Journal of Phytology 2011, 3(3): 53-62
Phytophysiology
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

EFFECT OF COPPER ON GROWTH, DRY MATTER YIELD AND NUTRIENT CONTENT OF *VIGNA RADIATA* (L.) WILCZEK

R. Manivasagaperumal[1*], P. Vijayarengan[1], S. Balamurugan[1], G. Thiyagarajan[1]
Environmental Biology lab, Botany wing (DDE), Annamalai University,
Annamalainagar-608 002, Tamil Nadu, India

SUMMARY

An attempt was made to study the influence of copper (Cu) on growth, dry matter yield and nutrient content of greengram (*Vigna radiata* (L.) Wilczek) in a glass house earthen pot experiment. Copper was applied to the soil in the form of copper sulphate ($CuSO_4.5H_2O$) in different concentrations (0, 50, 100, 150, 200 and 250 mg kg^{-1}) in which the greengram plants were grown. The plant samples were analysed 45 days after sowing. The results indicated that low level of copper concentrations (50 mg kg^{-1}) showed a significant increase in the overall growth, dry matter yield and nutrient content, while higher concentrations (100-250 mg kg^{-1}) decreased the growth, dry matter production and nutrient content of greengram.

Key words: Copper, Growth, Nutrient content, *Vigna radiata*

R. Manivasagaperumal et al. Effect of Copper on Growth, Dry Matter Yield and Nutrient Content of Vigna radiata (L.) Wilczek. J Phytol 3/3 (2011) 53-62.
*Corresponding Author, Email: rmvperumal1966@yahoo.co.in

1. Introduction

Soils with high heavy metal concentrations, including copper, have generally been contaminated due to the close proximity to natural metalliferous ore out crops, or as a result of mining, smelting or other industrial activities. Early studies of plants growing under stress environmental conditions posed intriguing questions about the nature, scale, and mechanisms of adaptation involved (Woolhouse, 1983; Ernst *et al.*, 1990; Masaka and Muunganirwa, 2007). Our study here was confined to copper as it is probably one of the most common contaminants of soils. Moreover, copper is also one of the essential micronutrients for plant growth. It is involved in numerous physiological functions as a component of several enzymes, mainly those which participate in electron flow, catalyze redox reactions in mitochondria and chloroplasts (Lolkema and Vooijs, 1986; Harrison *et al.*, 1999; Hansch and Mendel, 2009). However, in excessive quantities copper becomes toxic as it interferes with photosynthetic and respiratory processes, protein synthesis and development of plant organelles (Agarwala *et al.*, 1995; Upadhyay and Panda, 2009). Specifically excess copper can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability, leading to ion leakage (Ouzounidou *et al.*, 1992; Berglund *et al.*, 2002; Bouazizi *et al.*, 2010). Reports are also available on induced deficiency of various mineral content under copper toxicity (Mocquot *et al.*, 1996; Bouazizi *et al.*, 2010; Lequeux *et al.*, 2010). Apart from this, the information on plant metabolism is sporadic. Hence efforts have been made to establish the toxic level of copper on greengram plants in the present study.

2. Materials and Methods

A greenhouse experiment was conducted in polyethylene lined earthen pots containing 4 kg of well mixed air-dried soil. The pH of the soil was 6.2. Copper was applied at the rate of 0, 50, 100, 150, 200 and 250 mg kg^{-1} of soil in the form of copper sulphate ($CuSO_4.5H_2O$). The treatments were replicated five times in a completely randomized block design. Carefully selected uniform sized greengram seeds were directly sowed in each pot and thinned to five plants per pot, seven days after emergence. The plants were sampled 45 days after sowing and the various morphometric growth parameters were employed. Then the samples were kept in hot air oven maintained at 80°C for 48 hours. Dry weight of root and shoot was determined. Oven dried plants were digested in appropriate acid mixtures and the nutrient contents were measured. Using the acid digest, nitrogen was determined by micro-kjeldahl method and phosphorus was determined by molybdovanadate method measuring the absorbance at 460 nm by spectrophotometer. K, Na, Ca and Mg were determined by flame photometer. Fe, Mn, Zn and Cu were determined by atomic absorption spectrophotometer (AAS). The statistical analysis of the experimental data was carried out as per the procedure given by Gomez and Gomez (1984).

