture, pH, organic content, and the sensitivity of the crop species. The lower limit is more likely to exist in acidic coarse-textured soils and the upper limit in neutral fine-textured soils. Toxic levels on organic soils will be much higher than on mineral soils, probably around 1000 ppm or more. Organic soils which are low in iron will reach phytotoxicity sooner.

Soil testing should be done on a regular basis with a simple soil test that is effective on a wide range of mineral soils. The acid extractant, Mehlich 1 is effective and widely used on noncalcareous soil and Mehlich 3 is appropriate over a wide range of soil pH conditions. For organic soils, a chelating extractant such as EDTA or the HCl + $AlCl_3$ combination should be chosen. It is important to note that any change in cropping practice which affects either organic matter content or pH will affect soil solution levels of Cu^{2+}, which is the form of copper most available to plants.

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ecause they are not commonly used in Maine

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CALIFORNIA AGRICULTURE MARCH/APRIL 1985

7.

Controlling tadpole shrim

Albert A. Grigarick ☐ Joseph H. Lynch ☐ Michael O. Way

Copper sulfate works best on this rice-field pes

Tadpole shrimp, seen here from underside, utilizes strong mandibles to feed on bud sheath, roots, and leaves of rice seedlings.

Rice seed with bud sheath (coleoptile) chewed off by tadpole shrimp.

Buried tadpole shrimp eggs remain dormant until brought to the soil surface by cultivation and hatch when rice fields are flooded. (80X)

Use of chemicals to control the tadpole shrimp has resulted in some of the classic problems associated with this method: adverse effects on wildlife, ineffective control due to the wrong formulation or misapplication, phytotoxicity, and most recently, pesticide resistance.

The tadpole shrimp, *Triops longicaudatus* (LeConte), inhabits temporary fresh water bodies of the Americas, Japan, India, the West Indies, and a number of Pacific islands. The name tadpole was probably given to this shrimp because of its resemblance to the young of the bullfrog in color, swimming activity, and general outline. L. E. Rosenberg, University of California, Davis, first reported the tadpole shrimp as a pest of rice in northern California in 1946. Growers had seen it in rice fields earlier but didn't associate it with injury to the crop. These crustaceans may have existed in some of the first commercial rice plantings in 1912, since many of the fields are on land that was once part of temporary ponds in the flood plains of the Sacramento River.

Like a number of crustaceans, tadpole shrimp can survive drought or desiccation for several years in a resistant egg stage. The reddish eggs are laid at or close to the soil surface and hatch when flooded at temperatures above 59°F (15°C). Hatching may continue through a 2-week period, but most occurs within the first 2 to 3 days of flooding. Buried eggs remain dormant until they are brought to the soil surface by cultivation. After hatching, the larvae gradually increase in size with periodic molts; the hermaphroditic adults begin laying eggs in 9 to 12 days (over 500 eggs per individual have been recorded), and die in 20 to 30 days.

Microorganisms (fungi, bacteria, and minute animals) are the main food of tadpole shrimp during their early development (up to 4 to 6 days). As they grow, their diet includes higher plants and larger animals. With their strong mandibles, they can remove tissue from the bud sheath (coleoptile), roots, and leaves of rice seedlings. This feeding activity may also uproot seedlings, causing them to float to the water surface and eventually come to rest on the shoreline. The water of a rice field infested with shrimp is usually brown and turbid by the tenth day, because the shrimp stir the fine particles of the soil surface while feeding and laying eggs. The turbidity reduces light pen tration and may retard seedling growt

The tadpole shrimp is a pest of ri only from the time the seed germinat until the seedling emerges through t water. In California, rice is particular susceptible, because seed is common broadcast to semipermanent water. T shrimp is not a pest in countries where permanent flood is not applied until th seedlings are well developed or whe they are transplanted to flooded paddi from nurseries. In fact, several species o tadpole shrimp are considered by re searchers in Japan to be potential agent for biological control of small weeds i flooded paddies. The transplanted rice i these paddies is too large to be susceptibl to the shrimp.

Early control and wildlife

The first chemical control of tadpol shrimp in California was developed b Rosenberg in 1946: DDT formulated as a emulsifiable concentrate, sprayed at 0. pound active ingredient per inch of wate per acre, or copper sulfate (cupric sulfat pentahydrate) dust applied at 1 pound active ingredient per inch of water per acre Both chemicals controlled the shrimp, bu DDT became the most cost-effective an the most widely used.

In 1955, UC Davis scientists R. L. Rud and R. E. Genelly reported bird mortalit from DDT in California rice fields. The attributed this to airplane sowing o "DDT-coated" rice seed, which was eate by pheasants, blackbirds, and ducks. Mos aerially sown seed drops into flooded pad dies, but some is unavoidably scattered o the soil surface of levees and field mar gins. To reduce operating costs, grower had combined the sowing and preventiv chemical control phases. Rice did not re ceive "typical" seed treatment, but th chemical adhered to some of the seed when DDT granules (formulated as a wet table powder) were placed in the plan hopper with presoaked rice seeds. Th practice of combining sowing and treat ing was discontinued when the problem it caused for wild birds were discovered

In the early 1960s, several fish kills i rice-field drainage waterways also coin cided with DDT treatments for tadpol shrimp. In 1963, cooperative studies b California state agencies, the Universit of California, rice growers, and chemica ıpany representatives demonstrated t DDT spray treatments at recom- ɿded rates caused acute fish toxicity in inage waterways when water was al- ed to drain freely after treatment but when the water was held for 5 days ore release. Since other chemicals ·e available for tadpole shrimp con- , DDT was removed from University ɔmmendations in 1964; its general use ; banned in the United States in 1973.

ıer problems

Copper sulfate has been used by some : growers to control tadpole shrimp :e 1946, but during the late 1950s, ny reported a lack of control at recom- ɿded rates. The problem was not resis- ce, as had been thought, but the formu- on. Original control studies had used ɜly ground crystals of copper sulfate, crystals of later commercial formula- ıs were about the size of pea gravel (up 0 mm). This was more economical but vented the chemical from going into ıtion fast enough to be toxic to tadpole imp. Subsequently, copper sulfate was mulated as finely ground "rice" cry- ıs, and the problem disappeared.

Copper sulfate is sometimes also used ɜn algicide in rice fields. At one time, ɜliminate the cost of airplane applica- ı, the material was placed in metering ices at irrigation inlet boxes as a pre- tive treatment for algae and shrimp. : practice was stopped, because water rents distributed the chemical uneven- and because a high concentration of copper in water adjacent to the metering devices injured the rice.

Parathion at 0.1 pound active ingredient per acre was added to UC recommendations for tadpole shrimp control in 1965. Water spill is restricted from treated fields for 3 days, and no wildlife problems have been noted to date with this chemical at the recommended low rate of spray application. In the late 1970s and early 1980s, however, rice growers in several areas reported less than desirable control of tadpole shrimp with parathion. This was first attributed to improper treatment time or inadequate airplane coverage, since the practice was to spot-treat only turbid areas of the field or to spray in swaths that did not overlap.

We carefully monitored a commercial application of parathion for tadpole shrimp control in San Joaquin County in 1982. Although recommended time of treatment and application procedures were followed, the chemical was not effective.

Standardized laboratory tests, which we conducted in 1982, compared the susceptibility of tadpole shrimp from several localities with data from previous years. The test shrimp were reared from eggs collected in soil. When compared with 1964 and 1974 data from similar tests, the 1982 results indicated a reduction in level of control with parathion during the 18 years at the Butte County site (table 1). Tadpole shrimp from the Colusa County site were highly susceptible, but those from San Joaquin County showed a low level of mortality at exposure times comparable to those in other tests.

TABLE 1. Mortality of field-collected tadpole shrimp treated with parathion*

:ation	Year	Time after treatment	Percent mortality	
			Untreated	Treated
		hr		
te County	1964	18	0	100
te County	1978	19	6	81
te County	1982	18	0	65
usa County	1982	7	0	100
ı Joaquin County	1982	18	0	10

plication rate: 0.1 pound active ingredient per ½ acre-foot of water.

TABLE 2. Mortality of field-collected tadpole shrimp treated with copper sulfate

:ation	Year	Formulation	Rate*	Mortality after treatment†	
				4 hr‡	5 hr‡
			lb	%	%
te County	1978	Crystals	5	50	91
		Crystals	10	56	94
ı Joaquin County	1982	Crystals	5	88	100
		Crystals	10	100	100
		Liq.(chelated)	5	94	100
		Liq.(chelated)	10	100	100
ı Joaquin County	1984	Crystals	5	65	95
		Crystals	10	75	95
		Liquid	5	75	100
		Liquid	10	75	100

ıunds active ingredient per ½ acre-foot of water

ɔ mortality in untreated controls occurred at these time intervals.

me intervals in 1984 were 3 hours 45 minutes and 4 hours 45 minutes.

The most plausible conclusion from these tests is that tadpole shrimp have developed varying levels of resistance to parathion in different parts of the state. Several factors may affect the level of resistance at a particular site, but because tadpole shrimp are not usually transported in large numbers over great distances, the frequency of parathion use at the site is probably a major factor. Parathion is used at the same rate to control mosquitoes and the rice leafminer, so multiple applications may be made in some locations and resistance is likely to be greatest in these areas. Resistance to parathion also suggests that tadpole shrimp might be resistant to other organophosphates.

Present recommendations

Copper sulfate continues to be very effective in controlling the tadpole shrimp at recommended rates of 5 to 10 pounds of active ingredient per acre. Comparable rates of copper sulfate formulated in crystal form or as a liquid have resulted in similar mortality levels in replicated tests in the laboratory (table 2). Crystals are predominantly used now, but they may be replaced by the liquid formulation if it becomes economically advantageous.

Action guidelines for control of tadpole shrimp are given in the manual *Integrated Pest Management for Rice* (publication 3280, University of California Division of Agriculture and Natural Resources). Recommendations are based on the shrimp's presence during the critical period from planting to seedling emergence through the water, and the maximum number of plants desired per square foot. Seeding should be done as soon as possible after flooding, because most tadpole shrimp eggs hatch within 2 days after initial flooding, and the bigger the shrimp, the greater the potential for plant injury.

To order the IPM rice manual, send check or money order for $15 payable to **The Regents, University of California** to: ANR Publications (Dept. CA), University of California, 6701 San Pablo Ave., Oakland, CA 94608. Foreign orders: Request ordering information and pro forma invoice, indicating number of copies and method of shipment (surface or air) desired. Do not send payment.

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18.

From: erl@webspirs7.silverplatter.com
Date: Thu, 14 Jun 2001 20:00:09 -0400 (EDT)
Subject: copper

Record 1 of 1 in CAB Abstracts 1979-1981

TI: Studies on the use of chemicals to control algal surface blooms in rice floodwaters.
AU: Dunigan-EP; Hill-V
SO: 69th-Annual-Progress-Report,-Rice-Experiment-Station,-Crowley,-Louisiana-1977. 1978, 101-105.
PY: 1978
LA: English
AB: At Crowley, rice cv. Vista was sown on 25 May prior to flooding on 9 June 1977. Bluestone (hydrous copper sulphate) at 15, 30 and 45 lb/acre and K-lox and Ricetrine (both organocopper chelates) at 2 p.p.m. copper were applied in the floodwater on 31 June. Bluestone at 15 lb/acre was applied at other locations in south-western Louisiana. The predominant alga was Spirogyra. At Crowley, good control of algal surface blooms was obtained with all 3 chemicals with no detrimental effect on rice plant growth. At the other locations, the Bluestone treatment gave almost complete control of algal blooms. Copper concns. in the rough rice seed varied from 3.5 to 5.7 p.p.m. which was not considered to be high. It is noted that Bluestone is the only product so far cleared by the EPA for use as a rice-field algicide.
DE: control-; chemicals-; COPPER-SULFATE; cereals-; weeds-
OD: Algae-
GE: Louisiana-; USA-
ID: copper-chelates
RN: 7758-98-7
BT: plants; West-South-Central-States-of-USA; Southern-States-of-USA; USA; North-America; America; Delta-States-of-USA; Gulf-States-of-USA
CC: HH000; FF500
CD: Pathogen,-Pest-and-Parasite-Management-General; Weeds-and-Noxious-Plants
PT: Miscellaneous
UD: 950220
AN: 792327500

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HEAVY METALS INCIDENCE IN THE APPLICATION OF INORGANIC FERTILIZERS AND PESTICIDES TO RICE FARMING SOILS

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Abstract

The concentrations of Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn in different inorganic fertilizers (urea, calcium superphosphate, iron sulphate and copper sulphate) and in pesticides (two herbicides and one fungicide) are evaluated together with the contribution of these metals in soils from their use. The study was made in rice farming areas to the north of Albufera Natural Park (Valencia, Spain). The results obtained show that superphosphate is the fertilizer that contains the highest concentrations of Cd, Co, Cu and Zn as impurities. Copper sulphate and iron sulphate have the most significant concentrations of Pb, and are the only fertilizers in which Ni was detected. The three pesticides analysed show similar Cd contents and the highest levels of Fe, Mn, Zn, Pb and Ni are found in the herbicides. The most significant additions of heavy metals as impurities that soil receives from agricultural practices, are Mn, Zn, Co and Pb. Three contamination indexes have been applied to provide a basis for comparison of potential heavy metal toxicity. These results denote the potential toxicity of heavy metals in the studied soils.

Keywords: Heavy metals, fertilizers, pesticides, soil, contamination indexes.

INTRODUCTION

Addition of compounds to soils and crops has become a common practice in agriculture. The yearly consumption of fertilizers in Spain is about 2×10^6 tonnes (99.3 kg ha^{-1} of the land's cultivated surface) and the Valencia Community is the third largest in user (MOPT, 1991). The yearly consumption of pesticides is about 28 000 tonnes, which yields an average of 29.9 kg ha^{-1} of cultivated land (MOPT, 1991) and this represents a fifth of the national average. The main purposes of the use of chemicals are the improvement of the nutrient supply in soil (fertilizers) or crop protection and disease control (pesticides). These practices may cause chemical degradation of soil, as the result of accumulation of compounds at undesirable levels. Because fertilizers are usually not sufficiently purified during the processes of manufacture, for economic reasons, they usually contain several impurities, among them heavy metals. Also, heavy metals often form a part of the active compounds of pesticides.

A surplus of heavy metals in soils is frequently caused by using fertilizers, metallo-pesticides and sewage sludges, or by industrial activities, and the concentration of heavy metals in soils can increase by repeated and excessive fertilizer and pesticide applications.

Some countries have set tolerance limits on heavy metal additions to soil because their long-term effects are unknown (Barth & L'Hermite, 1987). These limits are usually established for the tillage layer of soil where most crop-root activity occurs. Controls on heavy metal concentrations in fertilizers and sewage sludges and their maximum total and annual loading rates to soil have been imposed in some countries (Häni, 1990; Celardin *et al.*, 1990), but regulations affecting fertilizers only include limits on Cd in phosphate fertilizers at present.

In this paper, a study on heavy metal contributions to soils through fertilizers and pesticides has been carried out in the rice farming areas of Albufera Natural Park. The concentrations of Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn in four inorganic fertilizers, two herbicides and one fungicide, as well as in soils, are evaluated. The heavy metals added to soil by this route have been assessed from the amount of these products commonly used in rice farming. Moreover, three contamination indexes (Zn Equivalent, Zn/Cd ratio and Extractable/Total ratio) have been applied to provide a basis for comparison of potential heavy metal toxicity.

MATERIALS AND METHODS

Site description

The study was conducted in a rice plot of 4000 m^2, located in the north zone of the Natural Park of La Albufera (Valencia, Spain). The Natural Park covers an area of 21 000 ha and 12 000 of these are used for rice crops. The Park has undergone a long series of

Table 1. Physical and chemical characteristics of the studied soil

	Horizons		
	Apl	Ap2	AB
pH H_2O	7.90	8.20	8.46
KCl	7.70	7.80	7.90
E.C.^{a} (dS m^{-1})	5.50	3.46	3.53
CO_3^{2-}(cmol kg^{-1})	1.10	0.96	0.00
HCO_3^- (cmol kg^{-1})	7.46	4.26	3.80
CO_3^{2-} tot^b (%)	32.87	33.53	36.72
O.M.^{c} (%)	2.92	2.35	1.99
Mg (cmol kg^{-1})	3.03	3.27	5.17
Ca (cmol kg^{-1})	11.95	10.75	9.90
K (cmol kg^{-1})	0.54	0.55	0.87
Na (cmol kg^{-1})	2.80	2.66	4.22
CEC^d (cmol kg^{-1})	16.21	16.19	19.08
S^e (cmol kg^{-1})	18.32	17.23	20.17
V^f (%)	100	100	96.57
ESP^g (%)	17.68	16.50	22.46
P_2O_5 (cmol kg^{-1})	25.62	22.19	12.48
Fe_2O_3 (cmol kg^{-1})	0.81	0.88	1.19
Al_2O_3 (cmol kg^{-1})	1.39	1.53	2.40
Fe/Al (%)	57.89	57.48	49.72
Total sand (%)	42.08	39.88	12.67
Total silt (%)	37.82	37.28	49.29
Total clay (%)	20.1	22.84	38.04
Textural classes	Loam	Loam	Clay–Silt–Loam

^{a}Electric conductivity measured in the extract paste.
^{b}Total carbonates.
^{c}Organic matter.
^{d}Cation exchange capacity.
^{e}Total bases.
^{f}Saturation in bases.
^{g}Exchangeable sodium percentage.

ecological attacks, beginning with the introduction of the rice farming during the last century. Other serious impacts are occurring because of the industrialization of the neighbouring areas.

The site studied is in a low lying area with a high water table, that comes to the surface at several points due to the topography and the vicinity of the marine water interface. Soils of this zone have been developed from organic black and gray silts and are affected intensely, at the surface, by agricultural practices. They show, as their most important physical and chemical characteristics, an impermeable profile, carbonated, with hydromorphic features, and high salinity level. According to FAO-UNESCO classification (FAO-UNESCO, 1988), the soils belong to the Calcareous-gleic Fluvisol type in saline phase. Table 1 shows some physical and chemical characteristics of the studied soils.

The analysed horizons are characterized by having a loamy (Apl and Ap2) and loam–clayey–silty (AB) texture with alkaline pH (between 7.9 and 8.5). The organic matter content varies between 2.9% in Apl and 2.0% for AB. All of them present high total carbonate levels (> 30%) and hydromorphic features. Moreover, they show high values of electric conductivity (5.50 dS m^{-1} in the extract of saturation), indicative of a salinization process that does not produce saturation by sodium as an exchange complex. This could be due to the action of the calcium carbonate, which is dominant in the soil solution. The soluble carbonate levels reflect the strongly alkaline character of the soil. Takiric features have been observed in the surface when the soil is dry and anthraquic characteristics because of soil flooding for the rice crop.

Table 2. Inorganic fertilizers and pesticides analysed, nutrient content and proportion added to soil

	Nutrient content	Added (kg ha^{-1})
Fertilizers		
Urea	46% N	300
Calcium superphosphate	18% P	600
Iron sulphate 7 hydrate	18.5% Fe	200
Copper sulphate 5 hydrate	25% Cu	35
Pesticides		
Ordram	Molinato 7.5%	60
Antracol	Propineb 70%	4
Saturn-G	Tiobencarb 10%	70

Sampling

Random sampling was carried out in this area at three depths (0–15, 15–30 and 30–45 cm) in 44 points. Sampling was performed when the soils were not flooded with an Eijkelkamp hydraulic sampler with acrylic tubes (120 mm diam., 600 mm long), and then the samples were stored in polyethylene bags hermetically sealed until analysis. Three samples of each depth were taken at each sampling point. In the laboratory, these samples were air-dried, passed through a 2-mm sieve and homogenized in an agate mortar. The three samples taken at each sampling point at different depth, were mixed and homogenized, and then four subsamples were taken for treatment with reagents.

The fertilizers and pesticides analysed were those used by the farmer and the amounts added to soil are given in Table 2. Fertilizers were: urea (46% N), calcium superphosphate (18% P), iron sulphate (18.5% Fe) and copper sulphate (25% Cu). The pesticides analysed were: Saturn-G (Tiobencarb 10%), Ordram (Molinate 7.5%) and Antracol (Propineb 70%). Three samples were taken of each of these commercial products, homogenizing them before analysis.

