Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

| ISSN: 0972-6268 | Vol. 15 | No. 3 | pp. 817-824 | 2016 |
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Original Research Paper

# Phytoextraction and Bioconcentration of Heavy Metals by *Spinacia oleracea* Grown in Paper Mill Effluent Irrigated Soil

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**Nat. Env. & Poll. Tech.**
**Website: www.neptjournal.com**

*Received:* 10-08-2015
*Accepted:* 07-10-2015

**Key Words:**
Bioaccumulation
Heavy metals
Paper mill effluent
*Spinacia oleracea*

## ABSTRACT

A comparative pot experiment was conducted in 2014 to study heavy metal contamination in soil and bioaccumulation in leaves and roots of *Spinacia oleracea* (spinach) on irrigation with paper mill effluent (PME). Study revealed that there was significant increase in the content of copper (Cu, +81.62 %) at (P<1%), iron (Fe, +79.62 %), zinc (Zn, +90.73 %), cadmium (Cd, +98.99 %), lead (Pb, +95.91 %), nickel (Ni, +98.40 %), chromium (Cr, +97.54 %) at (P<0.1%) in the soil irrigated with PME as compared to soil irrigated with pond water (PW) (control). Increasing PME doses from 10 % (10 % PME + 90 % PW) up to 80 % (80 % PME + 20 % PW) caused a progressive increase in the accumulation of metals, i.e. Fe, Zn, Cd, Cu, Pb, Ni and Cr in the roots and leaves of *S. oleracea*. The metals accumulated in the plant parts (root and leaves) of *S. oleracea* showed significant (p<5%) and a positive correlation with different concentrations of PME. Among various metallic concentrations high values of heavy metals such as Fe (10.97±0.02 mg/kg), Zn (8.45±0.10 mg/kg), Cd (7.63±0.08 mg/kg), Cu (12.12±0.09 mg/kg), Pb (6.47±0.04 mg/kg), Ni (8.48±0.10 mg/kg), and Cr (5.81±0.02 mg/kg) were found in roots compared to Fe (9.58±0.06 mg/kg), Zn (6.68±0.06 mg/kg), Cd (5.67±0.01 mg/kg), Cu (10.30±0.11 mg/kg), Pb (4.83±0.03 mg/kg), Ni (6.84±0.02 mg/kg) and Cr (4.50±0.09 mg/kg) in leaves of *S. oleracea* irrigated with 80 % (80 % PME + 20 % PW) dose. Thus, the practice of using undiluted PME as agro-based organic fertilizer for irrigation increased the concentration of heavy metals which were accumulated in *S. oleracea*, posing a potential threat to human health from this practice of irrigation. Therefore, proper dilution of PME appears to be necessary for irrigation purpose for the minimum accumulation of heavy metals in soil and plants.

## INTRODUCTION

Large areas of agricultural soils and aquatic bodies in India are contaminated with trace elements that mainly originate either from geogenic activities, bad agronomic practices, industrial emissions or the application of municipal waste water/industrial effluents (Kumar & Chopra 2012, Kashyap et al. 2015). Pulp and paper mills are major industrial sectors utilizing a huge amount of lingo-cellulogic materials and water during the manufacturing process and release chlorinated lingo-sulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbon in the effluents (Liss et al. 1997, Singh 2007). In India, there are 666 pulp and paper mills, out of which 632 mills are agro-residue based mills (Malla & Mohanty 2005, Kumar & Chopra 2012). Paper making process demands a large amount of freshwater and produces enormous quantities of wastewater. The industrial and municipal wastewaters are increasingly being utilized as a valuable resource for irrigation in urban and peri-urban agriculture, because of their easy availability and thus partially solving the problem of effluent disposal (Mishra et al. 2009, Gupta et al. 2010, Ghosh et al. 2011). Irrigation of crops with effluents is a very common practice in India due to scarcity of water for irrigation (Sharma et al. 2007, Arora et al. 2008). The concentration of heavy metals in paper mill effluents (PME) is usually low, but long-term use of these wastewaters on agricultural lands often results in buildup of the elevated levels of metals in soils (Rattan et al. 2002, Singh et al. 2010, Ghosh et al. 2011). Plants as essential components of natural ecosystems and agrosystems, representing the first compartment of the terrestrial food chain (Kimberly & William 1999). Wastewater irrigation is known to contribute significantly to the heavy metal contents in plants (Mapanda et al. 2005). The heavy metals taken into plants from soils are redistributed, i.e. uptake and accumulation of elements by plants may follow two different paths through the roots or foliar surface (Sawidis et al. 2001). The uptake of metals from the soil depends on different factors such as their soluble content in soil, soil pH, plant growth stages, types of species, fertilizers applied (Ismail et al. 2005, Sharma et al. 2006). Plant species have a variety of capacities in removing and accumulating heavy metals. It has been documented that some species may accumulate specific heavy metals, causing a serious health risk to human health when such plants based food stuffs are consumed (Wenzel & Jackwer 1999). The effect of effluent irrigation on various crops/vegetables also has been studied to observe the concentration of accumulated metals to which human beings are exposed (Ismail et al. 2005, Singh & Kumar 2006).