3. Results and Discussion

Growth response

Plants treated with low level of copper (50 mg kg^{-1}) showed a significant increase in root length, shoot length and leaf area, when compared to control (Table 1 and Fig.1). Higher concentrations showed a decrease in the root and shoot length and leaf area. These changes are also in consonance with previous observations (Lidon and Henriques, 1993; Moustakas *et al*., 1997; Xiong *et al*., 2006). Moreover, high concentrations of copper, the root and shoot elongation was poor with a concomitant decrease in root and shoot length (Bouazizi *et al*., 2008, 2010; Ahsan *et al*., 2007). Reduction of leaf area due to copper was also observed by Mocquot *et al*., (1996); Zeng *et al*., 2004. Significant increase in the growth, possibly due to copper is required by plants in trace amount (Reichman, 2002). The inhibitory action of excess copper in root and shoot length and leaf area may be due to reduction in cell division, toxic effect of heavy metal on photosynthesis, respiration and protein synthesis. These obviously contribute to the retardation of normal growth (Kupper *et al*., 1996; Sonmez *et al*., 2006).

Table-1. Effect of copper on growth and dry matter yield of greengram

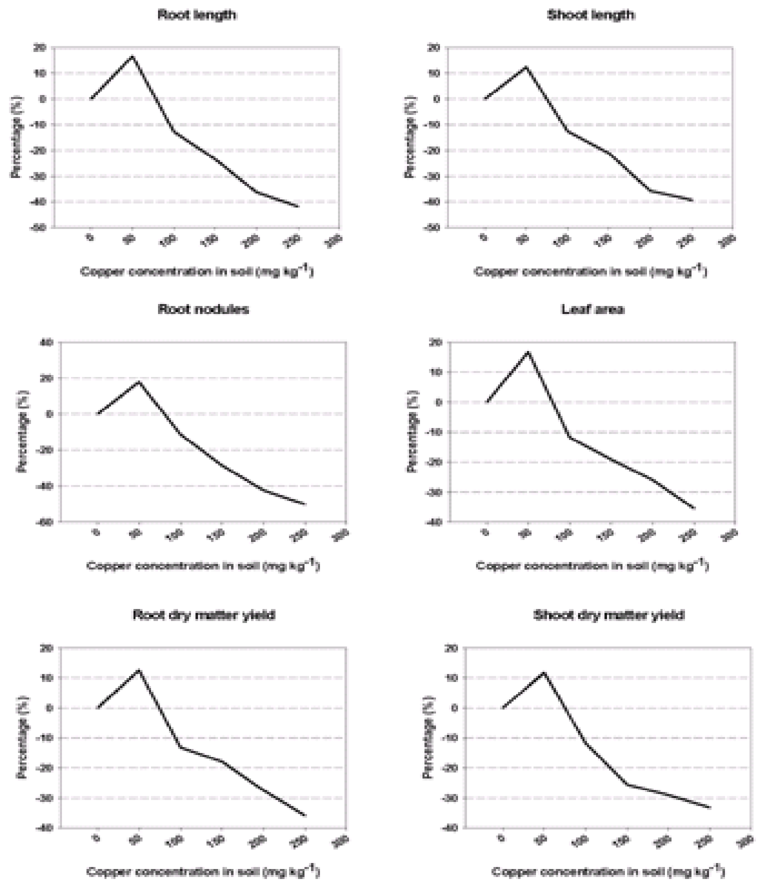
Copper added in the soil (mg kg^{-1})	Root length (cm $plant^{-1}$)	Shoot length (cm $plant^{-1}$)	Root nodules ($plant^{-1}$)	Leaf area (cm^2 $plant^{-1}$)	Dry matter yield (g $plant^{-1}$)	
					Root	Shoot
0	18.76	28.76	32.8	436.20	0.358	1.556
50	21.88 (+16.63)	32.32 (+12.37)	38.64 (+17.80)	508.95 (+16.67)	0.403 (+12.56)	1.740 (+11.82)
100	16.36 (- 12.79)	25.12 (-12.65)	29.1 (-11.28)	384.69 (-11.80)	0.311 (-13.12)	1.374 (-11.69)
150	14.36 (-23.45)	22.69 (-21.10)	23.46 (-28.47)	352.72 (-19.13)	0.294 (-17.87)	1.159 (-25.51)
200	11.94 (-36.35)	18.46 (-35.81)	18.90 (-42.32)	322.92 (-25.96)	0.260 (-27.37)	1.104 (-29.04)
250	10.92 (-41.79)	17.46 (-39.29)	16.38 (-50.06)	281.72 (-35.41)	0.230 (-35.75)	1.040 (-33.16)

Each value is the mean of the five replications
Figures in parenthesis – Percentage over to control
All the values are significant at $P < 0.01$

