Analysis on bioaccumulation of metals in aquatic environment of Beas River Basin: A case study from Kanjli wetland

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Abstract Wetlands, the biological filters of the Earth, play an important role in biochemical transformation of various pollutants. Wetland plants, in this direction, help in accumulating various contaminants from aquatic bodies. Considering this, the present study was planned to estimate different metals (Cd, Cu, Cr, Co, Fe, Pb, Zn, and Mn) in water, sediment, soil, and plant (4 aquatic and 12 terrestrial) samples of Kanjli wetland, Kapurthala, Punjab (India), and a Ramsar site. It was observed that the contents of Cd and Pb in water samples were higher than limits prescribed by Bureau of Indian standards. Bioaccumulation and translocation factors for various metals were also calculated. Although all the plant species were found to be hyperaccumulator for one or the other metal studied, maximum six metals (Cd, Co, Fe, Mn, Pb, and Zn) were bioaccumulated in Panicum antidotale among aquatic plant species while (Cd, Cu, Fe, Mn, Pb, and Zn) in Lantana camara and Ageratum conyzoids among terrestrial plants species. It is evident that all these plants have potential to phytoremEDIATE various inorganic pollutants and can act as bioindicators. The physicochemical characteristics revealed high biochemical oxygen demand (BOD) and nitrate (NO₃⁻) contents and low dissolved oxygen (DO) in water samples while the high content of phosphates in soil and sediment samples.

1. Introduction

Wetlands, often referred as “Earth’s kidney,” are transitional zone between terrestrial and aquatic system. Wetlands have been playing an important role in purifying the contaminated water for centuries. Majority of wetlands have been exploited for their natural cleansing capacity for assimilating various pollutants including heavy metals and pesticides [Joyce, 2012]. Wetland plants and sediments accumulate various contaminants that enter the water through natural and anthropogenic activities [Li et al., 2006; Adekola and Eletta, 2007; Lu et al., 2011]. Recent developments and industrial activities have resulted in discharging huge quantity of wastes including synthetic chemicals directly into wetlands. Aquatic plants uptake most of these pollutants through root and shoot structures because of their fast growth and high biomass [Bonanno and Lo Giudice, 2010; Matache et al., 2013]. Translocation to shoots is restricted, but magnification of heavy metals in roots reaches as high as 100,000 times more than the surrounding aquatic environment [Mishra et al., 2008]. Aquatic plants have been frequently used to assess wetland pollution all over the world and have been well documented to be indicator of pollution [Zayed et al., 1998; Zhu et al., 1999; Kamal et al., 2004; Souza et al., 2013].

India has a rich source of natural surface water bodies in the form of rivers, streams, ponds, and wetlands. There are atleast 26 wetlands in India which have the recognition of Ramsar site. Punjab, a Northern Indian state, has three such wetlands, viz., Harike, Ropar, and Kanjli, which are listed as Ramsar sites. Despite their importance in terms of economy and environment, various anthropogenic activities are resulting in deterioration of wetland ecosystems. Considering the degrading conditions of Kanjli wetland, the present study was aimed to estimate different metal contents along with other physicochemical characteristics of different samples like water, soil, and sediment of Kanjli wetland, Kapurthala, Punjab (India). The vegetation samples including 4 aquatic and 12 terrestrial plants (leaves) growing in vicinity of wetland were also analyzed for metal contents (Figure 1).

2. Materials and Methods

2.1. Study Area

Kanjli wetland (31°25′N and 75°22′E) is located near Kanjli village at distance of 4 km from Kapurthala Town (Figure 2). It covers an area of 183 ha and has been recognized as one of the wetlands of international
importance, as per Ramsar list since 2002. Kanjli wetland came into existence in 1870 due to the construction of barrage over Kali Bien (a tributary of Beas river), resulting in diversion of water towards Kapurthala. This wetland is thus a freshwater feature. Soil of the area is mainly alluvial in nature consisting of sand, silt, and clay. Maximum depth of water in wetland varies from 3.04 to 7.62 m depending upon the season and water inflow. Major catchment area of Kanjli is under agricultural practices.

2.2. Sample Collection
Sampling sites were systematically chosen for collection of water samples from upstream (approximately 1 km), wetland, and downstream (approximately 1 km). Water samples were randomly collected from 4 to 5 points of each site by immersing the sample bottle at least 15 cm below the surface level. The water samples were pooled in 1 L acid washed polyethylene bottles and brought to laboratory. Vegetation and soil samples were collected from both sides of the wetland belt lying between two extremes, i.e., upstream and downstream, whereas surface layer (0–10 cm) of bottom sediments was collected using handheld collector from the main wetland. Both aquatic and terrestrial plant/plant part samples growing under study area were collected, washed under running water, placed in plastic bags, and brought to laboratory. Collected plant samples were identified by comparing with preserved specimen in the herbarium of the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab. The accession numbers of the plant species were recorded during the identification of plant species (Table 1). In the case of two aquatic plants, viz., *Eichhornia crassipes* and *Panicum antidotale* (dominant macrophytes), different parts of plants
(roots, stem/petiole, and leaves) were separated and dried at 80°C till constant weight was attained. Dried plant parts were then crushed to make fine powder.

2.3. Estimation of Physicochemical Parameters

2.3.1. Heavy Metals

Different metals, viz., iron (Fe), copper (Cu), cadmium (Cd), chromium (Cr), lead (Pb), zinc (Zn), and cobalt (Co), were analyzed in water samples by direct filtration through Whatman filter paper No. 1. Soil and sediment samples were air dried at room temperature and digested with aqua regia (HCl:HNO₃ in 3:1). The solution was evaporated to 2 mL, filtered into 20 mL volumetric flask, and final volume was made up to mark with double distilled water. For plants and plant parts, metals were determined after acid digestion of dry samples with a triacid mixture (HNO₃:H₂SO₄:HClO₄ in 5:1:1) at 80°C till a transparent solution was obtained. Metal contents for all samples were determined using atomic absorption spectrophotometer (AAS 240 FS Agilent)

