RESEARCH ARTICLE

MANAGEMENT OF HEAVY METAL CONTAMINATED SOIL BY USING ORGANIC AND INORGANIC FERTILIZERS: EFFECT ON PLANT PERFORMANCE

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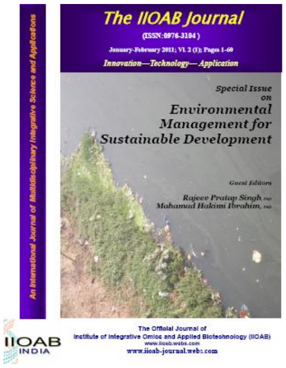
ABSTRACT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Heavy metal contamination leads to variety of harmful effects on soil and plant characteristics. In order to reduce the toxic effects of such substances, an experiment was conducted by FYM, N, NPK, FYM + NPK and FYM + N amendments in the soil from an area irrigated by waste water for more than 20 years. Soil and plant characteristics were compared between fertilizer (FYM, NPK, N and FYM + N, FYM + NPK) amended and non-amended control soil. As compared to the control, plants under FYM and FYM + NPK amendments showed lower accumulation of heavy metals and higher yield. Plants grown in NPK and N amended soil showed higher concentrations of heavy metals and lower yield compared to the control. Higher uptake of heavy metals in plants under NPK and N amendments, led to increase in the antioxidants enzymes, but reductions in photosynthesis rate, growth and yield. The results suggest that the application of FYM alone and in combination with inorganic fertilizers may be recommended as cost effective technique for reducing the availability of heavy metals in waste water irrigated soil.

Keywords: ***heavy metals; contamination; fertilizer; remediation; soil; plant***

[I] INTRODUCTION

Pollution of the biosphere by toxic metals has accelerated severely since the beginning of the industrial revolution. The primary sources of metal pollution include the burning of fossils fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, and waste water irrigation. Contamination of groundwater and soil by heavy metals leads to major environmental and human health problems. Plant metabolism is also affected negatively by the heavy metals [1]. Although some of the heavy metals act as micronutrient at lower concentration, but at higher concentration these are harmful for the normal functioning of plants [2]. Metals cannot be degraded to harmless products, such as carbon dioxide, but instead persist indefinitely in the environment, complicating their remediation.

Occurrence of oxidative stress in plants could be the indirect consequence of heavy metal toxicity [3, 4]. Molecular oxygen can accumulate in the leaves of plants under heavy metal stress, which can result into oxidation of cellular components. One of the most deleterious effects induced by heavy metals exposure in plant is lipid peroxidation, which can directly cause biomembrane deterioration [5]. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids of membrane is regarded as a reliable indicator of oxidative stress [6]. In response to generation of reactive oxygen species, plants induce the enzymatic and non enzymatic antioxidants that have ability to detoxify these species [7].

Exposure of plants to heavy metals causes reduction in photosynthesis, water uptake, and nutrient uptake [8]. Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips, and finally death [9, 10].

An effective and affordable technological solution is needed for marginal farmers to reduce the harmful effects of heavy metals in the area using waste water for irrigation. The practices that improve soil quality and sustainability of agricultural system may be the most important acceptable technique. In the present study, the amendments of organic and inorganic fertilizers alone and in combination in the soil was done to reduce the availability of heavy metals in the waste water irrigated soil and the consequent effects on heavy metal availability, biochemical, physiological, growth and yield responses of palak (*Beta Vulgaris L.*) were assessed.

[II] MATERIALS AND METHODS

2.1. Experimental site

The experiment was conducted in Dinapur situated at a suburban area in the north east of Varanasi (25º18' N latitude 83º01' E longitude and 76.19 m above the mean sea level) city in the eastern Gangetic plains of India, having long term uses of treated and untreated waste water for irrigation from Dinapur sewage treatment plant (DSTP) of 80 million liters per day capacity.

2.2. Field preparation and raising of plants

Experimental pots (radius = 12.5 cm) were prepared according to common agronomic practices. Soil was amended with farmyard manure (FYM) at 80 t ha^{-1}, urea (N) + superphosphate (P) + potash (K) at 80:40:40 kg ha^{-1}, respectively only urea (N) at 80 kg $ha^{-1}$and a combination of FYM (80 t ha^{-1}) + urea (80 kg ha^{-1}), and FYM at 80 t ha^{-1}+ NPK at 80:40:40 kg ha^{-1}, respectively. Control pots (C) were kept without any amendment. There were 10 replicate pots for each amendment. Genetically uniform seeds of palak (*Beta vulgaris* L. All green) obtained from Institute of Vegetable Research, Varanasi. Three seed were sown in the pot at 2 cm depth in triangle at equal distance. Waste water from DSTP was used for irrigating all the pots including control. After germination, only one plant was left in each pot.

2.3. Soil Sampling and preparation

Soil samples were collected in triplicate from pots of each amendment, dried at room temperature, crushed, sieved with 2 mm mesh size sieve and kept at room temperature for further analysis.

2.4. Sampling of plants

Plants were dug out along with intact root at random from each pot for various growth and yield analysis at the time of harvest i.e. 40 days after germination (DAG). These were thoroughly washed by placing them on a sieve of 2 mm mesh size under running tap water to remove soil particles adhering to the roots.