CD at 5%	1.23	1.34	0.90	0.91	.01	1.34

CD at 1%	1.64	1.78	1.21	1.21	.02	1.78

Fig. 1: Effect of copper on growth and dry matter yield of greengram



Nodule number

Number of nodules was high at lower concentration of copper (50 mg kg^{-1}). Further, the number of nodules decreased with a gradual increase in copper level (100-250 mg kg^{-1}) (Table 1 and Fig.1). Similar reduction in nodule number under copper treatment was reported for *Trifolium pratense* L. (Mc Ilveen and Coole, 1974) and *Vigna unguiculata* (Jain *et al.*, 1994). A decrease in the number of nodules in greengram plants, due to elevated level of copper, would be attributed to the reduction in the development of root system as well as the direct toxicity of copper on soil microbes. This would be evident from the previous studies suggested that most metal ions are toxic to soil microorganisms, even in small quantities (Tyler, 1981; Ginn *et al.*, 2006). Angle and Chaney (1991) also reported that lowest rhizobial population was found in the soil with the highest extractable metal concentration.

Dry matter yield

Plants treated with copper at low concentrations (50 mg kg^{-1}) showed a significant increase in dry matter production of root and shoot. But in higher concentrations (100-250 mg kg^{-1}), it showed a gradual decline in the dry matter production (Table 1 and Fig.1). This view was supported by previous findings indicated that application of copper slightly increased the dry weight at lower concentration, while excess of copper reduced the biomass (Lidon and Henriques, 1993; Mocquot *et al.*, 1996; Xiong *et al.*, 2006). Similar reduction in dry matter yield of greengram at higher concentration of heavy metals was observed by Vijayarengan and

Lakshmanachary (1995) due to nickel and by Madhavi and Rao (1999) due to cadmium. The decrease in biomass in excess copper treated greengram might be due to low protein formation, resulting in inhibition of photosynthesis, as well as hampered carbohydrate translocation (Wani *et al*., 2007; Samarakoon and Rauser, 1979).

Macronutrient content

The effect of copper on various macronutrient contents (N, P, K, Na, Ca and Mg) of greengram leaves indicated that the nutrient contents increased at low copper level (50 mg kg^{-1}) and decreased at high copper levels (100-250 mg kg^{-1}) (Table 2 and Fig.2,3). From this data it was also observed that the nitrogen content of greengram showed a progressive decline with the increase in copper level. However, 50 mg kg^{-1} of copper level produced positive effect on the nitrogen content of greengram leaves. The results are in close confirmation with the findings that the uptake of nitrogen was significantly increased at low level of copper, while higher concentration showed a declining trend of nitrogen (Lidon and Henriques, 1993; Xiong *et al*., 2006). Similarly, Seliga (1993) observed that the uptake of nitrogen from the soil is inhibited by the elevated level of copper in *Lupinus luteus* L. Sawhney *et al*., (1990) and Ureta *et al*., (2005) noticed that higher concentration of heavy metals affect not only the development of root nodules, growth and survival of rhizobia, but also nitrogen fixation and the activity of nitrogenase enzyme. Decrease in nitrogen content of greengram leaf due to copper could be attributed to poor development of nodules, reduced rate of nitrogen fixation and decreased uptake of nitrogen from the soil.

Table-2. Effect of copper on nutrient content of the leaves of greengram

Copper added in the soil (mg kg^{-1})	N	P	K	Na	Ca	Mg	Fe	Mn	Zn	Cu
	mg g^{-1}						µg g^{-1}			
0	54.6	5.34	32.9	1.74	13.6	4.16	325	86.55	55.16	25.27
50	60.16 (+10.18)	5.79 (+8.42)	36.97 (+12.37)	1.88 (+8.04)	14.87 (+9.33)	4.88 (+17.30)	342 (+5.23)	94.53 (+9.22)	61.95 (+12.10)	46.64 (+84.56)
100	50.18 (-8.09)	4.96 (-7.11)	30.35 (-7.75)	1.62 (-6.89)	13.00 (-4.41)	3.63 (-12.74)	299 (-8.00)	80.60 (-6.87)	49.17 (-10.85)	68.60 (+171.46)
150	41.58 (-23.84)	4.34 (-18.72)	27.51 (-16.38)	1.42 (-18.39)	12.30 (-12.50)	3.09 (-25.72)	247 (-24.00)	69.71 (-19.45)	42.87 (- 22.28)	89.52 (+254.25)
200	34.65 (-36.53)	3.85 (-27.90)	24.86 (-24.43)	1.28 (-26.43)	11.03 (-18.89)	2.60 (-37.50)	209 (-35.69)	64.16 (-25.86)	36.33 (- 34.13)	121.76 (+381.83)
250	32.05 (-38.10)	3.61 (-32.39)	22.8 (- 30.69)	1.14 (-34.48)	10.46 (-23.08)	2.44 (-41.34)	187 (-42.46)	55.06 (-36.38)	31.50 (-42.89)	150.00 (+493.58)

