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Evaluation of the phytoremediation potential of selected hydrophytes grown in wastewater. A case study in Sheikhupura, Pakistan

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ABSTRACT

Water pollution is a major environmental issue and human health risk worldwide. With rapid industrialization and economic growth, water bodies are contaminated at large scale. In the current study the concentration of four heavy metals (HMs) cadmium (Cd), copper (Cu), nickel (Ni), and manganese (Mn) was measured in water, sediment, and hydrophytes (*Eichhornia Crassipes, Pistia stratiotes* and *Lemna minor*) samples collected from wastewater stream in Sheikhupura, Punjab, Pakistan. The results revealed that the concentrations of Cd (0.23 mg L^{-1}), Cu (5.00 mg L^{-1}), Mn (6.00 mg L^{-1}), and Ni (3.00 mg L^{-1}) in water samples were above the permissible limits (APL) set by the World Health Organization (WHO). However, the concentration of Cd, Cu, Mn, and Ni in sediment samples was found to be below the maximum permissible limits (BMPL) set by WHO. The WHO permissible limits for Cd, Cu, Mn, and Ni in water is 0.01, 0.02, 0.20, and 1.40 mg L^{-1} respectively while in sediments is 3.00, 100, 2000, and 50 mg kg^{-1} respectively. In addition, Mn concentration was highest among all the tested hydrophytes, water, and sediment samples. HMs contents were higher in hydrophytes as compared to water and sediment samples. In addition, all three hydrophytes were found to absorb HMs from wastewater with bioaccumulation factor ranging from 2.52 to 216. Current data revealed that *Pistia stratiotes* have greater potential to absorb HMs from water as compared to *Eichhornia Crassipes,* and *Lemna minor*.

Keywords: Wastewater, heavy metals, hydrophytes, phytoremediation.

