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Efficiency of Biomonitoring through Macrophytes Present in the Aquatic Bodies of Dehradun and Haridwar

***Gunjan Sharma and Archana Sharma**
School of Environment and Natural Resources, Doon University, Dehradun (U.K.), India-248001.

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***Corresponding Author Email:** sharma007gunjan@gmail.com

Abstract
During last few decades, human activity posed a dreadful threat to the environment by adding totally new chemical substances that previously did not even exist viz; xenobiotics, radionuclides, persistent heavy metals etc. These confronting activities jeopardize the natural functioning of the environment and consequently add up the greatest pollutant burden on the natural ecosystems. Moreover, the situation will become more dramatic in the future but subsequently, a solution in this context is Biomonitoring of environmental pollutants through bioindicators of pollution to control the problem at its origin. In the present study three aquatic bodies of Dehradun and haridwar were taken, water analysis and heavy metal load of aquatic macrophytes from sites was done. Six metals (Pb, Fe, Cu, Cr, Mg& Ni) were investigated in the water samples and root and shoot parts of aquatic macrophytes. Among all, Ni, Cu and Fe from residential sites found to be within permissible limits as prescribed by WHO, whereas, Cr ranges from 0.15 – 0.22mg/l and Pb – 0.41 – 0.54mg/l which was far higher than the permissible limits. The variation was observed in average accumulation pattern of heavy metals by different plant part of the selected macrophytes. Pb ranges from 0.63-0.59 mg/l in roots of *Eichhornia*, roots of *Pistia* accumulates 0.57-0.49 mg/l of Pb from industrial and residential regions of Hardwar. Shoots of *Eichhornia crassipes* accumulates Cr in the ranges 0.48mg/l, 0.46mg/l, 0.59mg/l, 0.47mg/l from Dandaar, Dehradun. Shoots of *Pistia stratiotes* accumulates Pb in the range 0.29mg/l, 0.23mg/l,0.30mg/l, 0.33mg/l. Pb, Fe, Cu and Cr in Duckweed (*Spirodella* and *Lemna*) of Dandaar, Dehradun and Hardwar ranges from 0.24-0.35mg/l, 5.81-6.45mg/l, 0.10-0.13mg/l, 0.14-0.44mg/l of *Spirodella*and 0.38-0.53mg/l, 5.10-5.46mg/l, 0.05-0.09mg/l, 0.11-0.14mg/l of *Lemna* respectively.

Keywords
Biomonitoring, Bioindicators, Phytoremediation, Heavy metals.

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1. INTRODUCTION
"Population & Pollution", is the two most important words that are being used in today's world with utmost concern. These two '**P**'s have burdened the scientific society to a level that becomes difficult to handle. Rise in population is directly proportional to pollution increase. The wastes generated, ultimately find their way into environmental components i.e.

air, water and soil. The presence of these wastes in water bodies becomes undesirable, as it hampers the normal sustenance of life. Contamination of aquatic bodies by organic and inorganic pollutants has become a major concern for developing country like ours (India). Human actions are causing the slow extinction of flora, fauna & fungi in natural environment through toxic pollution owing to industrial and technological advancement in recent decades[1]. From last two decades, due to increase in amount of pollution, researchers from all over the world developed several techniques to combat the problem of aquatic pollution but at the same time it is difficult for the developing countries to implement these expensive and energy intensive modern technologies to treat the aquatic pollution. Thus, it is actually very difficult to adopt these modern technologies on ground level by developing nations. In fact, the developed world until now is not in a position to treat their whole waste generated by these efficient modern technologies.

As an alternative to this during recent past, utilization of aquatic macrophytes for the treatment of wastewater has been adopted as a feasible, easy to use, economical and very less energy intensive technique by the developing as well as developed world. It has been known for a long time that aquatic plants both, living & dead, are heavy metal accumulators & therefore, the use of aquatic plants for the removal of heavy metals from wastewater has gained high interest[2]. Aquatic macrophytes have great potential to accumulate heavy metals inside their plant body. These plants can accumulate heavy metals 100,000 times greater than in the associated water . Therefore, these macrophytes have been used for heavy metal removal from a variety of sources[3,4,5,6,7] .

This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate), or the air[8]**.** Biomonitoring has several advantages and is themost significant-one in study of sub lethal levels of bioaccumulatedcontaminants within the tissues of organisms,which indicate the net amount of pollutants integratedover a period of time [9]. Biomonitoring of pollutants using some plants as accumulatorspecies, which accumulate relatively largeamounts of certain pollutants, even from much diluted solutions without obnoxious effects [10].