Each value is the mean of the five replications
Figures in parenthesis – Percentage over to control
All the values are significant at $P < 0.01$

CD at 5%	1.23	1.46	1.23	0.88	1.23	1.61	1.69	0.89	1.23	1.33
CD at 1%	1.65	1.95	1.64	1.18	1.64	2.15	2.26	1.19	1.64	1.78

Fig. 2: Effect of copper on nutrient (nitrogen, phosphorus, potassium, sodium and calcium) contents of the leaves

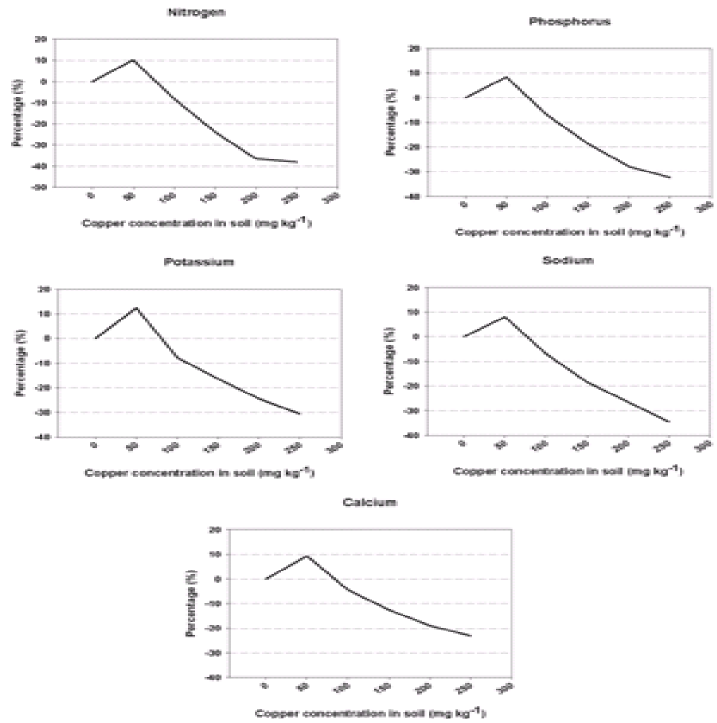
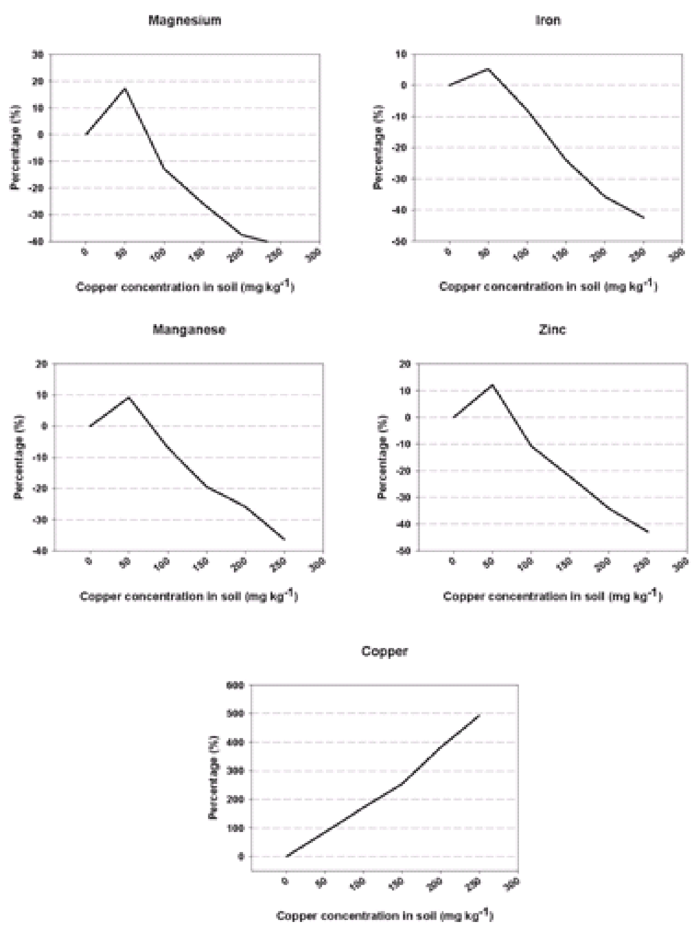


