JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH
Part B—Pesticides, Food Contaminants, and Agricultural Wastes
Vol. B37, No. 6, pp. 625–635, 2002

ASSESSING COPPER THRESHOLDS FOR PHYTOTOXICITY AND POTENTIAL DIETARY TOXICITY IN SELECTED VEGETABLE CROPS

Xiao-E Yang,[1,*] Xin-Xian Long,[1] Wu-Zhong Ni,[1] Zhen-Qian Ye,[1] Zhen-Li He,[2] Peter J. Stoffella,[2] and David V. Calvert[2]

[1]Department of Resource Science, Zhejiang University,
Huajianchi Campus, Hangzhou, China
[2]Indian River Research and Education Center, University of Florida,
Fort Pierce, FL 34945, USA

ABSTRACT

Copper pollution in soils is widespread, and its accumulation in crop products could pose a risk on human health. In this paper, bioavailability of added copper (Cu) and critical Cu concentrations in a vegetable garden soil was evaluated for Chinese cabbage (*Brassica chinensis* L.), pakchoi (*Brassica* chinensis L.), and celery (*Apium*g g*raveolens* L. *var. dulce* DC) based on human dietary toxicity. The availability of added Cu in the soil decreased with incubation time, and had minimal change after 10–12 weeks. After incubated for 12 weeks, about 60% of added Cu was not extractable by DTPA. The same crops were also grown in sand culture to determine their responses to solution Cu. Shoot growth was significantly inhibited at Cu concentrations above 10 mg kg^{-1} in the solution or above 150 mg kg^{-1} (DTPA-Cu) in the soil. The sensitivity of the crops to Cu toxicity differed among the three vegetable crops. Copper concentration in shoots and edible parts varied with Cu supply levels and type of the vegetables. Negative correlations ($r = -0.90–0.99^{**}$) were noted between Cu concentration in shoots and fresh matter yields, but Cu concentrations in the edible parts were

---

[*]Corresponding author. E-mail: xyang@mail.hz.zj.cn or xyang@mail.ifas.ufl.edu

DOI: 10.1081/PFC-120015443
0360-1234 (Print); 1532-4109 (Online)

www.dekker.com

positively correlated with available and total Cu in the soil ($r = 0.91-0.99^{**}$). The critical tissue Cu concentrations at 10% shoot DM reduction were 19.4, 5.5, 30.9 mg kg^{-1} for Chinese cabbage, pakchoi, and celery, respectively. Based on the threshold of human dietary toxicity for Cu (10 mg kg^{-1}), the critical concentrations of total and available Cu in the soil were 430 and 269 mg kg^{-1} for pakchoi, 608 and 313 mg kg^{-1} for celery, and 835 and 339 mg kg – 1 for Chinese cabbage, respectively.

Key Words: Copper; Toxic threshold; Vegetable crop; Genotypic difference; Human dietary toxicity

INTRODUCTION

Vegetables play an important role in the human diet, and production in suburban areas has increased as population has become more urbanized. Heavy metal pollution in soils has increased in these areas because of increased disposal of municipal and industrial solid and liquid wastes to the soils and precipitation of pollutants from air on the soils.[1,2] Retention of heavy metals by soil depends on factors such as the nature of the inorganic and organic constituents, the nature of metals, the composition of soil solution, and pH.[3] Heavy metal accumulation in soils is of concern in agricultural production due to adverse effects on food quality (safety and marketability), crop growth (due to phytotoxicity), and environmental health (soil flora/fauna and terrestrial animals).[4,5] Metal accumulation in vegetables may pose a direct threat to human health.

Copper, an essential micronutrient for plants, animals, and humans, plays an irreplaceable role in the function of a large number of enzymes that catalyse oxidative reactions in a variety of metabolic pathways.[6] Excess Cu is toxic to plants and humans and disturbs a wide range of biochemical and physiological processes, such as photosynthesis, pigment synthesis, protein metabolism, and membrane integrity.[6–8] Although a maximum Cu limit for human health has been established for edible parts of crops (10 mg kg^{-1}),[9] soil Cu thresholds for producing safe vegetables are not available. The objectives of this study were to examine excess Cu on growth and Cu accumulation among Chinese cabbage, pakchoi, and celery, the transformation of added Cu in vegetable garden soil, and to determine the critical Cu concentrations in the soil for Chinese cabbage, pakchoi, and celery based on human dietary toxicity.