Analysis

Materials used for experiments were previously soaked in 30% HNO_3 (v/v) for 24 h and rinsed three times with deionized water. All reagents used in this study were of analytical grade and were checked for possible trace metal contamination, which was not detected in any of them. Deionized water was used in preparing stock solutions.

A modification of Analytical Methods of Spanish Ministry of Agriculture, Fishing and Food (1986) was used for the fertilizers treatment: 10 g of urea, iron sulphate and copper sulphate and 2 g of calcium super-

Table 3. Linear ranges, detection and quantitation limits for the determination of the studied metals by F-AAS and GF-AAS

Element	Detection limit (mg litre^{-1})	Quantitation limit (mg kg^{-1})	Linear range (mg litre^{-1})
Co	0.003–0.15	0.023–1.15	0.05–4.00
Cu	0.051–2.55	0.138–6.90	0.05–2.00
Fe	0.065–3.55	0.11–8.05	0.10–6.00
Mn	0.033–2.18	0.62–8.47	0.20–3.00
Ni	0.044–2.20	0.08–4.00	0.05–5.00
Pb	0.047–2.35	0.159–7.95	0.10–6.00
Zn	0.087–4.35	0.25–12.5	0.20–7.00
Cd	0.005–0.22	0.008–0.39	0–50[a]

[a] mg μlitre^{-1}.

phosphate were dissolved in acid medium (with concentrated HCl, Panreac, p.a.).

The analysis of pesticides was made by acid digestion using Teflon reactors, this being a modification of the official methods of the Spanish Ministry of Agriculture, Fishing and Food (1986). In each reactor, 2 g of air-dried commercial pesticide were placed with 10 mlitre of concentrated HNO_3, 2 mlitre of concentrated H_2SO_4 and 10 mlitre of distilled–deionized water. The Teflon reactors were heated for 10 min at 120°C, and then the contents were transferred to volumetric flasks when cold. The flasks were heated and the contents slowly boiled but avoiding complete dryness. The residue was dissolved with 4 mlitre of concentrated HCl and 30 mlitre hot distilled–deionized water, and centrifuged at 5000*g* during 10 min at room temperature. The liquid was filtered through Whatman No. 9 filter paper and made up to a 50 mlitre volume with distilled–deionized water.

Total contents of metals were determined by the nitric–perchloric acid digestion method described by Andreu (1993). Soil samples were digested in a Tecator Digestion System 40, 1016 Digestor (40 places, tubes 2.5 cm diam.), using 1 g soil samples. The residue was dissolved in 4 mlitre of 6M HCl at 120°C, filtered and made up to a volume to 50 mlitre with deionized water. Three replicates were performed per sample.

To determine the extractable fraction of metals, 4 g of soil sample was treated with a solution of 0.05M EDTA at pH = 7.0, in a modification to the procedure proposed by the Agricultural Development and Advisory Service of England and Wales (ADAS, 1983). The agitation of soil-extractant mixtures was conducted in an INFORS AG CH-4130 shaker previously adapted. Again three replicates were performed per sample.

Presence of metals was determined by atomic absorption spectrophotometry (AAS). A Perkin–Elmer Model 2380 involving direct aspiration of the aqueous solution into an air–acetylene flame, equipped with a deuterium background corrector, was used to measure Cu, Ni, Pb, Zn, Fe and Mn. A Perkin–Elmer Model 1100B equipped with a Perkin–Elmer HGA 400 graphite furnace was used for absorbance measurements of Cd and Co by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS). For Cd a standard addition technique was employed because matrix interactions were observed.

To assess the quality of the applied methodology and the determinations by F-AAS and GF-AAS different aspects were studied. Matrix interferences were checked in soils, fertilizers and pesticides by the method of standard addition. No matrix interferences were observed for the determination of the studied metals except Cd total content in soils and fertilizers; it was necessary to apply the method of standard addition to make these measurements.

To estimate the necessity of the use of background correction factors to avoid non-specific interferences, recovery assays were carried out. Measures were made with and without the use of a deuterium lamp. Also, in the case of Ni, a Co lamp at $\lambda = 232$ nm was used and for Pb, the Pb lamp but at $\lambda = 220.4$ nm (non-absorbable line). Values were similar in both correction procedures.

Detection and quantitation limits were calculated according to the definition of the American Chemical Society (1980). Results are shown in Table 3 with the linear range for each element.

Precision of the methods, including the digestion process, was calculated by the analysis of seven homogeneous aliquots of a soil sample. Instrumental precision was

Table 4. Total metal content. Precision of the method and instruments

Element	Method			Instrumental		
	[a]X (mg kg^{-1})	[b]S_{n-1} (mg kg^{-1})	[c]RSD (%)	[d]Y (mg kg^{-1})	[b]S_{n-1} (mg kg^{-1})	[c]RSD(%)
Cd	1.17	2.24	7.77	0.62	3.70	5.96
Co	5.05	0.55	10.17	5.00	0.75	15.00
Cu	20.84	1.73	5.61	28.53	0.23	0.81
Fe	10578	123	28.00	10855	91.27	3.27
Mn	151.32	8.3	14.22	147.23	4.35	1.65
Ni	19.12	1.91	10.00	19.48	0.93	4.77
Pb	51.16	2.86	5.59	48.98	2.11	4.31
Zn	96.21	4.23	4.4	96.62	0.69	0.71

[a] Mean concentration, result of seven determinations of the same sample.
[b] Standard deviation.
[c] Relative standard deviation.
[d] Mean concentration of 12 consecutive measures of the same digested solution.

determined by 12 consecutive readings of a digested soil solution. Results are reported in Table 4.

Accuracy of the method was verified by means of recovery assays using certified reference material from the Community Bureau of Reference, CRM 141 Calcareous loamy soil. Sixteen aliquots of a homogeneous soil sample and the same of the BCR CRM 141, were taken and known amounts of each element were added to eight of them before applying the digestion procedure, to obtain a final concentration twice higher than the initial content of the sample. The recoveries obtained with 95% confidence intervals are: 102.51 ± 7.03% Cd, 86.01 ± 6.35% Co, 94.51 ± 1.22% Cu, 99.06 ± 3.19% Fe, 93.42 ± 2.56% Mn, 90.65 ± 5.84% Ni, 97.93 ± 7.51% Pb and 94.76 ± 5.01% Zn.

RESULTS AND DISCUSSION

The metal contents obtained for fertilizers, pesticides and soils are shown in Tables 5 and 6. For fertilizers, superphosphate contains the highest levels of Cd, Co, Cu and Zn as impurities. Copper sulphate and iron sulphate show the highest content of Pb; moreover, they are the only fertilizers in which Ni was detected. No fertilizers contain detectable amounts of Fe and Mn, except iron sulphate.

The results obtained for phosphate fertilizer are between the values obtained by other authors (Webber *et al.*, 1984; Alloway *et al.*, 1988) who found levels of 0.1–170 mg kg^{-1} Cd, 1–12 mg kg^{-1} Co, 1–300 mg kg^{-1} Cu, 7–38 mg kg^{-1} Ni, 7–225 mg kg^{-1} Pb, 50–1450 mg kg^{-1} Zn and 40–2000 mg kg^{-1} Mn. The same authors established that nitrate fertilizers contain between 0.05–8.5 mg kg^{-1} Cd and 5.4–12 mg kg^{-1} Co, whereas the results obtained for urea in this study are lower (0.008 and 0.051 mg kg^{-1} Cd and Co, respectively). Adriano (1985) found that urea Cu content was 0.6 mg kg^{-1}, a value similar to the 0.4 mg kg^{-1} found in this fertilizer (Table 5).

For pesticides, the highest concentrations of Fe, Mn, Zn, Pb and Ni (Table 5) were found in the herbicides (Saturn-G and Ordram). All the pesticides analysed showed similar concentrations of Cd (between 1 and 2 mg kg^{-1}). Antracol and Saturn-G have similar levels of Co (1.8 mg kg^{-1}), while the Ordram content was ten times lower. The most significative metal addition to soil is Zn, because this element is part of the active compound of the Antracol. The highest contents concentrations due to impurities are Mn, Ni, Pb and Fe.

Table 5. Heavy metal contents (mg kg^{-1}) in fertilizers and pesticides

	Cd	Co	Cu	Ni	Pb	Zn	Fe	Mn
Fertilizers								
Copper sulphate	0.21	0.06	255×10^3	0.60	11.0	21.4	ND	ND
Iron Sulphate	0.03	1.35	0.30	0.50	10.0	13.3	201×10^3	220
Urea	0.01	0.05	0.40	ND	ND	ND	ND	ND
Superphosphate	2.22	4.50	12.5	ND	ND	50	ND	ND
Pesticides								
Antracol	1.94	1.85	ND	0.75	5.00	274×10^3	0.275×10^3	15
Saturn-G	1.48	1.81	13.00	12.25	10.00	55.00	10.20×10^3	205
Ordram	1.38	0.17	ND	14.25	7.50	32.50	10.10×10^3	195

ND, Not detected by AAS-Flame.

Table 6. Total content and extractable fraction of metals (in mg kg^{-1}) in the studied samples, and Extractable/Total ratio

	Cd	Co	Cu	Ni	Pb	Zn	Fe	Mn
Total								
Ap1	1.832	5.291	20.35	22.82	45.96	55.80	12537	158.1
	(0.046)^a	(0.174)	(0.52)	(21.50)	(4.15)	(0.59)	(209)	(13.6)
Ap2	1.149	4.862	11.31	22.91	41.36	43.01	11582	183.5
	(0.001)	(0.064)	(1.14)	(2.09)	(4.56)	(0.48)	(144)	(2.7)
AB	1.256	5.647	22.58	24.78	35.08	54.90	19146	179.7
	(0.007)	(0.086)	(2.91)	(0.41)	(1.91)	(0.91)	(312)	(19.4)
Extractable								
Ap1	0.292	0.136	8.83	1.51	7.70	9.41	27.80	26.5
	(0.0004)	(0.004)	(0.18)	(0.08)	(0.001)	(0.30)	(3.02)	(0.8)
Ap2	0.261	0.039	3.57	0.45	7.05	4.27	91.92	28.2
	(0.002)	(0.006)	(0.15)	(0.001)	(0.001)	(0.30)	(7.99)	(0.9)
AB	0.249	0.241	1.41	0.38	6.08	0.65	93.40	39.3
	(0.001)	(0.002)	(0.03)	(0.001)	(0.31)	(0.001)	(3.07)	(0.3)
^{b}E/T	18.90	2.64	25.45	3.32	17.00	9.34	0.49	18.01

^{a}Standard deviation value appears in parentheses.
^{b}Extractable/Total ratio.

Table 7. Overall estimate of heavy metal additions to soils (mg ha^{-1} $year^{-1}$) from agricultural activities

	Cd	Co	Cu	Ni	Pb	Zn	Fe	Mn
Fertilizers								
Copper sulphate	7.14	2.24	8925[a]	21.00	385	749	—	—
Iron sulphate	6.20	270.20	60.00	100	2000	2600	40200[a]	44000
Urea	2.40	15.30	120	—	—	—	—	—
Superphosphate	1332	270	7500	—	—	30000	—	—
Pesticides								
Antracol (Propineb)	7.79	7.42	—	3.00	20.00	1100[a]	1.10	60.00
Saturn-G (Tiobencarb)	34.60	42.32	304	286.40	234	1.28[a]	238	4.79[a]
Ordram (Molinato)	82.80	10.40	—	855	450	1.95[a]	606	11.64[a]
Total (g^{-1} ha^{-1} $year^{-1}$)	1.47	3.05	8933	1.26	3.09	1137	40201	60.49

[a] g^{-1} ha^{-1} $year^{-1}$.

Table 6 shows the total and extractable concentrations of heavy metals in the soil samples. Total levels are lower than the maximum established by the European (CEC, 1986) and Spanish legislation for calcareous soils. When they are compared with those recommended in other legislation, such as Switzerland (Celardin *et al.*, 1990) and the United Kingdom (Barth & L'Hermite, 1987), the levels of cadmium and lead exceed the maximum allowed.

Values of Cd in the soil samples (Table 6) are higher than those obtained by Errecalde (1989) and Andreu (1993) which varied between 0.31 and 0.60 mg kg^{-1}, in studies carried out in the Albufera Natural Park. While for Co, Cu, Ni, Pb and Zn the values obtained are similar to those obtained by these authors.

Except for Mn (Table 6), all the values determined in the studied soils are, in general, within the range described by different authors for agricultural soils (Webber *et al.*, 1984; Kabata-Pendias & Pendias, 1984; Boluda *et al.*, 1988; Alloway, 1990; Crisanto & Lorenzo, 1993). These ranges are: Cd 0.01–2.40 mg kg^{-1}, Co 1–40 mg kg^{-1}, Cu mean value 20 mg kg^{-1}, Ni 1–100 mg kg^{-1}, Pb 30–100 mg kg^{-1}, Zn mean value 50 mg kg^{-1}, Fe 7000–550 000 mg kg^{-1} and Mn 20–10 000 mg kg^{-1} and a mean value of 1000 mg kg^{-1}.

The distribution of the values reported above, indicated the following order for the total content of metals in the analysed samples:

$$\mathrm{Fe} \gg \mathrm{Mn} > \mathrm{Zn} > \mathrm{Pb} \gg \mathrm{Ni} > \mathrm{Cu} \gg \mathrm{Co} \gg \mathrm{Cd}.$$

Cd, Cu, Pb and Zn show their maximum values in the superficial horizon (Ap1 0–15 cm). Cd and Pb are elements that accumulate more readily in the surface and show a significant decrease in the underlying horizons.

The extractable content of a heavy metal is an indicator of the quantity of metal available to plants. The proportion of this fraction relative to the total amount of the metal is also an indicator of its comparative mobility (He & Singh, 1993; Andreu, 1993). Table 6 shows the extractable amounts of heavy metals in the analysed soils. The following progression in the extractable content of metals in the studied area is demonstrated:

$$\mathrm{Fe} > \mathrm{Mn} \gg \mathrm{Pb} > \mathrm{Zn} > \mathrm{Cu} \gg \mathrm{Ni} > \mathrm{Cd} > \mathrm{Co},$$

which is different to the relationship observed for the total content.

Generally, the values of the extractable fraction coincide with the data reported by Errecalde (1989), Andreu (1993) and Andreu and Boluda (1995) for soils of the same area, but are lower compared with the values reported by several authors for similar soils (Klessa *et al.*, 1989; Berrow & Burridge, 1990). Except Co, the highest values have been obtained in the superficial horizons. Pb and Zn show a gradual decrease of their concentrations with depth. Mn and Fe (Table 6) are the most abundant elements.

The estimation of heavy metals added to soil by agricultural practices is shown in Table 7. The most significative additions are of Fe and Cu, which are added to soil as micronutrients. As impurities, the highest amounts correspond to Mn, Zn, Co and Pb, and the lower to Cd and Ni. Only Cu added to soil (9 kg ha^{-1} $year^{-1}$) is near to the limit established by European Union legislation (12 kg ha^{-1} $year^{-1}$).

To estimate the impact of heavy metal additions to the soil in comparison to the total heavy metal content, three contamination indexes have been used: *Zn Equivalent* (Chumbley, 1971; Greenland & Hayes, 1981; Webber, 1972), *Zn/Cd ratio* (Chaney, 1973) and *Extractable/Total ratio* (Andreu & Boluda, 1995).

The Agricultural Development and Advisory Service of England and Wales (Chumbley, 1971; Webber, 1972) proposed a general statement about the relative toxicity of Zn, Ni and Cu:

$$\mathit{Zn\ Equivalent} = (1 \times Zn) + (2 \times Cu) + (8 \times Ni)\,\mu g\ g^{-1}\ \mathit{dry\ matter}.$$

The *Zn Equivalent* has been calculated assuming that 1 ha of soil to a depth of 15 cm weighs 2×10^6 kg (Greenland & Hayes, 1981). The heavy metal addition from agricultural practices according to the *Zn*

Equivalent is 9.51 $\mu g\ g^{-1}\ year^{-1}$. This represents 3% of the total *Zn equivalent* (332 $\mu g\ g^{-1}$) of the soil surface horizon. It has been recommended that a total of 250 μg 'Zn Equivalent' g^{-1} soil is the maximum that can be safely added, if there have been no previous additions to the soil and the pH is maintained at not less than 6.5. This addition may be as a single, heavy application or the sum of small applications spread over a period of 30 years (Greenland & Hayes, 1981). Using this recommendation, the addition found is low (9.51 $\mu g\ g^{-1}$) but considering the time factor, the safety limit is surpassed (285 $\mu g\ g^{-1}$).

Zn/Cd ratio (Chaney, 1973) assesses the antagonism that the Zn exerts on the Cd assimilation by plants to decrease the toxicity of Cd. The ratio of Zn/Cd must be 200, although Chaney considers the ideal value, to obtain an effective antagonism between Zn and Cd in plants to be close to or over 1000. However, Diamant (1979) asserted that this ratio must be > 100. By considering Chaney's conclusions and applying the same procedure as above, the value obtained is 39.3 for the soil and 773 for the total fertilizer application. If we assume Chaney's first criterion, which agrees with the one established by legislation in Spain and the European Union ($Zn/Cd \geq 200$), then can say that this addition value is not dangerous. However, if the ideal value Zn/Cd ratio is considered (close to or over 1000), then the value obtained is worrying. Nevertheless, this ratio is very low for the soil and, theoretically, the Zn antagonism to Cd can be considered as not effective.

The third index applied, the *Extractable/Total ratio*, can be useful to obtain information on the bioavailability and mobility of these elements in soil. If the mean values of the total content and extractable fraction are considered, and the corresponding percentage of the extractable fraction calculated, an estimate of the comparative mobility of the studied metals is obtained (Table 6). According to these data, the order of comparative mobility is:

$$\mathrm{Cu} > \mathrm{Cd} \approx \mathrm{Mn} > \mathrm{Pb} > \mathrm{Zn} \gg \mathrm{Ni} > \mathrm{Co} \gg \mathrm{Fe}.$$

This progression shows that the elements potentially most toxics to humans (i.e. Cd and Pb), along with Cu, are the most mobile. Fe, Co and Ni are the elements most strongly retained by the soil. Generally, the extractable fraction is present in a low proportion in alkaline soils. Lake *et al.* (1984) observed that the extractable form of metals represents 10% or less of the total content. In Cd, the extractable fraction could reach 18% of its total content in this type of soils.

CONCLUSIONS

The results obtained for fertilizers show that superphosphate contains the highest concentrations of Cd, Co, Cu and Zn as impurities. Copper sulphate and iron sulphate have the highest contents in Pb, and moreover they are the only fertilizers in which Ni was detected.

Similar concentrations of Cd are found in the three pesticides analysed. The highest levels as impurities are of Fe, Mn, Zn and Ni.

The most significative heavy metals additions to soil as impurities are Zn, Mn, Co and Pb. Only the amount of added Cu is near to the limit established by European Union legislation for calcareous soils.

The results obtained from application contamination indexes denote a potential toxicity of heavy metals in the studied soil. The total content and the extractable fraction determined in the analysed soil samples are lower than the maximum values established by the European and Spanish legislation for calcareous soils. The percentage of the extractable fraction suggests that the highest relative mobility are for Cu, Cd, Pb and Mn.

The additions of fertilizers and pesticides to rice farming should be adjusted to real needs. If not, it could promote the accumulation of heavy metals in soils and plants giving rise to situations that could affect the food chain and human health. These situations could result from the special conditions in which this crop is grown (seasonal floods, processes of anaerobiosis, soil characteristics, etc.) that favour the availability of the metals to rice plants.

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Environmental Contamination and Toxicology

Copper in Rice and in Soils According to Soil Type in Japan, Indonesia, and China: A Baseline Study

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Copper is essential to human life and health because of its important role in various metabolic processes, although as with most heavy metals, excess amounts of copper are toxic. Many studies had been carried out on the effect of copper on the environment and on human health. However, there have been few studies on the copper content of rice and rice field soil (Rivai *et al.*, 1988; Ohmomo and Sumiya, 1981; Suzuki, 1980), and none of these studies have fully reported on the content of copper in rice grown in Indonesia or China or on the copper content of soil in these two countries.