Fig. 3: Effect of copper on nutrient (magnesium, iron, manganese, zinc and copper) contents of the leaves of green gram



Excess of copper resulted in lowering of phosphorus content in greengram and revealed a close relationship between phosphorus and copper. Phosphorus deficiency can be induced when copper is raised from 0.16 to 3.1 µM in maize roots and shoots (Lexmond and Van der Vorm, 1981). High concentration of copper suppresses phosphorus metabolism by lowering the content of inorganic phosphorus. This suggests a negative correlation between copper and phosphorus (Wallace and Cha, 1989; Mateos-Naranjo *et al*., 2008). The decreased content of phosphorus in greengram due to copper treatment could be attributed to P-Cu interaction mechanism. The decrease in potassium content of greengram due to elevated level of copper is in conformity with the reports of Lidon and Henriques (1993) and Ouzounidou (1994). In addition, previous reports noticed that high concentration of cadmium inhibited the uptake of potassium by inhibiting respiratory rates, ATP levels and ATPase (Lindberg and Wingstrand 1985; Veselov *et al*., 2003). The decrease in potassium content of greengram due to copper may be attributed to the toxic effect of copper on plant growth or competition by other ions, which in turn exercised a regulatory control on potassium uptake.

The reduction of sodium content in greengram at higher copper concentrations was in close confirmity with the findings of Moral *et al*., (1994) who suggested that the application of cadmium reduced the uptake of sodium. The decrease in sodium content as a result of heavy metal treatment might be a consequence of deterioration in the physiological state of the plant, which in turn resulted in a reduction in its uptake. Reduction of calcium content of greengram due to high levels of copper observed in the present study confirms the earlier reports (Lidon and Henriques, 1993; Gussarsson, 1994; Mateos-Naranjo, 2008). Similarly, Ouzounidou (1994) observed a sharp decline in calcium content in the roots and shoots of *Alyssum montanum*, when copper was applied in higher concentration. These changes are in consonance with the earlier reports that copper ions tend to displace Ca^{++} ions from exchange sites and are strongly bound in root free space (Jensen and Adalsteinsson, 1989; Mateos-Naranjo *et al*., 2008).

Decline in the magnesium content of greengram at high concentration of copper was in agreement with the previous reports that copper toxicity induced deficiency of magnesium (Ouzounidou, 1994; Lequeux *et al*., 2010). A decreased macronutrient content in the greengram leaves under high copper levels observed in the present study justifies the above statements.

Micronutrient content

Result on the effect of copper on micronutrient (Fe, Mn and Zn) content of greengram leaves is furnished in the Table 2 and Fig.3. Increased copper content of soil significantly decreased the micronutrient content (Fe, Mn and Zn) of greengram leaves. However, low level of copper (50 mg kg^{-1}) increased the Fe, Mn and Zn content of greengram plants over the control. The response of excess copper has frequently been attributed to an interference with iron metabolism (Yau *et al*., 1991; Ouzounidou *et al*., 1995). Earlier reports state that the interference of heavy metals in excess amount with normal iron metabolism was known to induce physiological iron deficiency (Kim *et al*., 1978; Gonçalves *et al*., 2009). The decrease in iron content of greengram with increase in copper level suggested a heavy metal induced iron deficiency. Excess copper induced manganese deficiency has been reported by Lidon and Henriques (1992). Zinc (Ruano *et al*., 1988; Wang *et al.,* 2009) and cadmium (Moral *et al*., 1994; Jiang *et al*., 2004) application also significantly decreased the manganese content of plants. Decrease in manganese content may be due to the competition of copper with manganese for transport sites in plasmalemma. Excess copper resulted in lowering of Zn content in greengram. The results are in close conformity with the findings that an elevated copper application decreased the zinc content (Lidon and Henriques, 1993; Luo and Rimmer, 1995). Aluminium (Smalley *et al*., 1993; Vitorello *et al*., 2005) and cadmium (Jalil *et al*., 1994; Gonçalves *et al*., 2009) application also significantly decreased the content of zinc, mainly because of the significant decrease in plant biomass.

Uptake and accumulation of copper

Maximum copper accumulation in the greengram leaves was recorded at 250 mg kg^{-1} level in the soil. The minimum copper accumulation of greengram leaves was observed in control. Increase in copper level in the soil resulted in the higher uptake and accumulation of copper by the plants. Ouzounidou (1994) made similar observations that uptake of copper increased with the increased application of copper in *Alyssum montanum* L. This view is also supported by Mocquot *et al*., (1996) and Cambrolle *et al*., 2011.