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family of Amaranthaceae. It is a cool-season crop that is easily grown here through winter and in early spring. It is a leafy vegetable and has greater potential of accumulating heavy metals in their edible parts than grain or fruit crops, and has hypoglycemic properties (Subhash et al. 2010, Gupta et al. 2010, Chopra & Pathak 2012). Keeping in view of its commercial essentiality, especially in northern India, appraisal of present investigation relates to the phytoextraction and bioconcentration of heavy metals in different parts of the spinach, which is growing on PME irrigated soil. Metal concentrations in PME, soil, *S. oleracea* (roots, leaves) were measured and compared to FAO/WHO standard limits. This study is expected to provide evidences for extraction, translocation, bioconcentration and resistance of *S. oleracea* plants to metals found in paper mill effluents.

## MATERIALS AND METHODS

**Study site and experimental setup:** A comparative pot study was conducted at the experimental Farm of the Department of Environmental Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry Nauni, Solan, Himachal Pradesh (30° 51' 29.21' N and 77°10' 07.13' E), India, during the period of October 2014-December 2014 to study the accumulation of heavy metals in *Spinacia oleracea* (spinach) irrigated with PME. The 12 kilogram pots without pores (dia. 60 cm), filled with a homogenous mixture of garden soil, compost and sand in 1:2:1 ratio, were used for growing *S. oleracea*. Seeds were sterilized with 0.01 % mercuric chloride and were soaked in distilled water for 12 hours. Ten seeds were initially sown in each pot. Each pot was irrigated twice a week with 1,000 mL of pond water (PW) till germination of 90 % plants.

The experiment was laid in a completely randomized design with six treatments, i.e. 10% (10% PME + 90% PW), 20% (20% PME + 80% PW), 40% (40% PME + 60% PW), 60% (60% PME + 40 % PW) and 80% (80% PME + 20% PW) of paper mill effluent were assessed along with 100% PW as control, separately in six replicates. Thus thirty-six (6 treatments × 6 replicates = 36) pots were used for cultivation of *S. oleracea*. The proper distance was maintained between each replicate (30 cm) between all treatments (60 cm) and plant to plant (5 cm) for the maximum performance of the crop. Plants were irrigated for 70 days with different treatments of PME and PW.

**Collection and preparation of PME doses:** Ruchira paper and pulp industry, Rampur Jatan, Kalaamb, Himachal Pradesh, India, was selected for the collection of effluent samples. The effluents (PME) were collected from the outlet of the secondary settling tank installed in the campus of the industry to reduce the biochemical oxygen demand and solids, using plastic containers of 50 litres. Before starting experiment each PME treatment/dose viz. 10%, 20%, 40%, 60% and 80% in the laboratory was prepared.

**Soil, plant and effluent analysis:** The soil was analysed for the certain physicochemical parameters and heavy metals before and after harvesting the crop which had been irrigated for 60 days with PME and PW separately. The standard methods, viz. pH and electrical conductivity by taking the soil: water ratio of 1:2 using glass electrode pH meter and electrical conductivity meter, respectively (Jackson 1973); organic carbon (OC %) by (Walkley & Black 1934) available calcium (Ava. Ca+) and magnesium (Mg) by ammonium acetate method (Merwin & Peech 1951), were used.

