



Research Article

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**Translocation of micronutrients in French bean (*Phaseolus vulgaris* L.) grown on soil amended with paper mill sludge**

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**ABSTRACT**

Translocation of micronutrients was investigated in *Phaseolus vulgaris* (var. Annapurna) grown on soil amended with paper mill sludge. The plants of *P. vulgaris* were grown in soil amended with 20%, 40%, 60%, 80%, and 100% paper mill sludge to study the accumulation and translocation of iron (Fe), zinc (Zn), cadmium (Cd), copper (Cu), chromium (Cr) and lead (Pb) in plant parts (shoot, root, leaves, fruits). The different rates of paper mill sludge showed significant ( $P < 0.01$ ) change in pH, electrical conductivity (EC), cation exchange capacity (CEC), organic matter (OM), nitrate nitrogen ( $\text{NO}_3^{2-}\text{-N}$ ), phosphate phosphorus ( $\text{PO}_4^{3-}\text{-P}$ ), Fe, Zn, Cd, Cu, Cr and Pb of the amended soil in comparison to their controls. The shoot, root length and yield showed significant ( $P < 0.05$ ) increase in lower amendment of sludge up to 20% to 40% in comparison to controls. The translocation of Fe was in order of leaves > shoot > root > fruits, for Zn, Cu and Pb it was Shoot > leaves > root > fruits and for Cd and Cr it was root > shoot > leaves > fruits of *P. vulgaris*. The order of accumulation of micronutrients in *P. vulgaris* was in order of Fe > Zn > Cd > Cu > Cr > Pb after amended with paper mill sludge. The translocation of different micronutrients was recorded to be plant parts specific. Significant positive correlation was recorded between metal accumulation in the soil and plants as well after amended with paper mill sludge.

**Keywords:** *P. vulgaris*, Paper mill sludge, micronutrients, Amendments, Accumulation, Translocation.

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**INTRODUCTION**

During last decade there is an increasing interest in the agricultural application of sludge obtained from wastewater treatment plants due to the possibility of recycling valuable components such as organic matter, N, P, K and other plant nutrients [31, 33, 37, 40]. Although, sewage sludge has been shown to increase crop production [27, 28] however, it may contain certain trace elements at levels injurious to plants and the food chain. The characterization of sewage sludge's metals is an important requirement prior to sludge disposal or application to farmland because there is a risk of toxic element accumulation in the soil [1, 36]. There are various reports available on the risk associated with the application of metal loaded sludge on agricultural land [16, 17, 40].

The nature of the soil is one of the most important factors in determining the heavy metal content of food plants [13]. However, the heavy metal content in plants can also be affected by other factors such as the application of fertilizers, sewage sludge or irrigation with wastewater [10, 12]. Thus the effect of water on soil and crops are of major concern to people when the irrigant is wastewater which may contain agents capable of inducing adverse effects on the soil media and the agricultural products. At present, there are 666 pulp and paper mills in India, of which 632 units are agro-residue and recycled fiber based units [9]. They generate a large amount of wastewater (black liquor) having high COD and BOD values. Effluent of Kraft mill is highly polluted and characterized by parameters unique to this waste such as color, absorbable organic halides and related organic compound [11]. The high chlorine contents of bleached plant react with lignin and its derivatives and form highly toxic and recalcitrant compounds that are responsible for higher biological and chemical oxygen demand. Trichlorophenol, trichloroguaiacol, dichlorophenol, dichloroguaiacol and pentachlorophenol are major contaminants formed in the sludge

of pulp and paper mill [5, 21]. *Phaseolus vulgaris* (French bean) is an annual herb of the leguminoseae family. Its seeds are used as spice and its leaves are used as leafy vegetables which are rich in vitamins and minerals. The seeds are protein rich. Seeds are used as spice and have medicinal values in the treatment of dyspepsia, rheumatism, asthma and constipation. It is also an important source of diosgenin. It is also a good source of cattle fodder. Keeping in view the reuse of wastewater effluent and the economic importance of *P. vulgaris*, the present investigation was undertaken to use the distillery effluents as a source of bio-fertilizer for more productivity of this crop [30, 40]. The utilization of industrial waste as soil amendment has generated interest in recent times. In recent past various studies have been made on the characteristics of sludge and their effect on soil characteristics [6, 8, 14, 18, 19, 20, 21]. Keeping in view of the above, a pot experiment is designed and the plants of *P. vulgaris* were grown on soil amended with different percentage of paper mill sludge in order to assess the translocation of micronutrients in the plant.

## EXPERIMENTAL SECTION

### Experimental design

A field study was conducted in the Experimental Garden of the Department of Zoology and Environmental Sciences, Faculty of Life Sciences, Gurukula Kangri University Haridwar (29°55'10.81" N and 78°07'08.12" E), for studying the effect of paper mill sludge on *P. vulgaris*. The poly bags having soil were used for the cultivation of *P. vulgaris*. The experiment was replicated by six times. Proper distance was maintained between each replicate (30 cm.) and between all treatments (60 cm.) for the maximum performance of the crop. Each poly bag was made porous for aeration and it was labeled for the various treatments viz. 20, 40, 60, 80 and 100%.