The copper content of rice, as a staple food in most of Asian countries, has been considered a good indicator of daily copper intake. The copper content of brown rice and paddy soil in Japan reported by Iimura (1981) was 2.9 μg/g and 32 μg/g, respectively, and the copper content of unpolished rice grown in Java, Indonesia, was reported by Suzuki *et al.* (1980) to be 3.41 μg/g. The daily copper intake of Japanese people estimated by multiplying the copper content of rice by daily rice consumption was 660 μg/person (Ohmomo and Sumiya, 1981). Toro *et al.*, (1994) conducted research on toxic heavy metals and other trace elements in foodstuffs from 12 different countries, but no data for Indonesia or China were available. Horiguchi *et al.* (1978) reported daily intake of several metals in foods in thirty countries but there were no data for Indonesia. Because of the existence of differences in food consumption and environmental contamination in different countries, the present study aims: 1) to determine copper content of rice and rice field soils in Japan, Indonesia, and China in order to identify the possibility of copper pollution with special reference on daily copper intake in rice from these three countries; 2) to determine the relationship between copper in rice and in soil.

MATERIALS AND METHODS

A total of 178 pairs of unpolished rice and soil samples were collected from Japan, Indonesia, and China. Soil samples were also collected from rice fields where rice plants were growing. Samples were taken from Hokkaido/Tohoku, Hokuriku, Kanto, Tokai, Chugoku, Shikoku, and Kyushu in Japan (N=111), from the east, northeast, and south portion of China (N=22), and from Java, Sumatra, Kalimantan, and Sulawesi in Indonesia (N=45). Sample sites recorded were usually on the prefecture or city level. An approximately 0.1 gram rice sample was placed in a test tube for pre-treatment, weighed, dried in an oven at 105^{0} C for 48 hours, and then weighed again to assay water content. The dried sample was ashed on a hot plate with 1.0 ml of concentrated nitric acid (metal free) until dry, and

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2.0 ml of 14% nictric acid was then added to dissolve the residue. Two methods were used to pre-treat the soil samples. The first method was extraction by HCl without ashing. Approximately 1 ml of a dried and filtered soil sample was placed in a tube and weighed, 50 ml of 0.1 N HCl was added, and the tube was then shaken in a 30° C water bath for 1 hour. The upper, clear solution was separated and centrifuged at 3,000 rpm for 5 minutes for copper analysis. The second method was extraction by ashing with nitric acid. An appproximately 1 gram dried and filtered soil sample was placed in a test tube and weighed, and then 5.0 ml of concentrated nitric acid (metal free) was added, and the sample was ashed on a hot plate. After adding 10.0 ml of pure water, the clear supernatant was taken for copper analysis. Each sample was analyzed two or three times with an autosampler AAS (Hitachi 2100) at a wavelength of 229 nm for rice samples and 326 nm for soil samples. Standard materials provided by the US National Bureau of Standards were used under the same conditions for reference to confirm accuracy: 0.1 gram of powdered rice (No. 1568) and 0.5 g of orchard leaves (No. 1571) for rice and soil samples. Concentrations of copper were determined with a flameless AAS (Hitachi 2100) under the recommended conditions.

Estimation of daily copper intake based on the amount consumed per day in a usual diet was calculated by the CIC method (copper level in rice multiplied by daily rice consumption). In addition, the ratios of copper level in rice to the level in soil were estimated by dividing the content of copper in rice by copper content in soil. Identification of soil type was based on the Soil Map of the World, Volume VIII (1978) and Volume IX by the FAO (Food and Agricultural Organization). A few samples on a borderline between two soil types were excluded.

RESULTS AND DISCUSSION

The arithmetic means and the standard deviation of the copper content of rice samples from Japan, Indonesia and China were shown in Table 1. Geometric means and the deviations were used when the data were skewed. The copper level of rice in Japan reported by Ohmomo and Sumiya (1981) was 2.81 μg/g, lower than in the present study. The level of copper in Indonesian unpolished rice from Java in a study of cadmium, copper, and zinc in rice produced in Java conducted by Suzuki *et al.* (1980) was higher, 3.41 μg/g, than in the present study, 2.69 μg/g. The copper content in Chinese rice (4.21 μg/g) was practically identical to the copper content of rice from Taiwan, 4.4 μg/g in a study by Masironi *et al.* (1977). Compared with other Asian country data reported by Masironi *et al.* (1977) for Philippines (3.9 μg/g), Bangladesh (4.7 μg/g), and Taiwan (4.4 μg/g) the copper content of rice seems to be about the same as that of Chinese and Japanese rice. However, compared with the copper content of rice from the Southern Catalonia, Spain (1.39 μg/g), according to Schuhmacher *et al.* (1994) and 2.49 μg/g for copper content of unpolished rice from Houston, Texas (Suzuki and Iwao, 1982) the copper content in the three countries seemed higher, but was in the same range as the copper content of wild rice from central Canada, 1.6—14.4 μg/g (Pip, 1993).

The arithmetic mean of copper content in the soil from Japan, Indonesia, and China was 7.35 ± 5.94, 1.98 ± 2.09, and 3.35 ± 2.37 μg/g, respectively, by the hydrochloric acid extraction method, and 20.20 ± 6.28, 11.57 ± 4.64, and 15.14 ± 1.44 μg/g, respectively, by the nitric acid ashing method. The level of copper content in soil from Japan, Indonesia, and China seemed much lower than in the paddy soils of Alcacer do Sal, Portugal, 49.4 μg/g (Fernandes *et al.*, 1990).

Table 5. Correlation coefficients between copper content in rice and in soil samples by country

	N	Cu in rice	Cu in soil[a]	Cu in soil[b]
JAPAN	111			
Cu in rice		1.00	0.15	0.15
Cu in soil[a]			1.00	0.80**
Cu in soil[b]				1.00
INDONESIA	45			
Cu in rice		1.00	-.06	0.04
Cu in soil[a]			1.00	0.75**
Cu in soil[b]				1.00
CHINA	22			
Cu in rice		1.00	0.36	0.33
Cu in soil[a]			1.00	0.37
Cu in soil[b]				1.00
Total	178			
Cu in rice		1.00	0.29**	0.29**
Cu in soil[a]			1.00	0.83**
Cu in soil[b]				1.00

Note: Cu, copper, a and b, see footnote Table 1, * & **, significant correlation at $p< 0.05$ & $p< 0.01$, respectively;

A positive Pearson's correlation coefficient was found between copper in rice and in soil extracted with hydrochloric acid (soil[a]) in Japan and China but a negative relationship was found for the samples from Indonesia ($r=-0.06$, $p>0.05$). None of the correlation coefficients for copper in rice and in soil ashed with nitric acid (soil[b]) from Japan, Indonesia, and China were significant. The correlation coefficient for copper level in soil[a] and in soil[b] was significant both for the samples from Japan and Indonesia ($r=0.80$ and $r=0.75$, $p<0.01$, respectively). When samples from the three countries were combined, there was a high correlation between copper content in rice and soil[a] ($r=0.29$, $p<0.01$) and between rice and soil[b] ($r=0.29$, $p<0.01$). The highest correlation was seen between copper in soil[a] to soil[b] ($r=0.83$, $p<0.01$). The amounts of copper in rice did not seem to be correlated with copper in soil. Similar results were also reported by Rivai *et al.* (1990), Suzuki and Iwao (1982), and Scuhmacher *et al.* (1994), whose reports all indicated no significant relationship between metals in rice and metal concentrations in soils. The reason for this is the very complicated interrelation between soil texture, humus content of soil, soil pH, etc., and metal absorption and assimilation by growing plants (Suzuki *et al.*, 1980; Gupta, 1979).

Daily copper intake from rice in the three countries is shown in Table 6. Daily copper intake in rice in China was 1.4 mg/person. The daily intake of copper by Indonesians was 1.1 mg/person, almost the same as the daily copper intake in rice in West Java, 0.9 mg/day for women and 1.3 mg/day for men, previously reported by Rivai *et al.* (1988). The daily copper intake in Japan was 1.1 mg/person, considerably higher than in the 0.66 mg/person reported by Ohmomo and Sumiya (1981). In addition, the daily copper intake in rice in the three countries seemed higher than the daily copper intake in rice of people in Southern Catalonia, Spain,

Table 6. Daily copper intake in rice by country (μ g/person)

Country	N	Cu in rice (μ g/g)	Daily Cu intake from rice (mg/person)
Japan (300.2)*	111	3.71	1.1
Indonesia (507.3)*	45	2.26	1.1
China (350.8)*	22	3.99	1.4

*, Average daily rice consumption (g/person) of 1979--1981 from the data of Food Balance Sheets by FAO (1984)

and than the daily copper intake in cereals (rice, noodles, sphagetti) of people in Tarragona Province, Spain, 19.04 and 346.4 μ g/person, respectively, according to Schuhmacher *et al.* (1994; 1993). Daily copper intake in rice seemed to depend on copper content in rice and daily rice consumption in each country.

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Toxicity of copper to lumbricid earthworms in sandy agricultural soils amended with Cu-enriched organic waste materials

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The average copper (Cu) content of Dutch arable sandy soils is 11 mg kg^{-1} (van Driel and Smilde 1981). Normal background concentrations of Cu may be increased appreciably by anthropogenic activities, for example in urban or industrial areas or in agriculture.

The use of copper sulphate as a feed additive to improve the efficiency of pig production has led in recent years to the spreading of Cu-enriched pig slurry on land. This has become a major emission source of copper in The Netherlands, with local inputs of between 1 and 10 kg Cu $ha^{-1} yr^{-1}$ in areas with intensive pig production. Furthermore, high local Cu inputs are generated in many countries by the spraying of copper oxychloride as a fungicide in fruit-growing areas. Other important pollution sources of Cu include dredged sludges, sewage sludges, and composted municipal wastes, which are often applied to sandy agricultural soils as organic fertilizer. Sewage sludge, for example, may contain Cu in concentrations between 200 and 8000 mg kg^{-1} dry matter (Berrow and Webber 1972). In The Netherlands, sewage sludge containing Cu at levels of up to 600 mg kg^{-1} dry matter is permitted for use in agriculture at annual rates of 1 and 2 tonnes dry matter per ha on grassland and arable soil, respectively. Similar guidelines are used for the application of composted municipal wastes.

Added Cu accumulates in the top 0–25 cm layer of arable land and in the top 0–5 cm layer of permanent grassland. In the long run, a level may be reached at which irreversible detrimental effects on plant and animal life occur. Since this accumulation of Cu can affect the ecological functioning of the soil and reduce soil fertility, it has become necessary to establish soil quality standards with respect to environmentally safe levels of copper. For this purpose, we need ecotoxicological data as a basis for environmental impact assessment leading to the regulation of copper use in agriculture and organic waste management.

Field observations (Niklas and Kennel 1978, van Rhee 1975) have suggested that copper is detrimental to lumbricid earthworms. This assumption is supported by laboratory studies, which showed that the toxicity of copper to earthworms is influenced by the pH and organic matter content of the soil (Streit and Jaeggy 1983, Streit 1984, Ma 1984). One of the objectives of the study reported here was to establish the specific contribution of copper to the toxicity to earthworms of organic waste materials such as sewage sludge, pig slurry, and composted urban wastes applied to sandy soils. In addition, the effect of pH was investigated further and a coherent analysis of various laboratory and field data was made to determine acceptable levels of soil Cu in relation to toxicity to earthworms.

Materials and methods

Laboratory and field experiments were carried out in sandy agricultural soils with pH 4.8–5.2, clay 2–4%, organic matter 4–6% and CEC 5–9 meq. (100 g dry $soil)^{-1}$. For a field experiment performed on arable land, composted urban waste was mixed into the 0–20 cm plough zone once a year at various rates. Sulphur or calcium carbonate was used to adjust the pH of the soil. Samples were taken after a lag period of three to four years during which no waste compost had been added. Sampling for earthworms was done in two to four replicate plots by digging and hand-sorting followed by additional formalin extraction.

Pot experiments were performed in four to six replicates at 15°C in 5-litre containers each provided with 6 kg (dry weight) soil and five worms. The median effective concentration at which a test parameter (e.g., reproduction) was reduced by 10% (EC_{10}) or 50% (EC_{50}) relative to the untreated control was determined by fitting generalized logistic response curves according to a computerized least-squares method. Behaviour experiments were conducted in 2-litre glass containers, one half of each was filled with Cu-amended and the other half with unamended soil. Five worms were placed on the surface of the soil in each jar, and the worms and all burrows made in both compartments were photographed at 24-h intervals to record the position of the worms and the length of the tunnels. Total burrow

Tab. 1. Effect of copper on earthworm cocoon production determined after 4 weeks of exposure to sandy soil with added $CuCl_2$ at 15°C.

Test species	Cu concentration in soil (mg kg^{-1})			
	EC_{10}	95% confidence interval	EC_{50}	95% confidence interval
Lumbricus rubellus	80	44–147	122	94–157
Aporrectodea caliginosa	27	17–45	68	55–83
Allolobophora chlorotica	28	21–38	51	46–57

Tab. 2. Distribution (in %) of total burrow length and frequency of occurrence of *Lumbricus rubellus* given a choice between a compartment containing amended ($CuSO_4$) soil and a compartment with unamended soil.

Test parameter	Soil	Cu conc. in amended soil (mg kg^{-1})			
		50	100	200	300
Burrow length	Unamended	56	59	75	92
	Amended	44	41	25	8
Worm distribution	Unamended	64	75	81	96
	Amended	36	25	19	4

length reached a peak level after about two days. Average burrow length and worm distribution were calculated for days 3 and 4. The experiment was done in four replicates.

The metal content of soil and worm samples in all laboratory and field experiments was determined by atomic absorption spectrophotometry after digestion with H_2SO_4-HNO_3 (1:1); concentrations are expressed on a dry matter basis.

Results and discussion

Laboratory studies

Tab. 1 shows the results of pot experiments performed to determine the effect of copper alone on reproduction rate of three species of earthworms. In *Aporrectodea caliginosa* and *Allolobophora chlorotica* cocoon production started to decline at a soil Cu level of about 30 mg kg^{-1} and in *Lumbricus rubellus* at 80 mg kg^{-1}. EC_{50} values ranged from about 60 mg kg^{-1} for *Aporrectodea caliginosa* and *Allolobophora chlorotica* to about 120 mg kg^{-1} for *L. rubellus*. The former two species thus appeared to be somewhat more sensitive to Cu. Furthermore, they showed an increased tendency to become quiescent when toxic environmental conditions were encountered.

Soil pH had a significant influence on the toxicity of Cu for lumbricids. Fig. 1 shows the effect of pH on the toxicity of Cu in two species, *Aporrectodea caliginosa* and *Lumbricus rubellus*. *A. caliginosa* seems to be a more acid-sensitive species than *L. rubellus*. The difference in acid sensitivity can be seen from the specific effect of pH on reproduction in unamended soil (12 mg Cu kg^{-1}), where pH-dependent reproduction was seen in *A. caliginosa* but not in *L. rubellus*.

In Cu-polluted soil, however, both species showed a pH response. This is due to the physico-chemical environment, which determines the chemical form of the

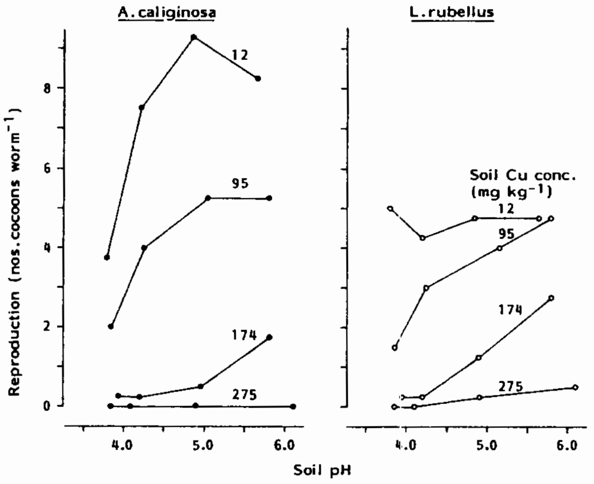
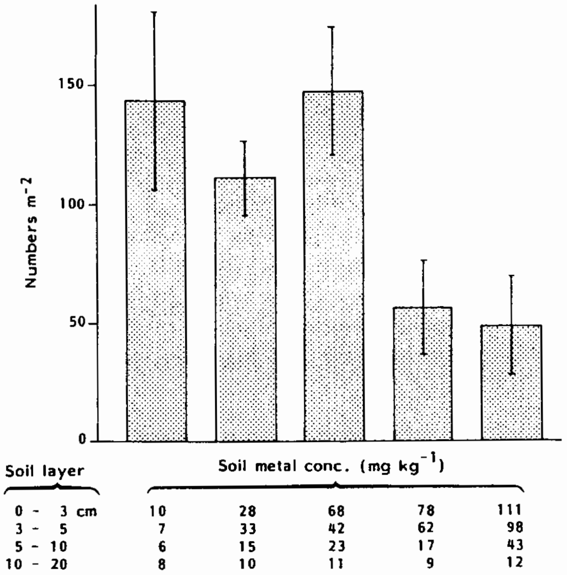
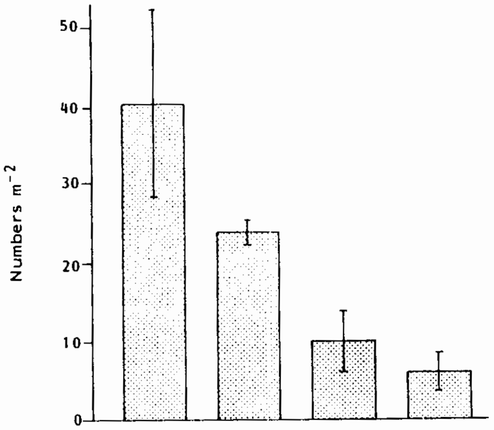


Fig. 1. Influence of pH on the effect of Cu in soil on the reproduction of two earthworm species.



Soil layer	Soil metal conc. (mg kg^{-1})				
0 - 3 cm	10	28	68	78	111
3 - 5	7	33	42	62	98
5 - 10	6	15	23	17	43
10 - 20	8	10	11	9	12

Fig. 2. Effect of frequent spreading of pig slurry on grassland on the distribution of Cu in the soil profile (lower part of Fig.), and the population size of *Lumbricus rubellus* (upper part of Fig.).



	Soil layer	Soil metal conc. (mg kg^{-1})			
Cd	0 - 3 cm	0.7	15	31	57
	3 - 5	0.5	10	21	31
	5 15	0.5	2.6	5.1	5.7
	15 - 25	0.3	0.5	0.7	0.8
Cu	0 - 3 cm	5.5	69	138	265
	3 - 5	4.7	50	99	150
	5 - 15	3.9	13	23	25
	15 - 25	3.0	4.5	5.4	6.0
Pb	0 - 3 cm	23	79	144	254
	3 - 5	22	147	288	438
	5 - 15	19	49	79	89
	15 - 25	16	21	22	24
Ni	0 - 3 cm	3.6	30	57	101
	3 - 5	4.7	24	44	69
	5 - 15	3.9	8.6	15	17
	15 - 25	2.7	4.1	3.5	3.9

Fig. 3. Effect of frequent spreading of sewage sludge on grassland on the distribution of some important heavy metals in the soil profile (lower part of Fig.) and the population size of *Lumbricus rubellus* (upper part of Fig.).

copper in the soil and hence the toxicity of the metal to organisms. Measurements of Cu at various pH levels in the soil show that the activity of cupric ions decreased with increasing pH, independent of the concentration of Cu present.