Table 1. Description of Vegetation Samples Collected From Kanjli Wetland, Kapurthala, Punjab (India)

| Sample No. | Botanical Name         | Common Name     | Family          | Habitat   | Accession Number/Date (DD-MM-YY) |
|------------|------------------------|-----------------|-----------------|-----------|----------------------------------|
| 1          | *Abutilon indicum*     | Indian mellow   | Malvaceae       | Terrestrial | 931/25-8-1982                    |
| 2          | *Achyranthus aspera*    | Chaff flower    | Amaranthaceae   | Terrestrial | 93/2-7-1982                     |
| 3          | *Adiantum caudatum*     | Maidenhair fern | Pteridaceae     | Terrestrial | 190                              |
| 4          | *Ageratum conyzoids*    | Chick weed      | Asteraceae      | Terrestrial | 4866/26-5-1993                   |
| 5          | *Anagallis arvensis*    | Red chick weed  | Primulaceae     | Terrestrial | 1023/26-8-1982                   |
| 6          | *Bignonia gracilis*     | Crossvine       | Bignoniaceae    | Terrestrial | 6133/19-1-2000                   |
| 7          | *Cannabis sativa*       | Bhang           | Cannabaceae     | Terrestrial | 6128/19-1-2000                   |
| 8          | *Eichhornia crassipes*  | Water hyacinth  | Pontederiaceae  | Aquatic    | 2661/23-4-1987                   |
| 9          | *Lantana camara*        | Wild sage       | Verbenaceae     | Terrestrial | 4580/31-3-1993                   |
| 10         | *Lemna minor*           | Duck weed       | Araceae         | Aquatic    | 2669                              |
| 11         | *Malvastum coromandelianum* | Broom weed | Asteraceae      | Terrestrial | 88/01-7-1982                    |
| 12         | *Panicum antidotale*    | Ghumur           | Poaceae         | Aquatic    | 6684/21-10-2011                  |
| 13         | *Parthenium hysterophorus* | Congress weed | Asteraceae      | Terrestrial | 1039/27-8-1982                   |
| 14         | *Potamogeton crispus*   | Curled pond weed | Potamogetonaceae | Aquatic    | 5963/04-8-1998                   |
| 15         | *Prosopis juliflora*    | Vilayati kikar  | Fabaceae        | Terrestrial | 6368/29-8-2001                   |
| 16         | *Sida acuta*            | Wire weed        | Malvaceae       | Terrestrial | 30/10-4-1980                     |
Technologies). The instrument was calibrated with standard solutions prepared from commercially available respective standards (Agilent Tech., Germany). Analytical blank was used before estimation of every metal. The analysis was performed in triplicates.

2.3.2. Other Physicochemical Parameters
Water samples were used for direct pH and electrical conductivity measurements. Prior to analysis, water samples were filtered using Whatman filter No. 1. Various physicochemical parameters such as alkalinity, total hardness, calcium (Ca), magnesium (Mg), chloride (Cl), nitrate (NO₃), phosphate (PO₄) and sodium (Na) were determined using standard methods for the examination of water and waste water [American Public Health Association, 2005]. Soil and sediment samples were dried at room temperature and passed through 2 mm sieve. A 1:5 soil/sediment:water (weight/volume) suspension was prepared, and pH and electrical conductivity (EC) were measured using hydromagnetic digital meter-COM-100 (New Delhi, India) and Equip-tronics EQ-614-A (Mumbai, India), respectively. A core cylinder was used for analysis of bulk density of soil samples [Jacob and Clarke, 2002], while bulk density of sediment was estimated using weight/volume method. Walkley-Black wet oxidation method was used for measuring organic carbon content and organic matter [Nelson and Sommers, 1982]. Ethylenediaminetetraacetic acid (EDTA) titration method was used for measuring calcium (Ca) and magnesium (Mg) [Lanyon and Heald, 1982]. Total nitrogen (N) was determined by Kjeldahl method [Bermner and Mulvaney, 1982], and available phosphorous (P) was measured by sodium bicarbonate extraction using spectrophotometric method [Olsen et al., 1954].

2.4. Metal Bioaccumulation Factor (BAF) and Translocation Factor (TF)
Bioaccumulation factor refers to the efficiency of a plant species to accumulate a metal into its tissue from the surrounding environment [Ladislas et al., 2012]. BAF of different metals from soil to terrestrial plants, sediment to rooted aquatic plants, and water to aquatic plants was calculated on the basis of dry weight of plant samples. It was calculated using the equation given by Wilson and Pyatt [2007].

\[
\text{BAF} % = \frac{C_{\text{plant tissue}}}{C_{\text{soil/sediment/water}}} \times 100
\]

where \(C_{\text{plant tissue}}\) is metal concentration in plant tissue and \(C_{\text{soil/sediment/water}}\) is metal concentration in soil, sediment, and water, respectively.

Translocation factor (TF) refers to the accumulation of metal concentration from roots to other plant parts. It was calculated using following equation [Zacchini et al., 2009].

\[
\text{TF} % = \frac{C_{\text{aerial parts}}}{C_{\text{root}}} \times 100
\]

where \(C_{\text{aerial parts}}\) is metal concentration in plant leaves/stem and \(C_{\text{root}}\) is metal concentration in roots.

2.5. Statistical Analyses
The data were presented as mean ± SE obtained from values for each sample. The experimental data was statistically analyzed using one way analysis of variance and Pearson correlation using STATISTICA 12. Pearson correlation coefficients were also calculated to examine the relationship between the metals content of in vegetation samples.

3. Results and Discussion
3.1. Physicochemical Parameters
3.1.1. Water Samples
Different water samples were analyzed for physicochemical parameters including metal contents (Tables 2–4). Most of the physicochemical characteristics of the studied samples were within the permissible range specified by Bureau of Indian Standards [2012a, 2012b] for drinking and surface water (Table 2). pH of the aquatic system is an important indicator of the water quality and the extent of the pollution in the watershed area. pH range of 6.5–8.5 is normally acceptable as per guideline suggested by Bureau of Indian Standards.
pH values of water samples of Kanjli wetland were found to vary from 7.23 to 7.25 and were within permissible limits. The alkaline pH could be because of the presence of carbonates and bicarbonate of Ca and Mg. The major source of which was the runoff from agricultural fields adjoining the wetland. Electrical conductance reflects inorganic pollution in terms of dominating cations as well as anion. Ionized species in water samples were found to vary between 0.18 and 0.21 mS/cm.