INTRODUCTION: Modernization and industrial revolution has gradually resulted in an increase of soil, water and air pollution at an alarming rate globally (Wang *et al.,* 2010). Anthropogenic activities, including agricultural chemical application (pesticides etc.), fertilizers applications, industrial operations such as tanning as well as mining, and municipal wastewater treatment plants are playing key roles in the escalation of this menace (Gałuszka *et al.,* 2016). The pollution of natural water bodies has a huge impact on sustainability of our natural resources and ecosystems including the health of living beings (Liyanage and Yamada, 2017). In many places of the world, running freshwater bodies, including rivers, canals and small drains have already been seriously contaminated with hazardous substances HMs (Kumwimba *et al.,* 2020). Accumulated HMs contaminated water bodies are un safe for drinking and washing, and are a continuous source of many diseases (Wang *et al.,* 2008). Such water bodies cost a lot to clean up (Sabry, 2015). Poor water governance nationally and internationally is a major issue in many places, especially in developing countries. Mostly the laws, policies and institutions meant to protect and manage water are not strong enough. Sometimes there is not enough money, or the necessary technology. Like many other developing countries, it is a very common practice in Pakistan, of dumping untreated waste waters of industries and municipal sewage into the running freshwater bodies which in turn are being used for irrigation of agricultural lands without any treatment (Azizullah *et al.,* 2011). In many cities municipal sewage (in combination with industrial waste) is being collected by some collection units and literally being sold out to farmers for irrigation of their fields to produce cereal as well as fodder crops (Qadir *et al.,* 2008). Farmers are happily using these heavily metals contaminated waters for irrigation purposes because of the following apparent advantages. Wastewaters having some beneficial organic and inorganic elements (nutrients) that surrogate fertilizers, and hence reducing the cost of fertilizers usage and increased crop production (Shepherd *et al.,* 2016). Being enriched with nutrients some polluted waters show some apparent positive effects on morphological appearance of plants. But in actual, scientific studies (Levidow *et al.,* 2014; Andresen *et al.,* 2018) have revealed that the use of wastewaters of unknown sources and composition usually have following disadvantages. Most of the waste waters contain HMs and toxic non-biodegradable organic compounds as well (Seleiman *et al.,* 2020). Absorption and accumulation of HMs and other toxic compounds by plants is the critical avowal of food chain contamination (Eid *et al.,* 2020). These contaminants are acting as slow poisons in our society and resulting into a sever health hazards by causing many diseases such as lung cancer, stomach problems, neurological disorders and adding discomforts in life of human beings (Patrick, 2003). HMs once they enter into any part of natural ecosystem they remain persistent for longtime because of their non-biodegradable properties (Habiba *et al.,* 2015). HMs are toxic for living systems, even when present in very trace amounts. So, monitoring of mobility of HMs in different parts of ecosystem is of highest importance. Water, once get polluted are not easy to clean and rehabilitate to their original version. Although, there are many remediation techniques, for decontamination of polluted water at laboratory level not applicable on large scale (Singh *et al.,* 2022). They are too costly and laborious. So it is very important to search for economical and practically feasible techniques to overcome this gradually mounting nuisance of water contamination. Phytoremediation proposes the use of living green plants for *in situ* risk reduction and removal of HMs contaminants from water bodies, and soil (Padmavathiamma and Li, 2007; Ashraf *et al.,* 2019). Phytoremediation is an eco-friendly, energy efficient, aesthetically pleasing method for remedy of low to moderate levels of HMs contamination and it can be used in combinations with other more traditional and scientific remedial methods as a finishing step to the remedial process (Song *et al.,* 2017). One of the main advantages of phytoremediation is that of it is relatively low cost compared to other remedial methods such as excavation. The cost of phytoremediation has been estimated as $25-$100 per ton of soil, and $0.60-$6.00 per 1000 gallons of polluted water (Kumari *et al.,* 2016; Wan *et al.,* 2016) investigated phytoremediation potential of plants growing in polluted areas in India. Results showed that aquatic plant species *Typha latifolia* was efficient metal accumulator of Fe, Cu, Zn, Ni, Al, Cd, and Pb, while *Azolla pinnata* was hyper accumulator to Cr. In terrestrial plant species *Croton bonplandium* showed maximum accumulation of Fe, Zn, Ni, Al, and Si. Similarly, Deepa *et al.* (2015) reported accumulation and distribution of As and Ni in *Polygonum glabrum* and *Lantana sp*. Othman *et al.* (2015) also explored phytoremediation potential of two aquatic plants *Glossostigmae latinoides* and *Hemianthus callitrichoides* for waste water remediation from aqua cultures. Nasser *et al.* (2014) examined the potential of *Helianthus annuus* L. as a phytoremediator for Cd and Pb soil remediation. Pollard *et al.* (2014) reviewed *Noccaea* (*Thlaspi*) *caerulescens* and *Arabidopsis halleri to* hyperaccumulate *zinc and cadmium.* Miguel *et al.* (2013), reported that *Calamagrostis Ligulata*, and *Juncus imbricatus* grown in Andean natural wetlands polluted by acid mine drainage, had shown efficient phytoremediation capabilities of HMs. Fawzy *et al.* (2012) investigated HMs such as Cd, cu, Pb and Zn bio-monitoring and phytoremediation potentials of six different aquatic vascular plant species in River Nile. The HMs accumulation capability of the investigated species was in order of *Ceratophyllum demersum*> *Eichhornia crassipes* > *Myriophyllum spicatum* > *Echinochloa pyramidalis* > Typha domingensis > Phrabmites *australis*. Batch experiments showed that dry plant biomass possess good potential to adsorb heavy metals such as Ni, Co, Cr, Fe, and Cd (Romeh, 2016). Broad leaf plantain plant (*Plantago major* L.) was used in phytoremediation of imidacloprid insecticide in water and effective results were found. Zhang *et al.* (2007) studied phytoremediation of urban wastewater by model wetlands

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with ornamental hydrophytes and found that most of the hydrophytes were fairly efficient in reducing HMs such as Cr, Pb, Cd concentrations in the wastewater.

OBJECTIVES: Keeping in mind the above mentioned facts the present study was designed from January to March, 2020 to probe into local case study in Shekhupora, Pakistan to evaluate a) concentrations of HMs in sediments, water and hydrophytes (*Eichhornia Crassipes, Pistia stratiotes* and *Lemna minor*) samples b) HMs phytoremediation potential of naturally grown hydrophytes.

MATERIAL AND METHODS: Experimental location and sampling: Sheikhupura is an industrial city near Lahore city, Pakistan and its geographical coordinates are 31° 42' 47" North, 73° 58' 42" East (figure 1). Drain Same nullah is located in Shekhupora, Punjab Province, Pakistan.

