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Bioaccumulation of heavy metals by freshwater algal species of Bhavnagar, Gujarat, India

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Abstract

The present study investigated copper, cadmium, lead and zinc accumulation in algal species *Oedogonium, Cladophora, Oscillatoria* and *Spirogyra* from freshwater habitats of Bhavnagar, India. Eight different locations were periodically sampled during August 2009 to March 2011. The general trend of heavy metal concentrations in all the algal species in present study (except at few stations), were found to be in the following order: Zn>Cu>Pb>Cd. Highest accumulation of Cu was recorded in *Oedogonium,* while *Cladophora* showed highest accumulation of Pb signifying a good bioaccumulator. *Oscillatoria* and *Oedogonium* were highest Zn accumulating algae which showed significant difference between the means at $P<0.05$. ANOVA was performed for comparing significance mean between the groups and within the group for heavy metals in water. The concentration of heavy metals in water was in the following order: Zn>Cu>Pb>Cd. The present study showed that *Oedogonium, Cladophora, Oscillatoria* and *Spirogyra* were excellent bioaccumulator and could be utilized as biomonitoring agents in water bodies receiving waste contaminated by metals.

Key words

Algae, ANOVA, Bio accumulation, Biomonitoring, Heavy metal

Introduction

Heavy metals endanger ecosystem biodiversity due to the development of mining, smelting and other industrial activities. Heavy metal pollution can diminish crop yield, affect water quality and pose threats to the health and life of animals and humans through food chain (Jarup 2003; Mohammad *et al.,* 2008; Sharma and Agrawal 2005). As a result of human activities, copper, zinc, nickel, lead, cadmium, cobalt, mercury, chromium and arsenic are easily released in the environment. Some of these heavy metals are essential in small concentrations (as micronutrients) for normal physiological activities in living organisms, but their accumulation in high becomes toxic to most life forms (Lasat 2002; Nies 1999; Tangahu *et al.,* 2011). Some metals such as Hg, have no known metabolic function and their presence even in trace amount is toxic for living organisms (Rodriguez *et al.,* 2003). Frequent changes in water current makes it difficult to monitor heavy metal contamination through conventional water analysis. Therefore, to provide a more insight into the extent of metal contamination, it is important to correlate the data of metals present in water with data from algal species in water bodies (Javed, 2006).

Biomonitoring of aquatic bodies includes macrophytes, zooplankton, insects, gastropod, bivalves, molluscs, fishes and amphibians as indicator species (Zhou *et al.,* 2008). Industrialization and exponential growth of human population has given rise to increased of metal content in the environment. Dwivedi *et al.* (2010) evaluated the accumulation potential of metals (Cr, Cu, Fe, Mn, Ni and Zn) and metalloid (As) by green and blue green microalgae from Cr-contaminated sites in Unnao and Kanpur districts Uttar Pradesh. Cu tolerance and accumulation potential of *Chlamydomonas reinhardtii* was investigated by Boswell *et al.,* (2002). Cyanophycean and green algae have been found to accumulate a significant amount of metals from contaminated surface water and are considered as a bioremediator of heavy metals in aquatic bodies (Dwivedi *et al.,* 2006). Bioaccumulation of Cu and Ni by *Chlorella* species was investigated by Doshi *et al.* (2006). Rai *et al.* (2008) confirmed that some algal forms growing in polluted environments and accumulating high amounts of toxic metals may be used as bioindicator species. Bhavnagar district in Gujarat, India has many small and medium scale industries pertaining to pharmaceuticals, textiles, chemical, paper and pulp and refineries which are important for the economic development of Gujarat. However, these industries cause contamination of aquatic ecosystems as they discharge effluents along with domestic sewage. The present study investigated the bioaccumulation potential of four selected algal species namely *Oedogonium, Cladophora, Oscillatoria* and *Spirogyra* from this region.

Materials and Methods

A total of eight stations (Fig. 1) were selected for the present study, comprising of Shetrunji River (Stations 1, 2 and 3), Sihor tributary of River Gautami (Stations 4, 5 and 6) and Bor Talav Lake (Stations 7 and 8). Sites at Shetrunji River were polluted by anthropogenic activities such as ship-breaking industries producing organic wastes. Sihor is a manufacturing hub for brass and copper wires, ceramics, pottery iron and steel plants. Rolling mills associated with Asia's largest ship-breaking industries at Alang are located here. Tributary of River Gautami was selected for the study which was heavily polluted from the effluent discharged from adjacent industries. Bor Talav Lake was polluted with domestic effluent and industrial effluent, especially from marine chemical processing industries such as magnesium carbonate, silica, calcium chloride, electroplating (negligible discharge-non cyanide process) and plastic recycling.