2.5. 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 tablets attached to an Ion analyzer (Model E.A 940, Orion USA). The organic carbon content was determined by using modified Walkley and Black's rapid titration method [11].

The total nitrogen (TN) content was determined by following the micro-Kjeldahl technique through the Gerhardt automatic analyzer (Model KB8S, Kjeldatherm, Germany). Available phosphorus ($NaHCO_3$ extractable) was determined by the method given by Allen et al. [12]. Exchangeable cations such as Na, K, Mg, Fe and Ca were extracted using ammonium acetate solution through repeated leaching technique (Jackson 1958), and contents were determined by atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

2.6. Digestion and analysis for heavy metals

Soil and plant samples (1g) 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 [12]. After cooling, the digested sample was filtered using Whatman no. 42 filter paper and the filtrate was finally maintained to 50 ml with distilled water. Concentrations of heavy metals in the filtrate of digested soil and plant samples were estimated by using atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA) fitted with specific lamp of particular metal using appropriate drift blank.

Phytoavailable heavy metals in the soil samples were extracted by the method given by Quevauviller et al. [13]. Sieved soil sample of 10 g was shaked with 20 ml of 0.05 N EDTA solution (pH = 7) for 1 hour and then kept for 24 hours before filtering. The concentrations of phytoavailable heavy metals in the filtrate were determined by using atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

2.7. Physiological characteristics

Portable Photosynthetic System (Model 6200, LICOR, Lincolin, NE, USA) was used for measuring photosynthetic rate, stomatal conductance, transpiration rate and water use efficiency at ambient climatic conditions on intact plants in the pot. 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 1100 to 1200 $\mu mol\ m^{-2}\ s^{-1}$.

Measurement of chlorophyll fluorescence was done with the help of Plant Efficiency Analyzer (Hansatech Instruments Ltd., England, PEA MK2, 9414) between 10.00 to 11.00 hours on the same foliage where photosynthetic rate was measured. Before taking measurements, leaves were dark adapted for 30 minutes. The adaxial surface of leaf was irradiated with red light and fluorescence signal was collected from the same surface. Chlorophyll fluorescence characteristics such as initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence ($F_v = F_m - F_o$) and F_v/F_m ratio were measured for different treatments.

2.8. Biochemical characteristics

Fresh plant leaves from three different pots were sampled at 40 Days after germination (DAG) for estimation of photosynthetic pigments, lipid peroxidation and different metabolites and enzymes. Ascorbic acid and proline contents were determined by the methods described by Keller and Schwager [14] and Bates et al. [15], respectively. Total phenol content was measured using the method of Bray and Thorpe [16]. The methods of Britton and Mehley [17] and Fahey et al. [18] were used for analyzing peroxidase activity and thiol content, respectively. The lipid peroxidation was measured as Malondialdehyde (MDA) concentration by following the protocol given by Heath and Packer [19].
Chlorophyll (Chl) and carotenoids were extracted with 80 % acetone and the amounts were estimated spectrophotometrically by the method of Machlachlan and Zalick [20] and Duxbury and Yentsch [21], respectively.

2.9. Growth parameters and yield of plant

Growth parameters were analysed with respect to root and shoot lengths, number of leaves per plant and leaf area. The leaf area was measured with the help of LI-COR leaf area meter (Model LI-COR 3000, LI-COR Inc., NE, USA). For biomass estimation, different plant parts were separated and oven-dried at 80°C till a constant weight was obtained. Oven-dried plant parts were weighed separately for biomass estimation.

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

2.10. Statistical analysis

The data under different amendments were subjected to ANOVA test for assessing the significance of differences in heavy metal concentrations, biochemical, physiological and growth parameters and yield of plants. All the statistical tests were performed using SPSS software (SPSS Ins., version 12).

2.11. Quality control analysis

Precision and accuracy of analysis was assured through repeated analysis of samples against National Institute of Standard and Technology, Standard Reference Material (SRM 1570) for all the heavy metals. The results were found within ± 2 % of the certified value. Quality control measures were taken to assess contamination and reliability of data. Blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were run after five determinations to calibrate the instrument. The coefficients of variation of replicate analysis were determined for different determinations for precision of analysis and variations were found to be less than 10 %.

[III] RESULTS AND DISCUSSION

3.1. Physico-chemical properties of soil

Among all the amendments, pH was maximum for FYM and minimum for NPK amendments in the soil at both the samplings time **[Table–1, –2]**. Soil pH is one of most important characteristic for determining the availability of heavy metals. Application of FYM increases the organic matter of soil that consequently increases the pH of soil due to the release of fumic and humic acid through the process of decomposition. Mgbeze and Abu [22] have also found higher pH in river sand amended with FYM compared to the control at Ugbowo campus of the University of Benin, Nigeria. Application of NPK showed lowest pH because phosphate increases the formation of slowly soluble Dicalcium phosphate (DCP) with a release of phosphoric acid which is responsible for decreasing the soil pH through its dissociation in to phosphorus and acidic hydrogen ion. Zheng Miao et al. [23] have also found lower soil pH under the application of super phosphate fertilizers.