The soil (n=36) and *S. oleracea* {leaves (n=36) and (roots n=36)} samples were dried in air at room temperature. The dried soil was sieved through 2-mm sieve and *S. oleracea* were ground using pestle and mortar. The 0.5 g samples were used for further processing. Soil (after/before 70 days), plant (after 70 days) and 5-10 mL of effluent (before 70 days) samples were digested in a mixture of concentrated nitric acid ($HNO_3$) and perchloric acid ($HClO_4$) (2:1, v/v) as per the standard method described in Association of Official Analytical Chemists (AOAC 1990) and Chaturvedi & Sankar (2006). In short, after digestion, all samples were diluted with double distilled water (1:3), filtered through Whatmann No. 42 filter paper, and in each case, volume was made to 50 mL. Heavy metals such as iron (Fe), zinc (Zn), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni) and chromium (Cr) were determined in the digested aliquot by Inductively Coupled Plasma Absorption Spectrometer-6300 DUO (ICAP- Model No. 6300 Duo) with the help of capillary tube of ICAP (Kashyap et al. 2015, APHA 2011). All the reagents and standards were of analytical grade.

**Statistical analysis of data:** Data were analysed for one way analysis of variance (ANOVA) for determining the difference between soil parameters before and after irrigation with different PME/PW treatments. Standard errors for each parameter were also calculated with the help of MS Excel- 2013. SPSS-20.0 was used for the calculation of coefficient of corelation (r-values) between heavy metals in paper mill effluent and different parts of *Spinacia oleracea*. The Turkey HSD test was used to compare the mean values among treatments, when a significant variation over all treatments was indicated.

## RESULTS AND DISCUSSION

**Characteristics of PME:** The mean values (±SE) of different physicochemical parameters viz. pH, EC, $Ca^{2+}$, $Mg^{2+}$, especially heavy metals (Fe, Zn. Cd, Cu, Pb, Ni, Cr) in PME at different concentrations are presented in Table 1. The results revealed that it was dark brown in colour with obnoxious odour and alkaline in nature. Among various parameters of effluent, (80%) dose was recorded with the highest pH (8.97±0.01), EC (16.94±0.02dSm$^{-1}$), $Ca^{2+}$ (355.83±0.17 mg/L), $Mg^{2+}$ (215.00±0.17 mg/L), Fe (19.14±0.03 mg/L), Zn (11.30±0.08 mg/L), Cd (9.25±0.08mg/L), Cu (14.13±0.05 mg/L), Pb (9.36±0.02 mg/L), Ni (12.22±0.05 mg/L) and Cr (8.54±0.01 mg/L), which were beyond the prescribed limits of Indian irrigation standards (BIS 1991). Kumar & Chopra (2012) also reported high inorganic load of heavy metals in paper mill effluents.

**Characteristics of Soil:** The mean values (±SE) and percentage increase/decrease of different physicochemical parameters viz. pH, EC, OC, $Ca^{2+}$, $Mg^{2+}$, especially heavy metals (Fe, Zn. Cd, Cu, Pb, Ni, Cr) in soil before and after 70 days of irrigation with PME at different concentrations and PW as control are given in Table 2. The irrigation of PME generally led to changes in the physico-chemical characteristics of soil and consequently heavy metal uptake by vegetables. There was a significant increase in each parameter of soil characteristics after irrigation with PEM, viz. soil pH was increased (8.67 % to 14.23 %) on irrigation with different doses of effluent. With an increase in concentration of PME in soil, it became alkaline (8.57±0.01) in nature significantly different (P<0.1%) from soil before and after irrigation with PW (8.05±0.01) in control (Table 2). High buffering capacity of the clay soil and nominal presence of any weak salts, namely carbonates or bicarbonates, which on dissolution release free cations, might be the possible causes for the stability of the soil reaction. This is the pH range of maximum nutrient availability in the soils (Brady & Weil 2005). The pH levels that resulted from the different levels of pollution appeared favourable to both biological and chemical reactions in the soils (Brady & Weil 2005).

The increase in the rate of PME (10-80 %) significantly (P<0.1 %) increased EC (Table 2), which was significant in comparison to control, i.e. PW irrigated soil. Similarly, electrical conductivity was maximum (8.94±0.01 dSm$^{-1}$) in 80 % of PME followed by 60 %, 40 %, 20 % and 10 %. Similar findings were also reported by Chonker et al. (2000) and Raverkar et al. (2000). The increase in EC from control irrigated soil (1.03 dS m$^{-1}$) to (2.26 dS m$^{-1}$) in 100% concentration of paper mill effluent irrigated soil was also reported earlier by Vinod et al. 2010.

