Accumulation and contamination of lead in different trophic levels of food chain in sewage-fed East Kolkata Wetland, West Bengal, India

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ABSTRACT

Lead (Pb) is one of the common hazardous heavy metals posing severe toxicity problems in aquaculture productivity and human health by bioaccumulation and biomagnification due to its uncontrolled discharge into ecosystem in the age of modern industrialization and agricultural growth. The study was carried out to monitor the contamination status of heavy metal Pb in different trophic levels of the food chain in the ecosystem of sewage-fed East Kolkata Wetland (EKWL), the plankton as primary producer which is consumed by highest level consumer fish, *Oreochromis niloticus*, is the most abundant species in EKWL. The concentration of Pb in water and accumulation in different trophic components of food chain was analyzed by Atomic Absorption Spectrophotometer (AAS). Study shows that the Pb concentration in water of EKWL was 0.573 mg/l and in plankton and small insects the concentration of Pb was 0.0021 µg/mg and 0.0023µg/mg respectively. Accumulation of Pb in different organ of studied fish (*Oreochromis niloticus)* was highest in intestine (0.0182 µg/mg) and lowest in fin (0.0004 µg/mg) with an order as intestine > liver > skin > muscle > scale > bone > fin. Besides this, bioconcentration factor among different species in the trophic level was also studied wherein plankton (0.037) and adult fishes (*O. niloticus*) (0.325) showed lowest and highest values, respectively. The concentration of Pb in water was alarming but in fish musels were below as per WHO permissible level. However, this monitoring study should be frequent practice for the conservation of EKWL and proper biosafety in aquaculture practices in order to control the Pb contamination as well as to save environmental and human health from the serious hazardous problems of Pb contamination.

1. Introduction

The East Kolkata Wetland comprises of large number of water bodies that consists of an area of 25,000 hectares which was once occupied by the Bidyadhari River. These wetlands receive different types of waste water from domestic and industrial sources. It acts as a natural purification system for incoming waste water. City wastewater from different pumping station distributed through open channel, networks into different fish ponds (Maiti and Banerjee 2012). It is a platform of pisciculture which receives toxic waste products due to the burgeoning population, and their several anthropogenic activities degrading the water quality (Mondal et al. 2015a) which is a concern since the last few decades.

Contamination of heavy metals may have adverse affect on ecological stability and organism diversity due to the entrance of pollutant into different trophic levels through food chain **(**Okocha and Adedeji 2011). Among several pollutants, heavy metal contamination is a growing concern due to its toxicity, bio-accumulation, long persistence and increasing contamination in water and soil (Fig. 1), which poses a serious threat to the environment as well as human beings (Tao et al. 2012). Approximately 50,000 m^3/d heavy metal contaminated effluent from domestic wastewater and industrial runoff are ending up in East Kolkata Wetland (Mondal and Ghosh 2015). Metal contamination in aquatic system mainly observed in fish, aquatic flora and fauna, sediment and water (Tirkey et al. 2012). Increasing concentration of metals among organisms is a strong evidence of bioaccumulation in different trophic level. Different species in aquatic system act as a bio-indicator of heavy metal toxicity (Akan et al. 2012). Lead (Pb) poisoning is an important health issue in many countries (Karrari et al. 2012). Some metals, known as trace metals are essential for organism and besides this some other heavy metals like mercury, lead, copper, cadmium, etc., are toxic to organism