An important question to investigate was whether earthworms would avoid migrating into sites polluted with Cu. In behaviour experiments a choice was given between untreated and treated soil. The results, which are summarized in Tab. 2, indicate that lumbricids are capable of distinguishing between Cu-polluted and unpolluted soil. The average burrow length made in polluted soil decreased with increasing Cu concentration, and the less frequent presence of worms reflected their reluctance to enter the polluted soil. This avoidance behaviour may have implications in the field, because it means that there is less chance of recolonization of Cu-polluted sites. It also suggests that caution should be exercised before concluding what chance earthworms have of surviving in Cu-polluted soil when the homogeneity of the pollution in the soil is uncertain and a refuge into unpolluted micro-sites remains possible.

Field studies

Figs 2 and 3 show the results of field experiments carried out in grassland receiving sewage sludge and Cu-enriched pig slurry, respectively. In both cases the sampled population showed *L. rubellus* as the dominant species. The data in these figures show that Cu accumulates strongly in the upper soil surface horizons. In the case of sludge-amended soil other metals accumulate as well; data on some of these metals are included in Fig. 3. The decline of the lumbricid populations was found to be correlated with an increase in the concentration of metals retained in the uppermost soil layer (0–5 cm). In both experiments a lag period of three to four years was allowed during which no treatment was applied before sampling took place. The outcome reflects the long-term consequences of soil treatment with sewage sludge or pig slurry.

Fig. 4 shows the results of a field experiment in arable soil with added urban waste compost and various pH levels. Sampling was done after an untreated lag period of four years. The population was composed predominantly of *Aporrectodea caliginosa*. The upper 0–20 cm layer of the soil showed an accumulation of Cu together with a number of other metals. The population decreased in numbers with increasing concentrations of metals in the soil. However, the toxic effect was substantially alleviated by liming of the soil. The influence of pH is presumably related to the modifying effect of pH on the available form of metals in metal-polluted soil (Emmerich et al. 1982). This influence of pH is similar to the effect of pH on Cu toxicity described

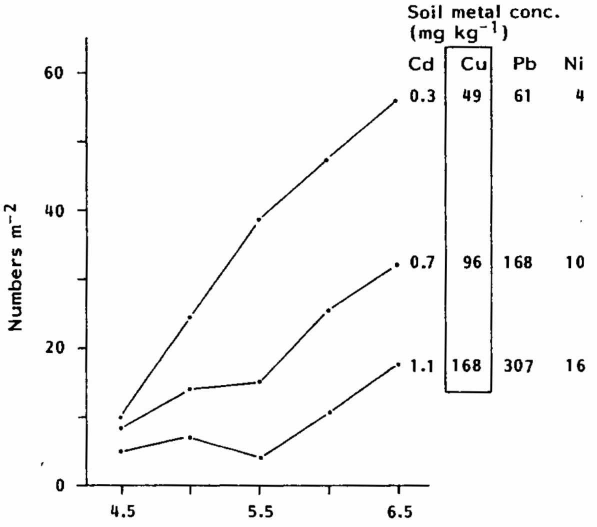
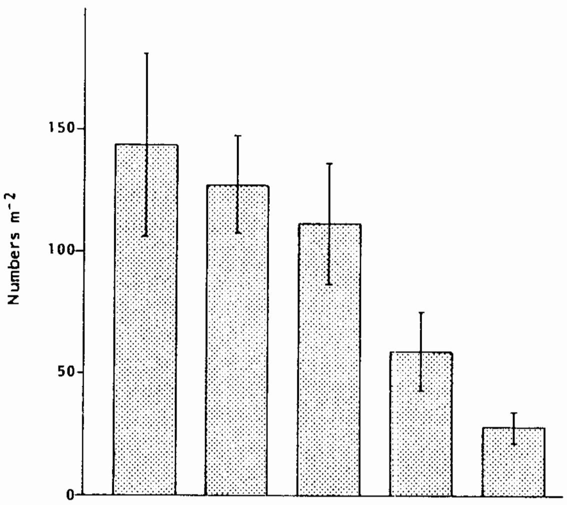


Fig. 4. Influence of pH on the effect of composted urban waste incorporated into the ploughed layer (0–20 cm) of arable soil on the populations size of *Aporrectodea caliginosa*. The accumulation of some heavy metals in the 0–20 cm layer is also shown.



Soil layer	Soil Cu conc. ($mg\ kg^{-1}$)				
0 - 3 cm	10	62	118	197	230
3 - 5	7	42	80	85	-
5 - 10	6	15	18	24	30
10 - 20	8	7	7	10	11

Fig. 5. Effect of frequent spreading of copper sulphate on grassland on the distribution of Cu in the soil profile (lower part of Fig.) and the population size of *L. rubellus* (upper part of Fig.).

above for reproduction of *Aporrectodea caliginosa* in the laboratory (see Fig. 1).

In plots treated with pig slurry, sewage sludge, or composted urban wastes the specific effect of Cu is difficult to assess because of the simultaneous presence of other metals. Sewage sludge and composted urban wastes contain many different kinds of heavy metals, and in pig slurry the salt concentration or the release of ammonia or other toxic substances may exert a harmful effect in addition to that of Cu. Nevertheless, the toxic effect of Cu alone could be assessed indirectly by comparing dose-response relationships obtained for sewage sludge or urban compost with those obtained in field experiments with copper as the only contamination source.

Fig. 5 shows the decrease in the size of a population of predominantly *L. rubellus* in grassland treated annually with only copper sulphate at rates comparable to those in the field experiments on Cu occurring in sewage sludge and pig slurry. The relative population decline associated with exposure to copper sulphate occurred at higher concentrations of Cu in the soil surface horizons than would have been expected from the results shown in Figs. 2 and 3 for Cu in sewage sludge or pig slurry. This suggested that although Cu was the main factor underlying the population decline, pig slurry and sewage sludge have other compounds that exert some additional toxic effect.

One of the consequences of frequent applications of Cu-enriched pig slurry is the formation of a mat of plant debris in the soil surface horizon (McGrath et al. 1980). The present results can explain this phenomenon, i.e., a strong reduction of populations of lumbricids by Cu leads to a diminished rate of breakdown of dead plant litter, which would then accumulate on the soil surface.

Concluding remarks

The present study has shown that the accumulation of Cu in the soil affects the reproductive potential of earthworms and at the same time deters earthworms from recolonizing Cu-polluted soils. The combined effect of these two responses would be a strong and sustained decline of lumbricid populations at Cu-polluted sites. Comparison of soils contaminated with single metal or multiple metals showed that Cu accounts for the greater part of the population decline occurring in soils treated with sewage sludge or composted urban wastes.

It was also found that in unlimed sandy agricultural soils (with about 4 to 6% organic matter and a pH of about 5) detrimental effects on earthworm populations can be expected to start at Cu concentrations above 50 mg kg^{-1} with a drastic decline above 100 mg kg^{-1}. Treatments to rehabilitate Cu-polluted soils with respect to toxicity for earthworms might benefit from the inclusion of liming to raise the pH.

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L12.

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Respiratory health of California rice farmers

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Rice farmers are occupationally exposed to agents that may affect respiratory health, including inorganic dusts and smoke from burning of agricultural waste. To assess respiratory health of this occupational group, we conducted a cross-sectional study, including a self- administered health and work questionnaire, spirometry, and chest radiography among 464 male California **rice** farmers. Mean age +/- SD was 48.3 +/- 15.2 yr; mean duration of **rice** farming was 25.7 +/- 14.3 yr. Prevalences for respiratory symptoms were: chronic bronchitis (6.3%), physician-diagnosed asthma (7.1%), and persistent wheeze (8.8%). Chronic cough was reported by 7.1% of respondents and was associated with reported hours per year burning **rice** stubble. Mean FEV1 and FVC were at expected values. FEV1 was inversely associated with years working in **rice** storage and use of heated **rice** dryers. Mean FEF25-75 was 93% of expected and was inversely associated with **rice** storage activities involving unheated **rice** driers. ILO profusion scores > or = 1/0 for small irregular opacities were seen in 18 (10.1%) of 178 chest radiographs. Study findings suggest increased asthma prevalence among California **rice** farmers. Radiologic findings consistent with dust or fiber exposure were increased compared with those of the general population, although no associations with specific farming activities were identified.

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Am. J. Respir. Crit. Care Med. Am. J. Respir. Cell Mol. Biol.

13.

Short-term Transformations of Copper in Copper-amended Soils[1]

W. P. MILLER, D. C. MARTENS, AND L. W. ZELAZNY[2]

ABSTRACT

Copper was added to three soils in the laboratory at a rate of 48 mg Cu kg^{-1} in the form of either $CuSO_4$ or a swine (*Sus scrofa domesticus*) manure obtained from swine fed a high-Cu ration (equivalent to 100 Mg manure ha^{-1} containing 1060 mg Cu kg^{-1}). In order to assess the potential transformations of the added Cu among various chemical forms in the soils, a sequential extraction methodology was used to determine types of soil-Cu associations in samples taken 1, 4, 12, and 29 d after Cu additions to moist soils. Copper added as high-Cu manure substantially increased water-extractable Cu in all soils through the entire incubation period, while $CuSO_4$ additions resulted in higher exchangeable Cu levels through days 4 to 12. Most of the Cu added in both forms was recovered in "specifically adsorbed" forms, with 20 to 25 mg $CuSO_4$-Cu kg^{-1} being Pb-extractable and 15 to 20 mg manure-Cu kg^{-1} being acetic acid-extractable throughout the incubation period. Significant amounts of Cu were also associated with soil organic matter ($K_4P_2O_7$-extractable), more so when the Cu was added as high-Cu manure (10–15 mg Cu kg^{-1}) than as $CuSO_4$ (5 mg kg^{-1}). Some additional added Cu was associated with Mn oxides and noncrystalline Fe oxides after 12 d of incubation, particularly in the two soils containing higher amounts of these oxides. These results suggest increases in short-term availability and mobility of Cu in these soils, particularly with high-Cu manure amendment, although longer-term reversion processes may reduce bioavailability over time.

Additional Index Words: **$CuSO_4$, sequential extraction, specific adsorption, trace metal fractionation, organically bound Cu, swine manure.**

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The fate of Cu applied to cropland either as a soil amendment to enhance crop growth, or as a component of a waste product, is of considerable interest both agronomically and environmentally. Copper has been suggested as a dietary supplement at high rates for swine (*Sus scrofula domesticus*) and poultry (*Gallus* sp.) (Stahly et al., 1980), with the potential result of substantial increases in manure Cu levels. The substitution of Cu for some antibiotics in animal feed in Europe has raised concern that soil contamination may result in the short- or long-term from land application of the resulting manure, which may contain 1000 mg Cu kg^{-1} (Lexmond and deHaan, 1977). Sewage sludges containing high levels of Cu and other trace metals pose a similar problem with respect to land disposal in this country (Allaway, 1977).

The potential detrimental effects of Cu added to soils in organic wastes is likely to be dependent upon the chemical transformations of Cu to more or less mobile or bioavailable forms (Page, 1974). Initially, Cu in both swine manure and sludges is in an alkali-soluble form that is most likely to be organic in nature, as the solid phase is dominated by organic surfaces (Stover et al., 1976; Miller et al., 1986b). The kinetics of the decomposition process, as well as inorganic reactions such as adsorption and precipitation of Cu, will determine changes in the chemical forms of the added Cu in soil, which ultimately control mobility. The assessment of solid-phase forms of trace metals in soils is characteristically accomplished with some form of selective extraction technique, often performed sequentially with reagents chosen to selectively solubilize metals from progressively less labile forms (McLaren and Crawford, 1973). While the results obtained by these methods are dependent upon specific conditions and are probably best considered semiquantitative, they do offer an opportunity to explain field observations of trace metal uptake or leaching and the possibility of predicting metal behavior (Jenne and Luoma, 1975).

A number of field studies have shown limited uptake of Cu by agronomic plants grown on soils amended with moderate to high levels of high-Cu swine manures (Batey et al., 1972; Mullins et al., 1982b; Sutton et al., 1983). Sequential extractions of Cu in sludge- and manure-amended field soils performed months or years after amendment have shown organic (alkali-soluble) forms to be predominant, although often more labile specifically adsorbed or dilute acid-soluble Cu was not determined (Emmerich et al., 1982; Sposito et al., 1983). Mullins et al. (1982b) found significant increases in salt- and dilute acid-soluble Cu in three manured soils, whereas the organic and oxidic forms (solubilized only by dissolution of these soil components) contained most of the added Cu. The Silviera and Sommers (1977) work is one of the few short-term studies of this kind, reporting that diethylenetriaminepentaacetic acid (DTPA)-extractable Cu in two soils increased over 30 d of incubation as sludge decomposition proceeded. While these studies begin to explain the limited plant uptake of Cu by demonstrating the strong binding of Cu by organic matter, it is not clear if these Cu-organic associations are a product of soil transformations, a residual effect of the original form of added Cu, or some combination of these factors. One limitation of previous work is the abbreviated extraction schemes used, often not determining more labile forms of metals prior to dissolution of whole soil components and associated Cu. The objective of the present study was, therefore, to examine the short-term transformations of Cu added as high-Cu manure to three soils of varying properties using an expanded, nine-step sequential extraction procedure devised previously (Miller et al., 1986a). The experiment was performed over a 29-d period in a controlled environment, with soils treated with equivalent amounts of $CuSO_4$ included in order to compare types and kinetics of Cu reactions with the organic-dominated, manure-amended soils. A previous report has detailed

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changes in soil chemical properties and forms of Fe and Mn oxides determined in the same series of experiments reported herein (Miller et al., 1985).

MATERIALS AND METHODS

The soils and experimental design used in collecting the data presented here were the same as those used by Miller et al. (1985). A Dyke clay (clayey, mixed, mesic Typic Rhodudults), a Guernsey silt loam (fine, mixed, mesic Aquic Hapludalfs), and a Bertie fine sandy loam (fine-loamy, mixed, mesic Aquic Hapludults) were sampled from the Piedmont, Appalachian, and Coastal Plain regions of Virginia, respectively. The Dyke sample was high in clay (450 g kg^{-1}) and Fe oxides (57 g kg^{-1}), the Guernsey was moderate (120 and 16 g kg^{-1}), and the Bertie, low (59 and 3 mg kg^{-1}, respectively). For each of the soils, pH (water) was in the range 5.3 to 5.6, and the soil organic matter was between 14 to 18 g kg^{-1}.

Bulk samples from the Ap horizons were air-dried, screened to <2 mm, and subsamples divided for treatment. High-Cu swine manure containing 1060 mg Cu kg^{-1} was applied in a freeze-dried form at the rate of 45 Mg ha^{-1}, resulting in a total Cu addition of 48 mg Cu kg^{-1} of soil. Equivalent amounts of Cu as $CuSO_4$ were added to separate samples by spraying the soil with 100 mg $CuSO_4$ L^{-1} solution using an atomizer. These samples, as well as untreated control soils, were brought to field capacity water content by spraying with deionized water, followed by thorough mixing. Three replications of each treatment on each soil were performed, and incubated at laboratory temperature in loosely tied plastic bags.

At 1, 4, 12, and 29 d after initiation of the experiment, triplicate subsamples were removed from each bag for analysis. Soil pH and redox potential were measured in a 10:1 water/soil slurry (Miller et al., 1985), and the centrifuged decantates analyzed for water-extractable Cu by atomic absorption spectrophotometry (AAS). These subsamples were then subjected to a sequential extraction procedure to selectively dissolve solid-phase Cu from the soils. The reagents used and approximate chemical forms of Cu dissolved are shown in Table 1. Solution/soil ratios were generally 40:1, with a wash step using 0.025 *M* $CaCl_2$ performed between successive extractions. Details of the method are given by Miller et al. (1986a).

RESULTS AND DISCUSSION

The 45 Mg ha^{-1} rate of manure application used in these experiments is equivalent to a field application of approximately 100 Mg ha^{-1} of wet manure slurry (250 mg solids kg^{-1}) and as such is a relatively high rate. It was chosen to represent a situation where problems with excessive Cu solubility may occur, and also to provide a sufficient level of added Cu for analytical determinations. Freeze-dried manure was used to ensure proper mixing with the soil, as preliminary testing had shown poor uniformity when slurries were used. Miller et al. (1986b) found that freeze-drying resulted in minimal changes in the behavior of Cu in the slurry, compared to more severe oven-drying methods.

Table 1. Sequential extraction method used for soil Cu†

Extractant	Conditions	Chemical form
0.5 *M* $Ca(NO_3)_2$	16 h, 22°C	Exchangeable (Exch.)
0.1 *M* $Pb(NO_3)_2$	16 h, 22°C	Pb displaceable: specifically sorbed (Pb-sp.)
0.44 *M* CH_3COOH, pH 2.2	8 h, 22°C	Acid soluble: specifically sorbed (Ac-sp.)
0.1 *M* $NH_2OH\cdot HCl$, pH 2	0.5 h, 22°C	Mn oxide-occluded (Mn-occ.)
0.1 *M* $K_4P_2O_7$, pH 10	24 h, 22°C	Organically-bound (Org.)
0.275 *M* $(NH_4)_2C_2O_4$, pH 2.5	4 h, 22°C	Noncrystalline Fe oxide occluded (Fe-NC)
0.275 *M* $(NH_4)_2C_2O_4$, pH 2.5	3 h, 85°C UV light	Crystalline Fe oxide occluded (Fe-crys.)
HNO_3/HCl/HF	2 h, 110°C Parr bomb	Residual (Res.) and Total

† 40:1 solution/soil ratio used.

The addition of high-Cu manure led to significant increases in water-extractable Cu in each of the three soils (Fig. 1). The high clay and oxide Dyke soil showed the lowest increase, presumably due to higher surface area and absorptive capacity, and levels declined further after 4 d of incubation. In a previous study (Miller et al., 1985), redox potentials were shown to reach a minimum value in manure-amended soils at day 4, when decomposition was probably at a maximum and large amounts of organics would be released into solution. These organics probably contained chelated Cu, as hypothesized by Sims and Patrick (1978) in explaining elevated soluble Cu levels in low redox soils. The manure itself contained significant amounts of water-extractable Cu, amounting to about 1 mg Cu kg^{-1} soil (Miller et al., 1986b). In the highly oxidic Dyke soil, redox increased more rapidly to oxidizing conditions than did the other two soils, perhaps speeding decomposition of soluble organics and thereby also reducing soluble Cu (Miller et al., 1985).

Copper added as $CuSO_4$ increased water-extractable levels only slightly in the three soils (Fig. 1), but this treatment did result in significant increases in salt-extractable Cu 1 to 4 d after addition, particularly in the sandy Bertie soil (Fig. 2). At the first sampling the soils contained 6 to 8 mg Cu kg^{-1} in the exchangeable form, but this declined to <2 mg kg^{-1} by day 12, and to near control levels

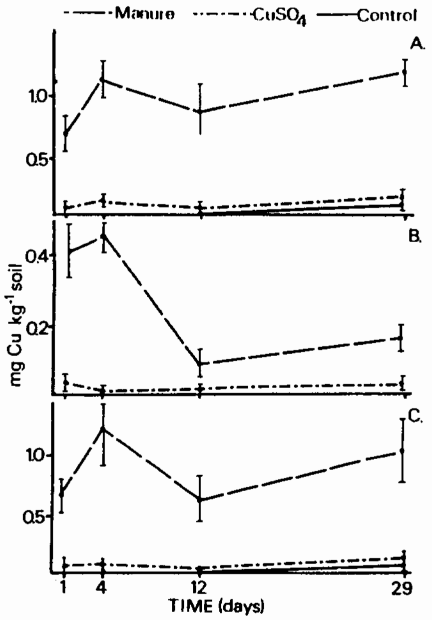

Fig. 1. Effect of Cu addition as either $CuSO_4$ or high-Cu manure on water-soluble (H_2O-extractable) Cu over 29 d of incubation for the Guernsey silt loam (A), Dyke clay (B), and Bertie fine sandy loam (C). Vertical bars indicate 95% confidence intervals.