The organic carbon (OC) content of soil was increased significantly (P>5 %) with application of PEM. The OC was maximum (1.50±0.03 %, +47.67 %) in 80 % PME irrigated soil and significantly different from control PW dose. However, minimum OC (1.15±0.01%, +31.94 %) was recorded in 10 % PME irrigated soil. Addition of organic matter through effluent and better crop growth with concomitant increase in root biomass could be the probable reasons for

Table 1: Characteristics of paper mill effluent.

| | Parameters paper mill effluent concentration (%) | | | | | BIS for irrigation water |
|---|---|---|---|---|---|---|
| | 10% | 20% | 40% | 60% | 80% | |
| pH | 7.90±0.01 | 7.93±0.01 | 8.09±0.03 | 8.59±0.01 | 8.97±0.01 | 5.5-9 |
| EC (dSm$^{-1}$) | 4.21±0.01 | 6.88±0.01 | 9.09±0.04 | 11.09±0.03 | 16.94±0.02 | N.A. |
| Ca (mg/L) | 52.77±0.18 | 72.52±0.13 | 152.30±0.14 | 230.83±0.40 | 355.83±0.17 | 250 |
| Mg (mg/L) | 30.18±0.09 | 50.51±0.14 | 105.33±0.21 | 180.08±0.08 | 215.00±0.17 | 200 |
| Fe (mg/L) | 3.83±0.01 | 6.48±0.11 | 11.16±0.01 | 16.11±0.02 | 19.14±0.03 | 10 |
| Zn (mg/L) | 2.28±0.07 | 3.50±0.08 | 6.14±0.01 | 8.00±0.02 | 11.30±0.08 | 15 |
| Cd (mg/L) | 1.97±0.02 | 3.68±0.10 | 5.15±0.06 | 7.66±0.01 | 9.25±0.08 | 2 |
| Cu (mg/L) | 3.85±0.01 | 7.31±0.07 | 9.13±0.03 | 12.13±0.03 | 14.13±0.05 | 3 |
| Pb (mg/L) | 1.29±0.02 | 2.14±0.01 | 4.12±0.04 | 7.85±0.01 | 9.36±0.02 | 1 |
| Ni (mg/L) | 1.47±0.02 | 2.98±0.03 | 5.84±0.02 | 9.68±0.08 | 12.22±0.05 | 2 |
| Cr (mg/L) | 1.23±0.01 | 2.01±0.02 | 4.51±0.02 | 6.00±0.03 | 8.54±0.01 | 2 |

Mean ± SE (standard error) of six values, BIS- Indian Standards for water quality the improvement in organic carbon content particularly in high PME treated plots. The results of Zalawadia & Raman (1994), Pathak et al. (1999) support these findings.

On irrigation with PME the exchangeable calcium and magnesium were changed with different concentrations of the effluent. The effluent concentrations 10 %, 20 %, 40 %, 60 % and 80 % of PME showed significant (P<0.1 %) change in the content of Ca and Mg in comparison to control soil irrigated with PW. It was quite interesting to note that the content of Ca and Mg were also recorded to be significantly (P<0.1%) different with 10 % concentration of PME (Table 2). The content of exchangeable calcium and magnesium were increased significantly from an initial (control) level of (38.43±0.14, +15.80 %) mg/kg to (112.83±0.40, +71.32 %) mg/kg, (41.49±0.13, +27.25 %) mg/kg to (104.67±0.21, +71.16 %) mg/kg in 80 % of PME, respectively. The similar increase in Ca and Mg from control irrigated soil in 100 % concentration of paper mill effluent irrigated soil was also reported earlier (Vinod et al. 2010).

**Heavy metals in soil:** The concentration of heavy metals viz. Fe, Zn, Cd, Cu, Pb, Ni and Cr were significantly (P<0.1%) affected by 20 % to 80 % concentration of PME (Table 2). The concentrations of heavy metals Fe (12.42±0.09, +79.62 %) mg/kg, Zn (9.40±0.05, +90.73 %) mg/kg, Cd (8.26±0.02, +98.99 %) mg/kg, Cu (13.19±0.04, +81.62 %) mg/kg, Pb (7.70±0.10, +95.91 %) mg/kg, Ni (9.37±0.13, +98.40 %) mg/kg and Cr (6.77±0.05, +97.54 %) mg/kg in soil irrigated with highest dose of PME was lower than the concentrations reported by Roy et al. (2007) for Fe (14,285±1244 mg/kg), Cr (197.76±12.83 mg/kg), and Zn (104.91±0.97 mg/kg) in the soil irrigated with tannery waste water; and the concentration reported by Chandra et al. (2009) for Zn (115.43± 7.75 mg/kg), Cr (53.70±12.37 mg/kg), Cd (11.42±4.63 mg/kg), Fe (1,715.80±421.33 mg/kg) in wastewater irrigated soil at industrial areas of Bangladesh. Under acidic conditions, elements such as Fe, Al, Mn and the heavy metals (Zn, Cu, Pb and Cr) become highly soluble and may create problems for vegetation (Charman & Murphy 1991). This is also in agreement with what was reported by other workers that organic wastes contain high amount of micronutrients and heavy metals (Roy et al. 2007). Physicochemical parameters and heavy metals in PME irrigated soil indicated that pH-OC, EC-Cu, OC-Cl, $NO_3$-Fe, $SO_4$-Fe, Fe-Mn, Zn-Cd, Cd-Pb, Cu-Cl, Pb-Cr, Mn-Cl, Cr and Cl had a strong positive correlation (Table 5).