The hardness of water body is regulated by the levels of Ca and Mg salts. Total hardness of all the water samples varied between 120 and 121.3 mg/L and was observed to be below the values (200–600 mg/L) given by Bureau of Indian Standard. Total alkalinity of all water samples was found to be in range of 200–213 mg/L and was within acceptable limits (200–600 mg/L) as prescribed by Bureau of Indian Standards (BIS). Dissolved oxygen (DO) content was 2.27, 2.66, and 1.87 for upstream, wetland, and downstream water samples, respectively. Low values of DO in all samples can be correlated to the fact that Kanjli wetland had heavy growth of *Eichhornia crassipes* and *Panicum antidotale* as well as decomposed matter. The decomposition of dead material plays an important role in consumption of DO in the wetland [Granier et al., 2000; Reddy et al., 2005; Sekomo et al., 2011]. Biochemical oxygen demand were observed to be 36.53 mg/L (upstream), 44.07 mg/L (wetland), and 63.33 mg/L (downstream) indicating high organic pollution. All the sampling sites were under the influence of anthropogenic stress in terms of receiving the effluents from domestic sources. Sutha et al. [2010] reported that BOD in the range of 27–51 mg/L in Hindon River was due to discharge of domestic and industrial effluents. Among inorganic nutrients, maximum nitrate content (83.44 mg/L) was observed to be in the sample collected from downstream of the wetland which was higher than the recommended standard value of 20 mg/L prescribed by BIS for drinking and surface water. Nitrate content in water sample collected upstream of the wetland was observed to be 5.58 mg/L. Phosphate content was maximum in wetland (1.02 mg/L) followed in downstream (0.09 mg/L) and (0.04 mg/L) in upstream site. Domestic waste and chemical fertilizer runoff from adjoining agricultural fields can be considered as the contributory factors for elevated levels

### Table 2. Physicochemical Characteristics of Water Samples Collected From Kanjli Wetland, Kapurthala, Punjab (India)

| Sample No. | Parameter (Units) | Upstream | Wetland | Downstream |
|------------|-------------------|----------|---------|-----------|
| pH         | 7.23 ± 0.015      | 7.23 ± 0.003 | 7.25 ± 0.003 | 6.5–8.5 |
| EC (mS/cm) | 0.18 ± 0.003      | 0.20 ± 0.007 | 0.22 ± 0.011 | - |
| Alkalinity (mg/L) | 200 ± 11.50  | 200 ± 0.000 | 213.3 ± 6.666 | 200–600 |
| Hardness (mg/L) | 120 ± 1.155      | 121.3 ± 1.763 | 120.0 ± 2.000 | 200–600 |
| Calcium (mg/L) | 33.46 ± 0.201    | 33.66 ± 0.066 | 43.68 ± 1.517 | 300 |
| Magnesium (mg/L) | 19.16 ± 0.206   | 19.32 ± 0.429 | 15.75 ± 0.071 | 100 |
| Nitrate (mg/L) | 5.580 ± 0.001    | 8.440 ± 0.110 | 83.44 ± 1.110 | 20–45 |
| Phosphate (mg/L) | 0.040 ± 0.002    | 1.020 ± 0.014 | 0.090 ± 0.002 | 20–50 |
| Chloride (mg/L) | 37.87 ± 1.893    | 37.86 ± 0.946 | 40.70 ± 2.504 | 250–600 |
| Sodium (mg/L) | 20.33 ± 0.333    | 17.26 ± 0.233 | 18.06 ± 0.586 | 200 |
| Dissolved oxygen (mg/L) | 2.270 ± 0.133 | 2.667 ± 0.133 | 1.879 ± 0.537 | 250 |
| Biochemical demand (BOD₅) (mg/L) | 36.53 ± 1.067 | 44.07 ± 0.033 | 63.33 ± 3.333 | 2–3 |

### Table 3. Physicochemical Characteristics of Sediment and Soil Samples Collected From Kanjli Wetland, Kapurthala, Punjab (India)

| Parameter (Units) | Sediment (Mean ± SE) | Soil (Mean ± SE) |
|-------------------|----------------------|------------------|
| pH                | 6.95 ± 0.192         | 8.85 ± 0.011     |
| EC (mS/cm)        | 0.73 ± 0.018         | 0.71 ± 0.011     |
| Bulk Density (g/cm³) | 0.96 ± 0.009       | 1.44 ± 0.022     |
| Calcium (meq/100g) | 9.35 ± 1.686        | 10.1 ± 0.673     |
| Magnesium (meq/100g) | 28.0 ± 2.534      | 22.6 ± 0.841     |
| Chloride (mg/100g) | 3.78 ± 2.987         | 4.02 ± 5.975     |
| Kjehldal nitrogen (mg/100g) | 48.0 ± 0.004 | 19.0 ± 0.001     |
| Available phosphate (mg/100g) | 2453 ± 0.062 | 694.9 ± 0.130 |
| Organic carbon (%) | 0.31 ± 0.006         | 0.11 ± 0.011     |
| Soil organic matter (%) | 0.55 ± 0.004       | 0.19 ± 0.001     |
| Sodium (mg/100g) | 25.2 ± 1.344         | 21.3 ± 0.294     |
of contaminants in water body. Excess of inorganic nutrients like nitrates and phosphates in water cause algal blooms that results in depletion of dissolved oxygen. Depleting level of oxygen in water can also be attributed to putrefaction of organic matter as well as death and decay of aquatic organisms. Both Cl and Na content were observed to be within permissible limits.

During collection of water samples, it was observed that various ritual activities were performed at downstream site of Kanjli wetland which led to accumulation of coconut shells, rags, and rotten flowers. Kanjli wetland complex was also found to be covered by thick layer of *Eichhornia crassipes*. All these factors could be the probable reasons for alteration of physicochemical characteristics of water. Variation in the physicochemical characteristics of water in upstream water could be attributed to the waste water discharges from domestic and industrial sources.