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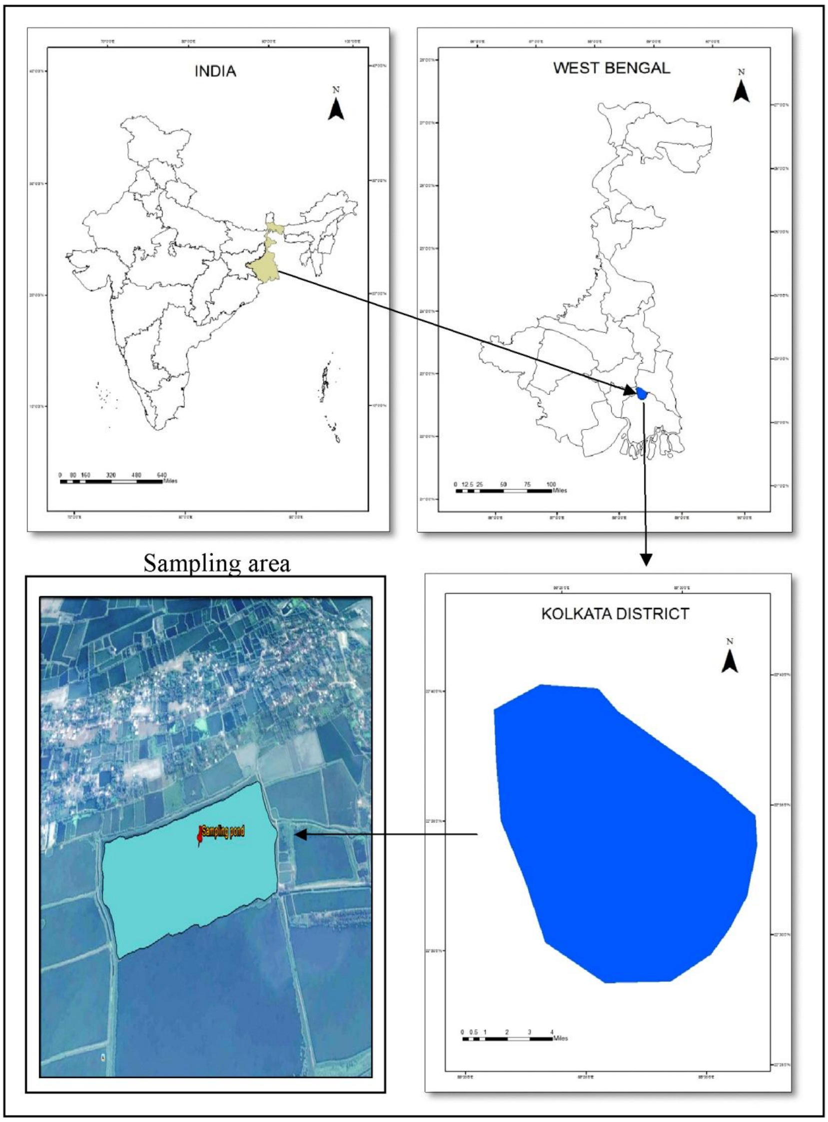
Fig. 1 Map of study area and sampling site



above certain concentration. According to the World Health Organization (WHO) maximum permissible limit of Pb in water is 0.05 mg/l and in plant and fish are 0.002 µg/mg and 0.005 µg/mg respectively. High levels exposure of Pb in human body causes several toxic biochemical effects in different organs (Bagul et al. 2015). Besides, different sorption through physicochemical attributes of clay soil of a system and ion exchange routes are the Pb subtraction mechanisms (Bhakta and Munekage 2013). Study revealed that some lactic acid bacteria also bioaccumulate Pb from aquatic medium (Bhakta et al. 2012). In natural processes, biotic and abiotic components of an ecosystem especially aquatic weed like *Pistia* sp. minimize the heavy metal toxicity to fish and other aquatic organisms through its removal from water column (Bhakta and Munekage 2008).

The objective of present study was to monitor the accumulation and contamination of Pb in different organs of fish and trophic level along with different parts of an aquatic weed *(Eichhornia* sp.*)* which serves as a metal accumulator and to correlate the affect of Pb accumulation on the quality of limnological parameters.