Sampling of water and algal samples were performed quarterly during the period of August 2009 to March 2011. Water samples were collected in precleaned 500 ml bottles. Filamentous algae *Oedogonium, Cladophora, Oscillatoria* and *Spirogyra* were selected for monitoring purpose, and were manually collected. Heavy metals (Cu, Cd, Pb and Zn) were determined by the method of Sharma *et al.* (2010). All the analyses were performed in duplicate. Each algal sample (0.5g of dry powder) was placed in a conical flask, and 12 ml of concentrated HNO_3 and $HClO_4$ (3:1) was added. Digestion was carried out on hot plate (200 °C) till solution was reduced to 1 ml. Solution was diluted with distilled water and filtered through 0.45μm membrane filter (Millipore) and filtrate was analyzed by flame/graphite furnace atomic absorption spectrophotometer (AA-680, Shimadzu). Values were expressed as mean of two subsamples for each sample. QA/QC calibration including interference check sample, calibration, verification, calibration standard, blank control and linear dynamic range were performed. Wavelengths used for measurement were 228.8, 324.8, 217.0 and 213.9 nm for Cd, Cu, Pb and Zn, respectively. To determine metal concentration in water samples were acidified by adding appropriate volume of concentrated HNO_3 to achieve a pH of 2. After acidification, samples were stored in refrigerator at 4°C. Water samples were mostly turbid with an appreciable load of suspended particulates; hence samples were digested to obtain the concentration of both dissolved and particulate metals. For this, HNO_3 digestion technique (APHA ,2005) was used. A volume of 100 ml of each acid preserved and well-mixed water sample was taken in a beaker; 5 ml of concentrated HNO_3 was added, and mixture was slowly evaporated on hot plate in a fume hood to a volume of 10 ml clear solution. Beaker walls were washed with double-distilled water and volume was made up to 100 ml in a

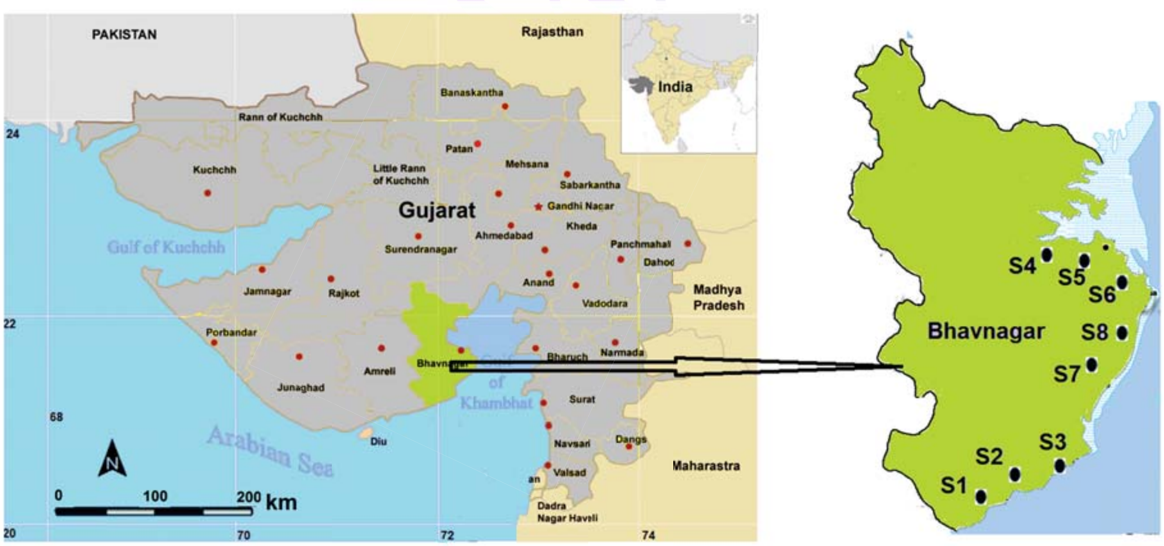

Fig. 1 : Location of sampling stations in Bhavnagar

volumetric flask, Cu, Cd, Pb and Zn concentration was estimated by Atomic Absorbtion Spectopholometer.