The aim was to: **(1)** evaluate the relationship among heavy metals concentrations in the water and aquatic macrophytes; through Bio concentration factor **(2)** determine the heavy metal selectivity of each plant species and of different organs; and **(3)** define which species and which plant organs exhibit the greatest accumulation. The use of these species for biomonitoring and phytoremediation programs has also been considered.

2. METHODS

2.1. Study Area

Dandaar, a freshwater lake present in the center of Dehradun city of Uttarakhand (30^0 19’ N & 78^0 40’ E) located around 11 km in north from the Doon University campus, was selected as a site for study. The lake is surrounded by residential apartments, car washing and repairing workshops, small scale stone crusher shops along with lots of construction work going on, though the aquatic body doesn’t receive any effluents directly from industries but all the domestic garbage, waste water generated from car washing and constructional waste ultimately find their way into the lake thereby facilitating the growth of macrophytes in abundance. The macrophytes present in the lake were utilized for heavy metal analysis and therefore assessed for their Biomonitoring potential.

The second site selected for study is Haridwar, a holy city that is now growing as industrial hub of the Uttarakhand state. Haridwar district lies between 29°33’ - 30°14’N latitude and 77°57’- 78°1’E longitude at an average altitude of 230m. Haridwar is the most densely populated district of Uttarakhand having a population of 19, 29,029 (Census, 2011). Due to large-scale operation and development of Integrated Industrial Estate in the city leads to cause adverse effect on the air, water and soil quality. Residential area (Rawali Mehdood) that comes under the vicinity of industrial area is affected badly. Site R1 and R2 are residential sites that receive effluents from industrial region as well as from residential regions. Site I1 and I2 are industrial sites from industrial region that receive effluents and discharges directly from the industries.