2. Materials and methods

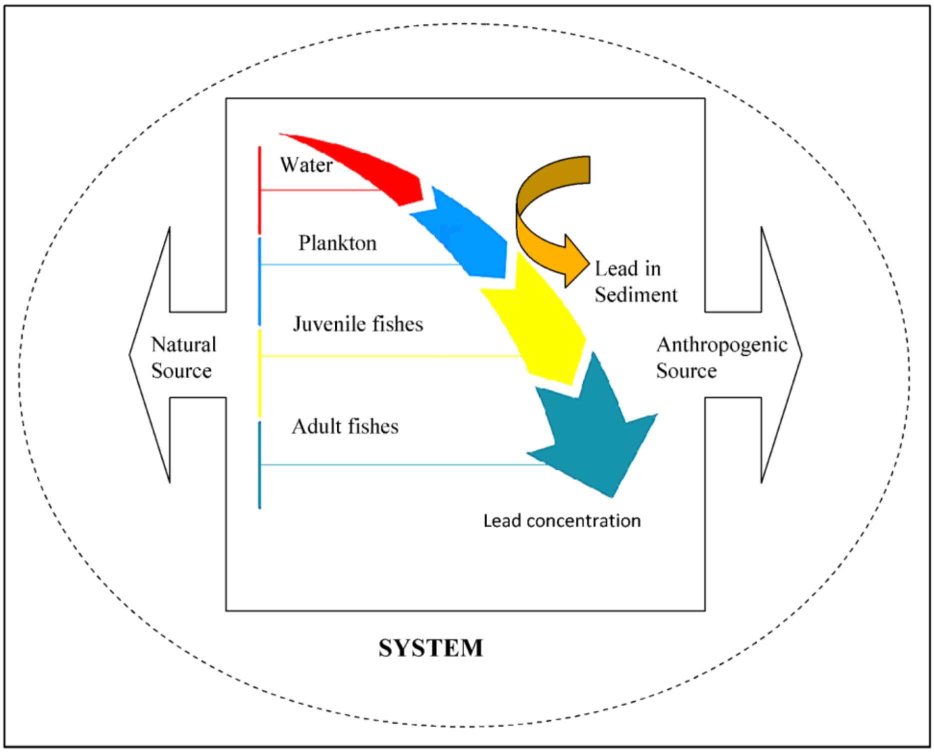
2.1. Study area

East Kolkata Wetlands ($22^0$27’ N $88^0$27’ E), which is located on the eastern fringes of Kolkata city is one of the largest assemblages of sewage fed fish ponds was selected for the study (Fig. 2).

2.2. Water quality analysis

Water samples were collected from three wetland ponds in different sampling points (n = 3) in seasons (summer, monsoon and winter). Analysis of water samples like pH, temperature, conductivity, total dissolved solid, dissolved oxygen, total hardness, total alkalinity, chloride, ammoniacal nitrogen, nitrate nitrogen, sulphate, phosphate, iron and sodium were carried out by APHA (2005) methods. Statistical analysis among different variables was carried out by MATLAB R2014a.

Fig. 2 Pathway of lead accumulation in different trophic levels of ecosystem



2.3. Heavy metal analysis

For the heavy metal analysis in water sample digestion method delineate by Gregg (1989) was adopted. 100mL of water sample was taken in a beaker containing 10ml of concentrated nitric acid (HNO_3) and heated slowly and evaporated on a hot plate to get the lowest possible volume of about 20 ml. After cooling additional volume of concentrated HNO_3 (5 ml) was added to it and heating was continued with added HNO_3 until digestion was complete. After that the sample was evaporated again and cooled, followed by addition of 5 ml of hydrochloric acid (HCl) solution (1:1 v/v). Then the solution was warmed and 5 ml of 5 M sodium hydroxide (NaOH) was added and filtered. The filtrate sample was transferred to 100 ml and volume was made up to the mark with distilled water for the elemental analysis.

Analysis of heavy metals from biological samples was carried out by following the method Smith and Windom, 1972. In this regard, required amount of biological sample like plant and animal tissues were taken in a teflon container. 4 ml of aqua regia was added and container was placed in microwave oven. The oven was adjusted at 450 W and the sample was digested for 7 min. Then 4 ml of hydrogen peroxide was added and was digested for another 7 min at 450 W. The solution was diluted with distilled water, and filtered in 25 ml of volumetric flask. Final volume was made up with distilled water. Reagent blank was prepared in the same way. Metal concentration was measured by Atomic Absorption Spectroscopy and calculated by following equation:

$$\text{Metal concentration (mg/g)} = \frac{(S-B)\times\left(\frac{25}{1000}\right)}{W}$$