**Phytoextraction of heavy metals in parts of *S. oleracea*:** The heavy metal concentrations in leaves and roots of *S. oleracea* after 70 days of effluent irrigation are shown in

Table 2: Characteristics of soil before/after PME and PW irrigation used for cultivation of *Spinacia oleracea*.

| Parameters | Before effluent irrigation | After pond water irrigation (Control) | After paper mill effluent irrigation Paper mill effluent concentration (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 10% | 20% | 40% | 60% | 80% | F calculated | CD |
| pH | 7.35±0.00 | 8.05±0.01 (+8.67%) | 8.16±0.01 (+9.85%) | 8.18±0.01 (+10.09%) | 8.28±0.01 (+11.19%) | 8.37±0.01 (+12.09%) | 8.57±0.01 (+14.23%) | 1999.11*** | 0.03 |
| EC ($dSm^{-1}$) | 2.62±0.01 | 2.84±0.02 (+7.80%) | 3.86±0.01 (+32.04%) | 4.81±0.05 (+45.47%) | 5.65±0.07 (+53.63%) | 6.93±0.01 (+62.18%) | 8.94±0.01 (+70.70%) | 4582.71*** | 0.10 |
| OC (%) | 0.79±0.01 | 1.07±0.01 (+26.52%) | 1.15±0.01 (+31.94%) | 1.17±0.01 (+32.91%) | 1.32±0.01 (+40.68%) | 1.38±0.04 (+43.25%) | 1.50±0.03 (+47.67%) | 155.84* | 0.05 |
| Ca (mg/kg) | 32.36±0.13 | 38.43±0.14 (+15.80%) | 42.33±0.76 (+23.56%) | 44.72±0.31 (+27.64%) | 55.00±0.82 (+41.17%) | 85.83±0.91 (+62.30%) | 112.83±0.40 (+71.32%) | 2588.80*** | 1.68 |
| Mg (mg/kg) | 30.18±0.09 | 41.49±0.13 (+27.25%) | 46.17±0.98 (+34.62%) | 50.32±0.10 (+40.02%) | 57.33±0.84 (+47.35%) | 74.50±0.43 (+59.49%) | 104.67±0.21 (+71.16%) | 2228.69*** | 1.16 |
| Fe (mg/kg) | 2.53±0.02 | 3.08±0.05 (+17.89%) | 3.57±0.07 (+29.02%) | 4.25±0.08 (+40.43%) | 6.03±0.08 (+58.04%) | 9.57±0.10 (+73.53%) | 12.42±0.09 (+79.62%) | 2535.96*** | 0.21 |
| Zn (mg/kg) | 0.87±0.00 | 0.87±0.02 (0.00%) | 1.10±0.04 (+20.76%) | 3.58±0.01 (+75.63%) | 5.31±0.02 (+83.59%) | 7.75±0.17 (+88.75%) | 9.40±0.05 (+90.73%) | 2463.31*** | 0.20 |
| Cd (mg/kg) | 0.08±0.00 | 0.08±0.00 (-2.04%) | 0.12±0.01 (+30.56%) | 2.17±0.01 (+96.17%) | 4.31±0.03 (+98.07%) | 6.52±0.11 (+98.72%) | 8.26±0.02 (+98.99%) | 5778.74*** | 0.13 |
| Cu (mg/kg) | 2.42±0.01 | 3.09±0.04 (+21.45%) | 5.17±0.31 (+53.10%) | 5.55±0.10 (+56.34%) | 7.94±0.01 (+69.47%) | 9.96±0.10 (+75.67%) | 13.19±0.04 (+81.62%) | 888.97** | 0.38 |
| Pb (mg/kg) | 0.32±0.01 | 0.15±0.01 (-114.77%) | 0.18±0.00 (-76.64%) | 2.67±0.13 (+88.20%) | 4.20±0.02 (+92.50%) | 5.31±0.04 (+94.07%) | 7.70±0.10 (+95.91%) | 2287.93*** | 0.18 |
| Ni (mg/kg) | 0.15±0.01 | 0.18±0.00 (+15.09%) | 0.22±0.01 (+31.82%) | 1.43±0.07 (+89.53%) | 2.35±0.06 (+93.62%) | 5.22±0.08 (+97.12%) | 9.37±0.13 (+98.40%) | 2679.26*** | 0.19 |
| Cr (mg/kg) | 0.17±0.01 | 0.18±0.00 (+6.54%) | 0.28±0.00 (+39.76%) | 2.20±0.06 (+92.42%) | 5.20±0.05 (+96.79%) | 5.76±0.01 (+97.10%) | 6.77±0.05 (+97.54%) | 6164.46*** | 0.11 |