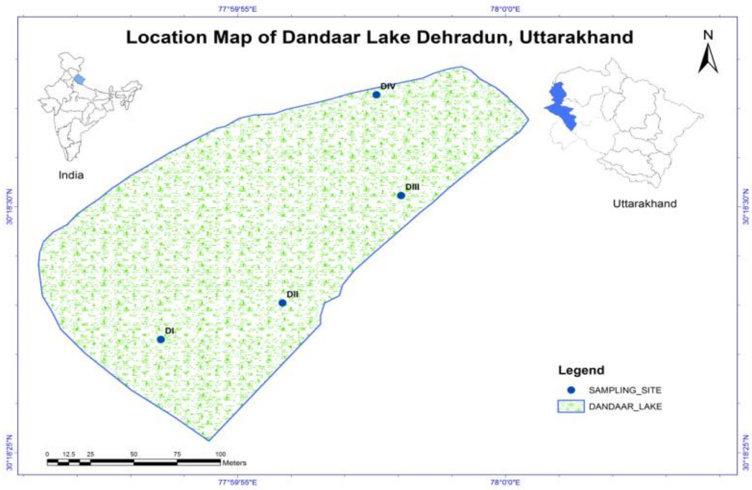

Fig-1: Study area of Dandaar Lake Dehradun

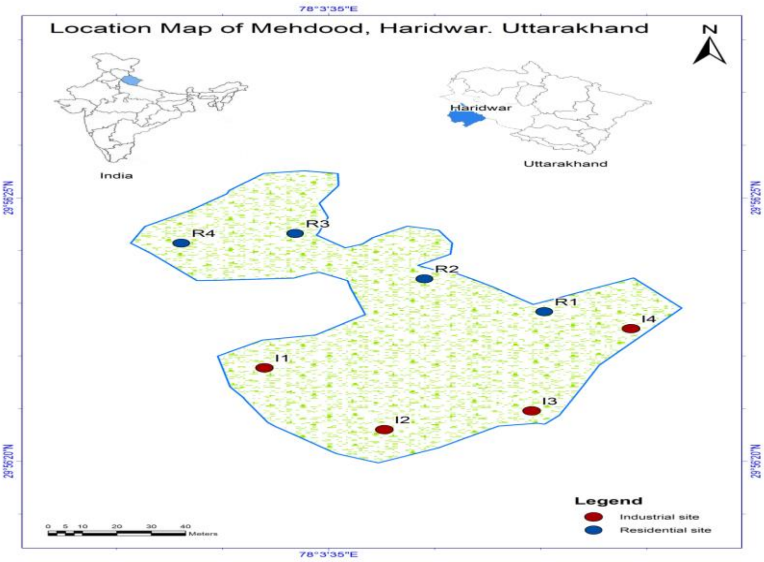

Fig:2- Study area of Mahdood Lake Haridwar

2.2. Sample collection

Surface water and four native aquatic plant species (*Eichhornia crassipes, Pistia stratiotes, Spirodellapolyrrhiza and Lemna gibba*) were sampled from two different cities Dehradun and Haridwar including all the study sites representing three different pollution sources. All samples of water and plants were collected in triplicates throughout the year (August 2015 to July 2016). All the samples were preserved in iceboxes throughout the field trip and during transportation to the laboratory. Each water sample was divided into two aliquots: one was acidified immediately in the field with nitric acid (1ml HNO_3 L^{-1}) for heavy metals determination, while the other half sample kept in a separate bottle and carried in ice box and upon reaching the lab refrigerated immediately until nutrient analysis.

After the screening of sensitive and tolerant macrophytes from the study area, among all macrophytes, four abundantly available existing macrophytes were selected for the estimation of heavy metals. *Eichhornia crassipes* (Mart.) Solms (water hyacinth), *Pistia stratiotes* L. (water lettuce), *Lemna gibba* L., *Spirodelapolyrrhiza*W. Koch (duckweed)have been selected. Healthy aquatic plants were collected by hand, washed with water, kept in plastic zipper bags and brought to laboratory. In lab, plants were again washed with tap water and then with distilled water to remove all the adhered dust and debris and then labeled carefully.

2.3. Analysis of Heavy Metals in Water and Plant Samples

The water samples around 45 ml were acidified with 5 ml of HNO_3, transferred to digestive tubes(vials), and then digested in Microwave digester. Later digested samples were filtered with Whatman no. 42 filter paper and a volume make-up was done. The

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metal analyses were carried out by means of Atomic Absorption Spectrophotometer (AAS, Perkin Elmer). Plant samples were dried in oven at 80^0C for 48 hours, after drying plant samples were grounded with the help of pestle- mortar and 0.5 gm were taken out of it and digested with 10ml of HNO_3, transferred in digestive tubes (vials) and digested in Microwave digester and diluted to 50 ml with de-ionized distilled water. The digested samples of plants were analyzed for heavy metals by the same procedure as it was for the water samples. Heavy metals were analyzed with procedures of US-EPA Guidelines 1998.

2.4 Estimation of Bioconcentration Factor

The Bioconcentration Factor (BCF) was calculated as the ratio of the trace element concentration in the plant tissues at harvest to the concentration of the element in the external environment [11, 12]. BCF is dimensionless. Bioconcentration of any chemical by aquatic organisms is described with bioconcentration factor [13]. Bioconcentration factor (BCF) was calculated using the formula as below [14].

BCF = Concentration of element in plant / Concentration of element in water

2.5. Statistical Analysis

Data analysis was carried out using SPSS software version 17.0 for statistical analysis. The significance was reported as $p<0.05$ level.

3. Results and Discussion

The present study aimed to investigate the status of the heavy metal pollution load of Dandaar Lake, Dehradun and two aquatic bodies of residential and industrial region of Haridwar. The results of the study carried out, focused on the assessment of heavy metals (Pb, Fe, Cu, Cr, Mg, Ni) in water and accumulation potentialities of native aquatic macrophytes (*Eichhornia crassipes, Pistia stratiotes, Lemna gibba and Spirodellapolyrrhiza*) growing naturally and found all over the aquatic bodies.