Where:
S = AAS reading (concentration) of sample (mg/l)
B = AAS reading (concentration) of reagent blank (mg/l)
25 = volume of digested sample (ml)
W = weight of sample (g)

Concentrations of metals in each trophic level were calculated in respect to the bioconcentration factors (BCF) using the following formula (Barron, 1995):

$$\text{Bioconcentration Factor (BCF)} = \frac{\text{Concentration in trophic level}}{\text{concentration in water}}$$

3. Results and discussion

Results of the limnological parameters have been represented in Table 1 are compared with the aquaculture standard. Analysis of correlation among different water quality parameters of East Kolkata wetland were carried out in this present study (Table 2). Some of the physico-chemical attributes of EKWL had a noticeable variation with the season. Maximum pH values observed in monsoon (8.45±0.16) than other seasons but remained within the aquaculture standard. Similar types of observation indicated in TDS and conductivity. But the temperature of the water body had a little variation over the seasons. In some sites of EKWL has a poor concentration of dissolved oxygen in winter season (2.96 mg/l) which was lower than aquaculture standard (IS 13891: 1994). Other chemical characteristic features like, total hardness, total alkalinity and chloride always remained within the permissible limits for aquaculture.

Table 1 Variation of water qualities (average ± Sd) in different seasons

Parameters	**Summer**	**Monsoon**	**Winter**	**IS 13891 : 1994 Aquaculture Standard**
pH	6.79±0.49	8.45±0.16	7.89±0.80	6.50-8.50
Temperature (˚C)	30.73±0.47	30.27±0.60	29.90±1.65	2.00 – 35.00
Conductivity (µs/cm)	792.00±31.05	829.33±0.58	794.00±162.01	-
TDS (mg/l)	582.00±7.00	585.00±4.58	563.33±114.08	-
Dissolved Oxygen (mg/l)	3.68±3.19	5.66±1.68	2.96±2.65	4.00
Total Hardness (mg/l)	182.67±32.58	180.00±6.93	197.33±37.17	-
Total Alkalinity (mg/l)	156.67±15.28	166.67±28.87	100.00±69.28	100. 00 – 300. 00
Chloride (mg/l)	143.29±16.07	143.29±32.14	124.96±15.00	-
NH_3 – N (mg/l)	0.04±0.03	4.23±1.56	1.09±0.67	-
NO_3–N (mg/l)	1.95±2.72	6.22±0.58	1.01±0.83	2. 00
Sulphate (mg/l)	24.13±10.24	31.58±7.68	16.17±11.11	-
Phosphate (mg/l)	1.50±1.31	0.23±0.04	0.42±0.17	-
Total iron (mg/l)	0.57±0.04	0.58±0.13	0.04±0.02	2. 00
Sodium (mg/l)	69.67±4.16	69.00±6.00	62.67±2.08	-
Potassium (mg/l)	15.67±1.53	16.00±2.65	19.33±1.15	-

Limnology plays an important role in maintaining the quality of an aquatic body and suitability for the production and distribution of fish and other organisms (Bhatnagar and Devi 2013). Values of pH was significantly ($F_{2, 6} = 7.14$; $P < 0.05$) higher in monsoon and lower in summer and was within the range of IS aquaculture standard. Statistical analysis reveals that pH has significant strong positive correlation ($P \leq 0.001$, $r = 0.759$) with ammoniacal nitrogen. Alkaline pH in monsoon may be due to the dilution by precipitation. These findings are in accordance with the studies conducted by Tara et al. (2011) and Abir (2014). In winter season, temperature was lower than other seasons and summer showed the highest value due to high solar radiation and clear atmospheric condition (Shinde et al. 2011). Temperature has significant strong positive correlation ($P \leq 0.01$, $r = 0.739$) with total dissolved solids. Among all the seasons, recorded conductivity and total dissolved solid (TDS) were highest in monsoon followed by summer and winter season, respectively. Conductivity is significant with strong positive correlation with total dissolved solids ($P \leq 0.001$, $r = 0.948$) and total alkalinity ($P \leq 0.01$, $r = 0.710$). Total dissolved solids shows significant strong positive relation with total alkalinity ($P \leq 0.001$, $r = 0.762$). Low conductivity in summer due to high temperature and input of high volume of waste with run-off from city may be the cause of high value in monsoon. Similar result was reported by Narayana et al.