Mean ± SE (standard error) of six values; Significant F - ***P<0.1%, **P<1%, *P<5% level, PW - Pond water, % increase/decrease in comparison to control given in parenthesis, CD - critical difference

Table 3: Heavy metals content in leaves and roots of *Spinacia oleracea* grown in PME and PW irrigated soil.

| Heavy metals | Plant parts | After pond water irrigation (Control) | After paper mill effluent irrigation Paper mill effluent concentration (%) | | | | | Limits | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 10% | 20% | 40% | 60% | 80% | Limit (a) | Limit (b) |
| Fe (mg/kg) | Leaves | 1.97±0.01 | 2.20±0.03 | 2.77±0.03 | 4.02±0.04 | 5.57±0.03 | 9.58±0.06 | 2.5 | 2.5 |
| | Roots | 2.48±0.05 | 2.98±0.05 | 3.51±0.03 | 5.40±0.07 | 7.61±0.05 | 10.97±0.02 | | |
| Zn (mg/kg) | Leaves | 0.56±0.02 | 0.64±0.01 | 1.55±0.10 | 3.41±0.07 | 5.24±0.01 | 6.68±0.06 | 0.3 | 1 |
| | Roots | 0.77±0.01 | 0.95±0.01 | 2.11±0.19 | 4.65±0.07 | 6.47±0.09 | 8.45±0.10 | | |
| Cd (mg/kg) | Leaves | 0.06±0.01 | 0.10±0.01 | 0.90±0.01 | 0.75±0.01 | 3.79±0.07 | 5.67±0.01 | 0.3 | 1.5 |
| | Roots | 0.07±0.01 | 0.96±0.02 | 1.25±0.03 | 0.96±0.02 | 4.35±0.07 | 7.63±0.08 | | |
| Cu (mg/kg) | Leaves | 0.88±0.07 | 1.40±0.06 | 2.30±0.07 | 4.27±0.05 | 6.22±0.02 | 10.30±0.11 | 0.05 | 0.5 |
| | Roots | 2.83±0.01 | 4.27±0.06 | 4.42±0.05 | 5.52±0.06 | 8.54±0.01 | 12.12±0.09 | | |
| Pb (mg/kg) | Leaves | 0.04±0.01 | 0.07±0.01 | 0.88±0.01 | 2.43±0.09 | 3.58±0.05 | 4.83±0.03 | 5 | 2.5 |
| | Roots | 0.14±0.01 | 0.16±0.01 | 1.15±0.01 | 3.13±0.07 | 4.03±0.09 | 6.47±0.04 | | |
| Ni (mg/kg) | Leaves | 0.09±0.01 | 0.15±0.01 | 0.76±0.01 | 0.90±0.02 | 2.80±0.05 | 6.84±0.02 | 2.0 | 1.5 |
| | Roots | 0.15±0.01 | 0.19±0.01 | 1.00±0.07 | 1.37±0.06 | 4.60±0.05 | 8.48±0.10 | | |
| Cr (mg/kg) | Leaves | 0.06±0.01 | 0.18±0.01 | 0.94±0.02 | 2.25±0.17 | 3.57±0.01 | 4.50±0.09 | 5 | 2.0 |
| | Roots | 0.16±0.01 | 0.23±0.01 | 1.96±0.01 | 3.91±0.03 | 4.70±0.07 | 5.81±0.02 | | |

Mean ± SE (standard error) of six values, (a) FAO/WHO standard (Codex Alimentarious Commission 1984) (b) Indian standard (Awashthi 2000).