Higher concentration of NO_3^-- N and NH_4^+- N in N amended soil was found at both the samplings **[Table–1, –2]**. Soil amended with N alone showed 36, 33, 55, 28, 29 % increments in NO_3^-- N concentration as compared to the control, NPK, FYM, FYM + NPK and FYM + N amended soil, respectively at the final sampling **[Table–2]**. Higher concentration of available forms of N (NO_3^-- N and NH_4^+- N) in N amended soil is due to the application of urea alone. Highest concentration of available P was found in NPK amended soil compared to other amendments at both initial and final sampling due to addition of phosphate fertilizer **[Table–2]**.

At both the samplings, FYM amended soil showed highest value of organic C and NPK amended soil showed lowest. At initial and final samplings, respectively, organic C increased maximally by 21 and 16 % in FYM amended soil and decreased by 8 and 9 % in NPK amended soil compared to the control soil **[Table–2]**. Application of organic matter in the form of FYM enhanced the organic C level of the soil and has direct and indirect effects on soil properties and processes [24]. Ruhlmann and Ruppel [25] have also found FYM to be a good source of organic matter.

Among different amendments, concentrations of exchangeable cations were highest in soil amended with FYM alone followed by the combination of FYM with inorganic fertilizer and least in those amended with inorganic fertilizer alone and control soil at the both the samplings **[Table–1, –2]**. Bhattacharyya et al. [26] have also found that FYM amended plots showed better supply of N, P, and K, and improved soil physical conditions compared to unamended ones. Soil amended with FYM + NPK showed higher concentrations of cations because the decomposition products of FYM in soil have arrested the fixation/adsorption of the applied fertilizer and resulted in to higher availability of these nutrients [27].

Table: 1. Physico–chemical properties of soil after various amendments at initial sampling (Mean ± 1SE)

Properties	Control	FYM	NPK	N	FYM + NPK	FYM + N
pH	$7.16^b \pm 0.01$	$7.60^a \pm 0.11$	$6.90^c \pm 0.01$	$7.17^b \pm 0.10$	$7.27^b \pm 0.28$	$7.33^b \pm 0.23$
Conductivity (ds cm^{-1})	$0.13^a \pm 0.01$	$0.14^a \pm 0.03$	$0.13^a \pm 0.005$	$0.12^a \pm 0.003$	$0.14^a \pm 0.005$	$0.13^a \pm 0.005$
Total N (%)	$0.23^a \pm 0.01$	$0.21^a \pm 0.01$	$0.25^a \pm 0.01$	$0.26^a \pm 0.01$	$0.24^a \pm 0.003$	$0.25^a \pm 0.003$
NO_3^- – N (µg g^{-1})	$13.0^b \pm 0.57$	$11.67^c \pm 0.88$	$13.33^b \pm 0.67$	$15.0^a \pm 0.57$	$14.0^{ab} \pm 1.15$	$13.67^b \pm 0.88$
NH_4^+– N (µg g^{-1})	$4.67^b \pm 0.17$	$4.33^c \pm 0.17$	$5.17^b \pm 0.16$	$5.50^a \pm 0.29$	$5.00^b \pm 0.29$	$5.10^b \pm 0.21$
Organic C (%)	$2.30^c \pm 0.12$	$3.17^a \pm 0.17$	$2.10^c \pm 0.06$	$2.17^c \pm 0.14$	$2.87^b \pm 0.08$	$2.83^b \pm 0.12$
Available P (µg g^{-1})	$87.53^b \pm 1.44$	$83.33^b \pm 1.67$	$90.10^a \pm 2.39$	$81.67^b \pm 1.67$	$89.33^b \pm 2.33$	$87.33^b \pm 1.45$
Na (µg g^{-1})	$158.67^b \pm 6.96$	$172.0^a \pm 3.05$	$168.0^b \pm 5.29$	$165.3^b \pm 2.96$	$171.0^{ab} \pm 3.78$	$170.67^{ab} \pm 2.33$
(µg g^{-1})	$175.0^a \pm 4.04$	$181.0^a \pm 3.21$	$178.60^a \pm 0.88$	$177.67^a \pm 4.33$	$184.0^a \pm 3.51$	$182.30^a \pm 3.38$
Ca (µg g^{-1})	$528.34^b \pm 15.89$	$582.60^a \pm 16.34$	$512.0^b \pm 14.04$	$539.56^b \pm 9.84$	$576.0^a \pm 14.0$	$576.30^a \pm 18.44$
Mg (µg g^{-1})	$64.30^b \pm 4.26$	$74.67^a \pm 4.37$	$65.67^b \pm 2.96$	$62.20^b \pm 3.92$	$75.67^a \pm 3.38$	$75.0^a \pm 5.04$
Fe (µg g^{-1})	$124.35^a \pm 8.68$	$148.0^a \pm 7.57$	$138.34^a \pm 4.41$	$139.40^a \pm 8.29$	$129.20^a \pm 4.67$	$130.0^a \pm 8.08$

Different letters in each row showed significant difference at $p \leq 0.05$

Table: 2. Physico–chemical properties of soil under various amendments at the time of harvest of palak (Mean ± 1SE)