Table 2 Correlations among limnological parameters

	pH	Temp	Cond	TDS	DO	TH	TA	Cl	NH_3-N	NO_3-N	SO_4	PO_4-P	Fe	Na
Temp	-0.576													
Cond	-0.121	0.585												
TDS	-0.384	**0.739**	**0.948**											
DO	0.350	-0.199	-0.379	-0.311										
TH	-0.116	0.257	0.665	0.541	**-0.882**									
TA	-0.257	0.578	**0.710**	**0.762**	0.028	0.182								
Cl	-0.220	0.566	0.283	0.437	0.405	-0.187	0.453							
NH_3-N	**0.759**	-0.313	0.195	0.021	0.521	-0.233	0.300	-0.047						
NO_3-N	0.591	0.044	0.329	0.160	0.269	0.013	0.545	0.213	**0.746**					
SO_4	0.457	-0.347	-0.205	-0.308	0.449	-0.357	0.327	0.041	0.566	**0.747**				
PO_4-P	-0.550	0.267	0.051	0.102	-0.499	0.253	0.195	-0.019	-0.581	-0.125	0.090			
Fe	-0.204	0.390	0.196	0.288	0.347	-0.349	**0.756**	0.410	0.297	0.545	0.564	0.291		
Na	-0.302	0.666	0.183	0.356	0.366	-0.305	0.493	**0.845**	-0.034	0.292	0.102	0.073	0.682	
K	0.184	-0.505	0.065	-0.089	-0.470	0.433	-0.423	**-0.757**	0.003	-0.396	-0.373	-0.111	**-0.702**	**-0.921**

Cell Contents: Pearson correlation (Temp, temperature; Cond, conductivity; TDS, total dissolved solid; DO, dissolved oxygen; TH, total hardness; TA, total alkalinity; Cl, chloride; NH_3–N, ammoniacal nitrogen; NO_3–N, nitrate nitrogen; SO_4^{2-}, sulphate; PO_4^{3-}, phosphate; Fe, iron; Na, sodium; K, potassium)

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2008; Jawale, and Patil, 2009. Concentration of dissolved oxygen (DO) was higher in monsoon and lower in winter season. DO concentrations depend on various factors like rainfall, temperature and photosynthesis. High DO concentration in monsoon may be due to the influx of fresh water and higher solubility (Anand and Kumarasamy, 2013). Dissolved oxygen is negatively correlated with total hardness ($P \leq 0.001$, $r = -0.882$). In summer and winter season DO concentration was below the level of IS aquaculture standard. Total hardness and total alkalinity showed the highest value in winter and monsoon season respectively and lowest in monsoon and winter respectively. Total alkalinity shows significant strong positive relation with iron ($P \leq 0.001$, $r = 0.756$). Higher value of total alkalinity in monsoon may be due to the addition of domestic waste water containing detergent and excessive run-off when compared with other season and lower alkalinity may be due to the consumption of bicarbonate by wetland biota which directly affects the total concentration of alkalinity (Harney et al. 2013). Chloride is one of the most important inorganic anion in water. Due to its high solubility, chloride ion is generally present in natural and all types of water. High concentration of chloride in water is an indication of pollution from domestic sewage (Abir, 2014; Shinde et al. 2011). In both seasons (summer and monsoon) chloride (Cl^-) concentration was higher than winter season. Similar trends were also found by Narayana et al. 2008; Reddy Vasumathi, 2009. Chlorine is significantly positively correlated with sodium ($P \leq 0.001$, $r = 0.845$) and significantly negatively correlated with potassium ($P \leq 0.001$, $r = -0.757$).