### Paper mill sludge collection and analysis

The paper mill sludge was collected from Star Paper Mill Saharanpur (Uttar Pradesh, India) in plastic bags. The paper mill sludge brought to the laboratory was analyzed for various physico-chemical and heavy metals viz. electrical conductivity, cation exchange capacity, organic matter, nitrate, and phosphate, Fe, Zn, Cd, Cu, Cr and Pb content following standard methods [2, 3] and further used for the amendment of the soil in different concentrations viz. 20, 40, 60, 80 and 100% for cultivation of *P. vulgaris*.

### Soil preparation, filling of pots, sampling and analysis

The soil used was collected at a depth of 0 – 15 cm, from the garden, air-dried and sieved to remove debris. Various amendments 20, 40, 60, 80 and 100% of paper mill sludge were prepared using garden soil along with 100% garden soil which served as control soil (C). Each pot (30x30cm.) was filled with 5 Kg well prepared soil. Borewell water (BWW) was used for irrigation of pot soils for 12-week (growing period of *P. vulgaris*). The soil was analyzed before and after sludge amendment as per effluent concentration for various physico-chemical parameters following standard methods [3].

### Cultivation practices of *P. vulgaris*

Seeds of *P. vulgaris* seeds (var. Annapurna) were procured from ICAR, Pusa, New Delhi and sterilized with 0.01 mercuric chloride and was soaked for 12 hrs. Ten seeds were initially sown in each pot. The plants were watered twice a week with 500 ml of Bore well water. Crop parameters viz. root, shoot leaves, biomass and fruits was noted by following Chandrasekar et al. [4] and chlorophyll content was estimated by following Porra [22].

### Micronutrients extraction and analysis

For the extraction of micronutrients, 0.5-1.0 g sample of air dried sludge/soil/plant was taken in digestion tube. 3 ml conc. HNO<sub>3</sub> was added and digested the sample on electrically heated block for 1 hour at 145° C. Then added 4 ml of HClO<sub>4</sub> and heated to 240° C for an additional hour. Aliquot was cooled, filtered through Whatman # 42 filter paper and made the volume 50 ml with double distilled water and used for analysis. The metals were analyzed by using Atomic absorption spectrophotometer (PerkinElmer Analyst 800 AAS) following standard methods [3, 4].

### Statistical analysis

Data was analyzed for one way analysis of variance (ANOVA) for determining the difference between soil parameters before and after sludge amendment with different concentrations. The mean and standard deviation for different parameters of the sludge and soil were also calculated with the help of MS Excel. The coefficient of correlation (r-value) was also calculated with the help of SPSS12.0 and graphical work was also carried out by using Sigma plot, 2000.

## RESULTS AND DISCUSSION

## Characteristics of paper mill sludge

The characteristics of paper mill sludge are given in Table 1. The results revealed that the paper mill sludge showed the higher content of Fe (19.25 mg Kg<sup>-1</sup>), Zn (12.42 mg Kg<sup>-1</sup>), Cd (9.98 mg Kg<sup>-1</sup>), Cu (10.47 mg Kg<sup>-1</sup>), Cr (9.86 mg Kg<sup>-1</sup>) and Pb (8.14 mg Kg<sup>-1</sup>) with 100% paper mill sludge. It was alkaline in nature with higher electrical conductivity, nitrate and phosphates.

Table1. Characteristics of Star paper mill sludge.

Parameter	Paper mill sludge concentration (%)				
	20	40	60	80	100
EC(dS m <sup>-1</sup> )	3.78±1.46	6.60±1.54	8.79±1.43	12.44±1.73	17.88±1.31
pH	7.65±1.24	7.91±1.21	7.95±1.6	8.12±1.73	8.48±1.27
NO <sub>3</sub> <sup>2-</sup> -N (mg Kg <sup>-1</sup> )	69.43±3.81	128.91±6.85	238.29±3.22	318.46±8.74	398.51±6.84
PO <sub>4</sub> <sup>3-</sup> -P (mg Kg <sup>-1</sup> )	22.37±3.26	46.79±3.17	89.81±3.81	136.87±4.14	188.25±5.76
Fe <sup>2+</sup> (mg Kg <sup>-1</sup> )	4.64±0.15	7.98±0.35	12.83±1.71	16.72±1.57	19.25±2.59
Zn (mg Kg <sup>-1</sup> )	3.64±0.15	5.54±0.18	7.19±0.72	9.65±0.49	12.42±1.22
Cd (mg Kg <sup>-1</sup> )	1.88±0.13	3.74±0.14	5.78±0.16	7.64±0.5	9.98±0.13
Cu (mg Kg <sup>-1</sup> )	2.23±0.18	4.58±0.13	6.42±0.17	8.69±0.15	10.47±1.16
Pb (mg Kg <sup>-1</sup> )	1.19±0.12	2.55±0.15	4.75±0.14	6.56±0.16	8.14±1.11
Cr (mg Kg <sup>-1</sup> )	1.18±0.10	3.55±0.13	5.56±0.12	7.58±0.13	9.86±1.18

