J. Agr. Sci. Tech. (2013) Vol. 15: 1553-1564

Reduction in Metal Toxicity by Applying Different Soil Amendments in Agricultural Field and Its Consequent Effects on Characteristics of Radish Plants (*Raphanus sativus* L.)

A. Singh[1], and M. Agrawal[1*]

ABSTRACT

***Raphanus sativus* L. (radish) plants were grown in waste water irrigated area of Dinapur, a suburban area in the north east of Varanasi, India. Soil and plant characteristics were compared between fertilized (farmyard manure (FYM), NPK, and FYM+N) and non-amended control treatments. Compared to the control, plants grown in FYM and FYM+N amended soil showed a decrease in concentrations of heavy metals by 14 to 32% and 14 to 17%, respectively, with corresponding yield increments of 60 and 49%. Plants grown under NPK amendment showed high availability of the heavy metals, which, consequently, induced the activities of antioxidant enzymes and reduced photosynthetic rate, growth, and yield of the plants. Therefore, application of a particular dose of FYM alone and in combination with N at regular intervals may be recommended as a cheap technique for reducing the availability of heavy metals in metal contaminated fields.**

Keywords: Amendment, Contamination, Heavy metals, Organic and inorganic fertilizer, Remediation.

INTRODUCTION

Due to increase in population, economic development, urbanization, and industrialization there is increased generation of solid waste and waste water. These are the major sources of heavy metal contamination (Singh *et al*., 2011). Heavy metals pose a critical concern to human health and environment (Handique and Handique, 2009). There is a need to remediate such areas to reduce the risk of food chain contamination. *In situ* immobilization techniques are particularly beneficial compared to the conventional techniques (Zeng *et al*., 2010). Among different techniques, application of organic and inorganic fertilizers reduced the heavy metal availability to the plants (Singh *et al*., 2010). Continuous application of inorganic fertilizers leads to an imbalance in the nutritional properties of the soil, but in combination with organic fertilizers, can mitigate loss of nutrients due to modification of the physico-chemical properties of the soil (Mahmoud *et al*., 2009).

Heavy metals stimulate various changes in the plants such as the formation of reactive oxygen species (ROS). The free radicals have negative effects on the membrane structure, causing loss of ions, protein cleavage, enzyme inactivation and DNA strand breakage (Keunen *et al*., 2011). The growth characteristics of *Solanum melongena* and *Luffa cylindrica* were found to be reduced as a result of lead and chromium toxicity, respectively (Purohit *et al*., 2003; Jiang *et al*., 2010). There are complex non-enzymatic and enzymatic antioxidant systems in the plant to provide protection against ROS. Measurement of antioxidants as stress markers is an important aspect for assessing the stress responses in plants (Keunen *et al*., 2011).

Plants can mitigate the metal toxicity up to certain extent, but, at higher concentration it

[1] Ecology Research Laboratory, Department of Botany, Banaras Hindu University, Varanasi 221005, India.
* Corresponding author; e-mail: madhoo58@yahoo.com

becomes very toxic for plants. Therefore, in order to reduce the metal contamination in the plants, various techniques should be developed. The present study aimed at the same objective by adding different forms of organic and inorganic fertilizers to the soils with long term waste water irrigation to evaluate their efficiency in reducing the availability of metals in the soil and its effects on plant performance.

MATERIALS AND METHODS

Plant Material and Growth Condition

Radish (*Raphanus sativus* L.) was selected as an experimental plant because it is an economically important horticultural crop due to presence of ascorbic acid, folic acid, and potassium, vitamin B6, riboflavin, magnesium, copper, and calcium.

The experiment was conducted at Dinapur, a suburban area situated in the north east of Varanasi city, India. Treated and untreated waste water from Dinapur sewage treatment plant (DSTP) of 80 million liters per day capacity is utilized for irrigation at the experimental site for about 20 years as clean water availability is limited. There were 10 replicate plots for each amendment and each plot was 1×1 m^2 with a margin of 0.3 m. There were three amendments of fertilizers along with a control (C: Without any amendment). First amendment was farmyard manure (FYM) alone at the rate of 80 t ha^{-1}, the second was urea (N)+superphosphate (P)+potash (K), at the rates of 80:40:40 kg ha^{-1}, respectively, and the third was combination of FYM (80 t ha^{-1})+N (80 kg ha^{-1}).

Seeds of radish (*R. sativus* var. Pusa Reshmi) obtained from Institute of Vegetable Research, Varanasi, were sown at 2 cm depth in rows in each plot. After seed germination, plants were thinned to one plant per 15×15 cm^2. Waste water from DSTP was used for irrigating all the plots including control ones. Flood irrigation was followed and care was taken to provide the same amount of water to each plot to maintain similar moisture level. Table 1 shows the characteristics of irrigation water from DSTP. Manual weeding was done thrice depending upon the weed intensity during the experiment.

Data Collection

Samples of soil (10×10×15 cm^3) collected from plots of each treatment at the time of sowing and harvest were dried at room temperature, crushed, sieved with 2 mm mesh size sieve, and kept at room temperature for further analysis. For plant sampling, monoliths of 10×10×25 cm^3 containing intact roots were carefully dug out at random from each plot i.e. 10 samples of each amendment. Sampling of the plants was done at the time of harvest i.e. 40 days after germination (DAG). These were thoroughly washed by placing them on sieves of 2 mm mesh size under running tap water to remove soil particles adhering to the roots.

Soil Analyses

Soil pH was measured in suspension of 1:5 (soil: water w/v) using a glass electrode standardized with pH 4, 7 and 9.2 buffer

Table 1. Physico-chemical properties of irrigation waste water.