Variation of nutrient parameters examined were a distinct alteration with the change of season. Ammoniacal nitrogen (4.23±1.56 mg/l), nitrate nitrogen (6.22±0.58 mg/l), sulphate (31.58±7.68 mg/l) and total iron (0.58±0.13 mg/l) were resulted the highest value in monsoon seasons where phosphate (1.50±1.31 mg/l) and sodium (69.67±4.16 mg/l) was observed in summer. Potassium concentration revealed marked concentration in winter (19.33±1.15 mg/l). Ammoniacal nitrogen (NH_3-N) and Nitrate nitrogen (NO_3-N) were significantly higher ($F_{2, 6} = 14.82$; $P<0.01$ and $F_{2, 6} = 8.24$; $P < 0.05$ respectively) in monsoon time followed by summer and winter having the lowest concentration. Ammoniacal nitrogen shows significant positive correlation with nitrate nitrogen ($P \leq 0.001$, $r = 0.746$). Nitrate nitrogen is significant with positive correlation with sulphate ($P \leq 0.001$, $r = 0.747$). During the monsoon season the nitrate level may be increase by surface run off and domestic waste water and in summer algal assimilation may reduce the nitrate concentration. Rajashekhar et al. 2007 and Shinde et al. 2011 also observed a similar trend in seasonal variation of nitrogen. Concentration of sulphate (SO_4^{2-}) and phosphate (PO_4^{3-}) ions were highest in monsoon and summer season respectively and were lowest in winter and monsoon season respectively. Similar types of result on sulphate were also found by Reddy Vasumathi, 2009 and Telkhade et al. 2008. Iron (Fe) concentration was significantly higher in monsoon season and lower in winter season ($F_{2, 6} = 41.25$; $P < 0.001$). Iron shows significantly strong negative correlation with potassium ($P \leq 0.1$, $r = -0.702$). Concentration of sodium (Na) and potassium (K) were higher in summer and winter season respectively and lower in winter and summer season. Sodium is significantly strongly negatively correlated with potassium ($P \leq 0.001$, $r = -0.921$).

There are a number of factors analyzed with Principal Component Analysis (PCA) extraction that control the physicochemical variables of EKWL water, including four factors (Eigen value > 1). The result showed that the two first components accounted for 59.27 % among variables and among these four factors responsible for 87.25% qualitative differences. Pb is allied with other factors like NH_3-N, DO, SO_4^{2-}, NO_3-N, Fe^-, Na^- and Cl^- attributable to the biological/anthropogenic source (Fig. 3). A major portion of organic matter also settles in the lower portion, with its decomposition being facilitated by DO and oxidizing agents