MATERIALS AND METHODS

Sand Culture Experiment

Seeds of three vegetable crops including Chinese cabbage (*B. Chinensis* L.), pakchoi (*B. Chinensis* L.), and celery (*A. Graveolens* L. *var. dulce* DC) were purchased from vegetable seed corporation of Hangzhou, China. Seeds were germinated on wetted filter paper for two days in the dark at 25°C. The germinated seeds were sown on quartz sand with a nutrient solution prepared for establishing seedlings.

Five uniform seedlings were transplanted into quartz sand with nutrient solution in a 3 L plastic container, the composition of the nutrient solution was modified from Yang et al.,[10] (in mmol L^{-1}): KNO_3 6.00, $Ca(NO_3)_2 \cdot 4H_2O$ 3.50, KH_2PO_4 1.33, $MgSO_4 \cdot 7H_2O$ 2.00, NaCl 0.48, and (in μmol L^{-1}): H_3BO_3 10.00, $MnSO_4 \cdot H_2O$ 0.50, $ZnSO_4 \cdot 7H_2O$ 0.50, $CuSO_4 \cdot 5H_2O$ 0.20, $(NH_4)_6Mo_7O_{24}$ 0.01, Fe-EDTA 200. After growth of 15 days, plants were exposed to different Cu levels as $CuSO_4 \cdot 5H_2O$: CK (0.01), 10, 20, 40 mg Cu L^{-1}. A randomized complete block design was used with each treatment replicated three times. The pH was maintained at 5.8 by daily adjusted with 0.1 mol L^{-1} HCl or 0.1 mol L^{-1} NaOH. The nutrient solution was replaced every seven days. Plants were harvested after 3 weeks of treatment. At harvest, roots of intact plants were rinsed with distilled water. Plant samples were separated into shoots (leaf blades and petioles-edible portion for celery) and roots, the base of stalks and roots were rinsed thoroughly with bi-distilled water, blotted dry, dried at 105°C for 30 min, then dried at 70°C. Fresh weight (FW) and dry weight (DW) of shoots and roots were recorded. Samples of plant dry materials were grounded with stainless steel mill and passed through a 60-mesh sieve for Cu analysis.

Incubation Experiment

Alluvial soil (Fluvio-marine yellow loamy soil) was collected from an old vegetable production area in Hangzhou suburb. The main agrochemical properties of the soil as analysed were: pH 7.15, organic C 38.7 mg g^{-1}, cation exchange capacity (CEC) 13.93 cmol kg^{-1}, total N 2.60 g kg^{-1}, total P 0.90 g kg^{-1}, total and available Cu 27.0 and 4.26 mg kg^{-1}, respectively. Based on Cu adsorption and desorption characteristics of the soil,[11] copper as $CuSO_4 \cdot 5H_2O$) was applied at the rates of 0, 200, 400, 600, and 800 mg Cu kg^{-1} soil, respectively. The mixed soil samples were then incubated in a plastic container (60 × 40 × 15 cm) at 70% of maximum field water capacity for 12 weeks. Soil DTPA-extractable Cu was measured at intervals of 0, 1, 2, 4, 8 and 12 weeks after incubation.

Soil Culture Experiment

The incubated and air-dried soil samples were used to grow Chinese cabbage, pakchoi, and celery. Each pot contained one kg of soil thoroughly mixed with 1.00 g of urea, 0.48 g of $Ca(H_2PO_4)_2$ and 0.30 g of KCl as basal fertilizers. The process of preparing seedlings was similar to the sand culture experiment and 5 plants of 20-day-old seedlings were transplanted. A randomised complete block experimental design was used with each treatment replicated four times. Soil moisture was maintained at 60–70% of the maximum field water capacity. Plants were harvested 30–35 days after transplanting. Shoots were separated from the roots, and fresh and dry weights were recorded. Samples of plant dry materials were ground with a stainless steel mill and passed through a 60 mesh sieve for Cu analysis.

Chemical Analysis

Soil agrochemical properties were analysed by SSICA.[12] Soil total Cu was measured by the method from Yuan.[13] Soil available Cu was extracted using 0.05 mol L^{-1} DTPA (soil:extractant ratio of 1:20), plant samples were ashed at 550°C, and dissolved with 1:1 HCl. Concentration of Cu in the solutions was determined using an Atomic Absorption Spectoscopy (AAS) (Hitachi, 8081).