Physico-chemical properties	Value
pH	6.89
Conductivity (Ds m^{-1})	0.78
Total dissolved Solid (μg ml^{-1})	596
Dissolved oxygen (μg ml^{-1})	5.00
Biological oxygen Demand (μg ml^{-1})	65.55
Ammonical nitrogen (μg ml^{-1})	5.30
Nitrate nitrogen (μg ml^{-1})	15.7
Exchangeable sodium ion (μg ml^{-1})	281.5
Exchangeable potassium ion (μg ml^{-1})	193.7
Exchangeable calcium ion (μg ml^{-1})	303
Total Cadmium (μg ml^{-1})	0.04
Total Copper (μg ml^{-1})	0.053
Total Lead (μg ml^{-1})	0.043
Total Zinc (μg ml^{-1})	0.117
Total Manganese (μg ml^{-1})	0.077
Total Nickel (μg ml^{-1})	0.020
Total Chromium (μg ml^{-1})	0.050

tablets attached to an Ion analyzer (Model EA 940, Orion USA). The organic carbon content was determined by using modified Walkley and Black's rapid titration method (Allison, 1986).

The total nitrogen (TN) content was determined by following the micro- Kjeldahl technique through the Gerhardth automatic analyzer (Model KB8S, Kjeldatherm, Germany). Available phosphorus ($NaHCO_3$ extractable) was determined by the method given by Olsen *et al.* (1954). Exchangeable cations such as Na, K and Ca were extracted using ammonium acetate solution through repeated leaching technique (Jackson, 1958), and the contents were determined by atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

Digestion and Analysis for Heavy Metals

Phyto-available heavy metals in the soil samples were extracted by the method given by Quevauviller *et al.* (1997). For total heavy metal concentration, the oven dried and sieved samples of root (1 g), were digested by adding tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio) at 80°C until a transparent solution was obtained (Allen *et al.*, 1986). The concentrations of total metals in digested part of the plants and phytoavailable heavy metals in soil were determined by using atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

Physiological and Biochemical Characteristics

Portable Photosynthetic System (Model 6200, LICOR, Lincolin, NE, USA) was used for measuring physiological characteristics in leaves of three intact plants of each treatment at ambient climatic conditions. The system was calibrated using a known CO_2 source of 509 ppm concentration. These parameters were measured between 9.00 and 10.00 hours on cloud free days. During the measurements, photosynthetically active radiation ranged between 1,100 to 1,200 µmol m^{-2} s^{-1}. Measurement of chlorophyll fluorescence (initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence (F_v= F_m–F_o) and F_v/F_m ratio was done with the help of Plant Efficiency Analyzer (Handy PEA, Hansatech Instruments Ltd., Norfolk, UK) between 10.00 to 11.00 hours on the same foliage where photosynthetic rate was measured.

Fresh leaves (3 replicates) were sampled from each plot at 40 DAG for estimation of photosynthetic pigments, lipid peroxidation, and different metabolites. Ascorbic acid, protein and proline contents were determined by the methods described by Keller and Schwager (1977), Lowry *et al.* (1951) and Bates *et al.* (1973), respectively. Total phenol content was measured using the method of Bray and Thorpe (1954). The methods of Britton and Mehley (1955) and Fahey *et al.* (1978) were used for analyzing peroxidase activity and thiol content, respectively. The lipid peroxidation was measured as melondialdehyde (MDA) concentration (Heath and Packer, 1968).

Chlorophyll (Chl) and carotenoid contents were extracted with 80% acetone and the amounts were estimated spectrophotometrically by the method of Machlachlan and Zalick (1963) and Duxbury and Yentsch (1956), respectively.

Yield

At 40 DAG, 10 plants of each treatment were harvested and yield was calculated as fresh weight of the edible part (root) of the plant.

Statistical Analysis

The data of heavy metal concentrations in the plants under different amendments were subjected to ANOVA followed by Duncan's test. All the statistical tests were performed using SPSS software (SPSS Inc., version 12; Armonk, New York, United States).

RESLUTS AND DISCUSSION

Soil Characteristics

Soil pH is the most important factor responsible for affecting metal availability. Kim *et al*. (2009) have found that with increase in soil pH, bioavailability of Cd decreased. In the present study, initial soil samples did not show significant difference in pH (Table2), but at the time of harvest, pH was significantly higher in FYM amended soil as compared to the NPK, FYM+N amended and the control soil (Table 3). Zaller and Kopke (2004) also recorded an increase in pH due to addition of FYM in the soil.

The lower soil pH under NPK amendment may be ascribed to superphosphate, where principal P component is monocalcium phosphate (MCP), forming dicalcium phosphate (DCP) and phosphoric acid in the soil after amendment. The phosphoric acid subsequently dissociates into P and hydrogen ions, leading to lowering of soil pH in NPK amended soil (Bolan *et al*., 2003). In case of NPK fertilizer, the source of N is urea, therefore, this is also one of the reasons for reducing the soil pH and increasing the availability of metals. It was observed that application of some fertilizers, such as NH_4^+ fertilizers including urea, ammonium sulfate, and mono ammonium phosphate (MAP), can enhance Cd availability by lowering pH (Zaccheo *et al*., 2006). The acidification occurs as a result of NH_4^+ nutrition due to the release of protons (H^+) by root cells or nitrification of NH_4^+, and this increases the mobilization of metals (Sarwar *et al*., 2010).

In both initial and final soil samplings, highest organic 'C' and available 'P' were recorded for FYM and NPK amended soil, respectively (Tables 2 and 3). At the time of harvest, organic C increased by 11% in FYM and 9% in FYM+N amended soil, but decreased by 11% in NPK amended soil compared to the control (Table 3). Organic farming systems using FYM as fertilizers stimulate the soil biological activity, which consequently, increases the organic C content in the soil (Zaller and Kopke, 2004). Hati *et al*. (2007) have also found that long-term application of balanced fertilizer alone or in conjunction with FYM induces increase in organic carbon status of the soil. Available P was highest in NPK amended soil due to addition of superphosphate fertilizer. Tables 2 and 3 show that in both initial and final soil samples, exchangeable

Table 2. Physico-chemical properties of the field soil under different amendments at the time of sowing (Mean±SE).