### 3.2. Sediment and Soil Samples

The physicochemical characteristics of sediment and soil samples are presented in Table 3. pH of the sediment was acidic (6.95) whereas soil pH was alkaline (8.85). The electrical conductivity (EC), considered as indicator of salinity, was observed to be 0.73 mS/cm for sediment and 0.71 mS/cm in soil sample. The bulk density is directly related to the porosity of the soil. Higher the porosity, less will be the bulk density. *Troeh and Thompson* [2005] stated that bulk density in range of 1.3–1.4 contained high sand content. Increase of organic matter in soil/sediment samples leads to reduced bulk density whereas inorganic material increases the bulk density. In the present study, bulk density of sediment was observed to be low (0.96 g/cm³) probably due to the reason that sediment was collected from the banks of the wetland which had deposition of the dead and decayed materials over it. The bulk density of 1.3–1.6 g/cm³ is considered best for agricultural practices. Bulk density of soil was 1.44 g/cm³ indicating suitability for agricultural activities. Among other essential nutrients, available P (2453 mg/100g) was maximum in sediment and (694.90 mg/100g) in soil samples. Kjehlal N content was observed to be 2.5-fold higher in sediments (15.0 ± 0.001) when compared to soil (19.0 mg/100g). The contents of other parameters for sediment samples were observed Ca (9.35 meq/100g), Mg (28 meq/100g), Na (25.23 mg/100g), Cl (3.78 mg/100g), and organic carbon (0.31%) whereas that of soil sample as Ca (10.15 meq/100g), Mg (22.66 meq/100g), Na (21.36 mg/100g), Cl (4.02 mg/100g), and organic carbon (0.11%). Organic matter was observed to be 0.55% and 0.19% for soil and sediments, respectively.

### 3.3. Metal Content

#### 3.3.1. Water, Soil, and Sediment Samples

Mean contents of different metals in Kanjli water, sediment, and soil samples are presented in Table 4. Metal contents of water was in the order as Co > Fe > Zn > Mn > Cd > Pb. The content of Cr and Cu were observed to be below detection limit. Maximum contents of different metals were observed as Fe (16.4 μg/L), Zn (24.1 μg/L), Mn (57.0 μg/L), and Cd (36 μg/L) in downstream water sample whereas Pb (20.0 μg/L) in upstream and Co (23.0 μg/L) in wetland sample. Metals either settle and accumulate in the sediments of aquatic system or enter soil through infiltration/irrigation/surface runoff. The accumulation and remobilization of metals in aquatic system are two important
mechanisms that regulate their concentration in water [Ishaq and Khan, 2013]. The decreasing trend of various metals in sediments was Fe > Mn > Zn > Cr > Cu > Pb > Co > Cd, while for soil, it was Fe > Mn > Zn > Cr > Co > Cu > Pb > Cd. The content of heavy metals in sediments was found to be considerably higher than those obtained in wetland water samples. The occurrence of heavy metals in wetland water, sediment, and soil samples can be attributed to discharge of untreated sewage and agrochemical runoff from nearby villages directly into wetland.

### 3.3.2. Plant/Plant Parts

The concentration of metals in 4 aquatic and 12 terrestrial plants collected from Kanjli wetland is presented in Table 5 and 6. Among different aquatic plants, maximum contents of metals were observed as Cd (9.13 mg/kg), Co (976.6 mg/kg), Cr (8.06 mg/kg), Cu (92.40 mg/kg), Pb (83.42 mg/kg), Zn (572.80 mg/kg) in *Eichhornia crassipes* roots, Fe (9766.60 mg/kg), and Mn (944.90 mg/kg) in *Panicum antidotale* stem. 

While minimum contents of metals was observed as Cd (5.20 mg/kg) and Zn (100.90 mg/kg) in *Eichhornia crassipes* petiole, Co (5.19 mg/kg), Cu (7.09 mg/kg), Fe (440 mg/kg), Mn (268.70 mg/kg), and Pb (67.99 mg/kg) were minimum for *Eichhornia crassipes* leaves. It was observed that uptake of metals was maximum in roots followed by leaves and petioles/stem for both samples.

Maximum contents of different metals in terrestrial plant samples studied were observed as Cd (9.59 mg/kg in *Achyranthus aspera*), Co (7.26 mg/kg in *Sida acuta*), Cr (7.42 mg/kg in *Malvestrum coromandiliana*), Cu (131.6 mg/kg in *Prosopis juliflora*), Fe (17502.1 mg/kg in *Malvestrum coromandiliana*), Mn (499.4 mg/kg in *Ageratum conyzoids*), Pb (83.39 mg/kg in *Lantana camara*), and Zn (258.4 mg/kg in *Ageratum conyzoids*) while minimum as Cd (6.75 mg/kg in *Malvestrum coromandiliana*), Co (2.33 mg/kg in *Anagallis arvensis*), Cr (2.33 mg/kg in *Anagallis arvensis*), Cu (3.02 mg/kg in *Bignonia gracilis*), Fe (675.47 mg/kg in *Prosopis juliflora*), Mn (121.1 mg/kg in *Prosopis juliflora*), Pb (23.47 mg/kg in *Prosopis juliflora*), and Zn (29.16 mg/kg in *Cannabis sativa*).

### 3.4. Bioaccumulation and Translocation Factor

To identify hyperaccumulator species BAF and TF can turn out to be important tools. BAF and TF for the metals investigated are shown in Tables 7 and 8. Plants with BAF and TF greater than 100 have potential to act as hyperaccumulator and indicator of pollution [Wilson and Pyatt, 2007]. BAF of different metals in aquatic plants followed an order as mentioned below.