RESULTS AND DISCUSSIONS

Bioavailability of Added Copper in a Vegetable Garden Soil

Total soil metals can be used to estimate the degree of soil exposure to heavy metal pollution, although this is not generally well corrected with metal mobility and bioavailability.[14] Plant availability, however, is better correlated with most extractable forms of Cu rather than total Cu concentration across a range of soil conditions.[15] Total Cu in soils includes six ‘pools’ classified according to their physical–chemical behaviour. The pools are soluble ions and inorganic and organic complexes in soil solution; exchangeable Cu; stable organic complexes in humus; Cu adsorbed by hydrous oxides of Mn, Fe and Al; Cu adsorbed on the clay–humus colloidal complex; and the crystal lattice-bound Cu.[16] When added to soil, Cu may react with soil constituent, changes its chemical form, then its availability to plants is also altered. The amount of soil Cu removed by a chelating agent like DTPA or EDTA is considered as the plant-available portion.[16] The DTPA extractable Cu decreased with incubation time, especially in the first 8 weeks. After a 12-week incubation, 60% of added Cu was not extractable by DTPA (Figure 1). These may have resulted from transformation of the added soluble Cu fraction to slowly available fractions of Cu in the soil.

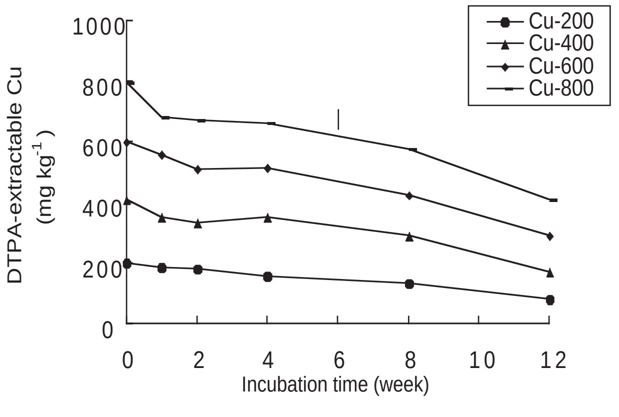

Figure 1. Changes of DTPA-extractable Cu with incubation time. Data are means of three replicates. The bar depicted $LSD_{(0.05)}$. Cu-200, Cu-400, Cu-600, and Cu-800 refer to Cu addition levels of 200, 400, 600, and 800 mg Cu kg^{-1} soil, respectively.

Excess Cu on Growth of Vegetable Crops

Excess Cu in growth media caused toxicity to all the three vegetable crops, resulted in chlorosis in new leaves, brown, stunted, coralloid roots, and plant growth was inhibited. Shoot fresh weight (FW) progressively decreased as the Cu in nutrient solution increased (Figure 2). Great differences in Cu tolerance were also noted among the three vegetable crops. Shoot fresh weight of celery, pakchoi and Chinese cabbage decreased to about 33%, 37% and 50% of the control, respectively, when grown with the Cu supply of 10 mg L^{-1}. Shoot growth of pakchoi decreased sharply at higher Cu supply levels, and its FW reduced to 90% of the control at Cu supply over 20 mg L^{-1} (Figure 2). Shoot growth between celery and Chinese cabbage was similar when external Cu levels were over 20 mg L^{-1} (Figure 2). The results indicate that celery is more tolerant to Cu toxicity than Chinese cabbage and pakchoi when grown under nutrient solution.

Significant negative correlations occur between shoot (or stem for celery) biomass and soil available or total Cu (Table 1). Shoot biomass of the three vegetable species was negatively and closely correlated with total soil Cu or soil available Cu, with the correlation coefficients being $0.95–0.99^{**}$. The critical soil available Cu concentrations at 10% reduction of dry matter yield were 50.6, 57.6, and 70.7 mg kg^{-1} for Chinese cabbage, pakchoi, and celery (stem), respectively. These results indicate that Chinese cabbage and pakchoi are less tolerant to Cu toxicity than celery when plants were grown in the soil, which showed a similar trend as those grown in nutrient solution.