Properties	Control ©	FYM	NPK	FYM+N
Soil pH	6.66^a ± 0.20	6.99^a ± 0.26	6.76^a ± 0.13	6.73^a ± 0.16
Organic C (%)	1.62^b ± 0.05	1.71^a ± 0.06	1.49^c ± 0.02	1.70^a ± 0.05
Total N (%)	0.23^a ± 0.01	0.20^a ± 0.02	0.29^a ± 0.02	0.22^a ± 0.02
Available P (µg g^{-1})	86.83^b ± 0.44	87.16^b ± 0.72	89.50^a ± 0.28	84.10^c± 0.23
Exchangeable Na^+ (µg g^{-1})	165.24^c ± 5.55	175.78^a ± 3.45	160.89^d ± 4.78	170.23^b ± 6.77
Exchangeable K^+ (µg g^{-1})	162.67^c ± 4.34	178.22^a ± 6.44	159.98^c ± 5.89	168.56^b ± 7.85
Exchangeable Ca^{+2} (µg g^{-1})	345.56^b ± 6.27	398.86^a ± 8.97	330.45^c ± 9.43	347.58^b ± 9.12
Phytoavailable Cd (µg g^{-1})	1.20^a± 0.21	1.23^a ± 0.15	1.22^a ± 0.16	1.24^a ± 0.18
Phytoavailable Cu (µg g^{-1})	5.39^a ± 0.16	5.20^b ± 0.02	5.33^a ± 0.07	5.17^b ± 0.08
Phytoavialable Pb (µg g^{-1})	7.15^a± 0.11	7.00^a ± 0.13	6.89^b ± 0.15	7.23^a ± 0.12
Phytoavailable Zn (µg g^{-1})	1.05^b ± 0.13	1.08^a ± 0.14	1.02^c ± 0.17	1.10^a ± 0.14
Phytoavailable Mn (µg g^{-1})	87.10^a ± 4.78	89.90^a ± 3.14	84.78^b ± 2.20	85.56^b ± 4.30
Phytoavailable Ni (µg g^{-1})	1.38^a ± 0.12	1.35^b ± 0.15	1.32^c ± 0.16	1.30^c ± 0.13
Phytoavailable Cr (µg g^{-1})	1.87^a ± 0.12	1.82^b ± 0.15	1.85^b ± 0.14	1.88^a ± 0.15

Table 3. Physico-chemical properties of the field soil under different amendments at the time of harvest (Mean±SE)[a].

Properties	Control ©	FYM[b]	NPK[c]	FYM+N
Soil pH	6.99[b] ± 0.04	7.08[a] ± 0.08	6.60[c] ± 0.05	6.93[b] ± 0.06
Organic C (%)	2.13[c] ± 0.06	2.67[b] ± 0.03	2.55[b] ± 0.17	2.97[a] ± 0.03
Total N (%)	0.26[a] ± 0.01	0.22[b] ± 0.02	0.29[a] ± 0.02	0.27[a] ± 0.02
Available P ($\mu g\ g^{-1}$)	73.73[b] ± 0.82	74.45[b] ± 0.29	76.62[a] ± 3.10	72.00[c] ± 0.68
Exchangeable Na^+ ($\mu g\ g^{-1}$)	170.37[c] ± 4.37	192.10[a] ± 5.44	170.20[c] ± 3.75	184.34[b] ± 22.34
Exchangeable K^+ ($\mu g\ g^{-1}$)	170.13[c] ±1 8.75	200.35[a] ± 7.92	167.22[d] ± 12.50	180.44[b] ± 22.32
Exchangeable Ca^{+2} ($\mu g\ g^{-1}$)	355.67[bc] ± 13.81	433.55[a] ± 8.75	365.47[b] ± 6.69	340.53[c] ± 15.96
Phytoavailable Cd ($\mu g\ g^{-1}$)	1.27[b] ± 0.01	1.08[d] ± 0.01	1.32[a] ± 0.01	1.15[c] ± 0.09
Phytoavailable Cu ($\mu g\ g^{-1}$)	6.37[b] ± 0.16	5.16[d] ± 0.01	6.61[a] ± 0.07	5.59 [c] ± 0.13
Phytoavailable Pb ($\mu g\ g^{-1}$)	8.07[b] ± 0.11	7.03[d] ± 0.01	8.57[a] ± 0.21	7.19[c] ± 0.06
Phytoavailable Zn ($\mu g\ g^{-1}$)	2.35[b] ± 0.03	2.16[c] 0.03	2.53[a] ± 10.08	2.18[c] ± 0.04
Phytoavailable Mn ($\mu g\ g^{-1}$)	115.16[b] ± 5.78	96.66[c] ± 6.52	123.78[a] ± 3.38	95.60[c] ± 0.11
Phytoavailable Ni ($\mu g\ g^{-1}$)	1.81[b] ± 0.02	1.48[d] ± 0.01	2.04[a] ± 0.01	1.78[c] ± 0.03
Phytoavailable Cr ($\mu g\ g^{-1}$)	2.03[a] ± 0.02	1.68[c] ± 0.01	2.09[a] ± 0.03	1.72[b] ± 0.08

[a] Different letters in each row showed significant difference at $P \leq 0.05$; [b] Farmyard manure, [c] Nitrogen, phosphorus and potassium.

cations (Na^+, K^+ and Ca^{2+}) were highest for FYM amended soil. As farmyard manure (FYM) contained higher levels of cations, hence, exchangeable cations were found highest in FYM amended soil compared to the control and other amendments (Kundu *et al*., 2007).

There was variation in phytoavailable metal concentrations under different amendments at the time of initial and final sampling (Tables 2 and 3). At the time of harvest, among all the heavy metals, the phytoavailability of Cd, Cu, Pb, Cr and Ni were lowest in FYM amended soil (Table 3). This may be due to more retention of metals under FYM amendment. Paulose *et al*. (2007) have also showed increase in retention of Zn, Cu and Ni in sewage irrigated soil under organic matter amendment. Among all amendments under FYM amendment, phytoavailable concentrations were maximally reduced by 14, 18, 12, 8, 16, 18, and 17% for Cd, Cu, Pb, Zn, Mn, Ni and Cr, respectively, compared to the control (Table 3). . High pH under FYM amended soil increases adsorption sites by increasing negative charges on the soil surface and reducing competing metal cations (Alamgir *et al*., 2011). Availability of all metals was significantly higher in NPK amended soil due to lower soil pH.