Fig. 3 PCA results among water quality variables in EKWL

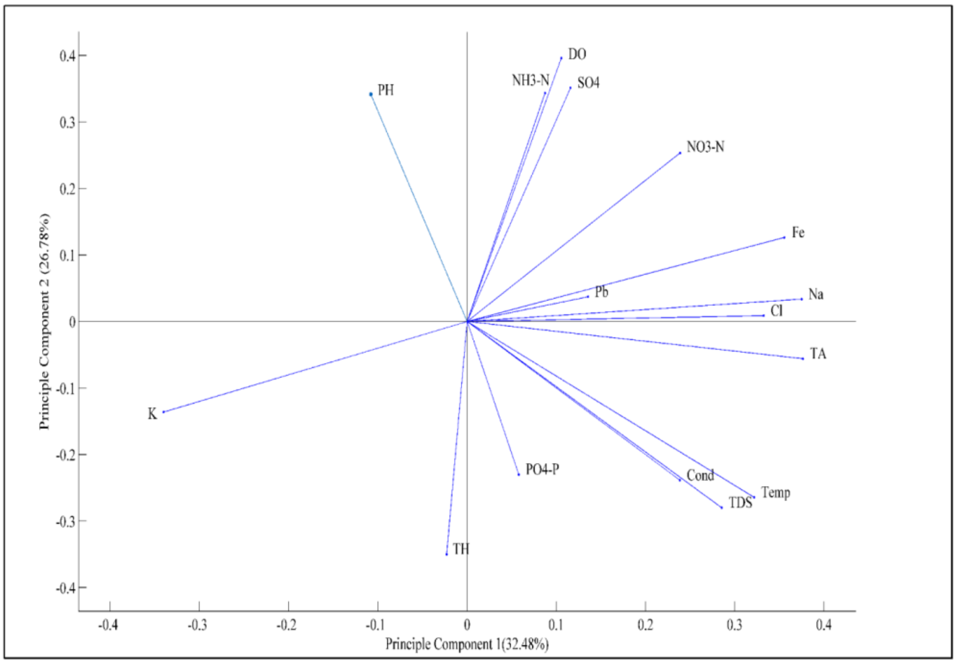
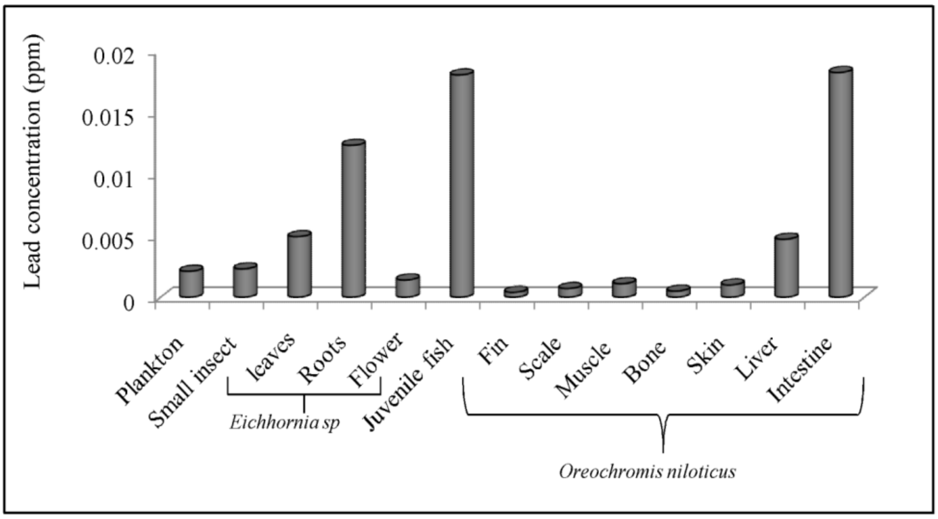


Fig. 4 Accumulation of lead in different organism in wetland ecosystem

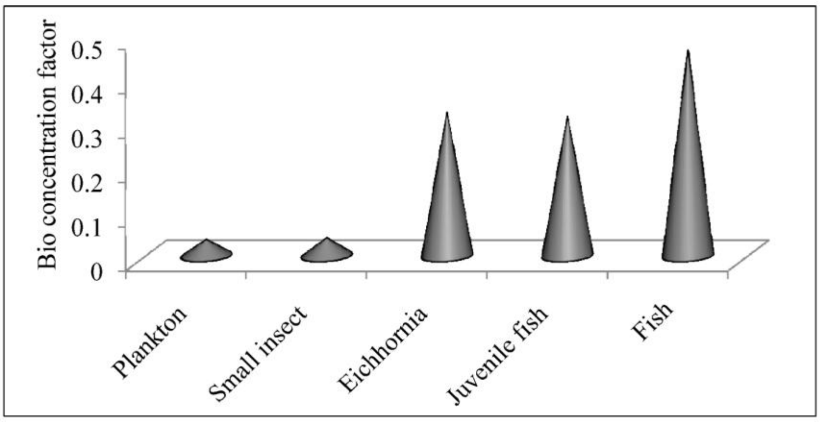


(Boehrer and Schultze 2008). Anthropogenic input includes agricultural run-off, animal byproducts are sources of SO_4^{2-} , NH_3-N, SO_4^{2-}, NO_3-N ions (Han and Liu 2004; Jalali 2009; Mondal et. al., 2015b) or byproducts of microbial activity while industrial and municipal sewage pollution are the major concern for Pb.

