International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs **Coden: IJARQG(USA)** **Volume 4, Issue 4 - 2017**

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.04.012

Changes in nutrients content of Radish (*Raphanus sativus*) under copper toxicity.

P.Vijayarengan* and M.Christhu Uthayam
Department of Botany, Annamalai University, Annamalai nagar - 608 002, Tamilnadu, India.
*Corresponding author: *drpvrengan@yahoo.com/axena30@gmail.com*

Abstract

In this experiment, we tried to explore the changes that are occurring in radish due to the treatment with copper. A pot culture experiment was conducted and copper was applied to radish (*Raphanus sativus*) plant. Macronutrients such as, nitrogen, phosphorus, potassium, sodium, calcium, magnesium were analysed for the treated plants. The results showed that the low concentrations of copper level (50 mg kg^{-1}) enhanced the macronutrients while higher concentration of copper(100 - 250 mg kg^{-1}) in the soil decreased these parameters.

Keywords: *Raphanus sativus*, nitrogen, phosphorus, potassium, sodium, calcium, magnesium.

Introduction

Heavy metals are very important environmental pollutants. Many of them are toxic even in low concentration. Pollution of soil and water with heavy metals creates serious problems for the environment and human health. Unlike many other pollutants heavy metals persists in ecosystems because they cannot be destroyed biologically.

Heavy metals such as Cu, Fe, Mn and Zn are essential minerals nutrients for higher plants. Copper which is relatively mild character is highly toxic to plants even at a micro molar range of exposers (Cabral, 2003). Heavy metals such as Cu and Zn are essential for normal plant growth and development since they are constituents of many enzymes and other proteins. However, elevated levels of both essential and non-essential heavy metals in the soil can leads to toxicity symptoms and growth inhibition in most plants (Hall, 2002). Among the myriad of heavy metals copper occupies the prominent position, since it plays a vital role in the growth and development of plants. Keeping these points in view the present investigation has been made to study the effect of copper on the nutrient status of radish.

Materials and Methods

The experimental plant, the radish belongs to the family Cruciferae (Brassicaceae) and is one of the important vegetables of the world. Certified seeds of radish (Cultivar Pusa Chetki) were obtained from TNAU, Coimbatore. Seeds with uniform size and weight were chosen for experimental purpose.

Pot culture experiments

Radish plants were grown in pots in untreated soil (control) and in soil to which copper had been applied (50, 100, 150, 200 and 250 mg kg^{-1} soil). The inner surfaces of pots were lined with a polythene sheet.

Each pot contained 6 kg of air dried soil. The copper as finely powdered ($CuSO_4 \cdot 7H_2O$) was applied to the surface soil and thoroughly mixed with the soil. Fifteen seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of five per pot, after a week of germination. Each treatment including the control was replicated seven times.

Sample collection

The plant samples were collected on 45th day for the measurement of macronutrient contents. Five plants from each replicate of a pot was analysed for its various nutrients. Nitrogen, phosphorus, potassium, sodium, calcium, magnesium, in plant materials were estimated by the following methods.

(i) Estimation of total nitrogen (Peach and Tracey, 1956)

Digestion

Hundred milligram of dried materials were taken in the Kjeldahl flasks and 5 ml of salicylic – sulphuric acid mixture (5 g salicylic acid in 100 ml concentrated sulphuric acid) was added. The flask was, rotated to mix and allowed to stand for 30 min. Approximately 0.3 g sodium thiosulphate was added and heated gently until fumes appeared. Then 5 ml of concentrated sulphuric acid and approximately 0.1 g of catalyst mixture (copper sulphate, potassium sulphate and selenium dioxide mixed in the ratio of 1:8:1) were added. Digestion was performed at low heat until frothing stopped and fumes of sulphuric acid were freely evolved. After 5-10 min heat was increased, so that the acid is boiled and condensed one third way up the neck of the flask. Digestion was continued for at least 3 h, till the digest has become colourless. On completion of digestion, the flask was cooled and 20 ml of water was added. The flask was again cooled and the content was transferred into a 50 ml volumetric flask and made up to the volume.

Distillation

Distilled water was boiled in the flask and clips were kept closed and opened respectively when steam passed through the funnel and the funnel was washed twice with 1 ml of water each time. The ground glass stopper was replaced and 8 ml of 40 per cent sodium hydroxide was added in funnel. The lower end of the condenser was kept dipped into 5 ml of 2 per cent boric acid and few drops of mixed indicator (6 ml methyl red solution (0.16 per cent in 95 per cent alcohol) and 12 ml bromocresol green (0.04 per cent in water) were mixed and 6 ml of 95 per cent alcohol was added to the mixture) contained in a 50 ml conical flask. When steam issued freely through the tube, the clip was closed and the ground glass stopper was lifted to allow sodium hydroxide to run into the digest. The stopper was immediately replaced and distillation was continued until 30 ml distillate had been collected. After a few ml of liquid had distilled over, the end of the condenser was raised above the level of boric acid. Heating was stopped after distillation was completed, so that the liquid in the distillation chamber was automatically sucked into the jacket. A few ml of water was added through the funnel and the stopper was replaced.