Statistical analysis was performed using XLSTAT 2012 software. Variability between sampling stations and algal species for each metal was analyzed by one-way ANOVA. Relationship between metal concentration in water samples and algae were evaluated by correlation analysis (at $P<0.05$).

Results and Discussion

The current study explored the accumulation potential of *Oedogonium, Cladophora, Oscillatoria* and *Spirogyra* for Cu, Cd, Pb and Zn from three freshwater habitats of Bhavnagar, Gujarat India. Metal concentration in water samples was observed in the following order : Zn>Cu>Pb>Cd (Table 1-8). Chatterjee *et al.* (2010) reported 3.95 mg l^{-1} of Cu concentration from contaminated water of Damodar River, India, whereas Cu concentration at stations 1 and 3 was 2.49 and 2.78 mg l^{-1} respectively in the present study. Damodharan (2013) reported that the mean concentration of Cd ranged from 0.011- 21.213 mg l^{-1} in contaminated river water of Uppanar, Cuddalore, South East Coast of India, whereas in the present study highest concentration was only 1.25 mg l^{-1} in Shetrungi River water. Pb concentration was in the range of 0.70-5.24 mg l^{-1} at Sihor tributary, which was lower than the concentration (0.57-40.47 mg l^{-1}) reported by Ustunada *et al.* (2011). In comparison to River Palar, which was contaminated with municipal waste and contained metals such as Cu (58.90 mg l^{-1}), Cd (6.90 mg l^{-1}), Pb (13.0 mg l^{-1}) and Zn (405 mg l^{-1}) (Govindasamy *et al.,* 2007), the sites analyzed in the present study appeared less contaminated by heavy metals.

In the present study maximum amount of Cu accumulation was observed in *Oedogonium* (118.5 $\mu g\ g^{-1}$) . Rai *et al.* (2008) reported Cu accumulation in *Oedogonium* (17.78±1.22 $\mu g\ g^{-1}$). Al-Homaidan *et al.* (2011) reported maximum of 138.28 $\mu g\ g^{-1}$ of Cu in *Cladophora* from Wadi Hanifah Stream, Riyadh, Saudi Arabia, whereas in the present study it was 116.56 $\mu g\ g^{-1}$. The concentration was very low when it was compared with macrophytes studied by Kumar *et al.* (2006), where Cu accumulation was 1325 $\mu g\ g^{-1}$. Rajfur *et al.* (2011) reported 47.5 $\mu g\ g^{-1}$ of Cu in *Spirogyra* for heavy metal polluted area in Poland, whereas in the present study it was found to be 22.11 $\mu g\ g^{-1}$ Cu in this species. Maximum accumulation of Cd was observed in *Oedogonium* (21.10 $\mu g\ g^{-1}$). The average concentration of Cd accumulation in *Cladophora* varied between 0- 4.14±3.02 $\mu g\ g^{-1}$, whereas Al-Homaidan *et al.* (2011) reported between 0.61 to 1.08 $\mu g\ g^{-1}$. Rajfur *et al.* (2011) reported 108.5 $\mu g\ g^{-1}$ of Cd accumulation in *Spirogyra,* whereas in the present study highest accumulation of Cd (9.23 $\mu g\ g^{-1}$) was found in spirogyra. A concentration of 2 $\mu g\ g^{-1}$ dry weight has been considered as maximum limit for non-polluted species (Al-Homaidan *et al.,* 2011; Lozano *et al.,* 2003). Therefore, it can be concluded that Cd content in all the algal species analyzed in present study were more than permissible limit, except at station 7. Highest Pb accumulation in *Cladophora* was 156.5 $\mu g\ g^{-1}$, whereas Eva and Jan (2001) reported only 7.9 $\mu g\ g^{-1}$ Pb in *Cladophora* from river Danube, Slovnaft, Bratislava, Croatia. Dwivedi *et al.* (2006) also reported Pb accumulation in *Oscillatoria* and highest concentration was 142.61 $\mu g\ g^{-1}$, whereas in the present study it was only 51.50 $\mu g\ g^{-1}$. Zn acts as an essential micronutrient for growth and development of algal; however can be toxic in higher amount (Rout and Das, 2009). Toxicity of this metal to algae led to chlorosis or death. Zn was highest accumulating heavy metal in the present study. Maximum concentration of Zn was observed in *Oscillatoria* (210.26 $\mu g\ g^{-1}$), whereas 277.06 $\mu g\ g^{-1}$ was observed by Dwevedi *et al.* (2006). The second largest Zn accumulating