Properties	Control	FYM	NPK	N	FYM + NPK	FYM + N
pH	$6.93^{bc} \pm 0.06$	$7.83^{a} \pm 0.17$	$6.76^{c} \pm 0.14$	$6.99^{b} \pm 0.17$	$7.13^{b} \pm 0.12$	$7.27^{b} \pm 0.12$
Conductivity (ds cm^{-1})	$0.15^{a} \pm 0.01$	$0.16^{a} \pm 0.003$	$0.15^{a} \pm 0.01$	$0.15^{a} \pm 0.003$	$0.16^{a} \pm 0.005$	$0.15^{a} \pm 0.005$
Total N (%)	$0.25^{a} \pm 0.01$	$0.28^{a} \pm 0.01$	$0.27^{a} \pm 0.01$	$0.30^{a} \pm 0.03$	$0.29^{a} \pm 0.03$	$0.27^{a} \pm 0.02$
NO_3^{-} – N (µg g^{-1})	$11.0^{b} \pm 0.57$	$9.67^{b} \pm 0.88$	$11.30^{b} \pm 0.60$	$15.0^{a} \pm 0.58$	$11.67^{b} \pm 1.20$	$11.60^{b} \pm 0.80$
NH_4^{+}– N (µg g^{-1})	$2.33^{b} \pm 0.16$	$2.16^{b} \pm 0.10$	$2.50^{b} \pm 0.28$	$2.83^{a} \pm 0.44$	$2.67^{b} \pm 0.17$	$2.66^{b} \pm 0.16$
Organic C (%)	$2.30^{b} \pm 0.11$	$2.67^{a} \pm 0.17$	$2.10^{c} \pm 0.05$	$2.27^{b} \pm 0.15$	$2.37^{b} \pm 0.08$	$2.33^{b} \pm 0.12$
Available P (µg g^{-1})	$81.67^{b} \pm 1.67$	$75.67^{c} \pm 1.76$	$83.0^{a} \pm 1.52$	$76.67^{c} \pm 1.76$	$77.0^{c} \pm 2.08$	$79.33^{b} \pm 3.17$
Na (µg g^{-1})	$138.60^{b} \pm 6.9$	$152.0^{a} \pm 3.05$	$148.0^{c} \pm 5.23$	$145.30^{c} \pm 2.90$	$154.0^{ab} \pm 3.08$	$150.57^{ab} \pm 2.00$
K (µg g^{-1})	$153.0^{a} \pm 4.00$	$159.00^{a} \pm 3.00$	$156.56^{a} \pm 0.80$	$155.64^{a} \pm 4.03$	$162.0^{a} \pm 3.05$	$160.30^{a} \pm 3.30$
Ca (µg g^{-1})	$505.3^{b} \pm 15.8$	$559.60^{a} \pm 16.00$	$489.0^{c} \pm 14.40$	$516.60^{b} \pm 9.0$	$553.0^{ab} \pm 14.10$	$553.30^{ab} \pm 18.0$
Mg (µg g^{-1})	$79.67^{b} \pm 5.48$	$86.0^{a} \pm 3.51$	$70.0^{c} \pm 1.15$	$66.33^{c} \pm 3.18$	$80.0^{ab} \pm 2.88$	$83.0^{ab} \pm 0.57$
Fe (µg g^{-1})	$139.6^{a} \pm 3.17$	$158.30^{a} \pm 7.76$	$146.60^{a} \pm 1.67$	$156.0^{a} \pm 11.0$	$138.30^{a} \pm 4.41$	$139.30^{a} \pm 5.81$

Different letters in each row showed significant difference at $p \leq 0.05$

3.2. Heavy metal concentrations in the soil

At initial sampling, total concentrations of heavy metal in the soil did not show significant difference among different amendments, whereas phytoavailable metals showed their minimum concentrations in FYM amended soil **[Figure–1a, –1b]**. There were 32, 47, 42, 21, 24, 47 and 38 % reductions in phytoavailable concentrations of Cd, Cu, Pb, Zn, Mn, Ni and Cr, respectively in FYM amended soil compared to the control soil **[Figure–1a, –1b]**. Phytoavialability of heavy metals depend upon various physico-chemical properties of the soil, such as pH and organic C [28]. Soil amended with the FYM showed more reduction in availability of heavy metals because of high organic C content of the soil. Organic C present in the FYM is responsible for the release of negatively charged ions that attract the positively charged heavy metals and consequently results into more retention of heavy metals in the soil with lower availability to the plant [29]. Singh et al. [30] have also found FYM application to the soil to be an effective measure for reducing Cr toxicity to spinach plants in Cr–contaminated soils at farm Punjab Agricultural University (PAU), Punjab, India. At final sampling the total concentrations of all heavy metals were higher in FYM amended soil **[Figure–2]**. This may be due to more retention of heavy metals in the soil under FYM amendment.