This liquid in the jacket was allowed to run as waste by opening the clip. Now the apparatus was ready for the next distillation. Then the whole distillate was titrated against standard 1/28 hydrochloric acids solution until the pink colour just reappear. Blank digestion, distillation and titration, were made using all the reagents without plant sample. The percentage of total nitrogen was calculated by the following formula.

Percentage of nitrogen = $(T\text{-}B) \times 5 \times N \times 1.4/S$

where,
T = Sample titrated (ml)
B = Blank titrated (ml)
N = Normality of hydrochloric acid (1/28 = 0.0357142)
S = Weight of plant material (g)
Aliquot factor = 5

(ii) Estimation of phosphorus (Black, 1965; quoted by Yoshida *et al.*, 1972)

One gram of dried and ground plant tissue was digested with 10 ml of acid mixture (nitric acid, 750 ml; sulphuric acid, 150 ml; perchloric acid 60 per cent, 300 ml). The digest was cooled and made up to 50 ml and filtered through acid washed Whatmann No.1 filter paper. One ml of digest was mixed with 2 ml of 2 N nitric acid and diluted to 8 ml. One ml of molybdovanadate reagent (25 g of ammonium molybdate in 500 ml water, 1.25 g ammonium vanadate in 500 ml of 1 N nitric acid; both were mixed in equal volumes) was added, make up to 10 ml, shacked and the absorbance was measured at 420 nm in a spectrophotometer, after 20 min of standing. Standard graph was prepared using potassium dihydrogen phosphate.

(iii) Estimation of potassium and sodium (Williams and Twine, 1960)

Dried and ground tissues weighing 0.5 g were digested in 100 ml Kjeldahl flasks using 15 ml of concentrated nitric acid, 0.5 ml of 60 per cent perchloric acid and 0.5 ml of concentrated sulphuric acid. Digestion was continued until the nitric and perchloric acids were driven-off. The inorganic residue was cooled and diluted with 15 ml of distilled water and filtered through Whatmann No.42 filter paper. The filtrate was made up to 50 ml with distilled water. The filtrate was used for potassium and sodium estimation by flame photometer and standards were prepared with potassium chloride and sodium chloride.

(iv) Estimation of calcium and magnesium (Yoshida *et al.*, 1972)

Two ml of the filtrate was mixed with 2 ml of 5 per cent lanthanum chloride solution and diluted with 10 ml of 1 N hydrochloric acid. The solution was fed into an atomic absorption spectrophotometer at 211.9 nm for calcium and 285.4 nm for magnesium. Standard curves were prepared by using calcium chloride and magnesium chloride.

Results

Macronutrients (mg g^{-1} dry wt.)

Nitrogen

Nitrogen content of radish leaves is presented in Table 1. Maximum nitrogen content of radish leaves was recorded at 50 mg kg^{-1} copper level (37.35). Minimum nitrogen content of radish was observed at 250 mg kg^{-1} copper level (18.07).

Phosphorus

Phosphorus content of radish plants is represented in Table 1. Results of phosphorus content showed the higher values at 50 mg kg^{-1} copper level (6.97). The lowest phosphorus content of radish plant (3.90) was recorded at 250 mg kg^{-1} soil level.

Table 1. Effect of copper on nutrients content (mg g^{-1} dry wt.) of radish (45th day)

Copper added in the soil (mg kg^{-1})	N	P	K	Na	Ca	Mg
Control	32.66	6.31	43.52	1.70	13.56	3.99
50	37.35 (+14.36)	6.97 (+10.45)	52.09 (+19.69)	2.11 (+24.11)	15.98 (+17.84)	4.91 (+23.05)
100	25.28 (-22.59)	5.71 (-9.50)	36.78 (-15.48)	1.39 (-18.23)	12.25 (-9.66)	3.48 (-12.78)
150	23.73 (-27.34)	5.06 (-19.80)	32.17 (-26.07)	1.28 (-24.70)	11.14 (-17.84)	3.05 (-23.55)
200	20.98 (-35.76)	4.81 (-23.77)	30.37 (-30.21)	1.16 (-31.76)	11.30 (-19.05)	2.75 (-31.07)
250	18.07 (-44.67)	3.90 (-38.19)	25.11 (-42.30)	1.10 (-35.29)	10.68 (-21.23)	2.11 (-47.11)

Average of five replications
Per cent over control values are given in parentheses

Potassium

A result on the potassium content of radish plants is compiled in Table1. Potassium content of radish plants was high at 50 mg kg^{-1} (52.09) copper level. 250 mg kg^{-1} copper level of radish (25.11) showed the minimum potassium content.