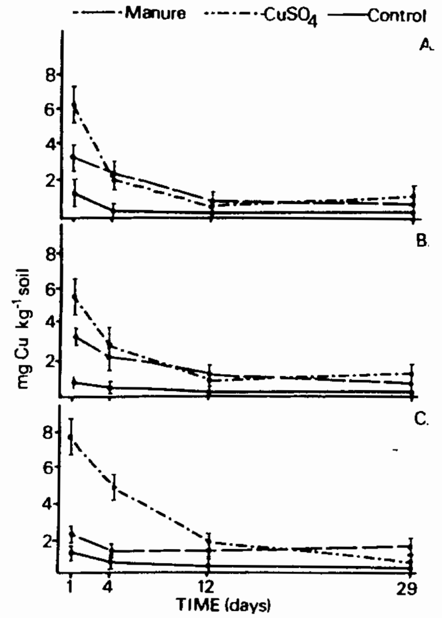

Fig. 2. Effect of Cu addition as either CuSO$_4$ or high-Cu manure on exchangeable [Ca(NO$_3$)$_2$-extractable] Cu over 29 d of incubation for the Guernsey silt loam (A), Dyke clay (B), and Bertie fine sandy loam (C). Vertical bars indicate 95% confidence intervals.

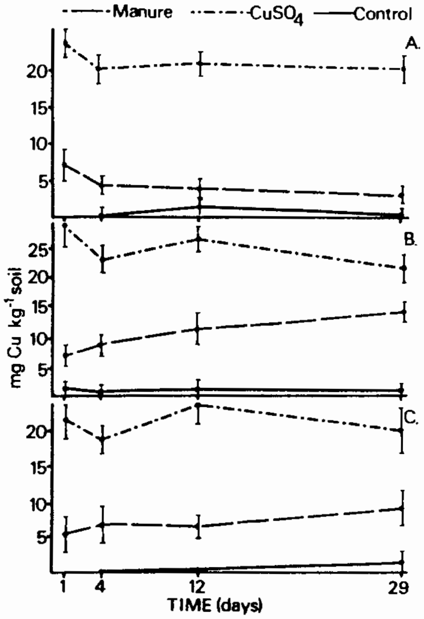

Fig. 3. Effect of Cu addition as either CuSO$_4$ or high-Cu manure on Pb-displaceable [Pb(NO$_3$)$_2$-extractable] Cu over 29 d of incubation for the Guernsey silt loam (A), Dyke clay (B), and Bertie fine sandy loam (C). Vertical bars indicate 95% confidence intervals.

by the last sampling. The manure-amended soils followed a similar trend, although initial concentrations were lower. As the exchangeable form of soil Cu is generally considered to be a mobile, plant-available form, observations on the low residual effects of fertilizer Cu (Brennan et al., 1980) may be traced in part to short-term reactions involving reversion of Cu to more insoluble forms, such as those documented here.

Trace metals are well-known to be adsorbed by soil surfaces in nonsalt-extractable forms, which are desorbed by dilute acid or replaced by other transition metals (McLaren and Crawford, 1973; Miller et al., 1986a). In the methodology used here, a Pb(NO$_3$)$_2$ extraction was used to desorb this specifically adsorbed Cu, followed by dilute acetic acid to solubilize other associated labile phases. Approximately 50% of the Cu added to the soils as CuSO$_4$ in this experiment was Pb-displaceable (Fig. 3). This 20 to 25 mg Cu kg^{-1} varied little among the soils or over the 29-d incubation period, with only a slight trend to decrease in all of the soils by day 29. This form of soil Cu is probably associated with covalent bonding to hydrous oxides and functional groups of soil organic matter, for which Pb has an affinity similar to that of Cu (Forbes et al., 1976; Kinniburgh et al., 1976). Much less of the Cu added as high-Cu manure was found in this fraction (5–10 mg kg^{-1}), suggesting that much of the Cu in the manure was strongly held in positions not accessible to Pb displacement. There is a trend toward increasing amounts of Pb-extractable Cu over time in two of the manure-amended soils (Fig. 3).

Dilute acetic acid-extractable Cu, suggested as an extractant for hydroxy and carbonate Cu phases as well as for specifically bound soil Cu (McLaren and Crawford, 1973; Miller et al., 1986a, b), was a major sink for Cu

Table 2. Sequential extraction data at day 29 for three soils with and without amendment with either CuSO$_4$ or high-Cu manure.

Soil	Treatment	Total Cu	Sol.†	Exch.	Pb-sp.	Ac-sp.	Mn-occ.	Org.	Fe-NC	Fe-crys.	Res.
		mg Cu kg^{-1} soil									
Guernsey	Control	29	<0.1	<0.1	0.1	0.1	0.5	2.2	0.3	2.6	24.8
	Cu-manure	72	1.2	0.6	3.3	17.5	3.8	13.6	7.0	4.2	21.3
	CuSO$_4$	66	0.1	1.0	19.4	11.3	1.7	5.7	2.8	5.1	19.7
	LSD‡	7	0.2	0.1	1.2	1.4	1.0	1.9	1.1	0.7	4.2
Dyke	Control	68	0.1	0.1	0.3	1.0	0.5	2.0	0.7	11.3	56.0
	Cu-mnaure	118	0.1	0.5	14.5	21.7	2.9	6.6	4.8	12.7	54.5
	CuSO$_4$	111	0.1	1.1	22.3	10.7	4.6	5.7	3.4	12.0	49.5
	LSD	12	0.1	0.1	2.6	1.3	0.9	1.0	0.8	2.1	8.5
Bertie	Control	14	<0.1	<0.1	0.2	0.4	0.3	1.1	0.2	1.1	10.7
	Cu-manure	57	1.0	1.5	9.4	14.4	1.0	7.8	3.2	4.5	11.3
	CuSO$_4$	51	0.1	0.7	19.9	10.8	1.3	4.5	2.2	2.3	9.3
	LSD	5	0.1	0.1	1.9	1.6	0.3	0.7	0.6	0.6	2.8

† Water-soluble; names and reagents used in remaining sequential extractions shown in Table 1.
‡ LSD = least significant difference, 95% probability.

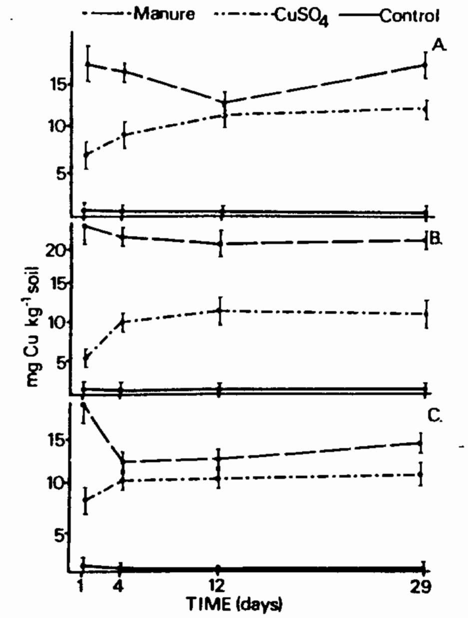

Fig. 4. Effect of Cu addition as either $CuSO_4$ or high-Cu manure on acetic acid-soluble (0.4 *M* CH_3COOH-extractable) Cu over 29 d of incubation for the Guernsey silt loam (A), Dyke clay (B), and Bertie fine sandy loam (C). Vertical bars indicate 95% confidence intervals.

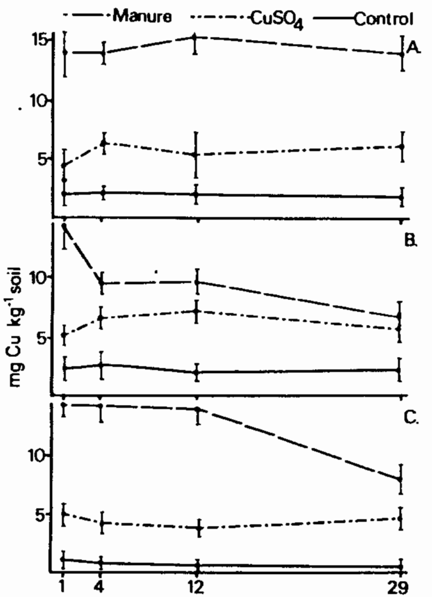

Fig. 5. Effect of Cu addition as either $CuSO_4$ or high-Cu manure on organically bound ($K_4P_2O_7$-extractable) Cu over 29 d of incubation for the Guernsey silt loam (A), Dyke clay (B), and Bertie fine sandy loam (C). Vertical bars indicate 95% confidence intervals.

added as high-Cu manure (Fig. 4). Amounts of Cu in this phase amounted to approximately 20 mg kg^{-1} for Dyke, 15 mg kg^{-1} for Guernsey, and 12 mg kg^{-1} for Bertie, with an irregular trend to decrease and then increase over time. This order is the same as that of decreasing surface area and redox buffering capacity of the soils, and may imply that Cu-containing resistant organics sorbed to soil surfaces constitute this phase. However, nearly 80% of the Cu in the original freeze-dried manure was also solubilized by this reagent, suggesting either a discrete, acid-soluble mineral or an acid-dissociable organic complex is involved (Miller et al., 1986b). In the case of $CuSO_4$-amended soils, about 10 mg kg^{-1} was found in this form in each of the soils, increasing slightly from day 1 to day 12.

Copper associated with Mn oxides (NH_2OH HCl-extractable) showed little regular variation over time, and only final 29-d concentrations for this form will be presented later in this paper. In the next extraction step, using $K_4P_2O_7$ to solubilize strongly bound organic metal, Cu did change over the incubation period, declining from approximately 15 mg kg^{-1} in the manure-amended soils on day 1 to < 10 by day 29 (except for the Guernsey soil; Fig. 5). This seems most likely due to decomposition processes, which may release Cu from the manure over time. The Guernsey soil was the slowest to reoxidize after manure additions, possibly thereby influencing the Cu in organic structural forms (Miller et al., 1985). Only 5 to 7 mg Cu kg^{-1} was alkali-extractable ($K_4P_2O_7$) in the $CuSO_4$-treated soils, which was approximately 3 mg kg^{-1} above levels in the untreated soils. Emmerich et al. (1982) determined roughly 50% of the Cu in sludge-amended soils to be alkali-soluble after longer incubation times, although they did not determine specifically adsorbed metals as in this study. Formation of strongly insoluble organic complexes is often cited as a mechanism for reduced availability of Cu fertilizer (Brennan et al., 1983), although this was not observed in this study.

Amounts of Cu found in association with structural forms of Fe (oxalate-soluble) and in nonextractable forms (residual) are shown for the last sampling time in Table 2, along with a summary of all the 29-d Cu fractions for

Table 3. Change in individual Cu forms of high-Cu manure- and $CuSO_4$-treated vs. control soils.†

Treatment	Series	H_2O		Exch		Pb-sp		Ac-sp		Mn-occ		Org		Fe-NC		Fe-crys		Sum§
		mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}
Cu-manure	Guernsey	1.1	3	0.6	1	3.2	8	17.3	41	3.4	8	11.4	27	6.7	16	1.9	5	42.6
	Dyke	0.2	1	0.5	1	13.3	30	20.8	47	2.4	5	2.4	5	4.2	9	0.6	1	44.4
	Bertie	1.0	2	1.5	3	9.4	22	14.1	33	3.7	9	6.9	16	3.0	7	3.5	8	43.1
$CuSO_4$	Guernesy	0.1	<1	1.1	3	20.6	47	11.8	27	1.2	3	3.7	8	2.7	6	2.6	6	43.8
	Dyke	<0.1	<1	1.1	2	21.1	48	9.6	22	4.1	9	3.5	8	2.7	5	1.9	4	44.1
	Bertie	0.1	<1	0.7	2	20.2	51	10.6	27	1.0	3	3.6	9	2.1	5	1.2	3	39.5

† Calculated as mg Cu kg^{-1} (treated)-mg Cu kg^{-1} (control), for $CuSO_4$ and manure treated samples; data from 29-d samples.
‡ Percent of added Cu ("Sum") found in each fraction.
§ Sum of mg Cu kg^{-1}, equivalent to Cu added to soil by treatments.

each of the treatments. The extraction with oxalate in darkness showed only small amounts of Cu associated with poorly crystalline Fe compounds in the untreated soils, but increases of 3 to 6 mg Cu kg^{-1} with manure treatment and smaller increases with $CuSO_4$. These values are slightly greater than for the Cu fraction associated with Mn oxides ($NH_2OH \cdot HCl$-extractable), which are also shown in Table 2. Both Mn and poorly crystalline Fe compounds were shown previously to be partially or wholly solubilized during the reduction phase that followed addition of the manure (Miller et al., 1985), and their reprecipitation upon reoxidation may have led to the occlusion of Cu in these phases. Additions of $CuSO_4$ did not result in redox changes in these soils, but some increases were noted in structural Fe- and Mn-occluded Cu, especially in the highly oxidic Dyke soil (Table 2). Additional Cu was also noted in the more crystalline Fe phases (oxalate-soluble under UV irradiation), more so in the Bertie and Guernsey soils where redox changes were most severe with manure additions. Copper not extractable with any of the reagents used (residual) was essentially unchanged by treatment, showing a 10 to 15% fluctuation that is most likely associated with cumulative experimental error (Table 2).

In order to quantify increases in individual fractions of Cu due to treatment with either Cu-manure or $CuSO_4$, amounts of Cu in untreated soil fractions were subtracted from those in treated soil at day 29 (Table 3). The results show the fate of the applied Cu, both in milligrams per kilogram and in the fraction of total Cu in each form. At the termination of the experiment at day 29, small amounts of added Cu were still water-soluble and exchangeable, but the largest fraction of that added was Pb-displaceable or acid-soluble. From 50 to 75% of the Cu added as Cu-manure was found in these two fractions (largely acid-soluble), while 70 to 80% of the $CuSO_4$-Cu was determined in these fractions (two-thirds of it Pb-displaceable). The increased organic matter and reducing conditions associated with manure additions provided conditions favorable for increases in organically bound and Fe oxide occluded forms of Cu. Total recovery of added Cu (sum of increases in individual fractions) was 80 to 90% for both treatments, the deficit is probably associated with cumulative analytical errors in the extraction and determination of individual fractions.

CONCLUSIONS

The major conclusion to be drawn from this study is that specific adsorption processes associated with Pb- and dilute acid-solubility are primarily responsible for Cu retention over a period of 29 d after Cu addition as either $CuSO_4$ or high-Cu manure. Initially, high levels of soluble and exchangeable Cu declined to near control concentrations over this time, suggesting accompanying decreases in bioavailability and leaching potential. With manure treatment the organic (alkali-soluble) form of Cu is of greater importance in Cu retention, as is Cu occluded within Fe oxides.

Longer-term studies of this kind have emphasized the importance of organic matter in binding Cu added as sewage sludge (Emmerich et al., 1982), high-Cu manure (Mullins et al., 1982a), and as $CuSO_4$ (Mullins et al., 1982b). It is likely that further transformation of adsorbed Cu to structural (organic, oxidic) forms will occur over time, as microbial processes and oxidation-reduction allow Cu to become incorporated into stable structural positions. Documentation of the kinetics of these reactions, as well as correlation with bioavailability, are yet to be accomplished.

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Soil Clogging During Subsurface Wastewater Infiltration as Affected by Effluent Composition and Loading Rate[1]

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ABSTRACT

The soil clogging effects of wastewater effluent composition and loading rate were investigated in pilot-scale infiltration cells installed in situ in the structured silty clay loam subsoil of a Typic Argiudoll (fine-silty, mixed, mesic). Over a 70-month period domestic septic tank effluent (DSTE), graywater septic tank effluent (GSTE) and tapwater (TW) were intermittently applied in an average of 5.2 doses/d to yield daily loading rates of 1.3, 2.6, and 5.2 cm/d. Soil clogging was negligible in all TW treatments and in the GSTE treatments at 1.3 and 2.6 cm/d. In the GSTE treatments at 5.2 cm/d and in all DSTE treatments, severe soil clogging led to continuous ponding of the soil infiltrative surfaces. A logistic model fit to the experimental data confirmed that soil clogging development was highly correlated with the cumulative mass density loadings of total biochemical oxygen demand and suspended solids. After 62 and 70 months of loading, soil properties were determined with depth. Clogged infiltrative surface zones exhibited significant accumulations of organic materials at the infiltrative surface and within the first few millimeters of the soil matrix. This field study demonstrated that higher quality wastewater effluents can be discharged to subsurface infiltration systems at hydraulic loading rates in excess of those utilized for DSTE without stimulating soil clogging development.

Additional Index Words: **septic tank systems, graywater, onsite soil absorption.**

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Clogging of soil infiltrative surfaces during intermittent infiltration of septic tank effluent (STE) can dramatically impact subsurface soil absorption system performance (Kristiansen, 1982; Otis, 1985). Some degree of clogging can enhance wastewater renovation through reactions within the clogged soil zone and the underlying unsaturated soil. However, excessive clogging can cause hydraulic dysfunction, anoxic soil conditions, and diminished wastewater purification. Although a wide variety of factors can influence soil clogging development, the applied wastewater composition and hydraulic loading rate are two that can be controlled through raw wastewater pretreatment or infiltrative surface area sizing. Prior research has suggested that soil clogging is generally accelerated under increasing hydraulic loading rates of a given STE, or under increasing concentrations of organic matter and suspended solids at a given hydraulic loading rate (Jones and Taylor, 1965; Laak, 1970; Hargett et al., 1982; Mitchell et al., 1982). Nevertheless, common practice has been to neglect consideration of effluent composition and loading rate interactions and simply apply STE to soil infiltrative surfaces at flux densities of 1 to 5 cm/d, with the rate based solely upon natural soil properties.

Reluctance to employ higher hydraulic loading rates for higher quality wastewater effluents has been due in part to a lack of reliable, long-term field data. To enhance the current state of knowledge and provide such data, a field investigation was conducted at the Univ. of Wisconsin-Madison between 1979 and 1985 (Siegrist, 1986). To facilitate this effort a research facility was established with pilot-scale wastewater infiltration systems installed in situ in a structured silty clay loam subsoil of a Typic Argiudoll. The research objectives were to determine the comparative soil clogging effects of different effluent composition and hydraulic loading rate scenarios and identify the causes of any soil clogging observed.

EXPERIMENTAL METHODS

A research facility was established at a rural Wisconsin home located 40-km north of Madison in the humid, continental climate zone. Graywater and domestic wastewater generated through standard water-use fixtures were separately treated in septic tanks prior to application to pilot-scale subsurface infiltration systems (hereinafter referred to as soil cells). The soil cells were installed in situ in the well-structured, silty clay loam argillic horizon of a pedon of the Plano series (fine-silty, mixed, mesic, Typic Argiudoll). Particle size analyses (Day, 1965) revealed this horizon to be comparised of an average of 9% sand, 51% silt and 40% clay. Percolation rates (Wisc. Adm. Code, 1972) ranged from 9 to 16 min/cm. Hydraulic conductivity properties measured in situ (Bouma et al., 1974) revealed a saturated hydraulic conductivity of 223 cm/d dropping off to < 1.0 cm/d at 2.5-kPa tension. Moisture retention data (Jaynes, 1978) revealed the soil moisture content to be 0.46 kg/kg at saturation decreasing to 0.36 kg/kg at 18-kPa tension.