In aquatic body, rapid removal and detoxification of metals are not easy and as a result metal accumulate in the animal and plant organs. Thus, accumulation of metals has become a growing concern to the biotic components in the ecosystem (Mohan et al. 2008). Bioaccumulation and biomagnification of heavy metals in living organisms deals with the process and flow of toxicity from one trophic level to another (Akan et al. 2012). In the present study, the concentration of Pb in water was observed to be 0.573 mg/l (Fig. 4). According to World Health WHO water standards, permissible limit of Pb is 0.05 mg/l. Hence in this study Pb concentration in water was found to be higher than the permissible limit which was in accordance a similar study conducted by Nazir et al (2015). Pb concentration in plankton and small insects' level was 0.0021 µg/mg and 0.0023 µg/mg respectively (Fig. 4). The accumulation of this heavy metal in leaves, roots, and flower of aquatic weeds *(Eichhornia* sp.) was 0.0049 µg/mg, 0.0123 µg/mg and 0.0014µg/mg respectively. As per WHO recommendation the permissible limit of Pb in plants is 0.002 µg/mg. In this regard, concentration of Pb in leaves was beyond the permissible limit while roots and flower were within the limit. In small fish the concentration was 0.018 µg/mg. Besides, Pb accumulates in different organs of *O. niloticus* in intestine > liver > skin > muscle > scale > bone > fin with the highest accumulation in intestine (0.0182 µg/mg) and lowest in fin (0.0004 µg/mg). Its accumulation in liver (0.0047 µg/mg) is also noticeable (Fig. 4). Comparing with the maximum permissible limit for Pb in fish (0.005 µg/mg) (WHO 1993), this present study represent small fish and intestine of big fish exceeds the allowed limit. Metal accumulation through different path way in fish include consumption of plankton, intake of water and metal ion exchange through gills and skin. Thus absorbed heavy metals are transported to different organs which are either accumulated or excreted (Akan et al. 2012).

Bioconcentration process depicts higher concentration of pollutant in organism than their surrounding environment and bioconcentration factor (BCF) which is the ratio of metal concentration in organism to its surrounding water, is the key element to characterize this process. Metal concentration in the tissue of a particular trophic level organism may be higher or lower than its lower trophic level (Jakimska et al. 2011). In the present study, the bioconcentration factor (BCF) of the plankton and insects which occupy the base of the aquatic trophic structure was observed to be 0.037 and 0.040 respectively. The BCF of juvenile fishes and adult fishes (*O. niloticus*) are 0.314 and 0.464 respectively (Fig. 5). Bioconcentration factor determination in plant is an important factor because it deals with the uptake,

Fig. 5 Bio-concentration factor of lead in different trophic level



immobilization and storage of metals into plant tissue and biomass. Metal accumulation in different parts of the plant like roots, stems and leaves vary upon the uptake ability of contaminants and hence are different in element concentration in different parts (Ndeba and Monohar, 2014). Aquatic weeds such as *Eichhornia* sp., although are not directly involved with other trophical groups, show the high BCF (0.325) than plankton, insects and juvenile fishes and lower than adult fishes (*O. niloticus*) (Fig. 5).

4. Conclusions

Pb concentration of EKWL system shows the increasing order of bioaccumulation from lower to higher trophic levels, which is a clear evidence of bioaccumulation of heavy metal. The concentration of Pb has a remarkable difference among the different trophic components of food chain having increasing pattern from lower to higher trophic organisms (plankton, small insects, juvenile fish and *O. nilotica*). In *O. nilotica*, its accumulation is relatively higher in intestine, primary absorptive site of gastrointestinal tract, followed by liver, detoxicification site of higher organisms. Pb in primarily edible portion, muscle, remains within the permissible limits of WHO. Accumulation potentiality of Pb by aquatic weeds (*Eichhornia* sp.) is also very high which can be proposed as an agent for removal of metals from wetland water. However, effluent containing heavy metal is discharged into the wetland which then gets distributed into different fish cultivation ponds. This leads to bioaccumulation of heavy metals in different trophic levels of food chain, especially in fishes that is consumed by human beings which in turn can cause a severe abiding damage and devastation on environmental and human health. Therefore, wetland should be monitored regularly to control the heavy metal pollution and for the conservation of biodiversity of wetland system.

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