The results with their respective tables are discussed under following heads –

3.1. Metal Concentration in Aquatic Bodies of Dehradun and Hardwar:

The mean values of six investigated heavy metals in the selected aquatic bodies of Dehradun and haridwar are presented in **Table 1.**The mean concentration of values of the heavy metals in these water bodies decreased according to following sequence:In site **Dand**- Mg > Fe >Pb > Ni > Cr > Cu,**Res**– Mg> Fe> Pb> Cr> Ni> Cu,**Ind** – Mg> Fe> Pb> Cr> Ni> Cu. The permissible limits of heavy metals in water according to Indian standards are mentioned in the table below.The maximum permissible limit for Ni in water is 0.2 mg/l. as per WHO recommendation. In all the samples from different sites, the concentration of nickel ranged between 0.05 to 0.19 mg/l, which is within the permissible limit. The maximum permissible limit for Cr in water is 0.1 mg/l. The concentration of Cr in all water samples ranged between 0.15 to 0.22mg/l. The concentration of chromium of all the water samples was found above the permissible limit set by WHO. The maximum permissible limit for Cu in water is 2 mg/l, the range of Cu concentration recorded was in between 0.005 to 0.06 mg/l. in the water samples of all the sites the concentration of copper was recorded below the permissible limit. The fate of elemental copper in water is complex and influenced by pH, dissolved oxygen and the presence of oxidizing agents and chelating compounds or ions. [15] Concentration of Pb in all the water samples ranged between 0.41 to 0.54 mg/l. As per the standards of WHO, permissible limit of lead in water is 0.05 mg/l. Accordingly, the concentration of Pb was found far higher than the permissible limit. The maximum allowed concentration of iron in drinking water is 1.0 mg/L according to WHO report [16].In the water samples collected from Site Dandaar, Dehradun and Industrial site, Hardwar, the concentration of iron was found within the permissible limit prescribed by WHO and rest of the water samples recorded iron above the permissible limit. Variation of individual metal was also observed among the selected sites. The average concentration of Pb ranged from 0.41 to 0.57mg/l and the highest concentration was recorded in Industrial site, Haridwar. The Fe content recorded lowest from Dandaar, Dehradun and highest from Hardwar Residential. Similarly, the content of Cu and Cr reported highest from Residential site of Haridwar. Furthermore, the average concentration of Mg and Ni reported highest from Residential and Industrial sites of Hardwar.

3.2. Metal Concentration in Different organs of Aquatic Macrophytes

The estimated heavy metals in macrophytes are Lead (Pb), Iron (Fe), Copper (Cu), Chromium (Cr), Magnesium (Mg) and Nickel (Ni). The variation in average accumulation pattern of heavy metals by different organs (roots and shoot) of selected aquatic macrophytes was observed. **Table 2** illustrates average metal concentration accumulation of heavy metals by root and shoot of *Eichhornia crassipes* and *Pistia stratiotes* and accumulation concentration by duckweed (*Spirodellapolyrrhiza and Lemna gibba*) of Dandaar, Dehradun and Residential, Industrial sites of Hardwar respectively. Accumulated concentration of heavy metals in naturally growing aquatic macrophytes recoded in the given decreasing sequence i.e., Fe, Mg, Pb, Cr, Cu and Ni. Pb is believed to be the metal of least bioavailability and the most highly accumulated metal in root tissue while Pb shoot accumulation is much lower in most plant species [17]. Similar results were obtained from the investigation of root and shootof *Eichhornia* and *Pistia.*In macrophytes *Eichhornia crassipes* (roots and shoots) and *Pistia stratiotes* (roots and shoots) are more promising accumulators than *Spirodella polyrrhiza* and *Lemna gibba*. Macrophytes accumulate in the given decreasing sequence *Eichhornia crassipes* > *Pistia stratiotes* > *Spirodella polyrrhiza* > *Lemna gibba.* In the present investigation, comparatively Industrial site of Hardwar was found more polluted with higher concentration of heavy metals than Residential site and aquatic body of Dehradun, Dandaar was less polluted among them. Cr is exception among the investigated metals as it was found higher in industrial site as well as in residential area and Dandaar. Cr is widely used in industry as plating, alloying, tanning of animal hides, inhibition of water corrosion, textile dyes and mordants, pigments, ceramic glazes and refractory bricks. [18]. Due to this wide anthropogenic use of Cr, the consequent environmental contamination increased and has become an increasing concern in the last years[11].

Table-1: Heavy Metals in water bodies of Dehradun and Haridwar

S.No.	Metals	Dandaar	Hardwar Res.	Hardwar Ind.	Indian Standard
1.	**Pb**	0.51±0.03	0.51±0.02	0.57±0.01	0.05
2.	**Fe**	1.35±0.06	4.41±0.43	2.17±0.04	0.3
3.	**Cu**	0.03±0.005	0.06±0.003	0.06±0.02	0.05
4.	**Cr**	0.18±0.005	0.19±0.01	0.20±0.007	0.05
5.	**Mg**	7.66±0.08	7.99±0.21	7.35±0.37	
6.	**Ni**	0.18±0.02	0.14±0.01	0.19±0.02	0.02

Values are Mean ± SE (n=3); Unit: - concentration in mg l^{-1}

Table-2: Accumulation Potential of Native Aquatic Macrophytes (Dandaar & Hardwar)