Mean ± SD of four values

Table 2. Characteristics of soil before and after amended with Star paper mill sludge used in the cultivation of *P. vulgaris*

Parameters	Before sludge amendment (Control)	After sludge amendment					F-calculated	CD
		Sludge concentration (%)						
		20	40	60	80	100		
BD (gm cm <sup>-3</sup> )	1.40±0.12	1.39±0.07	1.38±0.07	1.37±0.01	1.36±0.02	1.35±0.05	0.7 NS	0.1
pH	7.86±0.19	8.17±0.29	8.25±0.31	8.38a±0.29	8.42a±0.08	8.68a±0.21	2.72*	0.34
EC (dS m <sup>-1</sup> )	2.18±0.07	2.61a±0.06	2.73a±0.04	2.97a±0.11	3.27a±0.17	4.19a±0.19	32.54***	0.19
CEC (cmol kg <sup>-1</sup> )	14.56±1.03	16.68±2.59	23.56a±2.48	25.57a±4.58	28.56a±2.82	32.45a±2.73	16.77***	4.21
OC(mg Kg <sup>-1</sup> )	0.62±0.10	2.99a±0.52	4.28a±0.18	5.43a±0.20	7.69a±0.98	11.56a±0.78	319.72***	0.56
NO <sub>3</sub> <sup>2-</sup> -N (mg Kg <sup>-1</sup> )	42.36±4.34	54.07a±3.58	68.89a±4.48	72.54a±3.37	89.45a±2.74	97.61a±4.76	27.38***	5.72
PO <sub>4</sub> <sup>3-</sup> -P (mg Kg <sup>-1</sup> )	62.42±4.79	78.87a±3.60	97.44a±2.70	118.22a±4.23	127.61a±2.55	145.09a±3.48	256.44***	5.15
Fe <sup>2+</sup> (mg Kg <sup>-1</sup> )	2.63±0.85	5.69a±0.73	6.33a±0.43	7.51a±0.47	8.83a±0.42	10.99a±0.63	18.74***	1.02
Zn (mg Kg <sup>-1</sup> )	0.765±0.16	2.241a±0.12	3.419a±0.11	4.536a±0.09	5.673a±0.13	6.790a±0.29	78.69***	0.27
Cd (mg Kg <sup>-1</sup> )	0.040±0.06	0.117a±0.02	1.147a±0.01	2.148a±0.01	3.165a±0.01	4.176a±0.02	16.88***	0.04
Cu (mg Kg <sup>-1</sup> )	2.023±0.33	4.441a±0.41	5.205a±0.23	6.688a±0.47	7.185a±0.16	8.695a±1.02	55.23***	0.62
Pb (mg Kg <sup>-1</sup> )	0.016±0.01	1.125a±0.02	1.136a±0.05	2.150a±0.04	2.161a±0.07	2.171a±0.09	68.52***	0.02
Cr (mg Kg <sup>-1</sup> )	0.104±0.06	1.310a±0.05	1523a±0.05	2.609a±0.03	2.893a±0.07	3.933a±0.08	140.25***	0.09

Mean ± SD of four values; Significant F - \*\*P > 1% level, \*P > 5% level, a - significantly different to the control; NS - Not Significant

Table 3. Regression linear equation relating added Star paper mill sludge to soil characteristics

Effluent/soil characteristics	Regression equation	R <sup>2</sup>
Paper mill sludge versus soil BD	y = 1.4024 - 0.0007x	0.819
Paper mill sludge versus soil pH	y = 8.0896 + 0.0047x	0.956
Paper mill sludge versus soil EC	y = 2.3814 + 0.0089x	0.829
Paper mill sludge versus soil ECEC	y = 14.299 + 0.1274x	0.869
Paper mill sludge versus soil OC	y = 1.3029 + 0.0859x	0.914
Paper mill sludge versus soil NO <sub>3</sub> <sup>2-</sup> -N	y = 40.088 + 0.2411x	0.942
Paper mill sludge versus soil PO <sub>4</sub> <sup>3-</sup> -P	y = 65.675 + 0.6782x	0.883
Paper mill sludge versus soil Fe <sup>2+</sup>	y = 3.4739 + 0.0307x	0.789
Paper mill sludge versus soil Zn	y = 1.6955 + 0.0132x	0.582
Paper mill sludge versus soil Cd	y = 0.0936 + 0.001x	0.694
Paper mill sludge versus soil Cu	y = 3.0244 + 0.0419x	0.867
Paper mill sludge versus soil Pb	y = 0.0868 + 0.001x	0.621
Paper mill sludge versus soil Cr	y = 0.2085 + 0.0081x	0.947

## Characteristics of soil after amended with paper mill sludge

After the amendment of the soil with paper mill sludge the BD showed insignificant (P > 0.05) and negative linear relationship with the soil organic carbon (Table 2). Weil and Kroontje [32] also observed a negative linear relationship between soil organic matter content and BD on a soil amended with increasing rates of poultry manure application. The BD was minimum (1.35 g cm<sup>-3</sup>) in 100% of paper mill sludge followed by 80%, 60%, 40% and 20%. The BD was maximum (1.40 g cm<sup>-3</sup>) in control, which was insignificantly different (P > 0.05) with the concentrations of paper mill sludge (Table 2).