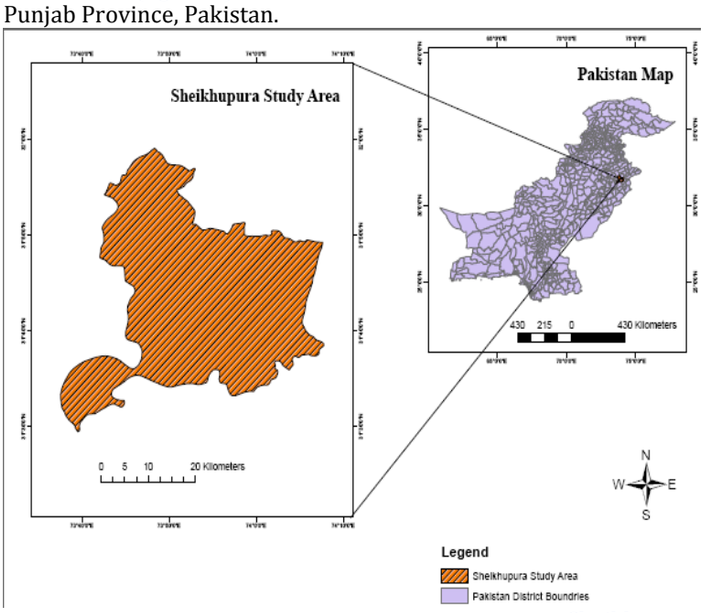

Figure 1: Wastewater sampling site "Same nullah" Sheikhupura, Pakistan.

Specific part was selected in Same nullah drain was selected for water, sediment, and hydrophytes collection. In addition, selected part of the drain was 900 m long with a prominent hydrophytes cover of about 600 m on surface water. Water, sediment and native hydrophytes such as *Eichhornia Crassipes, Pistia stratiotes* and *Lemna minor* were collected from 150 m intervals each. Hydrophytes were collected in polythene bags and transferred to lab. On arriving at lab plants were washed thrice immediately with plenty of tap water and then thoroughly rinsed with distilled water to remove all the debris and adhering soil particles. Extra moisture was removed by putting the washed plants onto blotting papers. In experimental setup C1 represents samples collected in January, C2 samples collected in February, C3 samples collected in March. Water, sediment, and hydrophytes samples were collected from the selected six sites indicated as S0 represents samples collected from upstream edge, S1 samples collected from mainstream at 150 m distance from upstream, S2 samples collected from mainstream at 300 m distance from upstream, S3 samples collected from mainstream at 450 m distance from upstream, S4 showed samples collected from mainstream at 600 m distance from upstream and S5 samples collected from downstream summit. First point was upstream and last point was downstream side. All samples were collected in triplicates. After collection samples were transported to the lab. Sediments samples were air dried in thin layers. Hydrophytes samples were washed thrice with double distilled water to remove any adhering soil. Physiochemical properties such as pH, EC of water and sediments were determined (Rayment and Higginson, 1992).

Chemical analysis: Water analysis: Water samples were collected in cleaned, washed and sterilized, air tight bottles and shifted to laboratory. Basic physiochemical properties such as EC**,** pH and TDS of water samples were measured immediately.

Sediment analysis: Sediment samples were collected in plastic bags and transported to the lab and immediately transferred to the glass containers for air drying. After air drying samples were oven dried. Samples were milled with pestle and mortar to fine powder to get homogeneous mixture. Subsamples (0.2 g) were digested (Ibrahim *et al.*, 2017) with Nitric acid (HNO_3) (GR, Merk, Germany) and Hydrogen peroxide (H_2O_2) (GR, Sinopharm, Shanghai, China) 1/1 *v*/*v*) in a microwave-accelerated reaction system and subjected to Atomic Absorption Spectrophotometer (Model: Perkin Elmer AAS400) for HMs analysis.

Hydrophytes analysis: Hydrophytes such as *Eichhornia Crassipes, Pistia stratiotes* and *Lemna minor* were dried in oven at 75 °C for 72 h. Dried plants samples were crushed with the help of pestle and mortar to a fine powder. From each plant sub sample of 0.2g was taken, digested with strong acids $HNO_3/HClO_4$ (mixture of 1:1 ratio). After digestion samples were filtered (0.2nm) and volumes were made up to 100ml with double distilled water. Filtered samples were subjected with Atomic Absorption Spectrometer (Model: Perkin Elmer AAS400) for Cd, Cu, Ni, and Mn analysis. Bioaccumulation factor (BAF) was calculated with the following formula (Usman *et al.*, 2019).

$$\text{Bioaccumulation factor} = \frac{\text{Metal conc. in medium water}}{\text{Metal conc. in medium water}}$$

Bioaccumulation factor= Metal conc. in medium water

Data analysis: Data was compiled in excel sheet, and was then statistically analyzed using SPSS (SPSS Inc., Chicago, IL, USA). For plotting figures and graphs, the Sigma plot (Systat Software, Inc., San Jose, CA, USA) was used.