Site	Species	Metals					
Dandaar		**Pb**	**Fe**	**Cu**	**Cr**	**Mg**	**Ni**
	E. crassipes S	0.41±0.01	8.93±0.24	0.03±0.005	0.48±0.04	7.80±0.41	0.09±0.05
	E. crassipes R	0.50±0.01	8.94±0.19	0.13±0.01	0.58±0.01	8.28±0.25	0.17±0.02
	P. stratiotes S	0.29±0.02	6.27±0.48	0.05±0.006	0.53±0.04	7.60±0.47	0.13±0.03
	P. stratiotes R	0.45±0.02	6.15±0.23	0.09±0.009	0.50±0.01	8.10±0.11	0.61±0.06
	Spirodella	0.31±0.04	5.81±0.41	0.13±0.01	0.44±0.30	7.78±0.32	0.02±0.01
	Lemna	0.38±0.06	7.17±0.66	0.12±0.04	0.06±0.02	5.47±0.60	0.009±0.01
Hardwar Res.							
	E. crassipes S	0.41±0.02	18.16±0.84	0.11±0.01	0.31±0.03	8.38±0.24	0.07±0.02
	E. crassipes R	0.63±0.03	12.03±1.45	0.11±0.01	0.47±0.03	8.63±0.19	0.11±0.02
	P. stratiotes S	0.41±0.06	7.42±0.19	0.06±0.01	0.33±0.02	7.99±0.10	0.18±0.006
	P. stratiotes R	0.49±0.005	7.80±0.01	0.12±0.002	0.39±0.01	9.07±0.14	0.20±0.007
	Spirodella	0.49±0.06	6.74±0.17	0.07±0.002	0.19±0.05	7.43±0.46	0.05±0.02
	Lemna	0.48±0.07	5.46±0.76	0.09±0.01	0.11±0.01	4.40±0.79	0.02±0.01
Hardwar Ind.							
	E. crassipes S	0.50±0.02	15.84±45	0.45±48	0.32±04	8.11±26	0.08±04
	E. crassipes R	0.63±0.04	15.14±44	0.15±03	0.47±04	8.06±17	0.14±03
	P. stratiotes S	0.42±0.05	8.02±17	0.06±02	0.35±03	7.87±32	0.46±47
	P. stratiotes R	0.56±0.04	8.09±29	0.12±01	0.39±02	8.92±34	0.20±03
	Spirodella	0.51±0.03	6.05±0.08	0.07±0.02	0.18±0.03	6.80±0.23	0.09±0.01
	Lemna	0.49±0.03	5.10±0.08	0.08±0.01	0.14±0.03	4.87±0.13	0.04±0.03

Values are Mean ± SE (n=3); Unit: - concentration in µg g^{-1}

Table-3: BCF Dandaar and Hardwar - Eichhornia, Pistia and Duckweed

Site	Species	Metals					
Dandaar		**Pb**	**Fe**	**Cu**	**Cr**	**Mg**	**Ni**
	E. crassipes S	0.8115	6.6296	0.8970	2.6064	1.0175	0.4887
	E. crassipes R	0.9843	6.6321	3.6684	3.1392	1.0804	0.9292
	P. stratiotes S	0.5790	4.6501	1.4246	2.8519	0.9915	0.7347
	P. stratiotes R	0.8860	4.5707	2.4729	2.6972	1.0578	3.3621
	Spirodella	0.6126	4.3165	3.6381	2.3829	1.0146	0.1543
	Lemna	0.7521	5.3267	3.3110	0.3252	0.7130	0.0502
Hardwar Res.							
	E. crassipes S	0.7956	4.1346	1.9539	1.6372	1.0483	0.5313
	E. crassipes R	1.2255	2.7672	1.9288	2.5156	1.0810	0.7822
	P. stratiotes S	0.8055	1.6906	1.1443	1.7725	1.0010	1.2866
	P. stratiotes R	0.9605	1.7804	2.0355	2.0744	1.1357	1.3947
	Spirodella	0.9550	1.5382	1.1818	1.0261	0.9296	0.3963
	Lemna	0.9421	1.2361	1.5096	0.6128	0.5495	0.1749
Hardwar Ind.							
	E. crassipes S	0.8808	7.2922	10.0046	1.5424	1.1050	0.4594
	E. crassipes R	1.1006	6.9717	2.7339	2.2733	1.0996	0.7631
	P. stratiotes S	0.7375	3.6945	1.1420	1.6731	1.0747	2.4878
	P. stratiotes R	0.9856	3.7244	2.1805	1.8769	1.2178	1.0489
	Spirodella	0.8880	2.7880	1.4006	0.8911	0.9286	0.4737
	Lemna	0.8641	2.3493	1.4818	0.6805	0.6640	0.2152