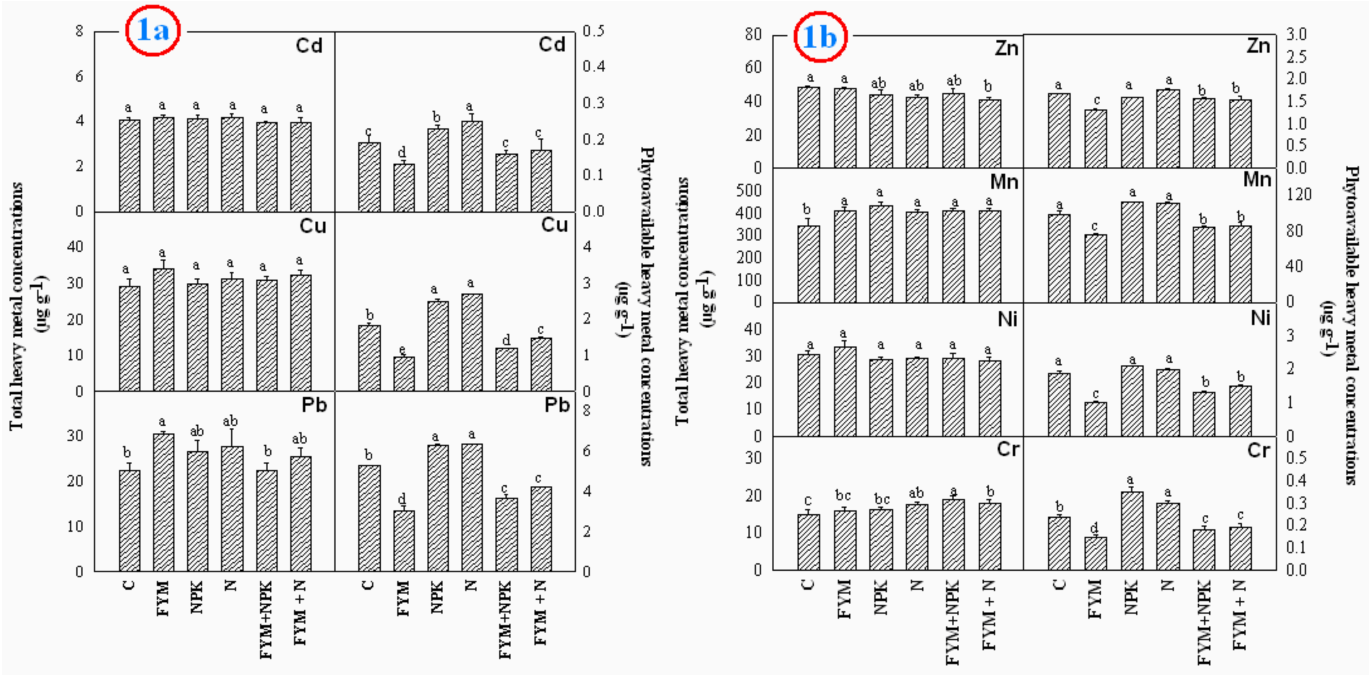

Fig: 1. (a) Total and phytoavailable concentrations of Cd, Cu and Pb in the soil at the time of initial sampling. (**b**). **Total and phytoavailable concentrations of Zn, Mn, Ni and Cr in the soil at the time of initial sampling**. Values are mean ± 1 SE. Bars with different letters in each group showed significant difference at $p \leq 0.05$.

Table: 3. Morphological characteristics and yield of palak at the time of harvest (Mean ± 1 SE)

Characteristics	Control	FYM	NPK	N	FYM + NPK	FYM + N
Root length (cm plant^{-1})	12.98^{a} ± 0.88	11.07^{b} ± 0.71	13.55^{a} ± 0.86	13.55^{a} ± 0.72	11.60^{b} ± 0.79	11.05^{b} ± 0.68
Shoot length (cm plant$^{-1)}$	9.81^{c} ± 0.62	14.24^{a} ± 0.72	11.18^{c} ± 0.57	11.20^{c} ± 0.77	13.11^{b} ± 0.88	12.75^{b} ± 0.52
Number of leaves (plant^{-1})	10.90^{b} ± 1.37	13.70^{a} ± 1.17	9.00^{c} ± 0.58	8.00^{c} ± 0.75	12.60^{b} ± 1.92	12.40^{b} ± 1.19
Leaf area (cm^{2}plant^{-1})	76.48^{c} ± 7.78	141.75^{a} ± 12.6	86.62^{c} ± 13.28	82.08^{c} ± 9.39	112.94^{b} ± 17.6	106.76^{b} ± 9.72
Shoot biomass (g plant^{-1})	0.80^{c} ± 0.02	1.47^{a} ± 0.05	0.99^{c} ± 0.06	0.83^{c} ± 0.03	1.16^{b} ± 0.05	1.13^{b} ± 0.08
Root biomass (g plant^{-1})	0.06^{a} ± 0.002	0.09^{a} ± 0.002	0.08^{a} ± 0.001	0.07^{a} ± 0.001	0.08^{a} ± 0.001	0.09^{a} ± 0.07
Yield (g plant^{-1})	2.99^{b} ± 0.13	3.43^{a} ± 0.15	2.97^{b} ± 0.14	2.88^{b}± 0.015	3.24^{ab}± 0.20	3.13^{ab} ± 0.17

Different letters in each row showed significant difference at $p \leq 0.05$

3.3. *Morphological characteristics*

Plants grown in FYM, FYM + NPK and FYM + N amended soil showed significant decrease in root length than those grown under control and N amended soil. Shoot length was significantly higher in FYM amended soil **[Table–3]**. Due to presence of higher concentration of nutrients in FYM amended soil, the root did not go deeper into the soil in search of nutrients and hence its length remained shorter. Number of leaves and leaf area were also significantly higher in FYM treated plants compared to other amendments and control soil. Mgbeze and Abu [22] have found higher shoot height, number of leaves and leaf area of African yam bean (*Sphenostylis stenocarpa*) grown in FYM amended river sand. Among all the amendments, shoot biomass was highest in FYM amended soil followed by FYM + NPK, FYM + N, NPK, N and then in control soil. Root biomass, however, did not vary significantly among different amendments. Yield of plant was highest in soil amended with FYM **[Table–3]**. Singh et al. [31] have also found higher yield of wheat on FYM amended soil compared to those grown in only NPK amended soil. This may be because of slow release of nutrients under FYM amendment. The plants showed higher yield in FYM + NPK and FYM + N amended soil than NPK amended soil because of residual effects of FYM. Similar to the results of the present study, the yield of wheat crop was found to be enhanced by 43 % and 71 % in N + FYM and NPK + FYM treatments, respectively, over NPK treatment [32].