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Availability and Form of Copper in Three Soils following Eight Annual Applications of Copper-Enriched Swine Manure

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ABSTRACT

The use of high levels of Cu in swine (*Sus scrofa domesticus*) production to stimulate growth and improve feed efficiency is presently being questioned due to the potential environmental hazard from repeated usage of the resulting Cu-rich manure in agricultural land. Three field studies were established in 1978 and continued through 1985 to determine the effects of long-term applications of Cu, as either $CuSO_4$ or Cu-enriched swine manure, at high rates on corn (*Zea mays* L.) production. The studies were conducted on Bertie fine sandy loam (Aquic Hapludults), Guernsey silt loam (Aquic Hapludalfs), and Starr-Dyke clay loam (Fluventic Dystrochrepts-Typic Rhodudults) in the Coastal Plain, Ridge and Valley, and Piedmont regions of Virginia, respectively. These soils have CEC ranging from 5.0 to 12.3 $cmol_c$ kg^{-1}. According to USEPA guidelines, which are based on the CEC values and pH levels ≥ 6.5, the three soils have a maximum safe Cu loading rate of 280 kg Cu ha^{-1}. After 8 yr, 890.4 t ha^{-1} of wet Cu-enriched manure had been applied to these soils. The manure contained up to 1550 mg Cu kg^{-1} and was produced by pigs fed diets with an average of 251 mg Cu kg^{-1}. Application of 336 kg Cu ha^{-1} as $CuSO_4$, or up to 264 kg Cu ha^{-1} as Cu-enriched manure, from 1978 through 1985 did not decrease corn grain yields on the three soils. The eight annual Cu applications increased Cu concentrations in ear leaf tissue by ≤ 2.1 mg kg^{-1}, but did not affect the Cu concentrations in the grain. The Cu levels in all plant tissue remained within normal ranges. Diethylenetriaminepentaacetic acid (DTPA)-extractable Cu was linearly related to the amount of Cu applied to the three soils (r = 0.93-0.98, α = 0.01) and was unrelated to Cu levels in grain or leaf tissue. Small increases, up to 1.6 mg Cu kg^{-1}, in extractable Cu in the B horizons of the Bertie and Starr-Dyke soils indicated little downward movement of the applied Cu. Larger increases in extractable Cu in the Guernsey subsoil were attributed to downward movement caused by plowing. Copper fractionation data indicated that a substantial portion of the applied Cu has reverted to forms not available to plants.

Supplementing feed of growing swine (*Sus scrofa domesticus*) with 125 to 250 mg Cu kg^{-1} has been shown to stimulate growth and to increase feed efficiency (1, 5, 8, 14). Feeding Cu-enriched rations to swine appears to have originated as an economical alternative to the use of certain antibiotics as growth stimulants or as a disease control measure (2, 6). The use of high levels of Cu in swine production has been questioned due to the concern about the potential environmental hazards associated with the repeated usage of the resulting Cu-rich manure in agricultural land (1, 8). Because as much as 95% of the dietary Cu is subsequently excreted in the wastes (27), the Cu concentration in the manure collected from swine fed Cu-enriched diets can be as high as that in sewage sludges (20, 38, 42). Copper concentrations in these manures generally are in the range of 600 to 2370 mg kg^{-1} of dry matter (3, 21, 31) and, unlike most sewage sludges, much of the Cu in the manure is in readily soluble forms (29).

Relatively few long-term field studies have been con-

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ducted to determine the effects of Cu-enriched swine manure applications on plant growth and composition. Batey et al. (3) found that three annual applications of 12.2 kg Cu ha^{-1} as a Cu-enriched pig manure slurry to a perennial ryegrass (*Lolium perenne* L.)-white clover (*Trifolium repens* L.) pasture increased the Cu concentration in the herbage, but not to a phytotoxic level. Much of the increase in Cu concentration was attributed to direct foliar contamination during manure application. Decreased yields occurred when foliage was smothered by the applied manure. They further concluded that the greatest risk to grazing livestock occurs from the direct ingestion of the slurry via the contaminated forage. Similar conclusions were made by McGrath (23) after applying Cu-enriched manure to a number of grass and clover pastures. Sutton et al. (35) found that four annual applications of 15.3 kg Cu ha^{-1} as Cu-enriched swine manure to a Crosby silt loam (fine, mixed, mesic Aeric Ochraqualfs) neither decreased corn grain yield nor increased Cu concentrations in corn tissues. Application of 102.9 kg Cu ha^{-1} as Cu-enriched pig manure over a 3-yr period on a Groseclose silt loam (clayey, mixed, mesic Typic Hapludults) resulted in the doubling of the Cu levels in corn (*Zea mays* L.) roots and slight increases n the ear leaves, but did not influence grain yield or Cu concentration (21). Mullins et al. (31) reported similar results following repeated applications of Cu, as either CuSO$_4$ or Cu-enriched swine manure, to three Virginia oils with a wide range of physical and chemical properies. They observed slight increases in the Cu concentraions in corn ear leaves and grain with no decrease in grain yield from three annual Cu additions. After applying up o 138 kg Cu ha^{-1} as Cu-enriched manure of CuSO$_4$ to he same soil over a 6-yr period, there still were no adverse ffects on corn grain yield or composition (12).

Analyses of subsoil samples taken from sites where Cunriched manure (3, 34, 39), sewage sludge (10, 42), or oluble Cu salts (30, 31) have been surface-applied inicate that there is little downward movement of the aplied Cu through the soil profile over considerable periods f time. This lack of leaching indicates the strong, rapid inding of applied Cu to various soil components, inluding clay minerals; Al, Fe, and Mn oxyhydroxides, and rganic matter (16, 26).

By performing soil Cu fractionation procedures before nd after applications of Cu, as either CuSO$_4$ or Cunriched swine manure, Mullins (31) demonstrated that oth plant-available and unavailable fractions increased s a result of the Cu additions. These results agree well ith reports that, with time, a portion of the applied Cu everts to forms unavailable to plants, probably due to he conversion to insoluble oxyhydroxide and organic orms (18, 21, 30).

The objectives of this study were to evaluate the efects of long-term applications of Cu-enriched swine anure and CuSO$_4$ on corn growth and composition nd to determine the form and availability of Cu present soils following eight annual applications of Cu. Exerimental results obtained through the sixth year of this udy have been reported previously (12, 31). Data from the seventh and eighth years of this study are reported herein.

MATERIALS AND METHODS

Field experiments were initiated in 1978 on three soils with diverse physical and chemical properties selected to ensure wide application of the experimental results. The soils used were a Bertie fine sandy loam (fine-loamy, mixed, thermic Aquic Hapludulfs), a Guernsey silt loam (fine, mixed, mesic Aquic Hapludalfs), and a Starr-Dyke clay loam (fine-loamy, mixed, thermic Fluventic Dystochrepts–clayey, mixed, mesic Typic Rhodudults) complex located in the Coastal Plain, Ridge and Valley, and Piedmont regions of Virginia, respectively. The Bertie, Guernsey, and Starr-Dyke soils had CEC values of 5.0, 10.4, and 12.3 cmol$_c$ kg^{-1}, respectively, which indicates that at pH levels ≥6.5 all three soils have a maximum safe Cu loading rate of 280 kg ha^{-1} based on USEPA guidelines (37).

Field Experimentation

Five experimental treatments were applied at each location on 3.7 by 6.1 m plots, which were arranged in a randomized complete block design with four blocks. *Treatment 1* was a control and did not receive Cu applications. *Treatment 2* received 33.6 t of wet Cu-enriched swine manure per hectare per year from 1978 through 1982, no Cu application in 1983, Cu as CuSO$_4$ at a rate sufficient to bring the total amount of applied Cu to 280 kg ha^{-1} in 1984, and 56 kg Cu ha^{-1} as CuSO$_4$ in 1985. *Treatment 3* received CuSO$_4$ applications each year equal to the Cu in the manure and CuSO$_4$ applied to treatment 2. *Treatment 4* received wet Cu-enriched swine manure at rates of 67.2 t ha^{-1} yr^{-1} from 1978 through 1980, 134.4 t ha^{-1} yr^{-1} in 1981 and 1982, 168 t ha^{-1} yr^{-1} in 1983 and 1984, and 84 t ha^{-1} yr^{-1} in 1985. *Treatment 5* received Cu as CuSO$_4$ at annual rates equal to the Cu in the manure applied to Treatment 4. The total amount of dry manure applied to the three soils ranged from 38.8 to 39.3 t ha^{-1} (168 t wet manure ha^{-1}) for Treatment 2 and from 194.0 to 200.6 t ha^{-1} (890.4 t wet manure ha^{-1}) for Treatment 4. Differences in moisture content of batches of manure collected from the same swine annually led to variations in the amount of solids applied for the two treatments of the three field experiments. The manure was plowed into the Bertie and Guernsey soils to depths of ~20 and ~22.5 cm, respectively, and was rotovated into the Starr-Dyke soil to a depth of ~12.5 cm.

The nutrient requirements of the plants grown during this study were met by applications of NH$_4$NO$_3$, KCl, triple superphosphate, Na$_2$B$_4$O$_7$, and MgSO$_4$ at rates based on the results of annual soil and plant analyses. Based on analyses of soil, leaf tissue, and manure samples from previous years, inorganic sources of N, P, and K were not applied to Treatment 4 in either 1984 or 1985. All plots received additional Zn applications as ZnSO$_4$, and the plots located on the Bertie fine sandy loam also received Mn applications as MnSO$_4$. The soil pH at all locations was maintained near 6.5 by applications of dolomitic limestone. All amendments were incorporated into the soils prior to planting. During the 1984 growing season, 'Pioneer 3192' corn was grown at populations of 54 530, 49 400, and 55 030 plants ha^{-1} on the Starr-Dyke, Bertie, and Guernsey soils, respectively. Populations of 'Pioneer 3192' were 61 650, 57 360, and 51 380 plants ha^{-1} on these soils in 1985, respectively.

Manure Collection and Analysis

The manure used during this study was collected from swine fed diets containing approximately 250 mg Cu kg^{-1} in feed as CuSO$_4$ and confined in protected pens on a concrete slab. Manure collection was started following a 3-d period in which the swine were equilibrated on the Cu-enriched rations. After the equilibration, the manure was collected daily and placed in plastic cans for transport to the experimental sites. After manure collection was completed, samples were taken from the manure to be used at each location. The Cu-enriched feed used to produce the manure also was sampled. Triplicate subsamples of each manure and feed sample were dried at 105° C for 24 h to determine percent dry matter and ground to pass a 0.84-mm sieve with a stainless steel Wiley mill. One gram subsamples were then ashed at 450° C for 2 h, the ash dissolved in 25 mL of 0.05 *M* HCl, and the mixture filtered prior to analysis for Ca, Cu, Fe, K, Mg, Mn, and Zn by atomic absorption spectrophotometry. Nitrogen concentrations in the feed and manure were determined by a micro-Kjeldahl procedure (24), P by a molybdovanadophosphoric acid method (19), and B by an Azomethine-H technique (32).

Soil Sampling and Analysis

Each spring, prior to application of treatments and fertilizers, soil samples consisting of 10 cores per plot were taken from the 0- to 15-cm depth. In 1985, samples also were taken from the top 15 cm of the B horizon at each location. Soil samples were air-dried and crushed to pass a 2-mm sieve prior to being analyzed. Extractable soil Cu and Zn were determined by the DTPA (diethylenetriaminepentaacetic acid) extraction procedure (7). Soil pH was determined in water with a 1:1 soil/solution ratio after a 1-h equilibration period, soil organic matter by a chromic acid oxidation procedure, and particle size distribution by the pipet method. Soil CEC was evaluated by saturation with 0.5 *M* Ca acetate (pH 7.0) followed by displacement with neutral 0.5 *M* Mg acetate.

The forms of Cu present in the 1985 surface soil samples were evaluated using a fractionation procedure modified from that given by McLaren and Crawford (25). The solution and exchangeable, specifically adsorbed, organically bound, oxide occluded, and residual Cu fractions were extracted using the procedure described below.

1. *Solution and exchangeable Cu (Ex-Cu):* 5 g of soil was weighed into a 100-mL plastic centrifuge tube to which 50 mL of 0.05 *M* $CaCl_2$ was added. The suspension was then shaken at 180 cycles min^{-1} in a 30°C water bath for 24 h.
2. *Specifically adsorbed Cu (HOAc-Cu):* The residue from the 0.05 *M* $CaCl_2$ extraction was shaken with 50 mL of 2.5% acetic acid for 24 h in a 30°C water bath (15).
3. *Organically bound Cu (Pyro-Cu):* Copper adsorbed by soil organic matter was determined by shaking a new 0.5-g subsample with 50 mL of 0.1 *M* $K_4P_2O_7$ for 16 h at 30°C. Pyro-Cu was calculated by subtacting the Ex-Cu and HOAc-Cu fractions from that extracted by the 0.1 *M* K_4P_2O.
4. *Oxide-occluded Cu (Ox-Cu):* The residue from the $K_4P_2O_7$ extraction was washed with 15 mL of deionized water. The residue was then tranferred into a 50-mL beaker and evaporated to dryness in a 85°C oven. Twenty-five milliliters of a solution containing 0.1 *M* oxalic acid and 0.175 *M* ammonium oxalate (pH 3.25) was added to the dried residue. After covering with plastic, the samples were placed in a 85°C water bath and exposed to UV light for 2 h (28).
5. *Residual Cu (Res-Cu):* Total soil Cu was determined by dissolving a 0.5-g sample with an aqua regia-HF mixture in a Parr digestion bomb as described by Bernas (4). Residual-Cu was than calculated by subtracting the sum of the Cu in the other fractions from the amount of total soil Cu. All extracts from the fractionation procedure were analyzed for Cu by atomic absorption spectrophotometry.

Plant Tissue Sampling and Analysis

At the early silk stage of growth, 10 ear leaves were taken from the two outside rows in each of the four row plots. Corn grain was harvested from 6.1 m of the center two rows at plant maturity and adjusted to 155 g moisture kg^{-1}. Plant tissue was dried at 70°C and ground in a stainless steel Wiley mill to pass a 0.84-mm sieve in preparation for laboratory analyses. Elemental analyses of the leaf samples were accomplished by the procedures described previously for the analysis of the manure and feed. One-half gram subsamples of the grain were digested in a HNO_3-$HClO_4$ mixture prior to determination of Cu concentration by atomic absorption spectrophotometry.

Statistical Analysis

All data were evaluated statistically by analysis of variance and LSD mean separation procedures using a 0.05 level of probability. The relationships between DTPA-extractable Cu, the cumulative amounts of Cu applied from 1978 through 1985, and Cu concentrations in plant tissues were evaluated by simple linear regression analyses.

RESULTS AND DISCUSSION

Copper concentrations in the swine feed used to produce the Cu-enriched manure for the three field studies ranged from 178 to 300 mg kg^{-1}, with an average of 251 mg Cu kg^{-1} for the 8-yr period from 1978 through 1985. The Cu concentrations in the feed were generally in the upper portion of the range considered to have growth stimulating effects (5, 27). Zinc, which is commonly added to Cu-enriched rations to lessen the risk of Cu toxicity in the swine (9, 13, 27), averaged 65 mg kg^{-1} in the feed during the 8-yr period. The average concentrations of other nutrients in the feed were 10.1 mg B, 9.4 g Ca, 7.4 g K, 1.6 g Mg, 37.2 mg Mn, 27 g N, and 6.0 g P kg^{-1}. The swine manure used in the field experiments also contained a considerable amount of plant nutrients. The average composition of the manure (dry matter basis) during the 8-yr period was 19 mg B, 39 g Ca, 17 g K, 7.9 g Mg, 241 mg Mn, 20 g P, 311 mg Zn, and 224 g solids kg^{-1}. The Cu concentration in the manure ranged from 899 to 1550 mg kg^{-1} and averaged 1310, 1292, and 1269 mg kg^{-1} for that applied to the Guernsey, Bertie, and Starr-Dyke soils, respectively. The concentrations of Cu and solids in the manure are comparable with those in manure collected from other pigs fed similar Cu-enriched diets (3, 21).

Elemental Composition of Plant Tissue

Nutrient concentrations in ear leaf tissue from all locations in 1984 and 1985 (Table 1) were within accepted sufficiency ranges (17), indicating that an adequate level of nutrients were supplied from the manure and inorganic fertilizers. Ear leaf composition, except Cu concentration (Table 2), generally was not related to $CuSO_4$ application. However, ear leaves from plots that received the high levels of manure tended to contain higher P and Mn concentrations and lower Zn concentrations (Table 1). Other researchers (35, 41) have also reported elevated P levels in corn leaves following application of swine wastes and indicated that a buildup of excessive quan-

Table 1. Effect of eight annual applications of Cu as either $CuSO_4$ or Cu-enriched swine manure on nutrient concentrations in ear leaves of corn grown on three soils.

Cu treatment		Macronutrients					Micronutrients	
Amount†	Source	Ca	K	Mg	N	P	Mn	Zn
kg ha^{-1}		g kg^{-1}					mg kg^{-1}	
		Guernsey silt loam						
0	Control	4.1b*	22a	4.9a	31ab	2.5bc	62bc	45a
336	Manure + $CuSO_4$	4.2b	22a	4.4a	31ab	2.6b	65b	42ab
336	$CuSO_4$	3.8b	23a	4.4a	29c	2.4c	54c	38b
264	Manure	5.6a	22a	5.3a	33a	3.0a	101a	41ab
264	$CuSO_4$	3.8b	22a	5.0a	30bc	2.6bc	64bc	39ab
		Bertie fine sandy loam						
0	Control	4.1a	20a	5.5ab	29a	2.7ab	25a	45a
336	Manure + $CuSO_4$	4.6a	20a	5.2ab	30a	2.9a	26a	50a
336	$CuSO_4$	4.4a	20a	5.7a	30a	2.6b	26a	44a
256	Manure	4.4a	20a	4.9b	31a	3.0a	23a	37b
256	$CuSO_4$	4.2a	20a	5.2ab	30a	2.7ab	28a	48a
		Starr-Dyke clay loam						
0	Control	5.7a	22a	6.0a	27a	2.3b	78b	25a
336	Manure + $CuSO_4$	6.0a	22a	5.8ab	28a	2.4b	84b	24a
336	$CuSO_4$	6.0a	23a	5.5ab	28a	2.3b	85b	26a
253	Manure	6.3a	23a	5.2b	29a	2.9a	101a	17b
253	$CuSO_4$	6.2a	22a	6.0a	28a	2.5b	88b	25a

* Column means for each soil followed by different letters are significantly different at the 0.05 probability level.

† Cumulative amount of Cu applied during the 8-yr period from 1978 through 1985.

Table 2. Effects of repeated applications of Cu as either $CuSO_4$ or Cu-enriched swine manure on Cu concentrations in corn ear leaves and grain and on corn grain yields.

Cu treatment			1984			1985		
Amount								
1984	1985	Source	Leaf Cu	Grain Cu	Grain yield	Leaf Cu	Grain Cu	Grain yield
kg ha^{-1}			mg kg^{-1}		kg ha^{-1}	mg kg^{-1}		kg ha^{-1}
			Guernsey silt loam					
0	0	Control	8.9c*	2.2a	10 320b	9.3b	2.8a	10 230a
280	336	Manure + $CuSO_4$	10.4ab	2.1a	9 660b	10.2ab	2.9a	10 080a
280	336	$CuSO_4$	10.7a	2.2a	9 320b	9.5ab	3.2a	9 760a
245	264	Manure	10.1b	1.9a	11 960a	10.7a	3.0a	10 710a
245	264	$CuSO_4$	10.7a	2.0a	10 280b	10.5a	3.0a	10 570a
			Bertie fine sandy loam					
0	0	Control	6.5c	1.9a	8 950a	9.1a	3.2ab	9 860ab
280	336	Manure + $CuSO_4$	7.1bc	1.9a	8 830a	9.9a	3.7a	8 850ab
280	336	$CuSO_4$	7.6b	1.9a	9 180a	10.0a	3.7a	8 220b
234	256	Manure	7.6b	1.5b	8 710a	10.1a	3.0b	9 540ab
234	256	$CuSO_4$	8.6a	1.9a	9 100a	9.2a	3.6ab	10 150a
			Starr-Dyke clay loam					
0	0	Control	10.5ab	1.7a	11 340b	8.8a	3.7a	7 910a
280	336	Manure + $CuSO_4$	10.5ab	1.5ab	12 680a	9.8a	3.7a	7 600a
280	336	$CuSO_4$	10.9ab	1.6ab	10 900b	9.3a	3.6a	7 370a
235	253	Manure	10.2b	1.3b	12 440a	9.2a	3.5a	9 100a
235	253	$CuSO_4$	11.1a	1.7a	11 830ab	9.0a	3.6a	7 980a

* Column means for each soil followed by different letters are significantly different at the 0.05 probability level.

Table 3. Effect of seven annual applications of Cu as either $CuSO_4$ or Cu-enriched swine manure on soil pH, organic matter, and DTPA-extractable Cu and Zn in the Ap and B horizons of three soils.