- **Cd**: *E. crassipes* > *L. minor* > *P. antidotale* > *P. crispus*
- **Cr**: *P. crispus*
- **Co**: *E. crassipes* > *P. crispus*
- **Cu**: *P. antidotale* > *P. crispus*
- **Fe**: *E. crassipes* > *L. minor* > *P. antidotale* > *P. crispus*
- **Mn**: *P. antidotale* > *P. crispus*
- **Pb**: *E. crassipes* > *L. minor* > *P. antidotale* > *P. crispus*
- **Zn**: *E. crassipes* > *L. minor* > *P. crispus* > *P. antidotale*

Bio accumulation factor for terrestrial plants is shown in Table 8 and order of BAF was observed as follows:
Table 6. Heavy Metal Contents (mg/kg) in Terrestrial Plant Samples Collected From Kanji Wetland, Kapurthala, Punjab (India)\(^a\)

| Sample No. | Plant sample                        | Cadmium | Cobalt | Chromium | Copper | Iron | Manganese | Lead | Zinc          |
|------------|-------------------------------------|---------|--------|----------|--------|------|-----------|------|---------------|
| 1          | Lantana camara                      | 8.08 ± 0.249 | 3.46 ± 0.401 | BDL      | 17.88 ± 0.648 | 815.82 ± 6.576 | 475.2 ± 1.587 | 83.39 ± 3.008 | 155.8 ± 0.555 |
| 2          | Bignonia gracilis                   | 7.55 ± 0.148 | BDL    | 302 ± 0.008 | 2,339.33 ± 4.147 | 237.9 ± 15.83 | BDL           | 180.6 ± 1.089 |
| 3          | Abutilon indicum                    | 8.80 ± 0.261 | BDL    | 103.3 ± 0.547 | 1,515.06 ± 6.469 | 533.1 ± 10.55 | BDL           | 105.4 ± 0.387 |
| 4          | Adiantum caudatum                   | 7.51 ± 0.526 | BDL    | 62.1 ± 0.233 | 987.86 ± 2.479 | 234.6 ± 2.154 | BDL           | 107.1 ± 0.703 |
| 5          | Malvestrum comordianum              | 6.75 ± 0.057 | 7.18 ± 1.127 | 7.42 ± 1.305 | 9.04 ± 1.203 | 17,502.1 ± 6.194 | 245.9 ± 6.508 | BDL           | 88.23 ± 0.235 |
| 7          | Parthenium hysterophorus            | 7.51 ± 0.148 | 5.66 ± 0.420 | 5.66 ± 0.420 | 32.94 ± 0.393 | 243.46 ± 1.941 | 319.1 ± 2.109 | BDL           | 123.3 ± 0.780 |
| 9          | Sida acuta                          | 7.59 ± 0.097 | 7.26 ± 1.354 | 7.26 ± 1.354 | 14.35 ± 0.951 | 960.31 ± 80.70 | 177.6 ± 2.462 | 30.82 ± 0.749 | 76.26 ± 0.351 |
| 10         | Prosopis juliflora                  | 8.04 ± 0.399 | BDL    | 131.6 ± 1.553 | 675.47 ± 1.467 | 121.1 ± 0.393 | 23.47 ± 1.346 | 31.39 ± 0.493 |
| 11         | Cannabis sativa                     | 7.19 ± 0.261 | 6.36 ± 0.252 | 4.69 ± 1.851 | 11.38 ± 0.223 | 732.53 ± 3.915 | 277.9 ± 0.934 | 31.42 ± 2.696 | 29.16 ± 0.287 |
| 12         | Achyranthus asperis                  | 9.59 ± 0.168 | 4.69 ± 0.740 | 5.02 ± 0.319 | 17.00 ± 0.631 | 944.66 ± 2.188 | 7.48 ± 0.769 | 28.62 ± 2.833 | 34.05 ± 0.016 |

F ratio: 8.864 |

HSD: 1.267 |

\(^a\)Critical value of F statistic (12, 26) at \(p \leq 0.05 = 2.15\). HSD, honestly significant difference; BDL, below detection limits.

Among terrestrial plants, five plant species (\(A. aspera\), \(A. conyzoids\), \(A. arvensis\), \(S. acuta\), and \(S. sativa\)) have shown bioaccumulation for all the metals studied. The bioaccumulation factor for metals varied as Cd: \(A. aspera > A. indica > L. camara > A. arvensis = P. juliflora > S. acuta > B. gracilis > P. hysterophorus = A. caudatum > A. conyzoids > C. sativa > M. coromadilianum\) Cr: \(M. coromadilianum > S. acuta > A. conyzoids > P. hysterophorus > A. aspera > C. sativa > A. arvensis\) Co: \(S. acuta > M. coromadilianum > C. sativa > A. conyzoids > P. hystorophorus > A. aspera > L. camara > A. arvensis\) Cu: \(P. juliflora > A. arvensis > P. hystorophorus > A. conyzoids > L. camara > A. aspera > S. acuta > C. sativa > A. indica > M. coromadilianum > A. caudatum > B. gracilis\) Fe: \(M. coromadilianum > S. acuta > A. arvensis > B. gracilis > A. conyzoids > A. indica > A. caudatum > A. aspera > L. camara > C. sativa > P. juliflora > P. hysterophorus\) Mn: \(A. arvensis > A. indica > A. conyzoids > L. camara > P. hystorophorus > C. sativa > M. coromadilianum > B. gracilis > A. caudatum > S. acuta > P. juliflora > A. aspera\) Pb: \(L. camara > A. conyzoids > C. sativa > S. acuta > A. aspera > A. arvensis > P. juliflora\) Zn: \(A. conyzoids > B. gracilis > L. camara > P. hystorophorus > A. caudatum > A. indica > M. coromadilianum > S. acuta > A. aspera > P. juliflora > C. sativa\)

Table 7. Bioaccumulation and Translocation Factor of Heavy Metals in Aquatic Plants Collected From Kanji Wetland, Kapurthala, Punjab (India)

| Element | Eichhornia Crassipes | Lemma Minor | Panicum Antidotale | Potamogeton Crispus |
|---------|----------------------|--------------|--------------------|---------------------|
|         | BAF                  | TF           | BAF                | TF                  | BAF                |
|         | Root/Water           | Leaf/Root    | Stem/Root          | Root/Sediment       | Leaf/Root          | Stem/Root    | Root/Sediment |
| Cadmium | 304,333.33           | 61.22        | 56.95              | 2,783,333.33        | 753.77             | 100.62       | 73.96         | 674.52       |
| Chromium| -                    | -            | -                  | -                   | -                  | -            | -             | -             |
| Cobalt  | 4,246,086.94         | 0.53         | -                  | -                   | -                  | -            | -             | -             |
| Copper  | 6,645,753.06         | 8.18         | 5.06               | 477,118.51          | 113.96             | 29.08        | 0.008         | 62.29        |
| Iron    | -                    | 81.20        | 94.46              | -                   | 333.26             | 118.41       | 119.98        | 93.48        |
| Manganese| -                   | 81.20        | 94.46              | -                   | 333.26             | 118.41       | 119.98        | 93.48        |
| Lead    | 2,780,666.67         | 81.50        | 95.88              | 25,140.00           | 306.03             | 102.92       | 98.45         | 302.67       |
| Zinc    | 11,416,000.00        | 29.60        | 17.61              | 2,062,000           | 132.25             | 110.83       | 69.26         | 124.91       |
Translocation factor for different metals in _E. crassipes_ and _P. antidotale_ was observed as follows:

- **E. crassipes**<sub>leaf</sub>: Pb > Mn > Cd > Zn > Fe > Co
- **E. crassipes**<sub>petiole</sub>: Pb > Mn > Cd > Zn > Fe
- **P. antidotale**<sub>Leaf</sub>: Mn > Zn > Pb > Cd > Cu > Fe
- **P. antidotale**<sub>Stem</sub>: Mn > Pb > Cd > Zn > Fe

_E. crassipes_ was observed to be the hyperaccumulator for Cd, Co, Fe, Pb, and Zn while _P. antidotale_ for Cu and Mn. Among all metals, Pb has shown maximum translocation in leaves and petiole of _E. crassipes_ whereas Mn in leaves and stem _P. antidotale_.

Cadmium is nonessential as well as highly toxic element that affects growth, metabolism, and water status of plants [Divan et al., 2009]. Cd also produces oxidative stress by releasing free radicals and reactive oxygen species which can cause death of plants via damaging membrane lipids, proteins, pigments, and nucleic acids [Foyer et al., 1994]. In the present study, the content of cadmium in different aquatic plant samples varied from 5.20 mg/kg (E. crassipes<sub>petiole</sub>) to 9.13 mg/kg (E. crassipes<sub>root</sub>) while in terrestrial plants from 6.75 mg/kg (M. coromandilianum<sub>leaf</sub>) to 9.59 mg/kg (A. aspera<sub>leaf</sub>). The content of cadmium (5–700 mg/kg) was previously documented to be in phytotoxic [Chaney, 1989]. Another metal, chromium beyond 0.5 mg/kg was reported to be toxic to plants [Allen, 1989]. In the present study, Cr content in vegetation samples ranged from 2.33 mg/kg to 8.06 mg/kg, indicating high phytotoxic threshold. Low content of cobalt has some favorable effects on plant growth but at higher concentrations; Co is considered as one of the most toxic elements [Bonanno, 2011]. The content of Co ranged from 2.33 mg/kg in A. arvensis<sub>leaf</sub> to 7.26 mg/kg in _S. acuta<sub>leaves</sub>_ among nine terrestrial plants. The average content of Co in leaves of terrestrial species was observed to be that of samples of unpolluted agricultural land where the observed value were 4 mg/kg [Vardanyan and Ingole, 2006; Bonanno, 2011].

Copper at low concentration is essential for plant nutrition and is required for various enzymatic activities but becomes toxic at higher levels [Fairbrother et al., 2007; Bonanno, 2013]. Kabata-Pendias and Pendias [2001] reported that content of Cu in various plants from unpolluted regions in different countries were less than 8.40 mg/kg. During the present study, Cu content was found to be maximum (92.40 mg/kg) in _E. crassipes<sub>root</sub>_ and 131.60 mg/kg in _P. juliflora<sub>leaf</sub>_ whereas minimum, i.e., below detection limits, in _P. antidotale_ and 3.02 mg/kg in _B. caperata<sub>root</sub>_ among aquatic and terrestrial plants, respectively. Most of the plant species had Cu content higher than the phytotoxic range, i.e., 25–40 mg/kg as given byChaney [1989].

Iron, the vital element for plant growth, plays a key role in processes of energy transformation needed for synthesis and other life processes of cell [Kabata-Pendias and Pendias, 2001]. In the present study, Fe content in plants was higher than the natural uncontaminated habitat. _P. antidotale<sub>roots</sub>_ and _E. crassipes<sub>roots</sub>_ showed maximum accumulation of Fe as 9766.66 mg/kg and 5383.06 mg/kg, respectively. The values were observed to be much higher than the natural content (370 mg/kg) as described by Brooks and Robinson [1998]. The reason for high Fe content in roots of plants can be due to the formation of iron hydroxide plaques that are

**Table 8.** Bioaccumulation Factor for Terrestrial Plants Collected From Kanjli Wetland, Kapurthala, Punjab (India)<sup>a</sup>

| Plant Sample          | Cadmium | Chromium | Cobalt | Copper | Iron | Manganese | Lead | Zinc |
|-----------------------|---------|----------|--------|--------|------|-----------|------|------|
| Abutilon indicum      | 758.62  | -        | -      | 140.92 | 423.13 | 194.84    | -    | 241.74|
| Achyranthus aspera     | 826.72  | 24.33    | 46.90  | 231.92 | 269.44 | 2.73      | 505.65| 78.09|
| Adiantum caudatum      | 647.41  | -        | -      | 84.72  | 281.16 | 85.74     | -    | 245.46|
| Ageratum conyzoides    | 628.44  | 28.25    | 57.60  | 441.47 | 622.86 | 182.52    | 591.34| 592.66|
| Anagalis arvensis      | 693.10  | 11.29    | 23.30  | 469.16 | 1,047.04| 262.13    | 493.99| 89.03 |
| Bignonia gracilis      | 650.86  | -        | -      | 41.20  | 667.23 | 86.95     | -    | 414.22|
| Cannabis sativa        | 619.80  | 22.73    | 63.60  | 155.25 | 208.93 | 101.57    | 555.12| 66.88 |
| Lantana camara         | 696.55  | -        | 34.67  | 243.92 | 232.67 | 173.68    | 1,473.32| 357.33|
| Malvestrum coromandilianum | 581.89 | 35.96    | 71.80  | 123.32 | 4992.04| 89.87     | -    | 202.36|
| Parthenium hysterosphorus | 647.41 | 27.43    | 56.60  | 441.47 | 622.86 | 182.52    | 591.34| 66.88 |
| Prosopis juliflora     | 693.10  | -        | -      | 1795.36| 192.66 | 44.26     | 414.66| 71.99 |
| Sida acuta             | 654.31  | 35.19    | 72.60  | 195.77 | 2738.54| 64.91     | 544.52| 78.09 |

<sup>a</sup> s/p, bioaccumulation factor soil to plant.
mobilized and precipitated onto root surfaces [Weis and Weis, 2004]. Kabata-Pendias and Pendias [2001] studied that high iron content inhibits copper uptake. Similar results were observed in the present study where plants had shown low Cu and high Fe contents indicating inhibition of Cu uptake due to high Fe content.