Table 4. Effect on growth of *P. vulgaris* plants grown on different amendments of Star paper mill sludge

Sludge concentration	Shoot length (cm.)	Root length (cm.)	Biomass	Chlorophyll content (mg/g.)	Yield (gm.)
0 (Control)	28.36±1.58	11.47±2.86	2.98±0.89	2.12±0.06	36.58±4.26
20	32.52±2.36	14.26±2.14	3.69±1.02	3.12±0.09	48.57±5.34
40	39.86±3.14	18.96±1.69	6.58±2.01	5.36±1.01	64.83±6.98
60	36.56±2.27	16.42±2.34	5.14±1.94	3.44±0.08	64.25±5.88
80	31.45±1.98	13.44±2.47	4.63±1.24	3.21±0.09	52.44±6.32
100	28.96±2.45	12.15±2.65	3.22±1.08	2.45±0.11	46.22±5.87
r-value	-0.64	-0.64	-0.43	-0.57	-0.39
F-calculated	4.28*	3.14*	1.98NS	1.74NS	6.94*
CD	1.34	0.16	0.47	0.28	3.77

Mean ± SD of four values; Significant F - \*\*P > 1% level, \*P > 5% level, a - significantly different to the control; NS - Not Significant

The soil pH was recorded initial level (7.86) alkaline and it was turned to more alkaline (8.68) with 100% concentration of paper mill sludge. The sludge concentration 60%, 80% and 100% of paper mill sludge showed significant ( $P < 0.05$ ) effect on soil pH in comparison to control soil (Table 2). The regression equation and  $R^2$  value, 95% of the variation in soil pH was recorded for by paper mill sludge (Table 3). The increase in the rate of application of paper mill sludge significantly ( $P < 0.001$ ) increased the EC of the soil (Table 2). It was recorded to be significantly different with 20% to 100% concentration of paper mill sludge in comparison to control soil. The sludge treated plots registered significantly higher EC ( $4.19 \text{ dS m}^{-1}$ ) than control ( $2.18 \text{ dS m}^{-1}$ ) this was due to very high salt load ( $17.88 \text{ dS m}^{-1}$ ) EC of the paper mill sludge. The regression equation and  $R^2$  value, 82% of the variation in soil EC was recorded for by paper mill sludge (Table 3). Similar findings were also reported by the authors Chonker *et al.* [7] and Raverkar *et al.* [23]. The ECEC was increased in the paper mill sludge amended soil were increased significantly from initial level  $14.56\text{--}32.45 \text{ cmol kg}^{-1}$  in 100% of paper mill sludge. The ECEC of the paper mill sludge amended soil was found to be significantly ( $P < 0.001$ ) different with 25% to 100% concentrations of paper mill sludge (Table 2). The regression equation and  $R^2$  value, 86% of the variation in soil ECEC was recorded for by paper mill sludge (Table 3).

The content of nitrate and phosphate were increased significantly from an initial (control) level of  $42.36\text{--}97.61 \text{ mg Kg}^{-1}$  and  $62.42\text{--}145.09 \text{ mg Kg}^{-1}$  in 100% of paper mill sludge respectively. The effluent concentrations 20%, 40%, 60%, 80% and 100% of paper mill sludge showed significant ( $P < 0.001$ ) change in nitrate and phosphate of the soil. The organic carbon content of the soil increased considerably with the application of paper mill sludge. It increased from an initial level of  $0.62\text{--}11.56 \text{ mg Kg}^{-1}$  in 100% of paper mill sludge. The soil organic carbon was found to be significantly ( $P < 0.001$ ) different with 20% to 100% concentrations of paper mill sludge (Table 2). The regression equation and  $R^2$  value, 91% of the variation in soil organic carbon was recorded for by paper mill sludge (Table 3). Addition of organic matter through effluent and better crop growth with concomitant increase in root biomass could be the probable reasons for the improvement in organic carbon content particularly in high paper mill sludge treated plots. The results of Singh *et al.* [24] support these findings. The concentration of micronutrients viz. Fe, Zn, Cd, Cu, Cr and Pb were recorded to be significantly ( $P < 0.001$ ) affected with 10% to 100% concentration of paper mill sludge (Table 2). The regression equation and  $R^2$  value, 78%, 58%, 69%, 86%, 62% and 94% of the variation in soil Fe, Zn, Cd, Cu, Cr and Pb were recorded for by paper mill sludge (Table 3).