RESULTS AND DISCUSSION: The present research was initiated to probe into a local case study, for planning some strategies required for monitoring and handling of food chain contamination (HMs) in industrial area Shekhupora, Punjab, Pakistan. Unplanned dumping of industrial wastewater, containing complex organic matter and different HMs, into freshwater bodies and then irrigation of crop plants is resulting in slow poisoning of whole food chain (Kumar *et al.*, 2019). Aquatic life has primarily been threatened because of rising level of HMs contaminants. Soil and water once get polluted are too much difficult to rehabilitate to their real virgin state. Phytoremediation is the only feasible technology for such intricate cases (Coetzee *et al.*, 2020). Although it is not as simple as it seems. For simplicity and comprehension, data related to the HMs content of sediments, water, and the three selected hydrophytes were shown and compared with each other. The concentration of pH was 6.91 units, EC 537 µs/cm, and TDS was 10 mg/L in water samples. After analysis of all samples, results were compared with maximum allowable limits of HMs in soil and irrigation water as established by the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) Guidelines (table 1).

Metals	Water	WHO limits		Sediment	WHO limits	
Cd	0.016-0.23	0.01	APL	0.117-2.33	3.00	BMPL
Cu	0.107 - 5.00	0.02	APL	23.97-57.69	100.00	BMPL
Mn	1.00-6.00	0.20	APL	207.00-370.00	2000.00	BMPL
Ni	0.103-3.00	1.40	APL	13.94-31.27	50.00	BMPL

Table 1: Comparison of the concentration of HMs (mg/L) found in the waste water, and sediment samples collected from wastewater stream with maximum allowable limits established by WHO (Chiroma *et al.*, 2014). WHO: World Health Organization. APL: Above Permissible Limit BMPL: Below Maximum Permissible Limit.

The allowable limits for Cd, Cu, Mn, and Ni in waster samples are 0.01, 0.02, 0.20, and 1.40 mg L^{-1}. But in wastewater samples concentration of Cd, Cu, Mn, and Ni was 0.23, 5, 6, and 3 mg L^{-1} respectively. It was evident from the results that HMs content in water samples were higher than WHO limits for irrigation of water. HMs were also accumulated in the bottom sediment of the selected drain. In sediment concentration of Cd, Cu, Mn, and Ni was 2.33, 57.69, 370, and 31. 27 mg kg^{-1} respectively. Although concentrations of HMs were high in sediment as compared to the concentrations in surface flowing water when the values were compared with the standard permissible limits (WHO and FAO) (table 1). This increase may be due to downwards movement and finally settle down of HMs at the bottom (sediment). A perusal of the above mentioned table indicates that hydrophytes absorbed HMs from water and accumulate them into their bodies, exhibiting bio magnifications. Bioaccumulation factor (BAF) is essential for feasibility of plant used for phytoremediation purposes. The highest BAF value (7.51) for Cd was shown by *Pistia stratiotes*. For Cu highest value (7.90) was shown by *Eichhornia cressipes.* In-addition Ni and Mn highest values (216.00 and 16.37) were shown by *Lemna minor* and *Eichhornia cressipes* respectively (table 2). It was amazing to find t similar trend for maximum bioaccumulation of different HMs in three collected hydrophytes as Mn>Ni>Cu>Cd. However, the trend of maximum concentration of different HMs in water was Mn>Cu>Ni>Cd.

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Metals	*Pistia sp*	*Lemna sp*	*Eichhornia sp*
Cd	7.51	2.52	6.78
Cu	7.59	4.40	7.90
Mn	215.80	216.00	209.83
Ni	16.34	12.39	16.37

Table 2: Bioaccumulation factor (BAF) of HMs in selected hydrophytes.