4. Conclusion

From these observations it can be concluded that, low copper concentration (50 mg kg^{-1}) had stimulatory effect on growth, dry matter yield and mineral nutrient content of greengram. Application beyond these levels (100-250 mg kg^{-1}) adversely affected the growth, dry matter yield and nutrient content.

References

Agarwala, S.C., Nautiyal, B.D., Chatterjee, C., and Nautiyal, N. 1995. Variations in copper and zinc supply influence growth and activities of some enzymes in maize. Soil Sci, Plant Nutr., 41: 329–335.

Ahsan, N., Lee, DG., Lee, SH., Kang, K.Y., Lee, J.J., Kim, P.J., Yoon, H.S., Kim, J.S. and Lee, B.H. 2007. Excess copper induced physiological and proteomic changes in germinating rice seeds. Chemosphere., 67: 1182–1193.

Angle, J.S. and Chaney, R.L. 1991. Heavy metal effects on soil population and heavy metal tolerance of *Rhizobium meliloti*, nodulation and growth of alfalfa. Water, Air, Soil Pollut., 57-58: 597–604.

Berglund, A.H., Mike, F., Quartacci, M.F., Calucci, L.C., Navari-Izzo, F., Pinzino, C. and Liljenberg, C. 2002. Alterations of wheat root plasma membrane lipid composition induced by copper stress result in changed physicochemical properties of plasma membrane lipid vesicles. Biochimica. et Biophysica. Acta. 1564: 466–472.

Bouazizi, H., Jouili, H., Geitmann, A. and El Ferjani, E. 2008. Effect of copper excess on H2O2 accumulation and peroxidase activities in bean roots. Acta. Biol. Hung., 59(2): 233–45.

Bouazizi, H., Jouili, H., Geitmann, A. and Ferjani, E.E.I. 2010. Copper toxicity in expanding leaves of *Phaseolus vulgaris* L.: antioxidant enzyme response and nutrient element uptake. Ecotox. Environ. Safe., 73: 1304–1308.

Cambrolle, J., Mateos-Naranjo, E., Redondo-Gomez, S., Luque, T. and Figueroa, M.E. 2011. Growth, reproductive and photosynthetic responses to copper in the yellow-horned poppy, *Glaucium flavum Crantz*. Environ. Exp. Bot. 71: 57–64.

Ernst, W.H.O., Schat H. and Verkleij, J.A.C. 1990. Evolutionary biology of metal resistance in *Silene vulgaris*. Evol. Trends Plants., 4: 45–51.

Ginn, T., Sengor, S.S., Barua, S., Moberly, J. and Peyton, B. 2006. Metal toxicity effects on microbial growth and degradation, in: Slovenia and U.S. Workshop on Environmental Science and Engineering, Ljubljana, Slovenia, pp. 39–40.

Gomez, K.A. and Gomez A.A. 1984. Statistical procedures for agricultural research, John Wiley and Sons, New York.

Gonçalves, J.F, Antes, F.G, Maldaner, J., Pereira, L.B., Tabaldi, L.A., Rauber, R., Rossato, L.V., Bisognin, D.A., Dressler, V.L., de Moraes Flores, E.M. and Nicoloso, F.T. 2009. Cadmium and mineral nutrient accumulation in potato plantlets grown under cadmium stress in two different experimental culture conditions. Plant Physiol. Biochem. 47: 814–821.

Gussarsson, M. 1994. Cadmium induced alterations in nutrient composition and growth of *Betula pendula* seedling; The significance of fine roots as a primary target for cadmium toxicity. J. Plant Nutr.,17(12): 2151–2163.

Hansch, R. and Mendel, R.R. 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe,Ni, Mo, B, Cl). Curr. Opin. Plant. Biol. 12: 259–266.

Harrison, M.D., Jones, C.E. and Dameron, C.T. 1999. Copper chaperones: function structure and copper-binding properties. JBIC., 4: 145–153.

Jain, A., Sood, I.S. and Sharma, K.K. 1994. Root nodulation in the presence of heavy metals in *Vigna unguiculata*. Poll. Res., 13(3): 233–236.

Jalil, A., Selles, F. and Clarke, J.M. 1994. Effect of cadmium on growth and the uptake of cadmium and other elements by d*urum* wheat. J. Plant Nutr., 17(11): 1839–1858.