Heavy Metal Concentrations in Plants

In edible portion of the radish (root part), trend of heavy metal concentrations was Mn> Zn> Cu> Pb> Ni> Cr> Cd (Figure 1). As compared to the control, plants grown in FYM amended soil showed maximum percent reduction for all the metals and it was 16, 22, 21, 19, 23, 13, and 32.8% for Cd, Cu, Pb, Zn, Mn, Ni, and Cr, respectively. The present findings support the study of Pichtel and Bradway (2008) reporting that FYM amendments to the metalliferous waste disposal contaminated soil resulted in reduction in the uptake of Pb, Cd, and Zn concentrations in Spinach (*Spinacea oleracea*) and cabbage (*Brassica oleracea*) plants. It was suggested that due to dense plant cover and extensive root formation under FYM amendment, leachate production decreased, which increased the retention of the metals in the soil (Pitchel and Bradway, 2008). Khan *et al*. (2013) have also reported that FYM was effective in reducing the phytoavailability of metals (Cd, Cu, Fe) to wheat crops irrigated with

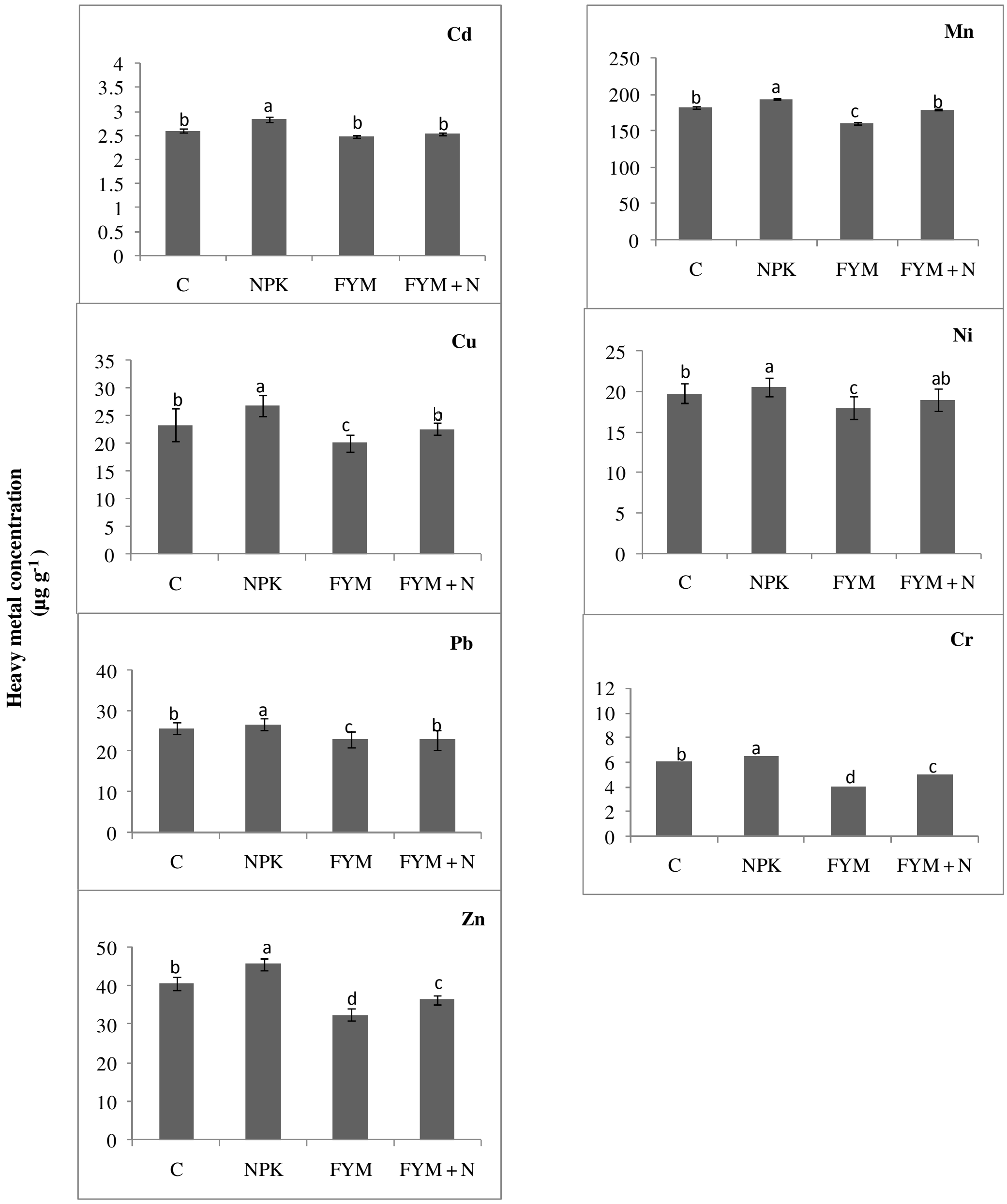

Figure 1. Heavy metal concentrations (µg g^{-1}) in edible portion of radish plants grown under different amendments at 40 DAG. Bars with different letters in each group showed significant difference at P≤ 0.05. C: Control; FYM: Farmyard manure, NPK: Nitrogen, phosphorus and potassium.

waste water generated from HIE (Hayatabad Industrial Estate, Peshawar, Pakistan) and municipal wastes by making insoluble chemical complexes with the metals. The presence of reactive groups such as hydroxyl, phenoxyl and carboxyl are responsible for controlling the adsorption and complexation of heavy metal in the soil (Mahmood, 2010). The addition of FYM resulted in increase in organic matter content in the soil and this organic matter acted as a primary sorbent of heavy metals in contaminated soils. It was found that the sorption affinity of soil for Cd was 30 times greater due to presence of organic matter as compared to mineral soil (Sauvè *et al*., 2003). Thus, in metal contaminated soils, use of various organic amendments such as FYM, composts, bio-solids, and bio-solid compost can effectively reduce metal availability to plants (Puschenreiter *et al*., 2005; Sarwar *et al*., 2010).