Cu treatment				DTPA-extractable Cu and Zn			
				Ap horizon		B horizon	
Amount†	Source	Soil pH	Soil organic matter	Cu	Zn	Cu	Zn
kg ha^{-1}			g kg^{-1}	mg kg^{-1}			
			Guernsey silt loam				
0	Control	6.7a*	20cd	1.6b	7.0b	0.5c	1.6b
280	Manure + $CuSO_4$	6.6a	21bc	29.9a	9.3b	4.8bc	2.5b
280	$CuSO_4$	6.6a	19d	35.9a	7.9b	6.0ab	1.6b
245	Manure	6.5a	24a	37.5a	17.3a	11.0a	4.4a
245	$CuSO_4$	6.7a	22ab	31.2a	5.9b	8.1ab	1.8b
			Bertie fine sandy loam				
0	Control	6.2a	21b	1.3d	6.1b	0.3c	0.3bc
280	Manure + $CuSO_4$	6.2a	20b	49.9a	8.1b	1.9a	0.5a
280	$CuSO_4$	6.2a	21b	36.8b	5.6b	0.7bc	0.2c
234	Manure	6.3a	26a	28.4bc	15.2a	0.9b	0.4ab
234	$CuSO_4$	6.1a	21b	23.8c	6.4b	1.0b	0.3bc
			Starr-Dyke clay loam				
0	Control	6.6a	16b	2.6b	5.1bc	0.7b	0.4b
280	Manure + $CuSO_4$	6.7a	17b	37.1a	6.7b	19.a	0.6ab
280	$CuSO_4$	6.6a	16b	32.6a	5.8bc	1.6ab	0.5ab
235	Manure	6.7a	23a	37.5a	17.2a	1.5ab	0.8a
235	$CuSO_4$	6.7a	16b	30.8a	4.7c	1.2ab	0.4b

* Column means for each soil followed by different letters are significantly different at the 0.05 probability level.

† Cumulative amount of Cu applied during the 7-yr period from 1978 through 1984.

tities of N and P represents one of the hazards associated with repeated applications of large amounts of manure.

The observed increases in Mn concentrations from manure application on the Guernsey and Starr-Dyke soils (Table 1) were probably due to Mn supplied by the manure and to Mn solubilized by reduction of Mn in insoluble soil oxyhydroxides during oxidation of components of the manure. Limited amounts of available Mn in the Bertie soil made supplemental Mn applications necessary. The lower levels of Zn in corn leaves from soils that received manure applications probably reflect the decreased Zn uptake at the higher levels of plant-available P and the Zn dilution from the greater vegetative growth by plants on the manure-treated soils, which was noted at the time the samples were taken. Enhanced corn growth resulting from manure applications is frequently observed and is generally attributed to improved soil physical properties and moisture relations (36). Increases in organic matter content ranging from 4 to 7 g kg^{-1} were observed in this study following seven annual applications of the Cu-enriched manure (Table 3) and are probably responsible for improvements in the physical properties and water relations in these soils. The N concentrations in the leaves from the manure-treated soils were equal to those from the control plots (Table 1), even though no inorganic sources of N had been applied to the manure-amended soils for several years.

Copper concentration in the ear leaf samples from the 1984 growing season were increased slightly by Cu applications on the Guernsey and Bertie soils, but not on the Starr-Dyke soil (Table 2). The Cu concentrations in all leaf tissue were within the accepted normal range of 6 to 20 mg kg^{-1} (17). The Cu levels in the leaves from the soils treated with the high rate of manure were below those found in tissue from soils to which an equivalent amount of Cu as $CuSO_4$ had been applied. This relationship may represent a lower availability of Cu from the manure compared with that from the $CuSO_4$ or more probably a dilution effect.

In 1985, although the total amount of applied Cu had reached 336 kg ha^{-1}, increases in Cu concentration in leaf tissue were observed only on the Guernsey soil treated with 245 kg Cu ha^{-1} as either $CuSO_4$ or Cu-enriched swine manure. No difference occurred between the sources of Cu with respect to Cu concentrations in ear leaves. These results are consistent with the finding that most of the Cu in the manure exists in a soluble form (27).

Copper concentration in corn grain was not increased by any Cu treatment on any of the three soils in either 1984 or 1985 (Table 2), even though the predicted maximum safe loading rate, 280 kg Cu ha^{-1}, for these soils (37) had been exceeded by $CuSO_4$ additions and closely approached by application of Cu-enriched swine manure. Compared with the control treatment, the manure applications resulted in a decrease in the Cu concentration in the grain from the Bertie and Starr-Dyke soils in 1984. In 1985, the Cu concentration in the grain from the manure-treated Bertie soil was lower than that in grain from plots that received an equivalent amount of Cu as $CuSO_4$ but was not different from that in the grain taken from the control plot. These results are consistent with the reports that Cu is accumulated in corn roots with little internal translocation. Similar small or negligible increases in Cu concentration in corn ear leaves and grain have been reported in a number of other studies following Cu additions as Cu-enriched pig manure (21), sewage sludge (18, 33, 34), and $CuSO_4$ (30).

Grain Yield

Corn grain yields were not adversely affected in 1984 nor in 1985 (Table 2) by applications of Cu as either $CuSO_4$ or Cu-enriched manure. In 1984, grain yields were increased on the Guernsey silt loam and Starr-Dyke clay loam soils that had received 806.4 t of wet, Cu-enriched manure per hectare since 1978. Yield was also increased on the Starr-Dyke soil treated with a lower amount of manure plus $CuSO_4$. These increases probably are attributable to improvements in the general tilth and water relations of these soils caused by the manure. Yields were not decreased, as compared with the control, by any Cu-treatment on any of the three soils in 1985. The levels of applied Cu in both 1984 and 1985 were well above the 200 kg Cu ha^{-1}, as $CuSO_4$, shown to decrease corn yields on an European soil (22). The levels of applied Cu were below those found to be toxic to bush bean (*Phaseolus vulgaris* L.) in a greenhouse pot study (40).

DTPA-Extractable Copper

The concentrations of DTPA-extractable Cu in the three soils used in the study exhibited a strong linear relationship with amount of added Cu (Table 3). Correlation coefficients determined through simple linear regression analyses using both the 1984 and 1985 data and assuming no difference in extractability between the two sources of Cu were 0.93, 0.96, and 0.98 ($\alpha = 0.01$) for the Guernsey, Bertie, and Starr-Dyke soils, respectively. The assumption of equal extractability between sources of Cu is substantiated by the data in Table 3. These results are consistent with other reports of linear changes in DTPA-extractable Cu following Cu additions (11, 12). Soils that received the high rate of manure also showed higher levels of extractable Zn in 1984 and in 1985 (Table 3). The amounts of DTPA-extractable Cu in the three soils were not significantly correlated ($\alpha = 0.05$) to the Cu concentrations in either corn ear leaves or grain sampled during the previous year. Similar results were reported during the early stages of this study with lower levels of applied Cu on these soils (12, 31). Since the soil samples were taken prior to application of treatments, no attempt was made to correlate the extractable Cu in the soil samples with the Cu concentrations in the plants grown during the same year.

Analysis of the subsoil samples taken in the spring of 1985 showed that Cu applications caused only slight increases in the Cu concentrations in the Bertie and Starr-Dyke subsoils (Table 3). No differences in subsoil Cu concentration were observed between Cu sources. These results indicate little downward movement of the applied Cu. Downward movement appears to be slightly greater in the Bertie soil compared with that in the Starr-Dyke. The Bertie soil contains lower concentrations of clay and Fe and Mn oxyhydroxides, which results in less adsorption of Cu (16, 26) and therefore greater leaching of the applied Cu. The relatively large increases in the levels of extractable Cu and Zn in the Guernsey subsoil do not represent downward movement due to leaching. Instead, the increases in extractable Cu and Zn reflect movement through plowing of the soil.

Copper Fractions

Samples taken from the Ap horizon of the three soils in the spring of 1985 were fractionated to determine the forms of Cu present in the soils following seven annual applications of $CuSO_4$ and Cu-enriched manure. Results of the procedure show increases in all fractions caused by Cu additions (Table 4). The magnitude of the changes was dependent on the amount of applied Cu and, to some extent, on the source of Cu. Only small increases in Ex-Cu were found in any of the three soils. The greatest increase in Ex-Cu was in the relatively coarse-textured Bertie soil, as would be expected due to the low amounts of clay and oxyhydroxides (31), which are known to bind Cu in soils. Higher amounts of Ex-Cu were found in the Guernsey and Starr-Dyke soils treated with manure compared with the same soils treated with an equivalent amount of Cu as $CuSO_4$. This may reflect chelation of the Cu by soluble organics in soils treated with manure. The HOAc-Cu fraction tended to follow the level of applied Cu with no consistent difference between Cu sources. The Pyro-Cu fraction had the largest increases due to Cu treatment. A greater amount of Pyro-Cu was found in the manure treatments than in the $CuSO_4$ treatments for the Bertie and Starr-Dyke soils. The Ox-Cu fraction also had large increases that followed the level of applied Cu. Changes in the level of residual Cu were also observed. The largest changes were generally found

Table 4. Distribution of Cu fractions in the Ap horizon of three soils following seven annual Cu applications as either $CuSO_4$ or Cu-enriched swine manure.

Cu treatment		Soil Cu fractions				
Amount†	Source	Ex-Cu	HOAc-Cu	Pyro-Cu	Ox-Cu	Res-Cu
kg ha^{-1}		mg kg^{-1}				
		Guernsey silt loam				
0	Control	0.04c*	0.2c	4.2b	5.4c	7.8b
280	Manure + $CuSO_4$	0.08b	14.5a	43.4a	32.8a	7.6b
280	$CuSO_4$	0.08b	14.8a	40.6a	34.8a	9.6ab
245	Manure	0.11a	11.5b	41.9a	29.3b	11.2a
245	$CuSO_4$	0.07b	10.7b	41.6a	33.0a	13.2a
		Bertie fine sandy loam				
0	Control	0.04c	0.3d	2.8d	1.8c	5.4d
280	Manure + $CuSO_4$	0.19a	25.7a	39.3a	29.2a	8.6c
280	$CuSO_4$	0.16a	19.1b	27.2b	20.5b	9.5c
234	Manure	0.08b	9.9c	27.7b	28.1a	11.6b
234	$CuSO_4$	0.09b	13.2bc	19.2c	20.9b	14.7a
		Starr-Dyke clay loam				
0	Control	0.03b	0.9d	6.4e	13.2e	32.5b
280	Manure + $CuSO_4$	0.04ab	20.7a	46.7b	50.1b	43.8a
280	$CuSO_4$	0.03b	14.6b	40.0c	62.9a	34.8b
235	Manure	0.05a	11.8c	51.3a	41.8d	35.7b
235	$CuSO_4$	0.03b	11.2c	33.8d	46.1c	44.7a

* Column means for each soil followed by different letters are significantly different at the 0.05 probability level.

† Cumulative amount of Cu applied during the 7-yr period from 1978 through 1984.

in soils to which the lower level of cumulative Cu had been applied. This may be due to the fact that a larger portion of the Cu had been added during the earlier stages of this study allowing for a longer reaction time.

Viets (39) concluded that the forms of Cu most available to plants were the Ex-Cu, HOAc-Cu, and Pyro-Cu fractions, which are in equilibrium with the soil solution. This conclusion is supported by another study conducted by McLaren and Crawford (25), who also indicated that the Ox-Cu and Res-Cu fractions generally do not represent a major source of Cu to plants. Therefore, both plant-available and plant-unavailable forms of Cu have been increased by the applications of Cu (Table 4). Copper concentrations in neither the corn ear leaves nor grain (Table 2) were related to the observed changes in the potentially available forms of Cu (Table 4), which may reflect Cu accumulation in plant roots rather than translocation to shoots.

SUMMARY AND CONCLUSIONS

Application of up to 264 kg Cu ha^{-1} as Cu-enriched swine manure and of up to 336 kg Cu ha^{-1} as $CuSO_4$ from 1978 through 1985 did not decrease corn grain yields on three soils with diverse properties. The Cu concentrations in corn tissues were within normal ranges where Cu-enriched manure and $CuSO_4$ were applied to the three soils. The cumulative amount of Cu applied over the 8 yr via Cu-enriched manure application approached the maximum tolerable loading rate of 280 kg ha^{-1} for the three soils; whereas, the cumulative amount of Cu applied as $CuSO_4$ over the 8 yr exceeded the maximum tolerable loading rate by 56 kg Cu ha^{-1}. The levels of DTPA-extractable Cu increased linearly with rate of Cu application and showed limited downward movement of the applied Cu. Sequential extraction data indicated that a substantial portion of the applied Cu reverted to forms unavailable to plants. The lack of adverse effects from the applied Cu can be attributed to the relatively high pH levels (≥ 6.1) maintained in the soils and to the conversion of a portion of the applied Cu to more stable forms that are not available to plants.

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ERRATUM

The Health and Future of Division A-5 and the *Journal of Environmental Quality*

G.A. O'Connor, T.J. Logan, and L.E. Sommers; J. Environ. Qual. 17(3):iii-v.

An error occurred in Table 1 of the above editorial. The mean value under the last column should have been 110 rather than 85. The correct Table 1 follows:

Table 1. Numbers of papers presented in Division A-5 and published in *Journal of Environmental Quality* since their inception.

Year	No. papers presented in Div. A-5	No. papers published in JEQ
1971	61	–
1972	72	104
1973	105	118
1974	133	97
1975	81	117
1976	99	109
1977	123	97
1978	120	117
1979	97	120
1980	96	136
1981	106	113
1982	104	141
1983	99	109
1984	116	122
1985	91	107
1986	87	81
1987	109	76
	Mean = 100	Mean = 110

115.

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Form and Availability of Copper and Zinc following Long-Term Copper Sulfate and Zinc Sulfate Applications[1]

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ABSTRACT

High levels of Cu and Zn application to agricultural land poses potential environmental hazards due to reduced plant growth and accumulations of undesirable levels of Cu and Zn in the food chain. The levels of Cu and Zn that can be safely added to cropland have yet to be established. This study was conducted on a Davidson silty clay (Rhodic Paleudult) to determine the response of corn (*Zea mays* L.) to the cumulative application of up to 415 kg Cu and 897 kg Zn ha^{-1} as $CuSO_4$ and $ZnSO_4$, respectively, over the 20-yr period from 1967 through 1986. Neither corn grain nor silage yields were affected by the metal additions, even though the cumulative added amounts of Cu and Zn greatly exceeded the maximum allowed Cu and Zn loading rates (i.e., 250 kg Cu and 560 kg Zn ha^{-1}) for this soil. Over the period from 1984 through 1986, Cu concentrations in leaf and grain tissue were generally unaffected by metal applications, whereas Zn concentration in ear leaves increased linearly with the cumulative level of applied Zn. Zinc concentration in corn grain was increased only by the highest rate of Zn application. Copper and Zn concentrations in the corn leaves and grain were within normal ranges for all treatments. Levels of DTPA-extractable Cu and Zn in the Ap horizon sampled annually from 1984 through 1985 increased linearly with the amount of metals applied (r^2 = 0.97 and 0.98, α = 0.001, for Cu and Zn, respectively). Extractable Cu was also influenced by Zn applications with a larger amount of extractable Cu being present when both Cu and Zn were added compared to when only Cu was applied. Analysis of subsoil samples indicated limited downward movement of Cu and Zn. Sequential extraction data revealed that the applied Cu and Zn was distributed among both potentially plant-available (exchangeable, specifically adsorbed, and organically bound) and plant-unavailable (oxide associated) fractions. The results of this study indicate that application of 135 kg Cu and 337 kg Zn ha^{-1} in excess of the limits set forth by USEPA guidelines was not detrimental to corn production on the Davidson soil with a pH > 6.5. The reasons for the lack of phytotoxicity of the metals include the relatively high soil pH maintained at the site, which limits the availability and the conversion of the applied Cu and Zn to plant-unavailable forms over time.

Copper and Zn are commonly added to agricultural soils in fertilizers, pesticides, animal wastes, and sewage sludge. Once in the soil, these elements are relatively immobile and can therefore persist for extended periods (2, 3, 9, 13). Consequently, there is major concern that repeated application of these metals may result in phytotoxic levels in the soil and accumulation of undesirable concentrations in the food chain (2, 3, 13). Copper and Zn are among the metals identified as most likely to become phytotoxic to crops (2, 3).

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The levels of Cu and Zn that can be safely added to cropland have yet to be accurately determined. Much of the research conducted to evaluate the effects of Cu and Zn on plant growth involves relatively large applications made over short periods of time. Walsh et al. (22) determined that a single application of 363 kg Zn ha^{-1} as $ZnSO_4$ decreased the yield of snapbean (*Phasealus vulgaris* L.) on a Plainfield loamy sand (Typic Udipsamment) during the first growing season following the addition, but did not affect yield during the subsequent year. Snapbean yields were decreased for two successive growing seasons on the Plainfield loamy sand following a single application of 486 kg Cu ha^{-1} as $CuSO_4$ (23).

Martens et al. (15) reported that application of up to 50 kg Cu and 67 kg Zn ha^{-1} to a Davidson clay loam (Rhodic Paleudult) and a Marlboro fine sandy loam (Typic Paleudult) over a 6-yr period did not adversely affect soybean [*Glycine max* (L.) Merr.] growth. Copper concentration in the leaves and seeds was not increased by Cu treatment, whereas Zn concentration in plant tissue varied directly with the amount of Zn applied.

Mullins et al. (17) found that neither corn (*Zea mays* L.) grain nor silage yield was affected by 15 annual applications of various rates of Cu and Zn sulfates, which provided up to 172 kg Cu and 290 kg Zn ha^{-1}. They also found that the Cu concentration in ear leaf tissue and grain was generally not influenced by Cu application, whereas the Zn concentration in leaf tissue and grain increased with increasing levels of Zn addition. Subsoil analysis indicated that after 13 yr of Cu and Zn application, the metals were accumulating in the surface horizon with little downward movement. Fractionation data showed that a substantial portion of the applied Cu and Zn had reverted to forms considered to be unavailable to plants.

The purpose of this study was to evaluate the effects of long-term applications of Cu and Zn sulfates on corn growth and composition and to determine the form and extractability of Cu and Zn present in a Davidson silty clay. Experimental results obtained through the 15th yr of this study have been reported previously (15, 17). Data from the 18th through the 20th yr of this field study are reported herein.

MATERIALS AND METHODS

A long-term field study was initiated in 1967 and continued through 1986 on a Davidson silty clay located in the Piedmont region of Virginia. In 1983, the soil at the experimental site had a CEC (pH = 7.0) of 13.9 cmol$_c$ kg^{-1} and contained 15 g organic matter and 59 g free iron kg^{-1}. According to USEPA regulations (21), which are based on CEC and assumes soil pH levels are maintained at or above 6.5, this soil has maximum allowable Cu and Zn loading rates of 280 and 560 kg ha^{-1}, respectively.

Field Experimentation

Five treatments consisting of various levels of Cu and Zn sulfates applied either alone or together were imposed annually during the 20-yr period from 1967 through 1986 (Table 1). The annual Cu and Zn application rates averaged up to 16.5 and 32.9 kg ha^{-1} for the period 1967 through 1983. Due to the lack of response to the applied metals, application rates were increased substantially until 1986, when the rates were reduced to allow a more accurate determination of the levels of Cu and Zn that could be added before detrimental effects were produced (Table 1). The treatments were arranged in a randomized complete block design with four replications using a plot size of 5.0 by 7.6 m.

The nutrient requirements of the plants grown during this study were met by applications of NH_4NO_3, triple superphosphate, KCl, $Na_2B_4O_7$, and MgO at rates based on the results of annual soil and plant tissue analyses. Average levels of nutrients applied were 1.1 kg B, 74 kg K, 168 kg Mg, 263 kg N, and 39 kg P ha^{-1} yr^{-1}. The soil pH was maintained near 6.5 by applications of dolomitic limestone. All soil amendments were incorporated to a depth of approximately 12 cm by plowing and disking prior to planting. 'Pioneer 3192' corn was grown at populations of 57 400, 80 700, and 77 500 plants ha^{-1} during the 1984, 1985, and 1986 growing seasons, respectively. During this 3-yr period, approximately 29.4 cm of supplemental water was supplied to the experimental area by irrigation as was necessary.