Jensen, P. and Adalsteinsson S. 1989. Effects of copper on active and passive Rb^+ influx in roots of winter wheat. Physiol. Plant., 75: 195–200.

Jiang, X.J., Luo, Y.M., Liu, Q., Liu, S.L. and Zhao, Q.G. 2004. Effects of cadmium on nutrient uptake and translocation by Indian Mustard. Environ. Geochem. Health., 26(2-3): 319–324.

Kim, B.Y., Kim K.S., Kim B.J. and Han K.M. 1978. Uptake and yield of heavy metal Cu, Ni, Cr, Co and Mn. Rep. Off. Rural Dev., 1–10.

Kupper, H., Kupper F. and Spiller M. 1996. Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants. J. Exp. Bot., 47: 259–266.

Lequeux, H., Hermans, C., Lutts, S. and Verbruggen, N. 2010. Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol. Biochem. 48: 673–682.

Lexmond, T.M. and Van der Vorm P.D.J. 1981. The effect of pH on copper toxicity to hydroponicaly grown maize. Neth. J. Agric. Sci., 29: 217–238.

Lidon, F.C. and Henriques F.S. 1991. Effects of copper on the ascorbate, diamine and O-diphenol oxidases activities of rice leaves. *Oyton-Inter*. J. Bot., 52: 97–104.

Lidon, F.C. and Henriques F.S. 1992. Copper toxicity in rice; a diagnostic criteria and its effect on Mn and Fe contents. Soil Sci., 154(2): 130–135.

Lidon, F.C. and Henriques F.S. 1993. Effect of copper toxicity on growth and the uptake and translocation of metals in rice plants. J. Plant Nutr., 16(8): 1449–1464.

Lindberg, S. and Wingstrand G. 1985. Mechanism of Cd^{+2} inhibition of (K^+ + Mg^{+2}) ATPase activity and K^+ ($^{86}Rb^+$) uptake in roots of sugar beets (*Beta vulgaris*). Physiol. Plant., 63: 181–186.

Lolkema, P.C. and Vooijs, R. 1986. Copper tolerance in *Silene cucubalus*: Subcellular distribution of copper and its effects on chloroplast and plastocyanin synthesis. Planta., 167: 30–36.

Luo, Y. and Rimmer D.L. 1995. Zinc-copper interaction affecting plant growth on a metal contaminated soil. Environ. Pollut., 88: 79–83.

Madhavi, A. and Rao A.P. 1999. Effect of cadmium on plant growth and uptake of nutrients by fodder sorghum, greengram and lucerne. J. Res. ANGRAU., 27(3): 15–23.

Masaka, J. and Muunganirwa, M. 2007. The effects of copper oxy chloride waste contamination on selected soil biochemical properties at disposal site. Sci. Total Environ. 387: 228–236.

Mateos-Naranjo, E., Redondo-Gomez, S., Cambrolle, J. and Figueroa, M.E. 2008. Growth and photosynthetic responses to copper stress of an invasive cordgrass, *Spartina densiflora*. Mar. Environ. Res. 66: 459–465.

Mc Ilveen, W.D. and Coole H.J.R. 1974. Influence of heavy metals on nodulation of red clover. Phytopath., 64: 583.

Mocquot, B., Vangronsveld, J., Clijsters, H. and Mench M. 1996. Copper toxicity in young maize (*Zea mays* L.) plants: Effects on growth, mineral and chlorophyll contents and enzyme activities. Plant Soil. 182: 287–300.

Moral, R., Gomez I., Pedreno J.N. and Mataix J. 1994. Effect of cadmium on nutrient distribution, yield and growth of tomato grown in soil less culture. J. Plant Nutr., 17(6): 953–963.

Moustakas, M., Ouzounidou G., Symeonidis L. and Karataglis S. 1997. Field study of the effects of excess copper on wheat photosynthesis and productivity. Soil Sci. Plant Nutr., 43(3): 531–539.

Ouzounidou, G. 1994. Copper-induced changes on growth, metal content and photosynthetic function of *Alyssum montanum* L. plants. Environ. Exp. Bot., 34(2): 165–172.

Ouzounidou, G., Eleftheriou E.P. and Karataglis S. 1992. Ecophysiological and ultrastructural effects of copper in *Thlaspi ochroleucum* (*Curciferae*). Can. J. Bot., 70: 947–957.

Ouzounidou, G., Ciamporova M., Moustakas M. and Karataglis S. 1995. Responses of maize (*Zeamays* L.) plants to copper stress I. Growth, mineral content and ultrastructure of roots. Environ. Exp. Bot., 35: 167–176.