Yang *et al.* [34] reported that metal content; pH, Eh, water content, organic substances, and other elements in the rhizosphere affect the bioavailability and plant uptake of heavy metals in the soils. They also emphasized that plant roots and soil microbes and their interaction can improve metal bioavailability in rhizosphere through secretion of protons, organic acids, phytochelators, amino acids, and enzymes. The observed increase in soil pH as paper mill sludge amendments increased was attributed to the alkalizing effect of paper mill sludge. Organic matter and pH are important factors that control the availability of heavy metals in the soil [15, 37, 38, 39]. Raising the soil organic matter can increase the soil CEC, a factor that may affect both soluble and exchangeable metal levels [35, 39], which was also found in the present study. However, in the current study, the effects of paper mill sludge on soil pH seemed to be one of the promising factors, which affects availability, and uptake of metals (Fe, Zn, Cd, Cu, Cr and Pb).

#### Effects on growth parameters and photosynthetic pigments

The effect of paper mill sludge on the growth of *P. vulgaris* is given in Table 4. The maximum shoot length, root length, biomass, chlorophyll content and crop yield of *P. vulgaris* were noted with 40% of paper mill sludge in comparison to control. The amendment with 40% paper mill sludge showed significant ( $P < 0.05$ ) change in shoot, root length and crop yield of *P. vulgaris*. Biomass and chlorophyll content of *P. vulgaris* was recorded insignificantly ( $P > 0.05$ ) different with paper mill sludge. Shoot length, root length, biomass and total chlorophyll

content (Table 4) increased from 20% to 40% of paper mill sludge and further decreased from 60% to 100% of paper mill sludge as compared to their respective controls.

The changes observed in the growth of fenugreek were consistent with the results reported by Singh and Sinha [25, 26]. They observed that the shoot length of *Brassica juncea* and *H. annuus* grown on 75% tannery sludge amendment have shown an increase in its length as compared to control, however, root length of the exposed plants increased up to 35% tannery sludge as compared to control.

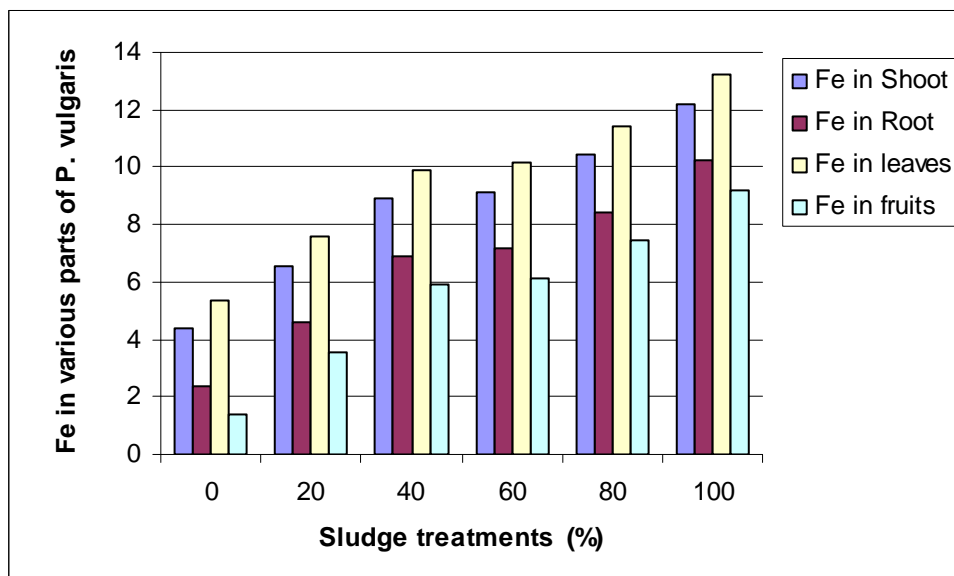


Fig. 1 Translocation of Fe in various parts of *P. vulgaris* cultivated in paper mill sludge amended soil.