Sodium

Sodium content of radish plants under copper stress is represented in Table 1. Maximum sodium content of radish was reported at 50 mg kg^{-1} copper level (2.11). Minimum sodium content of radish (1.10) was noticed at 250 mg kg^{-1}.

Calcium

Calcium content of radish plants is presented in Table 1. Calcium content of radish leaves was higher at 50 mg kg^{-1} copper level (15.98). 250 mg kg^{-1} plants of radish exhibited minimum calcium content (10.68).

Magnesium

Magnesium content of radish levels under copper stress is presented in Table 1. Maximum magnesium content of radish leaves (4.91) was recorded at 50 mg kg^{-1} copper level. Minimum magnesium content of radish (2.11) was noticed at 250 mg kg^{-1} copper level.

Discussion

Nitrogen

The nitrogen content of radish showed a progressive decline with increase in copper level. However 50 mg kg-^{1} copper level produced positive effect on nitrogen content of the radish. A well developed root system enhanced the uptake of nitrogen by plants. The reduction in nitrogen content under high copper treatments was comparable with the result of Lidon and Henriques (1993a; 1993b) under copper treatment and Stoyanova and Doncheva (2002) and Vijayarengan (2005) under nickel treatment.

Phosphorus

Phosphorus content of radish plants decreased with an increase in the copper content except 50 mg kg^{-1} in the soil. This is in accordance with the earlier reports of Bonnet *et al.* (2000) in rye grass and Stoyanova and Doncheva (2002) in pea under zinc treatment. The increased content of copper and decreased content of phosphorus in radish due to copper treatment could be attributed to P-Zn interaction mechanism (Mehra, 2003). Excess amount of trace elements usually affect the mineral nutrition of plants (Bollard, 1983). Metal toxicity may affect certain elements more than others and interaction among elements may occur (Davis *et al.*, 1978). Here the phosphorus content of radish plants decreased by copper stress.

Potassium

Copper level above 50 mg kg^{-1} significantly reduced the potassium content in radish plants. Many investigators have reported that heavy metals toxicity in general was associated with reduced absorption and accumulation of potassium (Clark *et al.*, 1981; Siddiqi and Glass, 1983; Lidon and Henriques, 1993a; Vijayarengan (2005). Potassium is one of the essential macro nutrients, taken up by the roots are generally transported to shoots through the xylem and this transport seems to be controlled by the shoot growth (Pitman and Cram, 1997). Decrease in potassium content of radish due 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 mineral nutritional status of plants can strongly affect the ‘‘quality’’ of crop plants and its organs. If potassium is inadequate, the synthesis of proteins is inhibited (Marschner, 1986). The decreased potassium and protein content in radish plants under copper stress in the present study justified the above statement.

Sodium

Sodium content of radish plant decreased with progressive increase in the copper level. However low level of copper (50 mg kg^{-1}) increased the sodium content. Similar decreases in sodium content with concomitant increase of heavy metals were also reported by Lidon and Henriques (1993a) due to copper and Moral *et al.* (1994) due to cadmium. The decrease in sodium concentration as a result of increase in the metal treatment may have been a consequence of deterioration in the physiological state of the plant, which in turn producing a reduction in its uptake.

Calcium

Calcium content of radish plants decreased with increase in copper level in the soil. However 50 mg kg^{-1} of copper treated plants showed higher calcium concentration. These results are in agreement with the results of Bonnet *et al.* (2000) and Stoyanova and Doncheva (2002) in zinc. The decrease in calcium content in excess of these metals might be due to antagonistic action of calcium with zinc. Zn: Ca interactions have been shown for bread and durum wheat cultivars (Hart *et al.,* 1998). Copper uptake in these plants was inhibited dramatically by Ca, which suggested Cu and Ca do not share a common transport mechanism. On the other hand, the decrease in the concentrations of Ca in response to higher Cu concentration was probably a result of osmotic adjustment.

Magnesium

Magnesium content of radish decreased with an increase in the copper level except 50 mg kg^{-1} of copper level in the soil. This is accordance with the earlier reports of Lidon and Henriques (1993a) and Ouzounidou (1994) under copper treatment, Dinev and Stancheva (1993) under aluminium treatment and Gussarsson (1994) under cadmium treatment. The decreased magnesium content due to copper treatment could be the replacement of magnesium in the chlorophyll by these metals (Agarwal *et al.,* 1987).