Table 1 : Heavy metals content at station 1

Metals	Water (mg l^{-1})	*Oscillatoria* sp. ($\mu g\ g^{-1}$ d.wt.)	*Cladophora* sp. ($\mu g\ g^{-1}$ d.wt.)	*Oedogonium* sp. ($\mu g\ g^{-1}$ d.wt.)	*Spirogyra* sp. ($\mu g\ g^{-1}$ d.wt.)
Cu	1.77±1.20(2.56-0.44)	35.72±6.40(42.36-28.2)	55.84±40.03(116.56-17.48)	13.72±1.73(15.26-10.52)	9.56±3.89(15.58-4.17)
Cd	0.56±0.22(0.85-0.26)	2.27±2.24(5.3-0.46)	4.03±3.28(8.25-0.58)	3.33±3.74(10.3-0.78)	1.23±0.69(1.40-0.94)
Pb	1.24±0.82(2.38-0.46)	44.19±35.54(51.50-10.06)	40.81±56.45(156.5-10.3)	43.12±26.74(85.24-14.50)	35.93±36.54(90.45-6.45)
Zn	4.92±1.17(6.74-3.11)	102.80±39.04(141.28-46.5)	88.48±17.52(118.5-59.27)	48.14±6.53(58.20-42.56)	31.96±16.62(60.54-13.2)

Mean ±SD (maximum to minimum range in parentheses)

Table 2 : Heavy metals content at station 2

Metals	Water (mg l^{-1})	*Oscillatoria* sp. ($\mu g\ g^{-1}$ d.wt.)	*Cladophora* sp. ($\mu g\ g^{-1}$ d.wt.)	*Oedogonium* sp. ($\mu g\ g^{-1}$ d.wt.)	*Spirogyra* sp. ($\mu g\ g^{-1}$ d.wt.)
Cu	1.33±1.25(3.67-0.33)	45.45±27.58(106.56-26.74)	50.34±28.86(97.51-34.27)	11.45±2.95(17.26-9.20)	10.04±4.06(15.56-4.38)
Cd	0.62±0.42(1.26-0.14)	2.43±1.74(4.38-0.783	3.05±2.10(6.17-0.74)	5.57±4.59(12.56-1.98)	2.64±2.13(5.36-0.53)
Pb	1.51±0.65(2.47-0.53)	32.61±8.73(46.56-17.50)	15.57±11.47(35.6-5.78)	28.96±17.90(55.17-9.22)	9.26±6.52(15.07-3.45)
Zn	4.65±1.36(6.24-2.46)	103.69±17.28(130.45-78.26)	85.54±30.97(141.5-41.25)	65.76±52.25(181.5-27.38)	36.32±13.86(56.59-20.32)

Mean ±SD (maximum to minimum range in parentheses)

Table 3 : Heavy metals content at station 3

Metals	Water (mg l^{-1})	*Oscillatoria* sp. (µg g^{-1} d. wt.)	*Cladophora* sp. (µg g^{-1} d. wt.)	*Oedogonium* sp. (µg g^{-1} d. wt.)	*Spirogyra* sp. (µg g^{-1} d. wt.)
Cu	2.39±1.28(3.73-0.50)	30.89±6.39(40.39-22.5)	41.16±22.30(90.43-25.53)	31.02±39.45(118.5-5.40)	25.40±36.97(108.5-3.27)
Cd	0.26±0.14(0.44-0.11)	4.70±5.30(13.5-0.97)	3.68±2.59(5.67-1.26)	9.52±6.45(14.68-1.59)	5.55±3.55(9.23-1.06)
Pb	0.52±0.20(0.68-0.28)	19.92±9.96(36.33-9.24)	10.34±6.87(17.09-0.95)	21.49±15.94(38.90-8.05)	7.07±4.55(12.74-4.89)
Zn	3.79±1.45(5.71-2.05)	102.29±51.69(210.26-51.98)	76.99±22.87(110.5-45.26)	67.73±27.82(112.54-46.58)	47.49±22.85(89.56-22.86)

Mean ±SD (maximum to minimum range in parentheses)