Physiological Characteristics of Plants

Plants showed higher photosynthesis rate and stomatal conductance under FYM and FYM+N amended soil compared to NPK amended and the control soil (Table 4). The lower uptake of heavy metals under FYM and FYM+N amendments led to lower stress to the plants and hence showed better physiological performance. Mandal and Sinha (2004) have found that photosynthetic activity, transpiration rates, stomatal conductance and growth increased under combination of FYM and NPK compared to NPK alone in *Brassica juncea* L.

Water use efficiency, refers to the efficient use of water in plant metabolism. There are two types of water use efficiency in plants: photosynthetic water-use efficiency and water-use efficiency of productivity. It as lowest in the plants grown under NPK amendment and the control soil due to lower photosynthetic rate compared to FYM and FYM+N amendments (Table 4). These parameters showed their impact on fluorescence activity of the plants. Demming and Bjorkman (1987) suggested that *Fv/Fm* ratio ranging from 0.78 to 0.85 denoted a healthy and unstressed condition in the plants. In the present study, ratio of Fv/Fm was lowest in plants grown under NPK amended and in the control soil that denoted stressed condition (Table 4). Defense response in plants grown under NPK amended and the control soil may not be able to counterbalance the damage caused by heavy metals, which, consequently, leads to adverse impact on photo systems in the form of lower quantum yield (Fv/Fm ratio).

Pigment concentration also varied with different amendments (Table 4). Chlorophyll a was significantly higher in plants grown in

Table 4. Physiological characteristics, pigment content, and yield of radish plants grown under different amendments at 40 DAG (Mean±SE)[a].

Characteristics	Control ©	FYM[c]	NPK[d]	FYM+N
Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1})	12.75^c± 0.07	15.30^a± 0.28	11.89^c± 0.18	13.73^b± 0.56
Stomatal conductance (m mol m^{-2} s^{-1})	2.25^b± 0.06	2.95^a± 0.23	1.61^c± 0.22	2.53^b± 0.14
Transpiration rate (mol H_2O m^{-2} s^{-1})	15.30^a± 0.75	15.10^a± 0.80	14.63^a± 0.67	15.40^a± 0.90
Water use efficiency (μ mol CO_2 m^{-2} s^{-1}/ mol H_2O m^{-2} s^{-1})	0.83^c± 0.07	1.01^a± 0.02	0.81^c± 0.08	0.89^b± 0.05
Fv/Fm[b]	0.70^c ± 0.01	0.78^b± 0.03	0.71^c± 0.03	0.83^a± 0.05
Chlorophyll a contents (mg g^{-1} dry wt)	0.770^c± 0.03	0.984^a± 0.02	0.909^b± 0.08	0.968^a± 0.01
Chlorophyll b contents (mg g^{-1} dry wt)	0.243^a± 0.01	0.240^a± 0.01	0.230^a± 0.03	0.25^a± 0.02
Carotenoids contents (mg g^{-1} dry wt)	0.451^b± 0.01	0.395^c± 0.04	0.502^a± 0.02	0.433^b± 0.02
Yield (g $plant^{-1}$)	60.4± 1.2	97±1.18	74.83± 1.16	90±1.12

[a] Different letters in each row showed significant difference at $P \leq 0.05$. [b] *Fv*= Variable fluorescence; *Fm*= Maximum fluorescence; [c]Farmyard manure, [d]Nitrogen, phosphorus and potassium.

FYM alone and FYM+N amended soil as compared to NPK and the control soil, whereas chlorophyll b concentration varied insignificantly among the amendments (Table 4). Application of FYM provides the micronutrients that may help to increase the chlorophyll synthesis in the plants (Anburani and Manivannan, 2002). Due to higher availability of heavy metals in plants grown under NPK amended soil, carotenoid content was highest (Table 4). The carotenoids are known to scavenge the damaging free radicals produced under heavy metal stress in the plants (Kenneth *et al*., 2000).

Biochemical Characteristics of Plants

Disintegration of bio-membrane by lipid peroxidation is a general process under metal stress condition and it is measured in terms of thiobarbituric acid reactive substance chiefly melondialdehyde (MDA) content. The content of MDA was lower in plants grown in FYM+N and FYM amended soil compared to the plants grown in NPK amended and the control soil due to presence of lower concentrations of heavy metals (Table 5). Proline content was highest in plants grown in NPK amended soil (Table 5). Accumulation of proline in plants under heavy metal stress provides protection against damage by ROS. It stabilizes the machinery of protein synthesis and it also acts as an effective singlet oxygen quencher (Alia and Matysik, 2001).

As the phytoavailability of heavy metals and their concentrations were higher in the control and NPK amendment, the antioxidants such as peroxidase, thiol, and ascorbic acid also showed higher levels in plants grown under these amendmemts (Table 5). High levels of heavy metals are responsible for generating free radicals and reactive oxygen species (Keunen *et al.*, 2011). Peroxidase enzyme oxidizes substrate by using H_2O_2, thus helps in maintaining the level of H_2O_2 in the plants and decreases the toxicity of heavy metals. Gopal and Rizvi (2008) have also reported increase in peroxidase activity of radish (*Raphanus sativus* cv. Jaunpuri) grown in nutrient medium containing Pb. The non-protein thiol and ascorbic acid are responsible for scavenging different types of ROS, thereby protecting potential cell injury (Gupta and Sinha, 2008). Non-protein thiols are involved in phytochelatin synthesis, which forms complexes with heavy metals and sequesters them in the vacuoles (Cobbett, 2000). Due to lower accumulation of heavy metals in the plants grown under FYM amended soil, reduction of 66% in thiol and 6% both for proline and ascorbic acid contents were recorded as compared to the control plants (Table 5).