Manganese is another essential micronutrient for plants, being involved in the enzyme activity and photosynthesis [Bonanno and Lo Giudice, 2010]. The content of Mn in leaves of all the plants species studied exceeded the natural content (52 mg/kg) as given by Brooks and Robinson [1998] as well as the toxic range (50–500 mg/kg) given by Allen [1989] and Bonanno [2013]. The presence of Mn is directly correlated to the use of fertilizers in the adjoining agricultural fields.

Lead is not an essential element for plant growth and is considered to be toxic. The content of lead in macrophytes was observed to be more than the normal values, i.e., 0.05–3.0 mg/kg [Kabata-Pendias and Pendias, 2001]. BAF for lead in all the aquatic plant species studied was above 100 whereas TF was < 100. The present study is in line with earlier reports indicating the immobile nature of lead from soil and sediments to aerial parts [Siedlecka et al., 2001]. Similarly, the lead content of terrestrial plants varied from 23.47 mg/kg (P. juli flora) to 83.39 mg/kg (L. camara). The observed content of Pb in present study was higher than the natural plant content (4.2 mg/kg) of uncontaminated sites.

Zinc is a vital plant nutrient which plays role in metabolism. However, Zn content above 500–1500 mg/kg is phytotoxic [Chaney, 1989]. In the present study, E. crassipes showed maximum (578.2 mg/kg) Zn content in roots. Among terrestrial plant species, Zn ranged from 29.18 mg/kg (C. sativa) to 258.4 mg/kg (A. conyzoids).

BAF for cadmium, chromium, and manganese in most of the terrestrial plants and all aquatic plants was more than 100 indicating high efficiency of plants to accumulate these metals from soil and water, respectively. TF for Cd, Fe, Pb, and Zn was > 100 in P. antidotale. Similar relationship between BAF and efficiency of plants to translocate metals from one part to another was given by Ma et al. [2001]. Heavy metals upon entering biological systems can cause various health effects (Figure 3). It is well documented that uptake of heavy metals in human beings can cause gastrointestinal disorder, kidney damage, nervous system disorder, and bone and lung injury [Duruibe et al., 2007; Peralta-Videa et al., 2009; Thakur et al., 2010; Jomova and Valko, 2011; Vila et al., 2012].

The present study reveals the tendency of native macrophytes to bioaccumulate heavy metals. Aquatic macrophytes are considered as biological filters which directly influence the movement of heavy metals in

Figure 3. Effects of heavy metals on living beings.
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Table 9. Pearson Correlation Matrix Between Heavy Metal Concentrations in Plant Samples

|       | Cd    | Co   | Cr    | Cu    | Fe    | Mn    | Pb    | Zn    |
|-------|-------|------|-------|-------|-------|-------|-------|-------|
| Cd    | 1     |      |       |       |       |       |       |       |
| Co    | 0.333 | 1    |       |       |       |       |       |       |
| Cr    | 0.257 | 0.398| 1     |       |       |       |       |       |
| Cu    | 0.320 | 0.473| 0.117 | 1     |       |       |       |       |
| Fe    | 0.050 | 0.108| 0.589*| –0.054| 1     |       |       |       |
| Mn    | –0.026| –0.085| –0.200| –0.153| –0.022| 1     |       |       |
| Pb    | –0.163| 0.277| –0.162| 0.130| –0.087| 0.445| 1     |       |
| Zn    | 0.184 | 0.866*| 0.298 | 0.287| 0.078 | 0.076| 0.370| 1     |

*Significance at p ≤ 0.05.

any aquatic ecosystem. Some plant species especially, hyperaccumulators are able to accumulate the metals in concentration exceeding their content in the aquatic ecosystem. Such plant species not only indicate the bioaccumulation potential of plant species but also are used as indicator of aquatic environmental hazards to evaluate the persistent and acute toxicity of metals [McGeer et al., 2003]. In the present study, all four aquatic plant species accumulated high contents of metals studied. However, E. crassipes and P. antidotale accumulated high metal contents as compared to L. minor and P. crispus. High metal content in the roots of E. crassipes and P. antidotale indicates that the metals were uptaken through the water and sediments directly by these indicator plants. The results are in line with previous studies which postulated that aquatic flora reflects the metal content of its environment [Demirezen and Aksoy, 2006; Harguinteguy et al., 2014]. The presence of metal in plants as well as aquatic ecosystem indicates the contamination of Kanjli wetland and strongly recommend the seasonal monitoring of water to conserve it.

Pearson correlation matrix measures the linear dependence between two variables. It has value between +1 and –1, where +1 is total positive linear correlation and –1 is total negative correlation. Table 9 describes the Pearson correlation matrix between heavy metal content in different plant samples. It was observed that only four metals have shown the significant correlations like Co was found to be correlated to Zn while Cr with Fe at p ≤ 0.05. No other metal has shown any significant correlation.

4. Conclusions
The study revealed the considerable variations in metal contents of water, sediment, and soil samples. High contents of cadmium and lead in wetland water samples exceeding the permissible limits of Bureau of Indian Standards [2012a, 2012b] due to direct discharges of industrial and domestic sewage at upstream points indicate the pollution of Kanjli wetland. Elevated levels of metal content in aquatic and terrestrial plants signify their hyperaccumulation potential. The present work paves the way for the use of wetland plants like E. crassipes and P. antidotale (aquatic) and A. aspera, A. conyzoids, A. arvensis, C. sativa, and S. acuta (terrestrial) as ecological indicators that show direct response to metal concentrations present in aquatic or soil ecosystem.

Apart from this, present study also recommends the harvesting of wetland plants at regular intervals to prevent transfer of toxic metals to higher trophic levels.