Reichman, S.M. 2002. The responses of plant to metal toxicity: A review of focusing on copper, manganese and zinc. Australian Minerals and Energy Environment Foundation; Melbourne, Australia, pp. 7.

Ruano, A., Poschenrieder Ch. and Barcelo J. 1988. Growth and biomass partitioning in zinc-toxic bush beans. J. Plant Nutr., 11: 577–588.

Samarakoon, A.B. and Rauser W.E. 1979. Carbohydrate levels and photoassimilate export from leaves of *Phaseolus vulgaris* exposed to excess cobalt, nickel and zinc. Plant Physiol., 63: 1162–1169.

Sawhney, V., Sheoran I.S. and Singh R. 1990. Nitrogen fixation, photosynthesis and enzymes of ammonia assimilation and ureide biogenesis in nodules of mungbean (*Vigna radiata*) grown in presence of cadmium. Ind. J. Exp. Biol., 28: 883–886.

Seliga, H. 1993. The role of copper in Nitrogen fixation in *Lupinus luteus* L. Plant Soil., 155/156: 349–352.

Smalley, T.J., Lasseigne F.T, Mills H.A. and Hussey G.G. 1993. Effect of aluminium on growth and chemical composition of marigolds. J. Plant Nutr., 16(8): 1375–1384.

Sonmez, S., Kaplan, M., Sonmez, N.K., Kaya, H. and Ilker, U.Z. 2006. High level of copper application to soil and leaves reduce the growth and yield of tomato plants. Sci. Agric. (Piracicaba, Braz.), 63(3): 213–218.

Tyler, G. 1981. Heavy metals in soil biology and biochemistry. In: A.E. Paul and J.N. Ladd. (eds.). Soil Biochemistry, Marcel Dekker, New York, 5: 371–414.

Upadhyay, R.K. and Panda, S.K. 2009. Copper-induced growth inhibition, oxidative stress and ultrastructural alterations in freshly grown water lettuce (*Pistia stratiotes* L.). C. R. Biologies., 332: 623–632.

Ureta, A., Imperial, J., Ruiz-Argueso, T. and Palacios, J.M. 2005. *Rhizobium leguminosarum Biovar viciae* symbiotic hydrogenase activity and processing are limited by the level of nickel in agricultural soils. Appl. Environ. Microb., 71(11): 7603–7606.

Veselov, D., Kudoyarova, G., Symonyan, M., Veselov, St. 2003. Effect of cadmium on ion uptake, transpiration and cytokinin content in wheat seedlings. Bulg. J. Plant Physiol., 353–359.

Vijayarengan, P. and Lakshmanachary A.S. 1995. Effects of nickel on growth and dry mater yield of greengram cultivars. Ind. J Environ. Hlth., 37(2): 99–106.

Vitorello, V.A., Capaldi, F.R. and Stefanuto, V.A. 2005. Recent advances in aluminum toxicity and resistance in higher plants. Braz. J. Plant Physiol., 17: 129–143.

Wallace, A. and Cha J.W. 1989. Interactions involving copper toxicity and phosphorus deficiency in bush bean plants grown in solutions of low and high pH. Soil Science. 147: 430–435.

Wang, C., Zhang, S.H., Wang, P.F, Hou, J., Zhang, W.J., Li, W. and Lin, Z.P. 2009. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. Chemosphere., 75(11): 1468–1476.

Wani, P.A., Khan, M.S., Zaidi, A. 2007. Cadmium, chromium and copper in greengram plants. Agron. Sustain. Dev. 27: 145–153.

Woolhouse, H.W. 1983. Toxicity and tolerance in the responses of plants to metals. In: O.L. Lange, P.S. Nobel, C.B. Osmond and H. Zeigler (eds.), Physiological Plant Ecology III.

Responses to the chemical and biological environment. A.P. Gottingen and Harvard M.H.Z. (eds.), Encyclopedia of Plant Physiology, N.S. Vol. 12C, pp. 245–300, Springer Verlog, Berlin.

Xiong, Z., Liu, C. and Geng B. 2006. Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis Rupr*. Ecotox. Environ. Safe., 64: 273–280.

Yau, P.Y., Loh C.F. and Azmil I.A.R. 1991. Copper toxicity of clove [*Syzygium aromaticum* (L.) Merr. and Perry] seedlings. Mardi. Res. J., 19: 49–53.

Zheng, Y.B, Wang, L.P. and Dixon, M.A. 2004. Response to copper toxicity for three ornamental crops in solution culture. Hortscience., 39: 1116–1120.