Table 4 : Heavy metals content at station 4

Metals	Water (mg l^{-1})	*Oscillatoria* sp. (µg g^{-1} d. wt.)	*Cladophora* sp. (µg g^{-1} d. wt.)	*Oedogonium* sp. (µg g^{-1} d. wt.)	*Spirogyra* sp. (µg g^{-1} d. wt.)
Cu	2.47±0.98(4.18-1.56)	7.45±8.45(21.24-0.58)	8.27±3.91(10.57-5.08)	18.83±10.79(25.65-11.70)	15.73±5.90(17.26-5.23)
Cd	1.28±0.78(2.46-0.20)	21.27±2.04(6.18-3.20)	3.25±1.97(4.81-2.36)	16.64±10.28(21.1-5.26)	2.96±1.81(3.83-1.46)
Pb	3.75±1.21(5.24-2.09)	1.65±1.17(3.18-0.55)	2.97±2.65(6.78-0.58)	4.35±3.16(8.23-1.35)	1.60±0.82(1.60-1.58)
Zn	4.14±1.35(5.38-2.58)	44.50±35.23(109.5-40.12)	73.22±32.80(90.56-56.2)	53.91±29.12(59.68-40.12)	76.09±23.40(110.5-38.5)

Mean ±SD (maximum to minimum range in parentheses)

Table 5 : Heavy metals content at station 5

Metals	Water (mg l^{-1})	*Oscillatoria* sp. (µg g^{-1} d. wt.)	*Cladophora* sp. (µg g^{-1} d. wt.)	*Oedogonium* sp. (µg g^{-1} d. wt.)	*Spirogyra* sp. (µg g^{-1} d. wt.)
Cu	1.64±1.19(3.46-0.42)	6.77±5.63(13.89-0.56)	10.28±5.88(15.18-5.36)	13.78±10.54(23.8-3.58)	15.03±5.75(20.18-6.75)
Cd	0.95±0.78(1.84-0.15)	3.94±2.28(5.30-3.26)	2.88±2.51(6.81-1.56)	5.18±4.11(10.5-1.56)	1.76±1.17(3.59-0.80)
Pb	1.21±0.48(1.87-0.70)	3.46±2.44(6.23-1.58)	4.25±2.60(6.10-2.38)	2.97±1.77(4.39-0.56)	0.87±0.73(1.09-0.32)
Zn	3.47±0.73(4.58-2.60)	69.22±40.10(102.86-50.53)	71.12±38.27(85.47-56.54	56.83±6.53(58.59-45.28)	77.57±18.37(98.23-45.27)

Mean ±SD (maximum to minimum range in parentheses)

Table 6 : Heavy metals content at station 6

Metals	Water (mg l^{-1})	*Oscillatoria* sp. (µg g^{-1} d. wt.)	*Cladophora* sp. (µg g^{-1} d. wt.)	*Oedogonium* sp. (µg g^{-1} d. wt.)	*Spirogyra* sp. (µg g^{-1} d. wt.)
Cu	2.18±1.13(2.89-0.52)	15.13±9.92(22.01-4.39)	8.34±4.04(12.16-5.86)	18.28±8.37(22.56-5.48)	13.59±5.95(22.11-8.07)
Cd	0.41±0.26(0.67-0.21)	4.77±2.57(6.12-4.09)	4.14±3.02(8.07-1.58)	2.07±1.90(5.09-0.59)	1.31±0.66(1.82-0.39)
Pb	3.06±1.12(4.58-1.54)	4.17±3.19(8.01-1.06)	1.76±1.11(2.8-1.23)	3.72±2.52(5.68-1.08)	2.08±1.87(5.26-0.32)
Zn	5.55±1.13(6.24-3.75)	66.27±37.87(97.32-52.63)	72.81±32.19(87.27-59.56)	64.18±28.85(85.18-50.74)	79.75±22.54(102.5-39.48)

Mean ±SD (maximum to minimum range in parentheses)

Table 7 : Heavy metals content at station 7

Metals	Water (mg l^{-1})	*Oscillatoria* sp. (µg g^{-1} d. wt.)	*Cladophora* sp. (µg g^{-1} d. wt.)	*Oedogonium* sp. (µg g^{-1} d. wt.)	*Spirogyra* sp. (µg g^{-1} d. wt.)
Cu	2.11±1.39(3.78-0.53)	15.06±8.79(21.56-5.56)	13.57±7.12(15.56-11.50)	17.69±6.77(20.81-10.18)	8.67±3.80(15.10-4.98)
Cd	0.38±0.17(0.58-0.25)	1.96±1.49(3.83-0.78)	0.00	1.92±0.83(1.79-1.39)	1.80±1.71(4.08-0.23)
Pb	1.14±0.63(1.74-0.46)	13.34±7.02(15.5-11.8)	7.37±3.84(8.21-6.53)	4.41±1.76(6.08-1.07)	6.11±2.41(10.33-3.45)
Zn	5.68±1.75(8.46-3.48)	159.30±86.02(199.1-119.5)	137.20±70.95(143.2-131.2)	168.43±74.89(191.6-110.53)	86.33±17.70(102.62-58.7)