References
Adekola, F. A., and O. A. A. Eletta (2007), A study of heavy metal pollution of Asa River, Ilorin, Nigeria; Trace metal monitoring and geochemistry, Environ. Monit. Assess., 125, 157–163.
Allen, S. E. (1989), Chemical Analysis of Ecological Material, 2nd ed., p. 368, Blackwell Sci., Oxford, U. K.
American Public Health Association (2005), Standard Methods for the Examination of Water and Waste Water, 21th ed., American Public Health Association, Washington, D. C.
Bureau of Indian Standards (2012a), Water quality Standards for classifying surface water sources. [Available at http://punenvis.nic.in/Printmains.aspx.]
Bureau of Indian Standards (2012b), Drinking water—Specifications, 2nd rev. [Available at http://cgwb.gov.in/Documents/WQ-Standards.pdf.]
Bermner, J. M., and C. S. Mulvaney (1982), Nitrogen total, in Methods of Soil Analysis, edited by A. L. Page, R. H. Miller, and D. R. Keeney, pp. 575–624, American Society of Agronomy, Madison, Wis.
Bonanno, G. (2011), Trace element accumulation and distribution in the organs of Phragmites australis (common reed) and biomonitoring application, Ecotoxicol. Environ. Saf., 74, 1057–1064.
Bonanno, G. (2013), Comparative performance of trace element bioaccumulation and biomonitoring in the plant species Typha domingensis, Phragmites australis and Arundo donax, Ecotoxicol. Environ. Saf., 97, 124–130.
Bonanno, G., and R. Lo Giudice (2010), Heavy metal bioaccumulation by the organs of Phragmites australis (common reed) and their potential use as contamination indicators, Ecol. Indic., 10, 639–645.
Brooks, R. R., and B. H. Robinson (1998), Aquatic phytoremediation by accumulator plants, in Plants that Hyper Accumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining, edited by R. R. Brooks, pp. 203–226, CAB International, Oxon.
Chaney, R. L. (1989), Toxic element accumulation in soils and crops: Protecting soil fertility and agricultural food chains, in Inorganic Contaminants in the Vadose Zone, edited by B. Bar-Yosef, N. J. Barrow, and J. Goldsmith, pp. 140–158, Springer, Berlin.

Demirezen, D., and A. Akoys (2006), Common hydrophobes as bioindicators of iron and manganese pollution, Ecol. Indic., 6, 388–393.

Divan, A. M., P. L. De Oliveira, C. T. Perry, V. L. Atz, L. N. Azzarini-Rostirola, and M. T. Raya-Rodriguez (2009), Using wild plant species as indicator for the accumulation of emission from a thermal power plant, Candidtia, South Brazil, Ecol. Indic., 9, 1156–1162.

Durulue, J. O., M. O. C. Ogwuegbu, and J. N. Egwurugwou (2007), Heavy metal pollution and human biotoxic effects, Int. J. Phys. Sci., 5, 112–118.

Fairbrother, A., R. Wenstel, S. Sappington, and W. Wood (2007), Framework for metals risk assessment, Ecotoxicol. Environ. Saf., 68, 145–227.

Foye, C. H., M. Lelandais, and J. K. Kunert (1994), Photooxidative stress in plants, Plantol. Plant. Physiol., 92, 696–717.

Granier, J., G. Billen, and L. Palfner (2000), Understanding the oxygen budget and related ecological processes in the river Mosel: The Riverstahler approach, Hydrobiologia, 410, 151–166.

Harguinteguy, C. A., A. F. Cirelli, and M. L. Pignata (2014), Heavy metal accumulation in leaves of aquatic plant Stuckenia filiformis and its relationship with sediment and water in the Suzuqia river (Argentina), Microchem. J., 71, 111–118.

Ishaq, F., and A. Khan (2013), Heavy metal pollution of river Yamuna and their relation with some physico-chemical parameters, Global J. Environ. Res., 7(2), 34–39.

Jacob, H., and G. Clarke (2002), Part 4. Physical method, in Inorganic Trace Elements in Soils and Plants, edited by A. L. Page, R. H. Miller, and D. R. Keeney, pp. 247–297, CRC Press, Boca Raton, Fla.

Li, Y., Z. Yu, X. Song, and Q. Mu (2006), Trace metal concentrations in suspended particles, sediments and clams from jiaozhou Bay of China, Environ. Monit. Assess., 121, 491–501.

Lu, Q., Z. L. He, D. A. Graetz, P. J. Stoffella, and X. Yang (2011), Uptake and distribution of metals by water lettuce ( Pistia stratiotes L.), Environ. Sci. Pollut. Res., 18, 978–986.

Matache, M. L., C. Marin, L. Rozylowicz, and A. Tudorache (2013), Plants accumulating heavy metals in the Danube River wetlands, Environ. Monit. Assess., 178, 491–501.

Mishra, V. K., A. R. Upadhyaya, S. K. Panday, and B. D. Tripathi (2008), Concentration of heavy metals and aquatic macrophytes of Gobind Ballah Pant Sagar an anthropogenic lake affected by coal mining effluent, Environ. Monit. Assess., 141, 49–58.

Nelson, D. W., and L. E. Sommers (1982), Total carbon and organic matter, in Methods of Soil Analysis (part 2), edited by A. L. Page, pp. 539–579, ASA-SSSA, Madison, Wis.

Olsen, S. R., C. V. Cole, F. S. Watanabe, and L. A. Dean (1954), Estimation of available phosphorous in soils by extraction with sodium bicarbonate, USDA circ. no. 939, US Department of Agriculture, Washington, D.C.

Olea europaea L., 696, 1–3.

Pak. J. Bot., 40, 390, 393, 789–798.

Pak. J. Bot., 40, 390, 393, 789–798.

Pak. J. Bot., 40, 390, 393, 789–798.
Zacchini, M., F. Pietrini, G. S. Mugnozza, V. Iori, L. Pietrosanti, and A. Massacci (2009), Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics, Water Air Soil Pollut., 197, 23–34.

Zayed, A., S. Gowthaman, and N. Terry (1998), Phytoaccumulation of trace elements by wetland plants: I. Duckweed, J. Environ. Qual., 27, 715–721.

Zhu, Y. L., A. M. Zayed, J. H. Qian, M. de Souza, and N. Terry (1999), Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth, J. Environ. Qual., 28, 339–344.