Protein content was also lower in plants under NPK amendment and the control compared to FYM and FYM+N amendments due to high concentrations of

Table 5. Biochemical characteristics of radish plants grown under different amendments at 40 DAG (Mean±SE) [a].

Characteristics	Control ©	FYM[b]	NPK[c]	FYM+N
MDA content (n mol ml^{-1} fresh leaf)	2.83^{a}± 0.48	1.23^{d}± 0.34	2.29^{b}± 0.11	1.65^{c}± 0.12
Peroxidase activity (µm purpurogallin min^{-1} g^{-1} fresh leaf)	11.32^{b}± 0.36	8.52^{c}± 1.28	12.39^{b}± 0.44	16.91^{a}± 0.65
Proline content (mg g^{-1} fresh leaf)	0.85^{b}± 0.02	0.79^{c}± 0.02	0.96^{a}± 0.01	0.88^{b}± 0.01
Phenol content (mg g^{-1} fresh leaf)	8.97^{a}± 0.25	6.37^{c}± 0.05	7.59^{b}± 0.04	8.16^{b}± 0.02
Ascorbic acid content (mg g^{-1} fresh leaf)	0.39^{a}± 0.004	0.36^{b}± 0.007	0.38^{a}± 0.009	0.37^{ab}± 0.007
Thiol content (µ mol g^{-1} fresh leaf)	9.45^{a}± 0.01	3.23^{b}± 0.37	8.93^{a}± 0.19	3.79^{b}± 0.01
Protein content (mg g^{-1} fresh leaf)	7.94^{c}± 0.17	10.14^{a}± 0.03	7.86^{c}± 0.47	8.62^{b}± 0.12

[a] Different letters in each row showed significant difference at $P \leq 0.05$; [b] Farmyard manure, [c] Nitrogen, phosphorus and potassium.

heavy metals (Table 5). Decrease in the protein content of *Brassica juncea* under Cd and Pb stress has been reported (John *et al*., 2009). These heavy metals may cause fragmentation of proteins due to reactive oxygen species, thus, leading to decline in the protein content. Due to lowest oxidative stress in FYM amended soil, plants protein content was highest under this amendment compared to others.

Yield

Due to more reduction in the availability of heavy metals under FYM amendment, plants showed highest yield compared to the control and other amendments (Table 4). Yield increment was 60, 49 and 24% in FYM, FYM+N, and NPK amended soils, respectively, as compared to the control (Table 4). Hutchinson (2011) reported that the application of FYM to the soil significantly increased the seed yields of *Solanum villosum* and *Cleome gynandra* as compared to plants grown in the control soil. Mandal *et al*. (2007) and Mahmoud *et al*. (2009) have also found that application of organic and inorganic fertilizer in combination led to higher yield of wheat and cucumber plants as compared to sole application of inorganic fertilizer. Under NPK amendment, plant showed lower yield compared to other amendments, because the amount of photosynthates produced in plants was utilized in the defense against heavy metals rather than contributing to the growth of the plants.

CONCLUSIONS

Availability of heavy metals in soil was highest under NPK followed by the control (without amendment), FYM + N, and the lowest, under FYM amendment. Heavy metals concentrations in plants also decreased in FYM and FYM+N amendments compared to NPK and the control. Due to higher heavy metal concentrations in plants under NPK amended soil, more antioxidants were inducted in order to reduce the oxidative stress caused by the heavy metals. Lower availability of heavy metals in plants under FYM amendment led to the improvement in physiological status and yield. The present study concludes that FYM alone and in combination with N may be used to reduce the phytoavailability of heavy metals in the soil to improve the yield of plants and reduce the food chain contamination. Therefore, application of FYM alone and in combination with N at regular intervals may be able to reduce food chain contamination by lowering the availability of heavy metals to the plants grown in metal contaminated area.

ACKNOWLEDGEMENTS

The Authors acknowledge the Department of Botany, Banaras Hindu University for providing necessary facilities and thanks are due to Center for Advanced Study (CAS) in Botany, Banaras Hindu University for providing Senior Research Fellowship to Dr Anita Singh.

REFERENCES

1. Alamgir, M., Kibria, M. G. and Islam, M. 2011. Effects of Farm Yard Manure on Cadmium and Lead Accumulation in Amaranth (*Amaranthus oleracea* L.). *J. Soil Sci. Environ. Manage*., **2(8)**:237-240.
2. Alia, M. P. and Matysik, J. 2001. Effect of Proline on the Production of Singlet Oxygen. *Amino Acids,* **21:** 95-200.
3. Allen, S. E., Grimshaw, H. M. and Rowland, A. P. 1986. Chemical Analysis. In: *"Methods in Plant Ecology*", (Eds.): Moore, P. D. and Chapman, S. B.. Blackwell Scientific Publication, Oxford, London, PP. 285-344.
4. Allison, L.E. 1986. Organic carbon. Part I. In: *"Methods of Soil Analysis*", (Ed.): Klute, A.. *American Society of Agronomy*, Madison, WI, PP. 1367-1381.