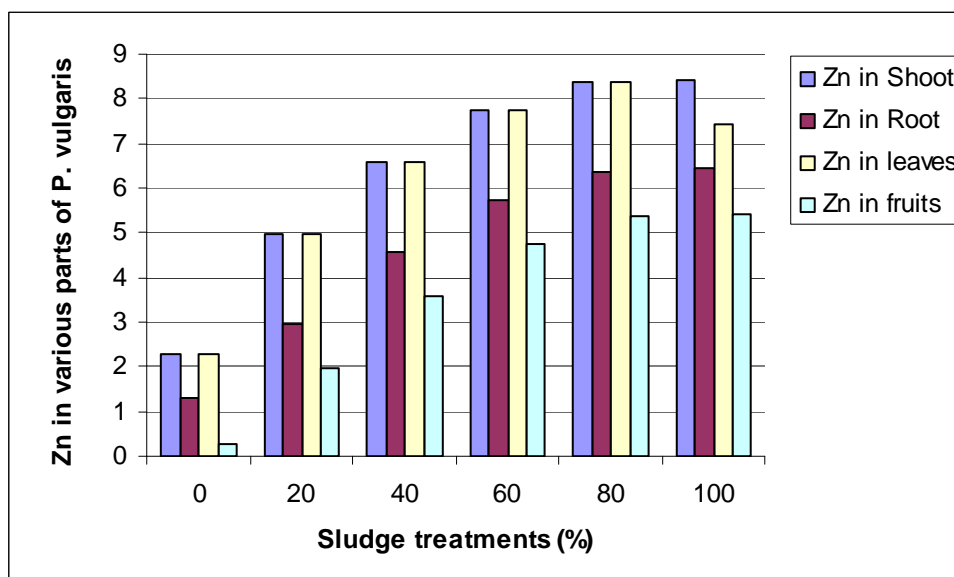


Fig. 2 Translocation of Zn in various parts of *P. vulgaris* cultivated in paper mill sludge amended soil.

#### Micronutrients translocation in *P. vulgaris*

Increasing sludge ratio caused a progressive increase in the accumulation of metals (Fe, Zn, Cd, Cu, Cr and Pb) in the roots and shoots, leaves and fruits of *P. vulgaris*. The 40% to 100% paper mill sludge showed significant ( $P < 0.05$ ) accumulation of Fe, Zn, Cd, Cu, Cr and Pb in root, shoot, leaves and fruits of *P. vulgaris* (Fig. 1, 2, 3, 4, 5, 6). The order of accumulation of metals was in order of  $\text{Fe} > \text{Zn} > \text{Cd} > \text{Cu} > \text{Cr} > \text{Pb}$  in *P. vulgaris* after amended with paper mill sludge. In the present study the maximum content of Fe was noted in the leaves ( $13.22 \text{ mgg}^{-1}$ ) of *P. vulgaris* while the minimum ( $9.22 \text{ mgg}^{-1}$ ) was in the fruits of *P. vulgaris*. The translocation of Fe was in order of leaves > shoot > root > fruits (Fig. 1). The content of Zn, Cu and Pb was found maximum in the shoot ( $8.44$ ,  $2.17$  and  $0.09 \text{ mgg}^{-1}$ ) of *P. vulgaris* and minimum was in the fruits ( $5.44$ ,  $0.87$  and  $0.02 \text{ mgg}^{-1}$ ) of *P. vulgaris* after amended

with paper mill sludge. The order of Zn, Cu and Pb translocation was Shoot > leaves > root > fruits of *P. vulgaris* (Fig. 2, 4, 5). Cd and Cr was recorded maximum in root (3.01, 0.58 mgg<sup>-1</sup>) of *P. vulgaris* and minimum was in fruits (1.88 and 0.41 mgg<sup>-1</sup>) of *P. vulgaris* and it was translocated in order of root > shoot > leaves > fruits of *P. vulgaris* after amended with paper mill sludge (Fig. 3, 6).

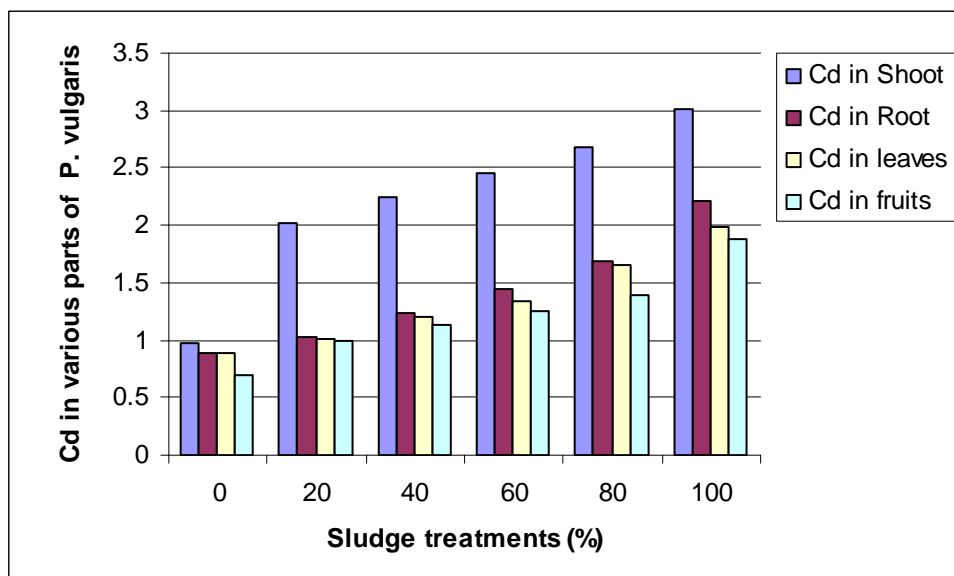


Fig. 3 Translocation of Cd in various parts of *P. vulgaris* cultivated in paper mill sludge amended soil.