3.4. *Physiological characteristics and pigment concentrations*

Photosynthetic rate (Ps) and stomatal conductance (g_s) increased maximally by 14 and 6 %, respectively in plants grown in FYM amended soil compared to the control **[Table–4]**.

Table: 4. Physiological characteristics of palak grown under various amendments at the time of harvest (Mean ± 1 SE)

Characteristics	C	FYM	NPK	N	FYM + NPK	FYM + N
Photosynthetic rate (µ mol CO_2 m^{-1}s^{-1})	16.67^{b} ± 1.20	19.0^{a} ± 0.58	14.0^{c} ± 0.57	14.33^{b} ± 0.88	15.33^{b} ± 0.67	16.67^{b} ± 0.67
Stomatal conductance (m mol m^{-2}s^{-1})	1.64^{ab} ± 0.14	1.74^{ab} ± 0.08	1.42^{b} ± 0.09	1.40^{b} ± 0.05	1.53^{ab} ± 0.03	1.45^{b} ± 0.02
Transpiration rate (mol H_2Om^{-2}s^{-1})	11.33^{a} ± 0.88	12.00^{a} ± 0.58	10.0^{b} ± 1.00	10.0^{b} ± 0.58	11.67^{a} ± 0.33	12.0^{a} ± 0.58
Water use efficiency	1.49^{a} ± 0.17	1.59^{a} ± 0.10	1.42^{a} ± 0.08	1.43^{a} ± 0.09	1.32^{a} ± 0.08	1.39^{a} ± 0.08
Fo (mv)	368.7^{b} ± 34.6	412.0^{a} ± 13.89	317.0^{c} ± 19.0	344.67^{b} ± 26.39	451.67^{a} ± 44.09	406.67^{a} ± 11.3
Fv (mv)	906.3^{b} ± 90.3	1053.0^{ab} ± 38.7	1146.7 ± 11.97	715.30^{b} ± 83.34	892.33^{a} ± 83.33	1015.7^{a} ± 61.3
Fm (mv)	1107^{b} ± 106.	1190.0^{ab} ± 61.4	1275.3 ± 37.35	935.0^{b} ± 28.87	1035.0^{b} ± 76.38	1015.7^{ab} ± 61.3
Fv/Fm	0.81^{a} ± 0.07	0.88^{a} ± 0.07	0.69^{b} ± 0.02	0.76^{a} ± 0.01	0.86^{a} ± 0.02	0.75^{b} ± 0.05

Different letters in each row showed significant difference at $p \leq 0.05$

Due to presence of higher concentration of heavy metals under N and NPK amendment soil, plants showed reduction in transpiration rate compared to other amendments and control soil. Fo, Fv and Fm showed highest values in plants grown in FYM amended soil. The ratio of Fv/Fm ranges from 0.78 to 0.85 under healthy and unstressed condition in the plants [33]. In the present study, Fv/Fm ratio varied from 0.69 to 0.88 among different amendments with the lowest in NPK amended soil **[Table–4]**. Lower value of Fv/Fm in NPK amended soil showed stress condition in the plant. Chlorophyll a and b contents were highest in plants grown in FYM amended soil **[Figure–3].** Plants grown in soil amended with combination of organic and inorganic fertilizers also showed increments in chlorophyll content compared to those where inorganic fertilizer was applied alone. Bokhtiar and Katsutoshi [34] have also found higher chlorophyll content in sugarcane plant grown in soil amended with organic fertilizer along with the inorganic fertilizer compared with inorganic fertilizer alone. Carotenoid content was highest in plants grown in NPK amended soil as compared to other amendments and control plants **[Figure–3]**. Carotenoids protect the plants by scavenging the free radicals generated under heavy metal stress. Although there was higher concentration of carotenoids in NPK amended soil, but it was not able to ameliorate the negative effect caused by excess heavy metal absorption in plants.