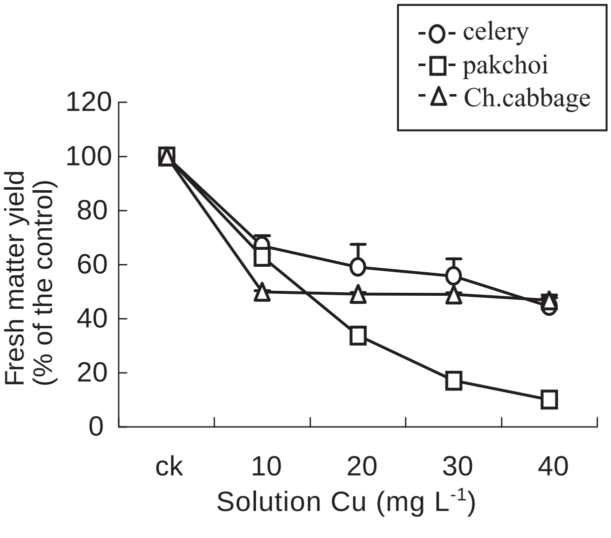

Figure 2. Growth response of different vegetable species to Cu levels in nutrient solution. All data are means of three replicates, vertical bars denote SE.

Table 1. Correlations Between Shoot Growth (DM) and Soil Total or Available Cu Among the Three Vegetable Species

	Soil Total Cu			Soil Available Cu		
Vegetable Specie	Regression Equation	R^2	$P <$	Regression Equation	R^2	$P <$
Ch.Cabbage (Shoot)	$Y = -0.0518X + 38.854$	0.922	0.05	$Y = -0.1266X + 37.858$	0.943	0.05
Pakchoi (Shoot)	$Y = -0.1111X + 105.93$	0.977	0.01	$Y = -0.2157X + 99.752$	0.976	0.01
Celery (Stem)	$Y = -0.0325X + 38.16$	0.991	0.01	$Y = -0.0636X + 36.451$	0.999	0.01
Celery (Leaf)	$Y = -0.0231X + 30.95$	0.973	0.01	$Y = -0.0454X + 29.771$	0.984	0.01
Celery (Shoot)	$Y = -0.0556X + 69.109$	0.989	0.01	$Y = -0.109X + 66.221$	0.998	0.01

Table 2. Copper Concentration (mg kg^{-1} DW) in the Shoots and Roots of Different Vegetables Grown in Nutrient Solution

Copper Levels (mg L^{-1})	Chinese Cabbage		Pakchoi		Celery		
	Root	Shoot	Root	Shoot	Root	Stem	Leaf
0.01	13.00	13.34	18.67	9.00	27.00	21.50	31.17
10	238.3	45.00	235.8	17.67	566.7	53.00	65.83
20	361.3	58.75	475.0	32.33	1312	81.50	101.0
30	565.6	71.13	528.3	128.3	1423	95.00	123.3
40	711.3	97.50	705.6	179.2	1557	145.0	166.7
LSD$_{0.05}$	536	3.58	8.34	6.67	11.23	9.47	10.32

Copper Concentration and Accumulation

Copper concentrations in shoots and roots varied both with different Cu levels and the type of vegetables (Table 2). Copper concentrations in both shoots and roots increased, but root Cu concentration raised more sharply than shoot Cu with increasing Cu supply levels (Table 2). For the three vegetable species tested, Cu was mainly accumulated in the roots, and a small proportion of absorbed Cu (10–20%) was transported to the shoots (Table 2). Large differences in shoot and root Cu concentrations were observed among the three species. Celery contained higher Cu in both roots and shoots than pakchoi and Chinese cabbage at external Cu levels below 20 mg L^{-1}. For example, Cu concentration in the roots of celery was 3-fold higher than that of pakchoi (Table 2).