5. Anburani, A. and Manivannan, K. 2002. Effect of Integrated Nutrient Management on Growth in Brinjal. *South Ind. Horticul.,* **50**: 377-386.
6. Bates, L. S., Waldran, R. P. and Teare, I. D. 1973. Rapid Determination of Proline for Water Stress Sstudies. *Plant Soil,* **39**: 205-207.
7. Bolan, N. S., Adriano, D. C., Natesan, R. and Koo, B. J. 2003. Effects of Organic Amendments on the Reduction and Phytoavailability of Chromate in Mineral Soil. *J. Environ. Qual.,* **32**: 120–128.
8. Bray, H. G. and Thorpe, W. V. 1954. Analysis of Phenolic Compounds of Interest in Metabolism. In: *"Methods in Biochemical Analysis",* (Ed.): Click, D.. Interscience Publ. Inc., New York, **1:** 27-52.
9. Britton, C. and Mehley, A. C. 1955. Assay of Catalase and Peroxidases. In: *"Methods in Enzymology, Assay of Catalase and Peroxidase",* (Eds.): Colowick, S. P. and Kalpan, N. O.. Academic Press Inc., New York, **II:** 764-775.
10. Cobbett, C. 2000. Phytochelatins and Their Roles in Heavy Metal Detoxification. *Plant Physiol.,* **123**: 825-832.
11. Demming, B. and Bjorkman, O. 1987. Comparison of the Effect of Excessive Light on Chlorophyll Fluorescence (77 K) and Photon Yield of O_2 Evolution in Leaves of Higher Plants. *Planta,* **171**: 171-184.
12. Duxbury, A. C. and Yentsch, C. S. 1956. Plankton Pigment Monographs. *J. Marine Res.,* **15**: 91-101.
13. Fahey, R. C., Brown, W. C., Adams, W. B. and Worsham, M. B. 1978. Occurrence of Glutathione in Bacteria. *J. Bacteriol.,* **133**: 1126 -1129.
14. Gopal, R. and Rizvi, A. H. 2008. Excess Lead Alters Growth, Metabolism and Translocation of Certain Nutrients in Radish. *Chemosphere,* **70**: 1539–1544.
15. Gupta, A. K. and Sinha, S. 2008. Antioxidant Response in Sesame Plants Grown on Industrially Contaminated Soil: Effect on Oil Yield and Tolerance to Lipid Peroxidation. *Bioresource Technol.,* **100**: 179 – 185.
16. Handique, G. K. and Handique, A. K. 2009. Proline Accumulation in Lemongrass (*Cymbopogon flexuosus* Stapf.) Due to Heavy Metal Stress. *J. Environ. Biol.,* **30**: 299–302.
17. Hati, K. M., Swarup, A., Dwivedi, A. K., Misra, A. K. and Bandyopadhyay, K. K. 2007. Changes in Soil Physical Properties and Organic Carbon Status at the Topsoil Horizon of a Vertisol of Central India after 28 Years of Continuous Cropping, Fertilization and Manuring. *Agric. Ecosys. Environ.,* **119**: 127–134.
18. Heath, R. L. and Packer, L. 1968. Photoperoxidation in Isolated Chloroplast. Kinetics and Stoichiometry of Fatty Acid Peroxidation. *Arch. Biochem. Biophys.,* **125**: 189-198.
19. Hutchinson, M. J. 2011. The Effect of Farmyard Manure and Calcium Ammonium Nitrate Fertilisers on Micronutrient Density (Iron, Manganese, Calcium and Potassium) and Seed Yields of *Solanum villosum* (Black Nightshade) and *Cleome gynandra* (Cat Whiskers) on Uetric Nitisol . *J. Agri. Sci. Technol.*, **13**: 35-52.
20. Jackson, M.L.1958. *Soil Chemical Analysis*. Prentice-Hall of India Pvt Ltd., New Delhi, 498 PP.
21. Jiang, N., Luo, X., Zeng, J., Yang, Z.H., Zheng, L. Y. and Wang, S. T. 2010. Lead Toxicity Induced Growth and Antioxidant Responses in *Luffa cylindrica* Seedlings. *Int. J. Agric. Biol.,* **12**: 205–210.
22. John, R., Ahmad, P., Gadgil, K. and Sharma, S. 2009. Heavy Metal Toxicity: Effect on Plant Growth, Biochemical Parameters and Metal Accumulation by *Brassica juncea* L. *Int. J. Plant Prod.,* **3**: 65-76.
23. Keller, T. and Schwager, H. 1977. Air Pollution and Ascorbic Acid. *Eur. J. Plant Pathol.*, **7**: 338–350.
24. Kenneth, E., Pallet, K. E. and Young, J. 2000. Carotenoids. In: *"Antioxidants in Higher Plants",* (Eds.): Alscher, R. G., and Hess, J. L.. CRC Press, Boca Roton, Florida, USA, PP. 60–81.
25. Keunen, E., Remans, T., Bohler, S., Vangronsveld, J. and Cuypers, A. 2011. Metal-induced Oxidative Stress and Plant Mitochondria. *Int. J. Mol. Sci.*, ***12:*** 6894-6918.
26. Khan, M.J., Jan, M.T., Khan, K. 2013. Effect of organic and inorganic amendments on the heavy metal content of soil and wheat crop irrigated with wastewater. *Sarhad J. Agric.* 29(1):49-57.
27. Kim, K. R., Owens, G. and Naidu, R. 2009 Heavy Metal Distribution, Bioaccessibility and Phytoavailability in Long-term

Contaminated Soils from Lake Macquarie, Australia. *Aust. J. Soil Res.,* **47**:166–176.