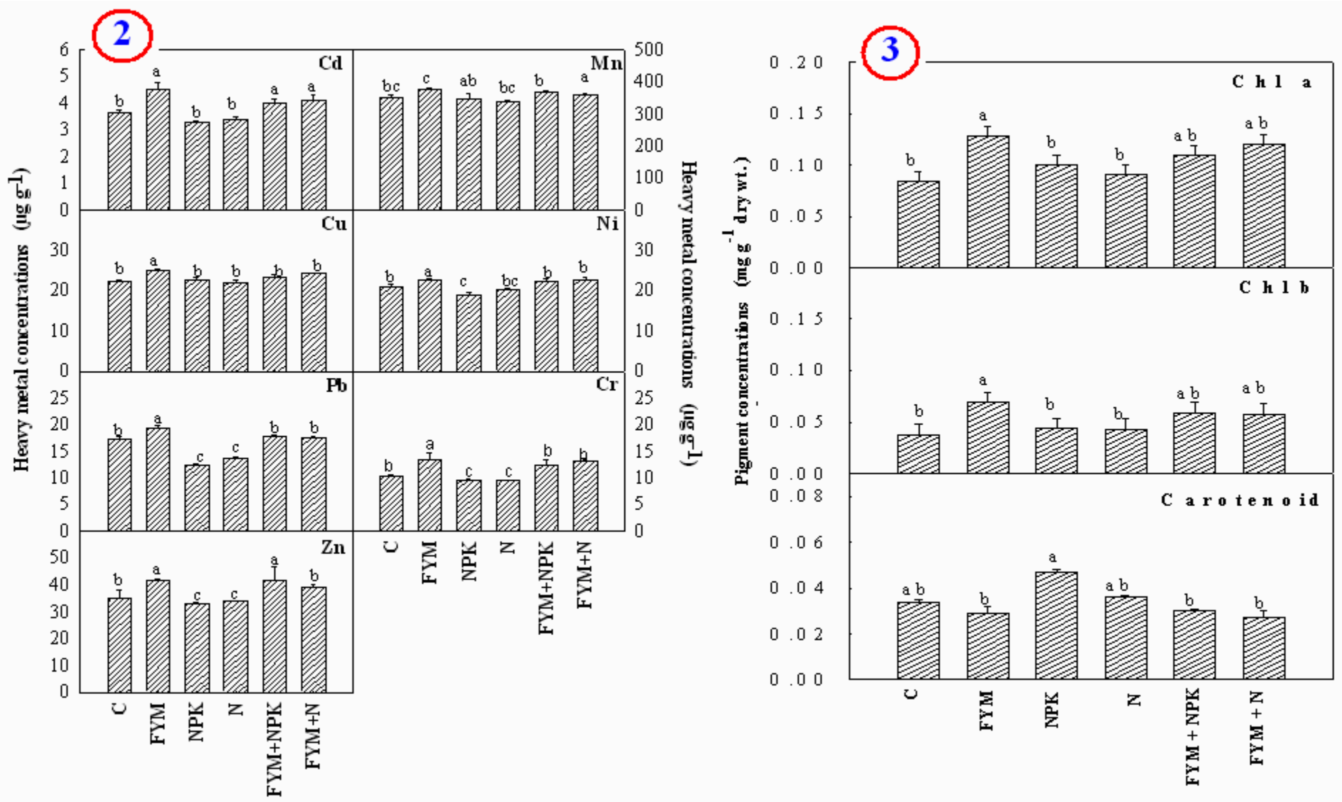

Fig: 2. Total heavy metal concentrations in the soil at the time of final sampling. Fig: 3. Pigment concentrations in palak at 40 DAG. In both the figures, values are mean ± 1 SE. Bars with different letters in each group showed significant difference at $p \leq 0.05$.

3.5. Biochemical characteristics

Palak plants grown in NPK amended soil showed maximum peroxidase activity and proline, ascorbic acid, phenol and thiol contents compared to the control **[Table–5]**. Antioxidants protect the plants by maintaining the balance between synthesis of free radicals and its detoxification [35]. Plants grown in NPK amended soil showed higher antioxidant levels, due to much higher availability of heavy metals compared to other treatments.

Heavy metal stress resulted into various harmful effects such as membrane damage that resulted into higher lipid peroxidation measured as MDA content in plants grown in NPK amended soil. Control plants also showed higher lipid peroxidation activity due to high heavy metal accumulation. Increase in lipid peroxidation under heavy metal stress is due to the generation of free radicals that distort the membrane architecture causing an oxidative damage [36].

Table: 5. Selected biochemical characteristics of palak grown under different amendments at harvesting time (Mean ± 1 SE)

Characteristics	C	FYM	NPK	N	FYM + NPK	FYM + N
LPO (nmol ml^{-1} fresh wt.)	0.73^{a} ± 0.01	0.22^{c} ± 0.02	0.72^{a} ± 0.14	0.60^{b} ± 0.41	0.41^{c}± 0.01	0.35^{c} ± 0.02
Peroxidase activity (µm purpurogallin min^{-1} g^{-1} fresh wt.)	7.90^{a} ± 0.51	5.17^{b} ± 0.14	8.81^{a} ± 1.65	5.18^{b} ± 0.37	8.40^{a} ± 0.51	5.43^{b} ± 0.43
Proline (mg g^{-1} fresh wt.)	0.28^{a} ± 0.03	0.22^{b} ± 0.01	0.29^{a} ± 0.03	0.26^{a} ± 0.02	0.23^{b} ± 0.02	0.18^{b} ± 0.01
Phenol (mg g^{-1} fresh wt.)	5.78^{ab} ± 0.74	4.38^{b} ± 0.63	6.14^{a} ± 0.49	5.88^{ab} ± 0.46	4.96^{ab} ± 0.44	4.31^{b} ± 0.28
Ascorbic acid (mg g^{-1} fresh wt.)	0.052^{c} ± 0.01	0.015^{d} ± 0.01	0.104^{a} ± 0.01	0.069^{bc} ± 0.01	0.055^{c} ± 0.01	0.079^{b} ± 0.01
Thiol (µmol g^{-1} fresh wt.)	5.54^{b} ± 0.36	3.73^{c} ± 0.39	8.73^{a} ± 0.97	4.02^{bc} ± 0.17	3.02^{c} ± 0.07	4.35^{bc} ± 0.37

Different letters in each row showed significant difference at $p \leq 0.05$

Plants grown in FYM amended soil showed percent reductions of 69 ,35, 21, 24, 71 and 33 in MDA content, peroxidase activity, proline, phenol, ascorbic acid and thiol contents, respectively as compared to the plants grown in the control soil. Due to lower availability of heavy metals in plants grown under FYM amended soil, the requirement of antioxidants was low and hence induction of antioxidants were also lower compared to other amendments and the control.