Copper accumulation coefficients (AF) in the shoots of the vegetable species were relatively small when grown at soil addition Cu levels of 200–400 mg kg^{-1}, and dramatically increased at soil addition Cu levels above 600 mg kg^{-1} (Table 3). When the soil Cu levels were below 400 mg kg^{-1}, copper AF in edible parts of the three vegetable species changed in the order: Chinese cabbage ≥ pakchoi > celery. These results are in agreement with other reports.[17,18]

Table 3. Copper Accumulation Coefficients (AF) of Different Vegetable Crops Grown at Various Soil Cu Levels

Soil Cu Added (mg kg^{-1})	Cu Accumulation Coefficient						
	Chinese Cabbage		Pakchoi		Celery		
	Root	Shoots	Root	Shoots	Root	Stem	Leaves
ck	0.223	0.060	0.438	0.074	0.117	0.005	0.090
200	0.081	0.016	0.091	0.013	0.062	0.008	0.071
400	0.081	0.014	0.071	0.010	0.134	0.009	0.084
600	0.091	0.019	0.072	0.017	0.318	0.015	0.107
800	0.167	0.030	0.157	0.036	0.324	0.017	0.150
LSD$_{0.05}$	0.007	0.003	0.006	0.002	0.017	0.003	0.009

AF = Cu in plant tissues/total Cu in soil.

Table 4. Regression Equations and Coefficients Between Cu Concentration in Plant Tissues and DM Yields Among the Vegetable Species[a]

Species	Regression	Equation	R^2	P<	Critical Cu[b] (mg kg^{-1})
Chinese cabbage	Shoot Cu vs Shoot DW	$Y = -91.70 \ln x + 573.2$	0.999	0.01	19.41
Pakchoi	Shoot Cu vs Shoot DW	$Y = -47.63 \ln x + 254.9$	0.947	0.05	5.47
Celery	Stem Cu vs stem DW	$Y = -35.64 \ln x + 217.5$	0.883	0.05	21.46
	Stem Cu vs Shoot DW	$Y = -57.96 \ln x + 364.3$	0.891	0.05	22.07
	Leaf Cu vs Leaf DW	$Y = -22.33 \ln x + 146.8$	0.900	0.05	23.07
	Leaf Cu vs Shoot DW	$Y = -63.65 \ln x + 403.5$	0.878	0.05	30.97

[a] Y-Dry matter yield (g pot^{-1}), x-Cu concentration (mg kg^{-1}).
[b] Copper concentration in plant tissue at 10% dry matter yield reduction.

Criteria for Cu Phytotoxicity

Copper plays an irreplaceable role in biological systems as a structural and catalytic component of proteins and enzymes.[6] Excess Cu is toxic to plants, and inhibits plant growth.[21] Phytotoxicity of heavy metals is related to the amount of toxicants taken up and accumulated in plant tissues.[22,23] Significant and positive correlation between shoot DW reduction (% the control) with tissue Cu concentration (based on DW) was observed for each of the three vegetable species grown in nutrient solution (Table 4). The critical tissue Cu concentrations at 10% reduction of DM yield (PT_{10}) were 19.4, 5.5, 22.1 and 30.9 mg kg^{-1} for Chinese cabbage, pakchoi, celery stem, and celery leaf, respectively. These results are in agreement with the findings by other scientists.[15,24–27] For instance, Hara et al.[25] reported an upper critical level (10% yield reduction) for Cu toxicity of 25 mg kg^{-1} in cabbage leaf, and Davis and Beckett[24] reported an upper critical level (10% yield reduction) for Cu toxicity of 17–21 mg kg^{-1} in lettuce leaf. Under soil culture conditions, significant and positive correlations were also noted between shoot Cu and soil available Cu levels, with $r = 0.96–0.99^{**}$ for Chinese cabbage and celery, and $0.86–0.90^{*}$ for pakchoi, respectively. These results are in agreement with the findings by other scientists,[19,20] showing that celery is more tolerant to Cu than the other two species, especially pakchoi under both solution and soil culture conditions.

Soil Thresholds of Potential Dietary Cu Toxicity

Soil threshold of heavy metal toxicity is an important factor affecting soil environmental capacity of heavy metal and determining heavy metal cumulative loading limit. As for soil-plant system, heavy metal toxicity threshold is the highest permissible content in the soil (total or bioavailable concentration) that does not cause any phytotoxicity (i.e., inhibit plant growth and decrease yield) or heavy metal in edible parts of the crops does not exceed Food Hygiene Standard. The critical food Cu threshold for human health has been established to be 10 mg kg^{-1}.[9] According to the

Table 5. Soil Cu Thresholds for Yield Reduction and Potential Dietary Toxicity in Edible Parts of the Vegetables