28. Kundu, S., Bhattacharyya, R., Prakash, V., Ghosh, B. N., Pathak, H., Gupta, H. S. and Ladha, J. K. 2007. Long-term Yield Trend and Sustainability of Rainfed Soybean-wheat System through Farmyard Manure Application in a Sandy Loam Soil of the Indian Himalayas. *Biol. Fert. Soils,* **43**: 271–280.
29. Lowry, O. H., Rosebrought, N. J. and Farr, A. L. 1951. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.,* **193**: 265-275.
30. Machlachlan, S. and Zalik, S. 1963. Plastid Structure, Chlorophyll Concentration, and Free Amino Acid Composition of a Chlorophyll Mutant on Barley. *Can. J. Bot.*, **41**: 1053-1056.
31. Mahmood, M. 2010. Phytoextraction of Heavy Metals: The Process and Scope for Remediation of Contaminated Soils. *Soil Environ.,* **29**: 91-109.
32. Mahmoud, E., Kader, N. A. E., Robin, P., Corfini, N. A. and Rahman, L. A. E. 2009. Effects of Different Organic and Inorganic Fertilizers on Cucumber Yield and Some Soil Properties. *World J. Agricult. Sci.,* **5**: 408-414.
33. Mandal, A., Patra, A. K., Singh, D., Swarup, A. and Masto, R. E. 2007. Effect of Long-term Application of Manure and Fertilizer on Biological and Biochemical Activities in Soil during Crop Development Stages. *Biores. Technol.,* **98**: 3585–3592.
34. Mandal, K. G. and Sinha, A. C. 2004. Nutrient Management Effects on Light Interception, Photosynthesis, Growth, Dry-matter Production and Yield of Indian Mustard (*Brassica juncea*). *J. Agron. Crop Sci.,* **190**:119-129.
35. Olsen, S., Cole, C., Watanabe, F., Dean, L. 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington, DC.
36. Paulose, B., Datta, S. P., Rattan, R. K. and Chhonkar, P. K. 2007. Effect of Amendments on the Extractability, Retention and Plant Uptake of Metals on a Sewage-irrigated Soil. *Environ. Pollut.,* **146:** 19-24.
37. Pichtel, J. and Bradway, D. J. 2008. Conventional Crops and Organic Amendments for Pb, Cd and Zn Treatment at a Severely Contaminated Site. *Biores. Technol.,* **99**: 1242–1251.
38. Purohit, S., Varghese, T. M. and Kumari, M. 2003. Effect of Chromium on Morphological Features of Tomato and Brinjal. *Ind. J. Plant Physiol.,* **8**: 17-22.
39. Puschenreiter, M., Horak, O., Friesl, W. and Hartl, W. 2005. Low-cost Agricultural Measures to Reduce Heavy Metal Transfer into the Food Chain: A Review. *Plant Soil Environ.,* **51**: 1–11.
40. Quevauviller, P., Rauret, R., Rubio, G., Lopezsanchez, J. F., Ure, A. M., Bacon, J. R. and Muntau, H. 1997. Certified Reference Materials for the Quality Control of EDTA- and Acetic Acid-extractable Contents of Trace Elements in Sewage Sludge Amended Soils (CRMs 483 and 484). *Fresenius J. Anal. Chem.,* **357**: 611-618.
41. Sarwar, N., Saifullah, Malhi, S. S., Zia, M.H., Naeem, A., Bibi, S. and Farida, G. 2010. Role of Mineral Nutrition in Minimizing Cadmium Accumulation by Plants. *J. Sci. Food Agric.,* **90**: 925–937.
42. Singh, A., Agrawal, M. and Marshall, F. 2010. The Role of Organic *vs.* Inorganic Fertilizers in Reducing Phytoavailability of Heavy Metals in a Wastewater-irrigated Area. *Ecol. Eng.,* **36:** 1733–1740.
43. Singh, J., Upadhayay, S. K., Pathak, R. K. and Gupta, V. 2011. Accumulation of Heavy Metal in Soil and Paddy Crop (*Oryza sativa*), Irrigated with Water of Ramgarh Lake, Gorakhpur, UP, India. *Toxicol. Environ. Chem.,* **93**: 462-473.
44. Sauvè S, Manna S, Turmel M. C., Roy, A. G. and Courchesne F. 2003. Solid Solution Partitioning of Cd, Cu, Ni, Pb, and Zn in the Organic Horizons of a Forest Soil. *Environ. Sci. Technol.,* **37**: 5191–5196.
45. Zaccheo, P., Laura, C. and Valeria, D. M. P. 2006. Ammonium Nutrition as a Strategy for Cadmium Metabolisation in the Rhizosphere of Sunflower. *Plant Soil,* **283**:43–56.
46. Zaller, J. G. and Köpke, U. 2004. Effects of Traditional and Biodynamic Farmyard Manure Amendment on Yields, Soil Chemical, Biochemical and Biological Properties in a Long-term Field Experiment. *Biol. Fert. Soils*., **40**: 222-229.
47. Zeng, Y. H., Shaw, W.S., Hung, Y. L., Horng, Y. G., Ting, C. C. and Zueng, S. C. 2010. Remediation Techniques and Heavy Metal Uptake by Different Rice Varieties in

Metal-contaminated Soils of Taiwan: New Aspects for Food Safety Regulation and Sustainable Agriculture. *Soil Sci. Plant Nutr.,* **56**: 31–52.

کاهش مسمومیت فلزات سنگین در مزارع کشاورزی با کار برد مواد بهساز و اثرات آن روی ویژگی های گیاه تربچه (*Raphanus sativus*)

ا. سینگ، و م. آگراوال

چکیده

در تحقیق حاضر، گیاه تربچه(*Raphanus sativus* L.) در زمین هایی که با فاضلاب آبیاری می شد کاشته شد. محل اجرا در محدوده حاشیه نشین ناحیه دیناپور در شمال شرقی واراناسی در هندوستان قرار داشت.در این مطالعه ، ویژگی های خاک وگیاه در تیمارهای آزمون که شامل مصرف سه کود مختلف (کود حیوانی به تنهایی، NPK، و کود حیوانی بعلاوهNPK) بود با تیمار شاهد(بدون مصرف کود) مقایسه شد. اندازه گیری ها نشان داد که غلظت فلزات سنگین در تیمار های کود حیوانی به تنهایی و کود حیوانی بعلاوهNPK به ترتیب ۱۴تا۳۲ درصد و ۱۴ تا ۱۷ درصد کمتر از شاهد بود ولی عملکرد محصول افزایشی برابر ۶۰ درصد و ۴۹ درصد نشان داد. تجزیه گیاهان تیمار NPK حاکی از بالا بودن فراهمی جذب فلزات سنگین بود که در نتیجه باعث تحریک کنش آنزیم های آنتی اکسیدانت و کم شدن نرخ فتو سنتز، رشد گیاه و عملکرد می شد. بنا بر این برای کاهش فراهمی فلزات سنگین در مزارع آلوده می توان افزودن مقدار معینی از کود حیوانی به تنهایی یا همراه با N را به خاک در دوره های منظم توصیه کرد.