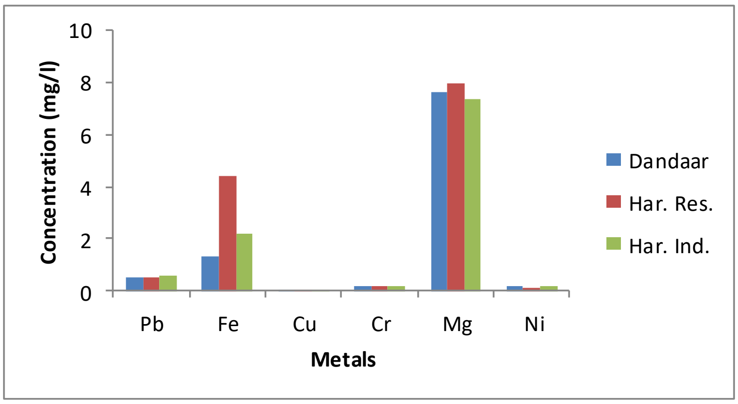

Fig:3-Metal concentration of different water bodies of Dehradun and Hardwar

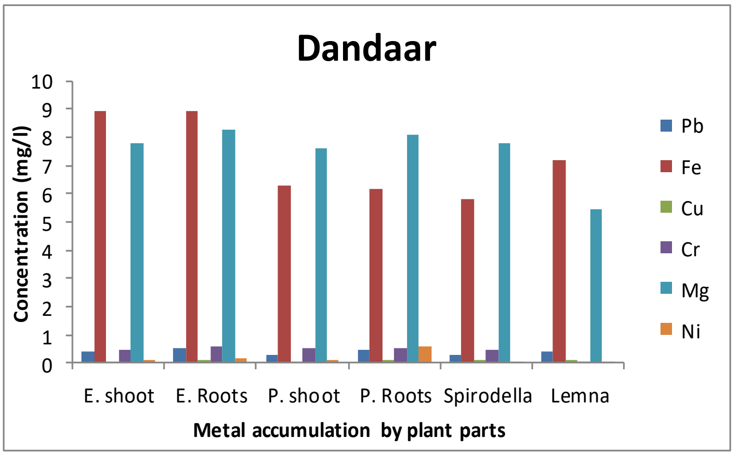

Fig-4: Metal accumulation by different aquatic plants

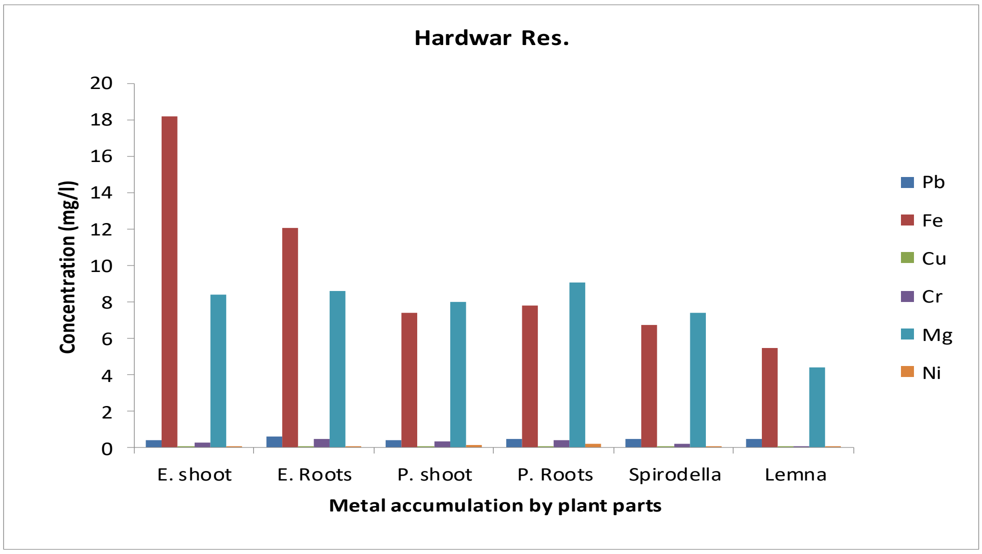

Fig-5: Metal accumulation by different aquatic plants

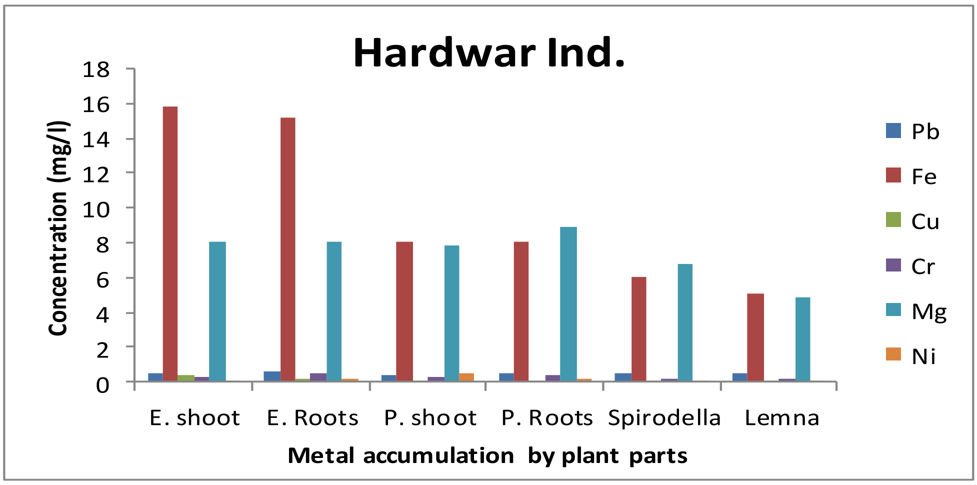

Fig-6: Metal accumulation by different aquatic plants

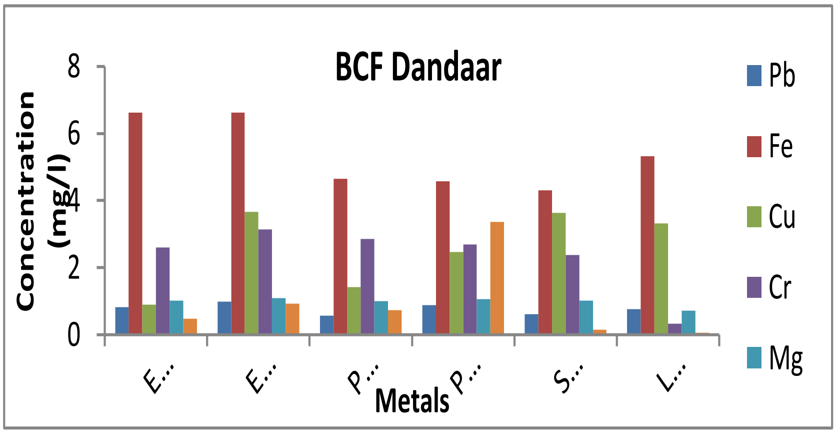

Fig-7: shown Bio-accumulation factor at Dandaar Lake

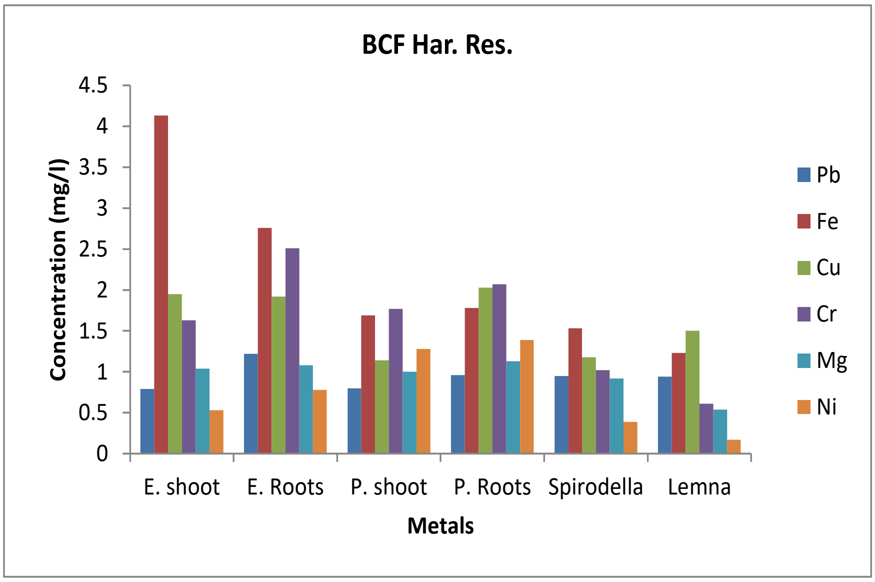

Fig-8: shown Bio-accumulation factor at Hardwar residential areas

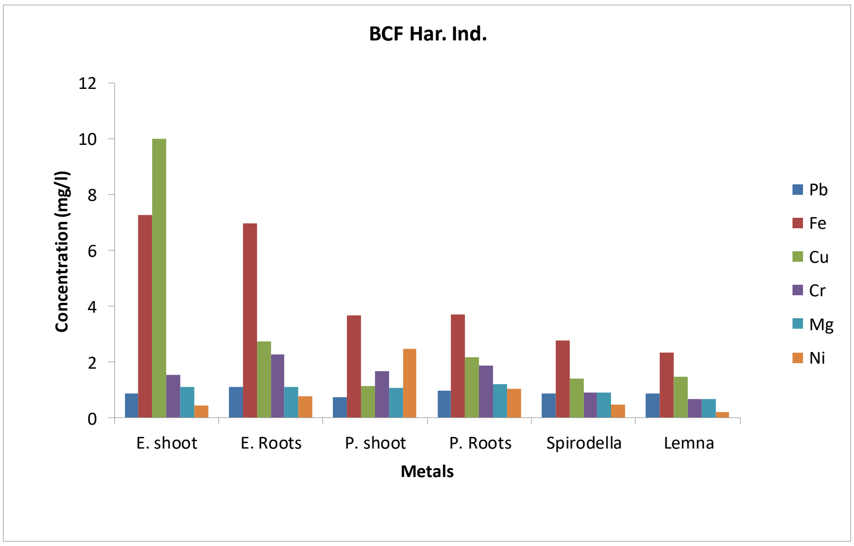

Fig-9: shown Bio-accumulation factor at Hardwar Industrial area

3.3. Bioconcentration Factor (Macrophytes/Aquatic bodies)

The respective bioconcentration factor (BCF) is tabulated in the **Table 3** of *Eichornia crassipes, Pistia stratiotes, Spirodella Polyrrhiza and Lemna gibba* of Dandaar, Dehradun and Hardwar respectively. More the BCF and