	Total Cu (mg kg^{-1})		Available Cu (mg kg^{-1})	
Crop Species	PDT[a] $\leq$ 10mg kg^{-1}	SDMYR[b] by 10%	PDT $\leq$ 10mg kg^{-1}	SDMYR by 10%
Chinese cabbage	835.56	142.84	338.98	50.58
Pakchoi	429.93	167.52	269.14	57.64
Celery (stem)	608.22	174.18	312.72	70.68
Celery (leaf)	161.07	232.03	57.22	92.09

[a] PDT = Potential Dietary Toxicity.
[b] SDMYR = Shoot Dry Matter Yield Deduction.

regression equations between shoot DM yields and Cu concentration in plant tissues or soil, soil Cu thresholds for phytoxicity (10% yield reduction) and potential dietary toxicity in edible parts of the vegetables could be calculated. Soil total and available Cu thresholds for potential dietary toxicity in the edible parts of the vegetable crops were 5-fold higher than those for phytoxicity (at 10% yield reduction) (Table 5). Among the three vegetable crops, pakchoi had much lower soil total and available Cu thresholds, as compared with other two vegetable species.

Currently, most soil quality criteria for heavy metals are based on the total amount in the soil. This approach is excessively conservative as it does not take into account the complex interactions between metals and soils, and does not distinguish between the various forms of metals in the soil.[28] To ensure adequate environmental protection at the most economical cost, soil quality guidelines should be based on the soil metal pools that are actually posing a threat to organisms: the bioavailable portion. Researchers have attempted to predict this 'bioavailable' portion by correlating data on plant tissue concentrations or biomass yield decreased with amounts found in various soil pools as determined by a wide variety of extraction reagents and methods. Unfortunately, most of the data derived from these studies tend to be inconsistent.[29,30] Future studies are needed to elucidate the interaction of phytoavailability and crop species for establishing soil Cu thresholds for potential dietary toxicity.

ACKNOWLEDGMENTS

This study was, in part, financed by a grant (#29977017) from the National Natural Science Foundation of China (NNSFC) and an International Cooperative program (China-US) through NNSFC (#00119). Florida Agric. Exp. Stn. Journal Ser. No: R-08771.

REFERENCES

1. MacNicol, R.D.; Beckett, P.H.T. Critical tissue concentrations of potentially toxic elements. Plant Soil **1985**, *85*, 107–109.

2. Jinadasa, K.B.P.N.; Milham, P.J.; Hawkkins, C.A.; Cornish, P.S.; Williams, P.A.; Kaldor, C.J.; Conroy, J.P. Survey of cadmium levels in vegetables and soils of greater sydney, Australia. J. Environ. Qual. **1997**, *26*, 924–933.
3. Msaky, J.J.; Calvert, R. Adsorption behavior of copper and zinc in soils: Influence of pH on adsorption characteristics. Soil Sci. **1990**, *150*, 513–522.
4. Fergusson, J.E. *The Heavy Elements: Chemistry, Environmental Impact and Health Effects*; Pergamin Press: Oxford, 1990; 382–399.
5. McLaughlin, M.J.; Hamon, R.E.; McLaren, R.G.; Speir, T.W.; Rogers, S.L. Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. Aust. J. Soil Res. **2000**, *38*, 1037–1086.
6. Marschnar, H. *Mineral Nutrition of Higher Plants*, 2nd Ed.; Academic Press: London, 1995; 33–347.
7. Miller, W.P.; McFee, W.W.; Alston, A.M. Distribution of cadmium, zinc, copper and lead in soil of industrial northwestern Indiana. J. Environ. Qual. **1983**, *12*, 29–35.
8. Merry, R.H.; Tiller, K.G.; Alston, A.M. The effects of contamination of soil with copper, lead, and arsenic on the growth and composition of plants: I. Effects of season, genotype, soil temperature and fertilizers. Plant Soil **1986**, *91*, 115–128.
9. PAC (Protect Academy of China). *Thresholds for Food Hygiene (In Chinese)*; China Standard Press: Beijing, China, 1995.
10. Yang, X.; Baligar, D.C.; Martens, D.C.; Clark, R.B. Influx, transport, and accumulation of cadmium in plant species grown at different Cd (II) activities. Environ. Sci. Health **1995**, *B30* (4), 569–583.
11. Long, X.X.; Ni, W.Z.; Yang, X.E. Characteristics of Cu adsorption–desorption of vegetable garden soils. Rural Eco-Environ. (in Chinese) **2000**, *16* (3), 39–41.
12. SSICA (Soil Sci. Ch. Acad.). *Physical and Chemical Analyses of Soils (In Chinese)*; Shanghai Academic Press: Shanghai, China, 1980.
13. Yuan, D.W. Compared study on the pre-treatment methods for measuring soil total copper, zinc, lead, cadmium, nickel, and manganese. Agro-Environ. Protect. (In Chinese) **1988**, *7* (1), 34–36.
14. Kuo, S.; Heilman, P.E.; Baker, S. Distribution and forms of copper and zinc in soils near a copper smelter. Soil Sci. **1983**, *135*, 101–109.
15. Borkert, C.M.; Cox, F.R.; Tucker, M.R. Zinc and copper toxicity in peanut, soybean, rice, and corn in soil mixtures. Commun. Soil Sci. Plant Anal. **1998**, *29*, 2991–3005.
16. Baker, D.E. Copper. In *Heavy Metals in Soils*; Alloway, B.J., Ed.; John Wiley & Sons Inc.: New York, 1990; 261–279.
17. Kim, S.J.; Chang, A.C.; Page, A.L.; Warneke, J.E. Relative concentration of cadmium and zinc in tissue of selected food plants grown and sludge-treated soils. J. Environ. Qual. **1988**, *17*, 568–573.
18. Dowdy, R.H.; Larson, W.E. The availability of sludge-borne metals to various vegetable crops. J. Environ. Qual. **1975**, *4*, 278–282.
19. Singh, B.R.; Narwal, R.P. Plant availability of heavy metals in a sludge-treated soil: II. Metal extractability compared with plant metal uptake. J. Environ. Qual. **1984**, *13*, 344–348.