References

Agarwal, K., A. Sharma and G. Talukder, 1987. Copper toxicity in plant cellular systems. *Nucleus,* 30**:** 131-158.

Black, C.A., 1965. Method for soil analysis. Part II. Chemical and microbiological properties. Amer. Soc. Agron. Inc., Madison, Wisconcin, pp. 242.

Bollard, E.G., 1983. Involvement of unusual elements in plant growth and nutrition. *In:* Lauchli, A. and R.L. Bieleski (eds.), Inorganic plant nutrition, Encyclopedia of plant physiology, New Series, Vol.15 B, Springer Verlag, Berlin, pp. 695-744.

Bonnet, D., O. Camares and P. Veisseire, 2000. Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll ‘a’ fluorescence and antioxidant enzyme activities of rye grass (*Lolium perenne* L. cv. Apollo). *J. Exp. Bot.,* 51: 945-953.

Cabral, J.P., 2003. Copper toxicity in five Parmelia lichens *in vitro*. *Environ. Exp. Bot.,* 49: 237-250.

Clark, R.B.M.P., A. Piper, K. Knudesen and J.W. Maeanville, 1981. Effect of trace elements deficiencies and excesses on mineral nutrients in sorghum. *J. Plant Nutr.,* 3: 367-374.

Davis, R.D., P.H.T. Beckett and E. Wollan, 1978. Critical levels of twenty elements in young spring barley. *Plant Soil*, 49: 395-408.

Dinev, N. and I. Stancheva, 1993. Effect of aluminium on growth of wheat, rye and triticale. *J. Plant Nutr.,* 16: 461-467.

Gussarsson, M., 1994. Cadmium induced alternation in nutrient composition and growth of *Betula pendula* seedlings. The significance of the roots as primary target for cadmium toxicity. *J. Plant Nutr.,* 17: 2151-2163.

Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.,* 53: 1-11.

Hart, J.J., W.A. Norwel, R.M. Welch, L.A. Sullivan and L.V. Kochian, 1998. Characterization of intact seedlings of bread and durum wheat cultivars. *Plant Physiol.,* 118: 219-226.

Lidon, F.C. and F.S. Henriques, 1993a. Effects of copper toxicity on growth and the uptake and translocation of metals in rice plants. *J. Plant Nutr.,* 16: 1449-1464.

Lidon, F.C. and F.S. Henriques, 1993b. Copper mediated inhibition on protein synthesis in rice shoots. *J. Plant Nutr.,* 16: 1619-1630.

Marschner, H., 1986. Mineral nutrition of higher plants. 2nd ed. Academic press, New York.

Mehra, R.K., 2003. Effect of application of zinc and phosphorus on the yield and nutrient uptake (N, P, Zn) by wheat (*Triticum aestivum* L.). *Indian J. Agri. Chem.,* 36: 1-2.

Moral, R., I. Gomez, J.N. Perdreno and J. Matrix, 1994. Effects of Cd on nutrient distribution, yield and growth of tomato grown in soils culture. *J. Plant Nutr.,* 17: 953-962.

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

Peach, K. and M.V. Tracey, 1956. Modern methods of plant analysis, I: Springer Verlog, Berlin, p. 477.

Pitman, M.G. and W.J. Cram, 1997. Regulation of ion content in whole plants. In. D.H. Jennings (ed.). Integration of activity in the higher plant. *Symp. Soc. Exp. Biol.,* 31: 391-424

Siddiqi, Y.M. and A.D.M. Glass, 1983. Studies on growth and mineral nutrition of barley varieties. II Potassium uptake and its regulation. *Can. J. Bot.,* 61: 1551-1558.

Stoyanova, Z. and S. Doncheva, 2002. The effect of zinc supply and succinate treatment on growth and mineral uptake in pea plant. *Braz. J. Plant Physiol.*, 14: 1-9.

Vijayarengan, P., 2005. Nitrogen and potassium status of greengram (*Vigna radiata*) cultivars under nickel stress. *Nature Environ. Poll. Tech.*, 4: 65-69.

Williams,C.H. and V. Twine, 1960. *In*: Modern Methods of Plant Analysis (Ed). K. Peach and M.V. Tracy. Vol. V. Springer Verlog, Berlin, pp. 3-5.

Yoshida,S., D.A.Forno, J.Cock and K.A.Gomez, 1972. Laboratory Manual for Physiological Studies of Rice, IRRI, Philippines.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Agricultural Sciences
DOI:10.22192/ijarbs.2017.04.04.012	

How to cite this article:
P.Vijayarengan and M.Christhu Uthayam. (2017). Changes in nutrients content of Radish (*Raphanus sativus*) under copper toxicity. Int. J. Adv. Res. Biol. Sci. 4(4): 88-93.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.04.012