less the TF [19] indicates that the respective species do possess good phytoremedial potential and are good phytostabilizers [20]. The BCF orders of heavy metals in macrophytes with their respective sites are given below:

1. *E. crassipes* (root)- Fe> Cu> Cr> Mg> Pb> Ni - **(D)**
2. *E. crassipes* (shoot)- Fe> Cr> Mg> Cu> Pb> Ni
3. *P. stratiotes* (root)- Fe> Ni> Cr> Cu> Mg> Pb - **(D)**
4. *P. stratiotes* (shoot)- Fe> Cr> Cu> Mg> Ni> Pb
5. *Spirodella*- Fe> Cu> Cr> Mg> Pb> Ni - **(D)**
6. *L. gibba*- Fe> Cu> Pb> Mg> Cr> Ni - **(D)**
7. *E. crassipes* (root) - Fe> Cr>Cu>Pb>Mg>Ni**(Res)**
8. *E. crassipes* (shoot)- Fe>Cu>Cr>Mg>Pb>Ni
9. *P. stratiotes* (root) - Cr>Cu>Fe>Ni>Mg>Pb**(Res)**
10. *P. stratiotes* (shoot) - Cr>Fe>Ni>Cu>Mg>Pb
11. *Spirodella*- Fe>Cu>Cr>Pb>Mg>Ni**(Res)**
12. *L. gibba*- Cu>Fe>Pb>Cr>Mg>Ni**(Res)**

13. *E. crassipes* (root)- Fe>Cu>Cr>Pb>Mg>Ni**(Ind)**
14. *E. crassipes* (shoot)- Cu>Fe>Cr>Mg>Pb>Ni
15. *P. stratiotes* (root)- Fe>Cu>Cr>Mg>Ni>Pb**(Ind)**
16. *P. stratiotes* (shoot)- Fe>Ni>Cr>Cu>Mg>Pb
17. *Spirodella*- Fe>Cu>Mg>Cr>Pb>Ni**(Ind)**
18. *L. gibba* - Fe>Cu>Pb>Cr>Mg>Ni**(Ind)**

4. CONCLUSION

Macrophytes found in Dehradun and Haridwar region of Uttarakhand are of significant importance as some of them are sensitive and others are tolerant to the pollutants present in the aquatic bodies. From the results, it is concluded that among the four selected plant species *Eichhornia crassipes* and *Pistia stratiotes* (root & shoot) could be more suitable species for phytoremediation and moreover, considered as potent biomonitors of pollutants present in the aquatic bodies. Hyperaccumulators are the model plants for phytoremediation as they are tolerant to heavy metals. Selected plants in the present study shown a wide range of tolerance to all the selected metals and therefore could be used for large scale removal of heavy metals from the aquatic bodies and hence remediate pollution in a green and clean way.

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