20. Adriao, D.C. *Trace Elements in Terrestrial Environment*; Springer-Verlag Inc.: New York, 1986.
21. Ouzounidou, G. Root growth and pigment composition in relationship to element uptake in Silene compacta plants treated with copper. J. Plant Nutr. **1994**, *17*, 933–943.
22. Chang, A.C.; Granato, T.C.; Page, A.L. A methodology for establishing phytotoxicity criteria for chromium, copper, nickel, and zinc in agricultural land application of municipal sewage sludges. J. Environ. Qual. **1992**, *21*, 521–536.
23. Bould, C.; Hewitt, E.J.; Needham, P. *Diagnosis of Mineral Disorders in Plants*; Chemical Publishing Principles: New York, 1984; Vol. 1.
24. Davis, R.D.; Beckett, P.H.T. Upper critical levels of toxic elements in plants: II. Critical levels of copper in young barley, wheat, rape, lettuce, and ryegrass, and of nickel & zinc in young barley and ryegrass. New Phytol. **1978**, *80*, 23–32.
25. Hara, T.; Sonoda, Y.; Iwai, I. Growth response of cabbage plants to transition elements under water culture conditions. II. Cobalt, nickel, copper, zinc, and molybdenum. Soil Sci. Plant Nutr. **1976**, *22*, 317–325.
26. Howeler, R.H.; Edwards; Asker, C.J. Micronutrient deficiencies and toxicities of cassava plants grown in nutrient solutions. I. Critrical tissues concentration. J. Plant Nutr. **1982**, *5*, 1059–1076.
27. Mocquot, B.; Vangronsveld, J.; Clijsters, H.; Mench, M. Copper toxicity in young maize (zea mays L.) plants: Effects on growth, mineral and chlorophyll contents, and enzyme activities. Plant Soil **1996**, *182*, 287–300.
28. Sauve, S.; Cook, N.; Hendershot, W.H.; McBride, M.B. Linking plant tissue concentrations and soil copper pools in urban contaminated soils. Environ. Pollut. **1996**, *94*, 153–157.
29. Davies, B.E. Inter-relationships between soil properties and the uptake of cadmium, copper, lead and zinc from contaminated soils by radish (*Raphanus sativus* L.). Water Air Soil Pollut. **1992**, *63*, 331–342.
30. Qian, J.; Wang, Z.J.; Shan, X.Q.; Tu, Q.; Wen, B.; Chen, B. Evaluation of plant availability of soil trace metals by chemical fractionation and multiple regression analysis. Environ. Pollut. **1996**, *91*, 309–315.

Received February 8, 2002