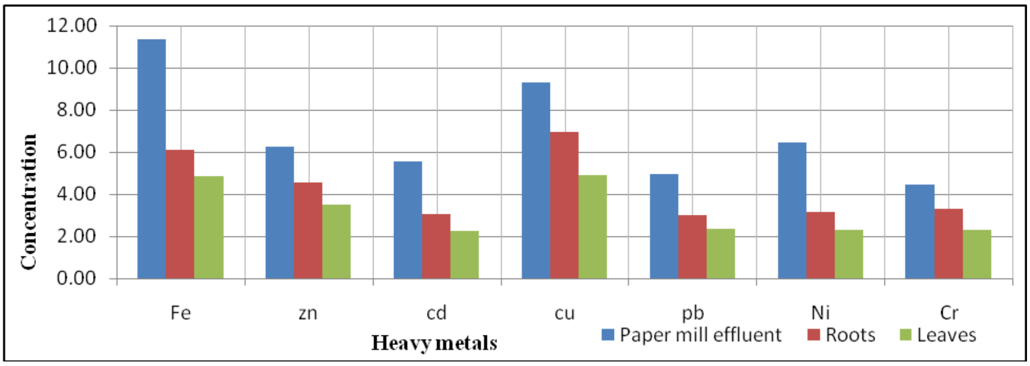


Fig. 1: Mean concentration of heavy metals in paper mill effluent (mg/L) versus roots and leaves (mg/kg) of *Spinacia oleracea.*

Table 3 and Fig. 1. The present study revealed that high concentrations of heavy metals such as Fe (10.97±0.02 mg/kg), Zn (8.45±0.10 mg/kg), Cd (7.63±0.08 mg/kg), Cu (12.12±0.09 mg/kg), Pb (6.47±0.04 mg/kg), Ni (8.48±0.10 mg/kg), and Cr (5.81±0.02 mg/kg) were in roots as compared to Fe (9.58±0.06 mg/kg), Zn (6.68±0.06 mg/kg), Cd (5.67±0.01 mg/kg), Cu (10.30±0.11 mg/kg), Pb (4.83±0.03 mg/kg), Ni (6.84±0.02 mg/kg), and Cr (4.50±0.09 mg/kg) in leaves of *S. oleracea* irrigated with 80 % PME dose. The concentrations of heavy metals in edible parts of *S. oleracea* irrigated with PME were found above the permissible limits of Indian Standard (Awashthi 2000, FAO/WHO Standard Codex Alimentarious Commission 1984). Increasing PME ratio caused a progressive increase in the accumulation of metals, i.e. Fe, Zn, Cd, Cu, Pb, Ni and Cr in the roots and leaves of *S. oleracea*. Metals accumulated in the plant parts (root and leaves) of *S. oleracea* showed significant (P <5 %) and a positive correlation with different concentrations of PME (Table 4). It was quite interesting to know that the accumulation of metals in roots as well in leaves of *S. oleracea* followed same trend in the order of Cu>Fe>Ni>Zn>Cd>Pb>Cr after amendment with different doses of PME. The accumulation of heavy metals in plant parts was in accord-

Table 4: Coefficient of correlation (r) between heavy metals in PME and different parts of *Spinacia oleracea.*

| Paper mill effluent versus heavy metals | Roots | Leaves |
|---|---|---|
| Paper mill effluent versus iron (Fe) | +97 | +93 |
| Paper mill effluent versus zinc (Zn) | +99 | +99 |
| Paper mill effluent versus cadmium (Cd) | +90 | +95 |
| Paper mill effluent versus copper (Cu) | +91 | +95 |
| Paper mill effluent versus lead (Pb) | +96 | +98 |
| Paper mill effluent versus nickel (Ni) | +95 | +91 |
| Paper mill effluent versus chromium (Cr) | +96 | +99 |

Table 5: Coefficient of correlation (r-values) between physicochemical parameters and heavy metals irrigated with PME