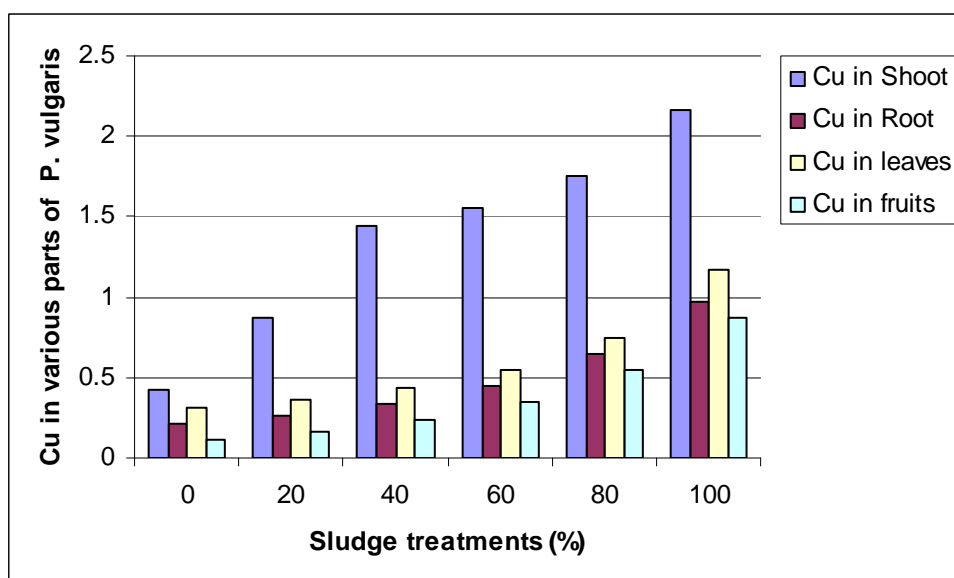


Fig. 4 Translocation of Cu in various parts of *P. vulgaris* cultivated in paper mill sludge amended soil.

The availability and bioaccumulation of metal is governed by several environmental factors viz. pH, solubility, and chemical speciation of the metal, presence of humic substances, presence of other metals, salinity, soil mineralogy, texture, and amorphous Fe and Al content. However, the observed differences in the metal accumulation in the different parts of the plant suggest different cellular mechanisms of bioaccumulation of metals, may control their translocation and partitioning in the plant. Poor translocation of Cr to the shoots could be due to sequesterization of most of the Cr in the vacuoles of the root cells to render it non-toxic which may be a natural protective response of this plant. It must be noted that Cr is a toxic and non-essential element to plants and hence the plants may not possess any specific mechanism to transport the Cr [24].

Recently, Singh *et al.* [27, 28] reported the accumulation of metals in different parts of the plants of tomato and *Helianthus annuus* grown on tannery sludge amended soil, which increased in a concentration and duration-dependent approach. The lowest accumulation of Cr was found in the edible parts of the tomato and BDL in seed of

*H. annuus*. Recently, Sinha *et al.* [30] reported that fenugreek plants have shown healthy growth when grown on soil irrigated with treated tannery wastewater. These authors have also reported the accumulation of Cr in root ( $117.24 \text{ mgg}^{-1}$ ), leaf ( $54.79 \text{ mgg}^{-1}$ ), and flower ( $35.42 \text{ mgg}^{-1}$ ).

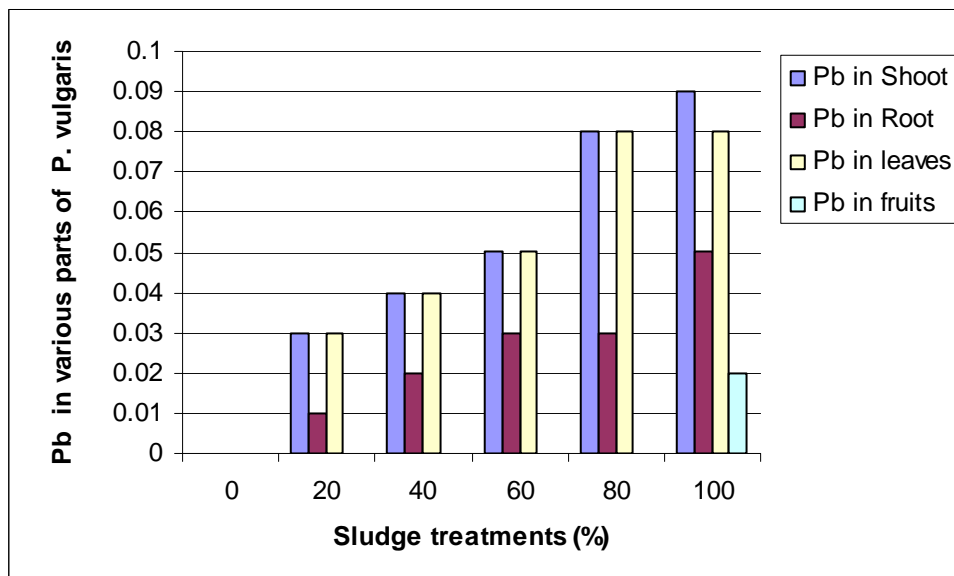


Fig. 5 Translocation of Pb in various parts of *P. vulgaris* cultivated in paper mill sludge amended soil.