Plant Sampling and Analysis

At the early silk stage of growth, 10 ear leaves were taken from rows two and five of each of the six-row plots. Corn grain and stalks were harvested from 6.1 m of the center two rows at plant maturity. Grain yields were adjusted to 155 g moisture kg^{-1} and stalk yields were reported on a dry-weight basis.

In preparation for analysis, all plant tissue was dried at 70°C and ground in a stainless steel Wiley mill to pass a 0.85-mm sieve. One-gram subsamples of the leaf tissue were then ashed at 450°C for 2 h with the ash being equilibrated with 25 mL of 0.5 *M* HCl for 2 h. The suspensions were then filtered prior to analysis for Cu and Zn by atomic absorption spectrophotometry. Copper and Zn concentrations in the grain were determined by atomic absorption spectrophotometry after 0.5-g subsamples were digested in a HNO_3–$HClO_4$ mixture.

Soil Sampling and Analysis

Each spring, prior to application of soil amendments, soil samples consisting of 10 cores per plot were taken from the 0- to 20-cm depth. In 1984 and 1986, samples were also taken from the subsoil (25- to 35-cm depth). Soil samples were air-dried and crushed to pass a 2-mm sieve prior to determination of DTPA (diethylenetriaminepentaacetic acid)-extractable Cu and Zn (12). Cation exchange capacity was evaluated by saturation with 0.5 *M* Ca acetate (pH 7.0) followed by displacement with neutral 0.5 *M* Mg acetate. Soil pH was determined in water with a 1:1 soil/solution ratio after a 1-h equilibration period.

Copper and Zn in the 1984 surface soil samples were sequentially extracted to determine solution and exchangeable Cu and Zn (Ca–Cu and Ca–Zn), specifically adsorbed Cu and Zn (HOAc–Cu and HOAc–Zn), pyrophosphate-extractable Cu and Zn (Pyro–Cu and Pyro–Zn), oxide-associated Cu and Zn (Ox–Cu and Ox–Zn), and residual Cu and Zn (Res–Cu and Res–Zn) fractions. The sequential extraction procedure of McLaren and Crawford (16) as modified by Mullins et al. (18) was used except that the residual Cu and Zn fractions were evaluated by subtracting the sum of the Cu or Zn in the other fractions from the amount of total soil Cu or Zn. Total soil Cu and Zn was determined by dissolving a 0.5-g sample with an aqua regia-HF mixture in a Parr digestion bomb as described by Bernas (1). All extracts from the fractionation procedure were analyzed for Cu and Zn by atomic absorption spectrophotometry.

Statistical Analyses

Corn grain and stalk yields, Cu and Zn concentrations in corn ear leaf and grain tissue, DTPA-extractable Cu and Zn, and the Cu and Zn fractionation data were evaluated statistically by analysis of variance techniques. Mean separations were performed by linear contrasts when the *F* value of the analysis of variance was significant at the 0.05 probability level. The relationship between DTPA-extractable Cu and Zn, the cumulative levels of Cu and Zn applied from 1967 through 1986, and the Cu and Zn concentrations in plant tissues were evaluated using simple linear regression analyses. All regression analyses were performed using data from all 3 yr (1984, 1985, and 1986) covered herein.

RESULTS AND DISCUSSION

Twenty annual applications of Cu and Zn sulfates made to a Davidson silty clay from 1967 through 1986 supplied up to 415 kg Cu and 897 kg Zn ha^{-1} (Table 1), which exceeds the maximum allowable Cu and Zn loading rates for this soil (280 kg Cu and 560 kg Zn ha^{-1}) as set forth by the USEPA (21). Despite the large cumulative amounts of Cu and Zn applied, neither corn grain nor silage yields were influenced by the metal additions over the period from 1984 through 1986, so only data averaged over treatments are shown in Table 2. The amount of Cu applied during this study is slightly lower than the level found to be toxic to snapbean on a Plainfield loamy sand (23), but well above the 200 kg Cu ha^{-1} as $CuSO_4$ shown to decrease corn yields on a European soil (11). The quantity of Zn applied is more than double that reported to be toxic to snapbean in a single application on the Plainfield soil (22). Other studies have been reported in the literature in which the predicted maximum safe Cu and Zn loading rates have been exceeded by applications of animal manure (19), sewage sludge (2, 3, 24), and inorganic salts (22) without adverse effects on plant growth.

Copper and Zinc concentrations in Corn Tissue

Copper concentrations in corn grain and ear leaf tissue were generally unaffected by the 18 to 20 annual applications of $CuSO_4$, so data averaged over treatments are shown in Table 2. The only significant treatment effect occurred in 1984 when the cumulative application of 334 kg Cu ha^{-1} increased ear leaf concentration (10.8 mg kg^{-1}) as compared to the control (10.0 mg kg^{-1}). Zinc concentration in both ear leaves and grain was increased by Zn additions in 1984, 1985, and 1986. Over the 3-yr period from 1984 through 1986, the concentration of Zn in the ear leaves increased linearly ($r^2 = 0.96$, $\alpha = 0.001$) with increasing levels of applied Zn. Zinc concentration in the corn grain was increased only by the highest Zn application rate during each of the 3 yr (695, 830, and 897 kg Zn ha^{-1} in 1984, 1985, and 1986, respectively). No consistent trend between Zn level in the grain and the cumulative amount of Zn applied was observed during the 3-yr period. The lack of correlation is possibly due to differences in plant population and growth conditions among years. These results are consistent with reports (2, 9, 14) that Cu accumulates in plant roots with little internal translocation, whereas Zn is mobile within the plant and can therefore accumulate in the aerial portion of the plant.

Table 1. Cumulative amounts of Cu and Zn applied as sulfates from 1967 through 1986 to a Davidson silty clay.

Treatment no.	1983		1984		1985		1986	
	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
	kg ha^{-1}							
1	0	0	0	0	0	0	0	0
2	0	560	0	695	0	830	0	897
3	143	189	151	200	160	211	168	222
4	280	0	334	0	388	0	415	0
5	280	560	334	695	388	830	415	897

Concentrations of both Cu and Zn in all plant tissue were within accepted normal ranges (8, 9). The linear contrasts comparing Treatments 4 and 5 for Cu and Treatments 2 and 5 or Zn indicate that there was no Cu-Zn interaction with respect to the metal concentrations in either corn ear leaves or grain during the 3 yr.

DTPA-Extractable Copper and Zinc

The level of DTPA-extractable Cu and Zn in the surface 20 cm of the Davidson soil exhibited a strong linear relationship with the cumulative amount of Cu and Zn added (Table 3). Correlation coefficients determined through simple linear regression analysis using the soil test results from all 3 yr were 0.97 and 0.98 ($\alpha = 0.001$) for Cu and Zn, respectively. These findings agree with other reports of increases in DTPA-extractable Cu and Zn following metal additions (6, 10, 18, 19).

The level of DTPA-extractable soil Cu was also influenced each year by Zn application (Table 3). A larger amount of the applied Cu remained in an extractable form when Zn was applied with the Cu than when Cu was applied alone. A similar phenomenon was observed in 1986 with extractable soil Zn where Cu had been added in conjunction with Zn. Similar results were reported by Mullins et al. (17) with lower cumulative applications of Cu and Zn sulfates on the same study site. These findings

Table 2. Corn yields and tissue Cu concentration averaged over treatments and Zn concentrations following up to 20 annual applications of Cu and Zn sulfates.

Treatment no.	1986 Cumulative Cu	1986 Cumulative Zn	1984 Leaf	1984 Grain	1985 Leaf	1985 Grain	1986 Leaf	1986 Grain
	kg ha^{-1}		mg Zn kg^{-1}					
1	0	0	19.6	16.3	19.2	25.4	19.9	21.5
2	0	897	41.9	24.9	60.0	35.0	52.6	28.1
3	168	222	25.0	17.0	27.6	29.2	28.0	22.0
4	415	0	21.1	17.1	20.4	24.4	21.2	17.3
5	415	897	41.8	26.4	52.9	33.4	54.3	23.4
F value			64.9**	14.5**	26.4**	4.6**	138.9**	6.2**
Linear contrasts								
1,4 vs. 3			7.6*	NS	NS	NS	18.1**	NS
3 vs. 2, 5			99.6**	30.3**	40.2**	NS	208.1**	NS
1, 4 vs. 2, 5			243.3**	48.5**	79.1**	18.1**	523.2**	16.7**
2 vs. 5			NS	NS	NS	NS	NS	NS
			mg Cu kg^{-1}					
Mean Cu conc.			10.4	2.2	9.7	3.4	9.7	2.6
			Grain	Silage	Grain	Silage	Grain	Silage
			kg ha^{-1}					
Mean yield			11 950	8 550	12 870	9 920	13 680	11 060

*,** Significant at the 0.05 and 0.01 levels of probability, respectively; NS = nonsignificant at the 0.05 level of probability.

Table 3. DPTA-extractable Cu and Zn in a Davidson silty clay sampled in the spring of 1984, 1985, and 1986 following 17, 18, and 19 annual applications of Cu and Zn sulfates, respectively.

Treatment no.	1986 cumulative		1984		1985	1986	
	Cu	Zn	0-20 cm	25-35 cm	0-20 cm	0-20 cm	25-35 cm
	— kg ha^{-1} —		mg Cu kg^{-1}				
1	0	0	2.1	0.9	1.6	1.8	1.0
2	0	897	2.8	0.8	3.0	4.1	1.9
4	415	0	19.2	1.3	19.7	25.4	4.5
5	415	897	22.8	1.6	24.0	28.5	3.7
F value			448.1**	4.7*	76.4**	141.1**	NS
Linear contrasts							
1, 2 vs. 4, 5			1318.9**	12.2**	284.6**	520.8**	--
4 vs. 5			24.4**	NS	7.0*	4.5*	--
	— kg ha^{-1} —		mg Zn kg^{-1}				
1	0	0	2.5	0.4	1.7	1.4	0.7
2	0	897	39.6	3.6	42.8	53.9	11.8
4	415	0	2.5	0.5	1.7	2.0	0.8
5	415	897	39.3	2.0	45.3	59.9	7.2
F value			194.6**	4.5*	174.5**	229.8**	4.9*
Linear contrasts:							
1, 4 vs. 2, 5			583.7**	10.9**	631.6**	815.4**	13.0**
2 vs. 5			NS	NS	NS	4.8*	NS

*,** Significant at the 0.05 and 0.01 levels of probability, respectively; NS = nonsignificant at the 0.05 level of probability.

suggest that there is competition between Cu and Zn for adsorption sites on the soil. This hypothesis will be explored in greater detail below when the fractionation data are discussed.

The amounts of DTPA-extractable Cu did not correlate ($\alpha = 0.05$) with the Cu concentrations in either the corn ear leaves or grain sampled during the following year. Since the soil samples were taken prior to application of treatments, no attempt was made to correlate extractable Cu in the soil samples with Cu concentrations in the plants grown during the same year. The lack of correlation between extractable soil Cu and the levels found in plant tissues is probably a result of the limited translocation of Cu within the plant. There was a strong linear relationship between DTPA-extractable soil Zn and Zn concentration in ear leaf tissue ($r^2 = 0.94$, $\alpha = 0.001$). DTPA-extractable Zn also correlated positively with the level of Zn in the corn grain ($r^2 = 0.65$, $\alpha = 0.05$). Similar relationships were reported during the earlier stages of this study with lower levels of applied Cu and Zn (17).

Downward Movement of Applied Copper and Zinc

Analysis of subsoil samples taken from the 25- to 35-cm depth in the spring of 1984 and 1986 indicate slight downward movement of both Cu and Zn in the Davidson silty clay over the 20-yr period of 1967 to 1986 (Table 3). The Zn concentration in the subsoil of the plots that received the high rate of Zn was substantially increased compared to the plots that received no Zn, with the largest increases being observed in the 1986 samples. Subsoil Cu levels were increased by the high rate of Cu addition in 1984, but the increase was not statistically significant in 1986. The concentration of Zn in the subsoil was increased to a greater extent by metal additions than was Cu, which reflects the higher amount of Zn applied and the greater mobility of Zn in the soil, which has been reported by other researchers (4, 5, 24).

Table 4. Forms of Cu and Zn in a Davidson silty clay following 17 annual applications of various rates of Cu and Zn sulfates.

Treatment			Fraction				
No.	Cu	Zn	Ca	HOAc	Pyro	Ox	Res
	— kg ha^{-1} —		mg Cu kg^{-1}				
1	0	0	0.09	1.0	5.7	10.9	40.7
2	0	560	0.08	1.5	5.4	11.6	43.3
3	143	189	0.09	5.3	14.5	20.6	43.8
4	280	0	0.12	15.8	28.9	32.3	44.2
5	280	560	0.15	20.8	27.0	32.1	43.5
F value			10.8**	247.8**	26.2**	37.3**	NS
Linear contrasts							
1, 2, vs. 3			NS	193.7**	26.1**	19.6**	--
3 vs. 4, 5			15.4**	132.2**	10.2**	30.6**	--
1, 2, vs. 4, 5			36.6**	968.7**	103.2**	148.7**	--
4 vs. 5			NS	5.0*	NS	NS	-
	— kg ha^{-1} —		mg Zn kg^{-1}				
1	0	0	0.9	1.0	5.3	22.6	107.3
2	0	560	4.5	42.8	38.9	63.9	118.7
3	143	189	1.7	4.4	17.8	32.6	112.2
4	280	0	1.5	1.3	6.8	22.3	110.4
5	280	560	5.5	40.2	38.3	61.9	116.4
F value			12.2**	238.9**	79.0**	80.8**	NS
Linear contrasts							
1, 4 vs. 3			NS	74.8**	75.3**	13.1**	-
3 vs 2, 5			21.5**	267.3**	32.5**	114.7**	-
1, 4 vs. 2, 5			42.1**	937.7**	310.0**	307.8**	-
2 vs. 5			NS	NS	NS	NS	-

*,** Significant at the 0.05 and 0.01 levels of probability, respectively; NS = nonsignificant at the 0.05 level of probability.

Forms of Copper and Zinc

The soil samples taken from the Ap horizon of the Davidson silty clay in the spring of 1984 were subjected to a fractionation procedure to determine the forms of Cu and Zn present following 17 annual applications of $CuSO_4$ and $ZnSO_4$. All fractions, except residual Cu and Zn, were increased by the Cu and Zn additions (Table 4). The concentration of Ca-Cu and Ca-Zn was increased only by the highest application rate of the respective metal, whereas levels of HOAc-, Pyro-, and Ox-Cu and -Zn varied more directly with the cumulative levels of Cu and Zn applied. Comparison of data collected from the control and the high Cu treatments indicates that the Cu concentration in the fractions increased in the order: Pyro-Cu > Ox-Cu > HOAc-Cu > CaCu. The same comparison made between the control and the high Zn treatment indicates the order of increase among the Zn fractions was Ox-Zn = HOAc-Zn > Pyro-Zn > Ca-Zn. The greatest increase among the Cu fractions occurred in the Pyro-Cu fraction, which is in agreement with the knowledge that soil humic substances have a high affinity for soil Cu. Zinc, on the other hand, has higher affinity for soil oxides and hydroxides, which is corroborated by the large increases in the Ox-Zn and HOAc-Zn fractions (2, 7, 9, 16). The increase in the specifically adsorbed, organic, and oxide-associated fractions for both metals indicates that the applied Cu and Zn can be adsorbed by the same or similar sites in the soil. The large increase in the Ox-Cu and Ox-Zn fractions shows that a substantial portion of the applied Cu and Zn have reverted to forms that are considered to be unavailable to plants. Conversion of Cu and Zn applied in a soluble form to plant-unavailable forms over time has also been reported by researchers using other soil types (13, 18, 19, 20).

The amount of specifically bound Cu increased with increasing Cu application rate and was also influenced by Zn application (Table 4). The level of HOAc-Cu was higher when both Cu and Zn were added at the highest rate compared to when Cu was applied alone. A possible explanation for this relationship is that a portion of the Cu that was held in the more stable Ox-Cu and Pyro-Cu forms when Cu was applied alone could not be held in these forms when Zn was added, due to competition between the two metals for adsorption sites. An interaction did not occur between the two metals with respect to the level of Cu and Zn in the other fractions. However, the amount of Cu and Zn in both the Pyro- and Ox-Cu and -Zn fractions tended to be lower when the two metals were added together than when only one was added, whereas the level of Cu and Zn in the $CaCl_2$-soluble fraction tended to be higher when both metals were added compared to when only one was supplied. These results lend some support to the hypothesis of competition between Cu and Zn for adsorption sites on the organic and oxide soil components.

SUMMARY AND CONCLUSIONS

Application of up to 415 kg Cu and 897 kg Zn ha^{-1} as $CuSO_4$ and $ZnSO_4$, respectively, to the Davidson silty clay from 1967 through 1986 did not reduce grain or silage yields compared to control plots; neither were the concentrations of Cu and Zn in corn tissues increased above normal ranges as a result of the metal additions. The cumulative amounts of the metals applied over the 20-yr period greatly exceeds the predicted maximum allowable Cu and Zn loading rates for this soil. The levels of DTPA-extractable Cu and Zn increased linearly with the amount of metals applied with limited downward movement of the applied metals. Sequential extraction data indicated that a substantial portion of the applied Cu and Zn had reverted to forms unavailable to plants. The lack of adverse effects from the applied Cu and Zn can probably be attributed to the relatively high soil pH (pH > 6.5) maintained and the conversion of the applied metals to more stable forms that are not available to plants.

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L16.

From: erl@webspirs7.silverplatter.com
Date: Thu, 14 Jun 2001 19:59:14 -0400 (EDT)
Subject: copper

Record 1 of 1 in CAB Abstracts 1979-1981

TI: Toxicity of chemicals used in California rice fields to third instar hydrophilid larvae.
AU: Zalom-FG; Grigarick-AA; Way-MO; Grant-CD (ed.)
AD: Department of Entomology, California University, Davis, CA 95616, USA.
SO: Proceedings and papers of the Forty-seventh Annual Conference of the California Mosquito and Vector Control Association, Inc. January 28-31, 1979, held at the Airport Marina Hotel, Burlingame, California. 1979, 18-19; 6 ref.
PB: CMVCA Press.; Visalia, California; USA
PY: 1979
LA: English
AB: Chemical preparations registered for use in rice-fields against weeds and insect and other pests were tested in the laboratory in California in order to determine whether they had any adverse effects on the larvae of Hydrophilus triangularis Say and Tropisternus lateralis (F.), which are natural predators of culicid and ceratopogonid larvae [see RAE/B 68, 100]. In the laboratory, Flit MLO, MCPA and parathion proved to be the most toxic to the hydrophilids and propanil and carbaryl less toxic, and copper sulfate was not toxic at all; it is pointed out however that, under field conditions, the effects of each compound could be modified by ecological factors such as water depth, plant cover, release of water into the fields before and after application, and the density of the hydrophilid population at the time of application.
DE: MCPA-; propanil-; pesticides-; toxicity-; parathion-; carbaryl-; sulfuric-acid; fields-; mosquito-nets; habitats-; rice-
OD: Culicidae-; Diptera-; Oryza-
ID: triangularis,-Hydrophilus; lateralis,-Tropisternus; N-(3,4-dichlorophenyl)propanamide; Hydrophilus-triangularis; Tropisternus-lateralis; Flit-MLO; copper(2+)-salt-(11); not-toxic; Hydrophilidae
RN: 94-74-6; 709-98-8; 56-38-2; 63-25-2; 7664-93-9
BT: phenoxyacetic-herbicides; phenoxy-herbicides; herbicides; pesticides; anilide-herbicides; amide-herbicides; organothiophosphate-insecticides; organophosphorus-insecticides; insecticides; carbamate-insecticides; carbamate-pesticides; Diptera; insects; arthropods; invertebrates; animals; Gramineae; Cyperales; monocotyledons; angiosperms; Spermatophyta; plants
CC: TT300; HH700; LL300
CD: Medical-and-Veterinary-Entomology-Records-Discontinued; Other-Control-Measures; Animal-Behaviour
PT: Miscellaneous
UD: 950220
AN: 790567820