| | pH | EC | OC | $NO_3$ | $SO_4$ | Fe | Zn | Cd | Cu | Pb | Mn | Cr | Cl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.000 | | | | | | | | | | | | |
| EC | 0.811 | 1.000 | | | | | | | | | | | |
| OC | 0.968 | 0.916 | 1.000 | | | | | | | | | | |
| $NO_3$ | 0.744 | 0.965 | 0.852 | 1.000 | | | | | | | | | |
| $SO_4$ | 0.816 | 0.977 | 0.898 | 0.987 | 1.000 | | | | | | | | |
| Fe | 0.752 | 0.977 | 0.870 | 0.996 | 0.982 | 1.000 | | | | | | | |
| Zn | 0.751 | 0.981 | 0.880 | 0.951 | 0.942 | 0.973 | 1.000 | | | | | | |
| Cd | 0.739 | 0.978 | 0.874 | 0.956 | 0.944 | 0.978 | 0.998 | 1.000 | | | | | |
| Cu | 0.825 | 0.995 | 0.929 | 0.967 | 0.978 | 0.979 | 0.972 | 0.972 | 1.000 | | | | |
| Pb | 0.739 | 0.980 | 0.868 | 0.940 | 0.943 | 0.962 | 0.993 | 0.992 | 0.967 | 1.000 | | | |
| Mn | 0.696 | 0.962 | 0.814 | 0.991 | 0.981 | 0.987 | 0.949 | 0.955 | 0.955 | 0.952 | 1.000 | | |
| Cr | 0.741 | 0.948 | 0.880 | 0.889 | 0.887 | 0.925 | 0.979 | 0.981 | 0.946 | 0.978 | 0.887 | 1.000 | |
| Cl | 0.839 | 0.993 | 0.943 | 0.949 | 0.963 | 0.968 | 0.979 | 0.978 | 0.996 | 0.975 | 0.937 | 0.966 | 1.000 |

ance with the findings of the other workers (Mishra & Tripathi 2008). The availability and bioaccumulation of metals are governed by several environmental factors such as 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 contents (Gu et al. 2012). However, the observed differences in the metal accumulation in the different parts of the plant suggest different cellular mechanisms of metal bioaccumulation, which may control their translocation and partitioning in the plant (Bradford et al. 1975). Fe, Zn and Cu are associated with the photosynthesis (Porra 2002).

The content of Fe and Zn was also high in the leaves of *S. oleracea* as that in roots and it could be due to the higher content of these metals in the PME amended soil. Fe is essential for the survival and proliferation of all plants. There is a high demand for Fe in the photosynthetic apparatus as a result plants accumulate and translocate Fe in their photosynthetic parts (Porra 2002). Cd content of the roots of *S. oleracea* was higher than that of the leaves. The low Cd value of the leaves may be due to the increased soil pH (8.57±0.01) which is alkaline in nature (Chaoui et al. 1997). The accumulation of Cd in the leaves of *S. oleracea* represents that the translocation of Cd was effectively made from root to leaves. As for the accumulation strategy, plants accumulated lower amounts of Cd in their tissues, and high amount of Cd is stored in the roots due to toxic nature of element (Sun et al. 2008). The content of Cu and Pb were less in the leaves of *S. oleracea* and it is likely due to increase in the organic carbon (OC) of the soil, which will decrease the uptake of Cu and Pb into plants (Zarcinas et al. 2004). The contents of Ni and Cr were higher in the roots than in the aerial parts, indicating that the roots act as barrier for translocation and protect the edible parts from toxic contamination (Mishra & Tripathi 2008). Poor translocation of Cr to the leaves could be due to the 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 (Chaoui et al. 1997).

It must be noted that Ni is a toxic and non-essential element to plants and hence the plants may not possess any specific mechanism to transport Ni (Hall 2002). Thus, the tolerant mechanism of plants appears to be compartmentalization of metal ions, i.e. sequestration in the vacuolar compartment, which excludes them from cellular sites where processes such as cell division and respiration occur, thus proving to be effective protective mechanisms (Pathak et al. 2011, Gu et al. 2012).

## CONCLUSION

It can be concluded that less diluted effluent irrigation of PME for 70 days increased the nutrient status of the soil, including an increase in heavy metals like Cd, Cu, Pb, Ni and Cr which in turn was responsible for the increase of these heavy metals in leaves/roots of *S. oleracea.* Thus, the practice of using less diluted PME as agro-based organic fertilizer for irrigation may accumulate the heavy metals in vegetables posing a potential threat to human health. Farmers can use effluents, especially PME, with more dilution for irrigation of their vegetables. The regular monitoring of these toxic heavy metals in soil and parts of vegetables is essential to prevent their excessive buildup in the food chain. Therefore, proper dilution of PME appears to be necessary for irrigation purpose for the minimum accumulation of heavy metals in soil and plants.

## ACKNOWLEDGEMENTS

The first author is thankful to the Head, Department of Environmental Science, Dr Y S Parmar U H F, Solan, Himachal

Pradesh, India for providing all the necessary facilities to carry out field and laboratory work. The authors declare that they have no conflict of interest.

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