Mean ±SD (maximum to minimum range in parentheses)

alga was *Oedogonium* (191.59 µg g^{-1}). Javed (2006) studied Zn accumulation in planktonic biomass and reported 290.13 µg g^{-1} Zn, whereas Lokeshwari and Chandrappa (2007) reported only 82.0 µg g^{-1} in weed plant. High concentration of heavy metals in algae were not usually found in these algae in unpolluted water and also much lower concentration has been reported by other

Table 8 : Heavy metals content at station 8

Metals	Water ($mg\ l^{-1}$)	***Oscillatoria*** **sp.** ($\mu g\ g^{-1}$ d. wt.)	***Cladophora*** **sp.** ($\mu g\ g^{-1}$ d. wt.)	***Oedogonium*** **sp.** ($\mu g\ g^{-1}$ d. wt.)	***Spirogyra*** **sp.** ($\mu g\ g^{-1}$ d. wt.)
Cu	0.96±0.86(2.56-0.42)	15.07±8.98(22.16-7.98)	8.59±5.22(12.28-4.27)	19.64±9.66(28.6-16.23)	8.83±4.59(16.7-3.09)
Cd	0.70±0.36(0.96-0.25)	4.06±1.65	1.56±0.64	2.3±0.94	1.38±1.74(4.9-0.40)
Pb	2.68±0.96(4.27-1.56)	11.84±6.21(13.56-10.12)	8.10±4.69(10.23-5.47)	5.02±2.70(6.47-1.67)	5.58±1.93(5.39-4.23)
Zn	6.51±3.23(9.87-1.80)	116.26±60.09(120.5-112.02)	128.87±71.24(140.13-111.53)	154.84±35.51(191-105.1)	97.27±39.14(99.58-56.90)

Mean ±SD (maximum to minimum range in parentheses)

worker from different countries/locations (Alkhalifa *et al*., 2012). A wide variation of heavy metal concentration among the studied algal species showed that anthropogenic discharge including domestic and industrial, agricultural discharge and other kind of pollutant in the studies stations were significantly higher. Usually, algae found in the field and the amount of metals accumulated metals reflects both population-specific accumulation capability and various metal bioavailabilities depending on environmental conditions (Skowronska, 2003). The present study includes both intra and intercellular accumulation of heavy metal in the studied algal species.

The trend of heavy metal in water was Zn>Cu>Pb>Cd. The results of ANOVA showed variation of mean for all the metals in water $P<0.05$. Large difference between the means were observed for all the heavy metals studied in *Spirogyra* at $P>0.05$. In *Oscillatoria,* significant difference between the means were observed for all the metals, except Cd. In ANOVA of *Oedogonium,* significant difference between the means were observed only for Pb and Zn at $P<0.05$. However, in *Cladophora* significant difference between the means was observed for Pb and Zn. *Oscillatoria* showed significant correlation ($r=0.944$; $P<0.05$) with Cu at station 5, whereas lowest correlation was observed with Zn ($r=0.178$; $P>0.05$) at station 4. Cu is the only metal which showed significant positive correlation ($P>0.05$) of 0.664, 0.528 and 0.347 with *Oscillatoria* at station 3, 4 and 6, respectively. In *Cladophora*, positive correlation ($P>0.05$) was observed with Cd at station 2 and 6, (0.568 and 0.552). *Oedogonium* showed highest significant correlation ($r=0.955$; $P<0.05$) with Pb at station 6. Further, it also showed positive correlation ($P>0.05$) with Cd (0.398, 0.523, 0.504) at station 1, 6 and 7, respectively. *Spirogya* showed significant correlation ($r=0.936$; $P<0.05$) with Zn at station 1 and also with Cd ($r=0.806$; $P<0.05$) at station 7.

The present study concluded that higher concentration of metals in algal biomass with respect to the metal concentration in water signifies the accumulation potential of these algae.

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