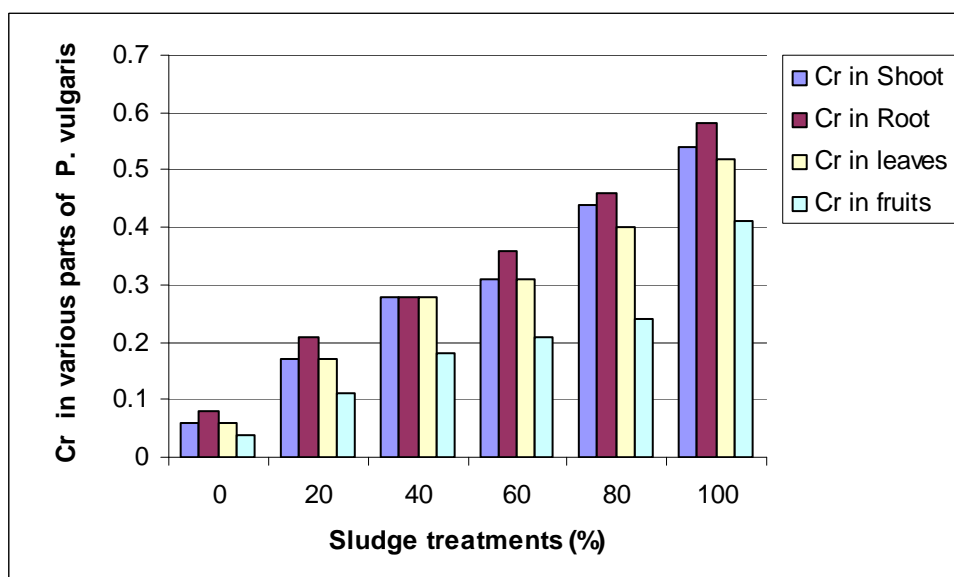


Fig. 6 Translocation of Cr in various parts of *P. vulgaris* cultivated in paper mill sludge amended soil.

#### Pearson Correlation for micronutrients accumulated in plants

The correlation coefficient ( $r$ ) of micronutrients accumulation in plant parts (shoot, root, leaves, fruits) showed significantly ( $P < 0.05$ ) positive correlation with different concentration of paper mill sludge (Fig. 1, 2, 3, 4, 5, 6). The accumulation of Fe in the shoot, root, leaves and fruits ( $r = +94$ ) of *P. vulgaris* showed positive correlation with different concentration of paper mill sludge. The accumulation of Zn showed positive correlation for Shoot ( $r = +88$ ), root and fruits ( $r = +91$ ) and leaves ( $r = +80$ ) of *P. vulgaris* with 20% to 100% concentration of paper mill sludge. The correlation of Cd in the shoot and leaves ( $r = +99$ ), root ( $r = +88$ ) and fruits ( $r = +96$ ) of *P. vulgaris*. Cu also showed the positive correlation for the shoot ( $r = +94$ ), root and fruits ( $r = +98$ ) and leaves ( $r = +96$ ) of *P. vulgaris*. The accumulation of Pb for shoot ( $r = +97$ ), root ( $r = +96$ ), leaves ( $r = +76$ ) and fruits ( $r = +71$ ) was also recorded to be positively correlated with different concentrations of paper mill sludge. Cr showed positive correlation for shoot, root ( $r = +98$ ), leaves ( $r = +97$ ) and fruits ( $r = +96$ ) of *P. vulgaris* after amended with paper mill sludge.

## CONCLUSION

The present investigation concluded that the plants of *P. vulgaris* grown on different amendments of paper mill sludge are well adapted due to increased level of antioxidants, which minimized the damage caused by toxic oxygen species. The accumulation of metals was increased with the increase in sludge treatment concentration. The order of accumulation of micronutrients was in order of Fe > Zn > Cd > Cu > Cr > Pb in *P. vulgaris* after amended with paper mill sludge. The translocation of metals was recorded to be plant parts specific. For Fe the translocation was in order of leaves > shoot > root > fruits, for Zn, Cu and Pb it was Shoot > leaves > root > fruits and for Cd and Cr it was root > shoot > leaves > fruits of *P. vulgaris*. Further, it is important to note that the least amount of toxic metals was translocated up to the fruit part of French bean at all the amendments of paper mill sludge. It is of the utmost importance to assess the level of metals in these plants before use in edible purposes.

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