3.6. Heavy metal concentrations in plant parts

In both root and shoot portions, of concentrations of metals were minimum in FYM amended soil **[Figure–4a, –4b]**. Plants grown in FYM amended soil showed reductions of 42, 34, 17, 17, 33 and 41 % for Cd, Cu, Pb, Zn, Ni and Cr, respectively in shoot portion compared to the plants grown in the control soil. Singh et al. [28, 30] have also reported reductions in heavy metals in the plant parts under FYM amendment of contaminated soil. Plants grown in FYM+ NPK and FYM + N amended soil showed less concentrations of heavy metals compared to plants grown in NPK and N amended soil because the phyto availability of heavy metals was lower in the soil under combined treatment **[Figure –4a, –4b]**. The residual effect of FYM along with the inorganic fertilizer is responsible for decreasing the concentrations of heavy metals in the plants.

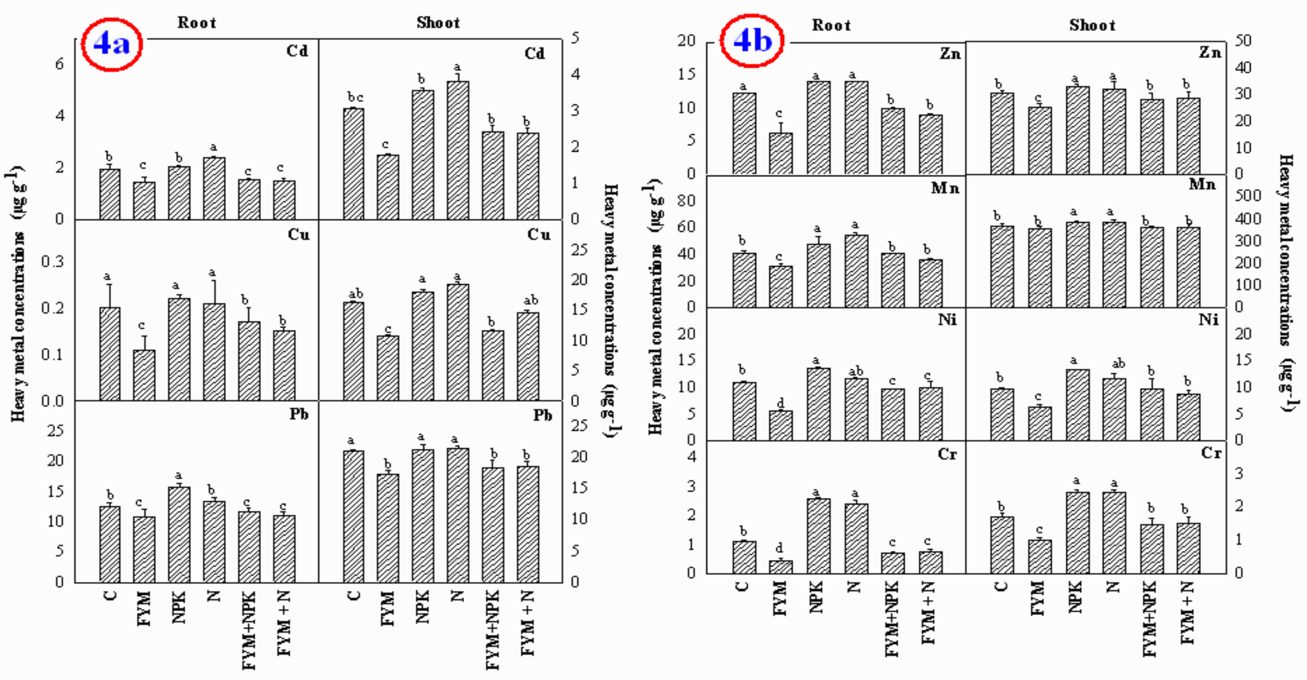

Fig: 4. (a). Cd, Cu and Pb concentrations in root and shoot portions of palak at the time of harvest. (b). Zn, Mn, Ni and Cr concentrations in root and shoot portions of palak at the time of harvest. Values are mean ± 1 SE. Bars with different letters in each group showed significant difference at $p \leq 0.05$.

[IV] CONCLUSIONS

Results of the present study conclude that among all the amendments, availability of heavy metals was higher in NPK and N amended soil and lower in soil amended with FYM alone and in FYM + NPK and FYM + N. Higher accumulation of heavy metals under NPK and N amendments led higher induction of antioxidants in the plants. Plants grown in FYM alone and FYM in combination with NPK and N showed better growth compared to other amendments and the control. The present study suggests that FYM alone and in combination with N and NPK (inorganic fertilizer) may be used to reduce the phytoavailability of heavy metals in the soil and consequently to maintain the physiological vitality and to improve the growth and yield of the plants at contaminated sites utilized for agriculture and horticulture purposes.

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