Concentration of cadmium (Cd) in water, sediments and hydrophytes: Origin of Cd contamination in environment is from diverse sources including power stations, metal processing e.g., electroplating industries, nickel-cadmium batteries, pigments and urban traffic (Juśkiewicz and Gierszewski, 2022). It is a non-essential element for plants and due to its high toxicity and large solubility in water it is being recognized as a serious contaminant (Ibrahim *et al.*, 2024). Cd concentration in the range of 0.04 to 0.32 mM are being considered non-toxic and between 0.32 to 1 mM are moderately contaminated but higher than this concentration is alarming (Asgharipour *et al.*, 2011). Data related to concentration of Cd are presented in figure 2A.

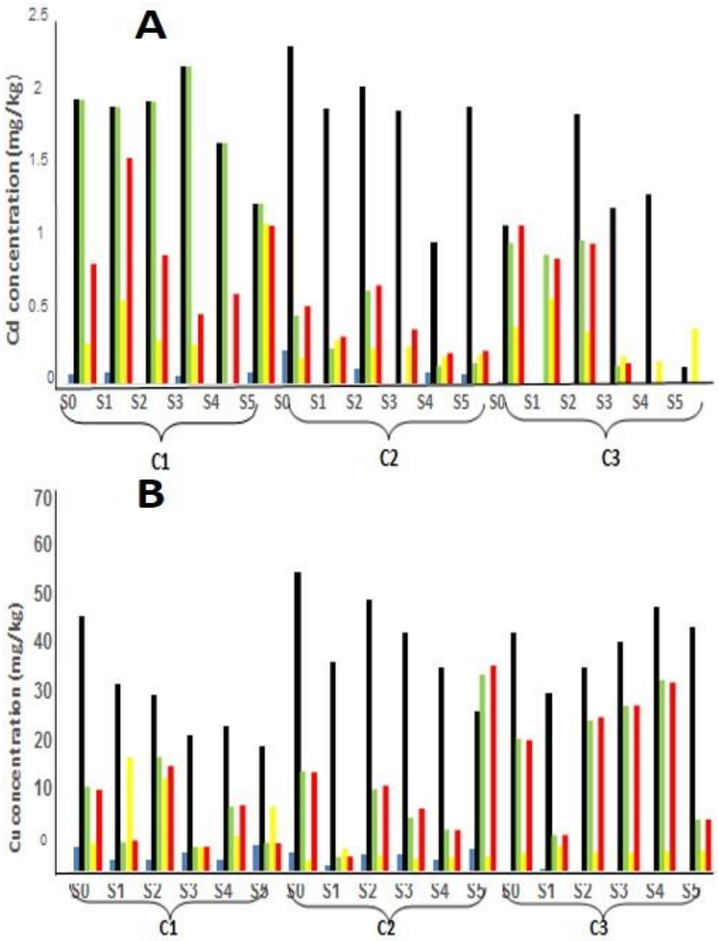

Figure 2: (A) Concentration of Cadmium (Cd), and (B) Cupper (Cu) in water (blue bar), sediment (black bar), Pistia (green bar), Lemna (yellow bar), Eichhornia (red bar) collected from wastewater stream.

S0= Samples collected from upstream edge, C1= Samples collected in January, S1= Samples collected from mainstream at 150m distance from upstream, C2=Samples collected in February, S2= Samples collected from mainstream at 300m distance from upstream, C3=Samples collected in March, S3= Samples collected from mainstream at 450m distance from upstream, S4= Samples collected from mainstream at 600m distance from upstream, S5= Samples collected from downstream summit.

The analysis showed that Cd concentration in many water samples was below detectable limit and where present its range0.016 mg/L-0.23 mg/L. It was observed that at different time intervals there was different Cd concentration in drain water. And in overall view highest Cd level was found after 2nd interval (C2) of sampling. Whereas Cd level in one sediment sample was below detectable range but in rest of the samples it was in range of 0.117 mg/kg-2.330 mg/kg. It was revealed that there was no significant change in Cd concentration at different time intervals (January to march). And in plants *Pistia*, *Lemna* and *Eichhornia* the Cd concentration was in the following ranges 0.123-1.7 mg/kg, 0.15-0.58 mg/kg and 0.14-1.56 mg/kg respectively (including some samples with below detectable Cd content). Results revealed that that *Pistia* can uptake higher concentration Cd ascompared with other two hydrophytes. Sample means in concentration x sample x time (C x S x T) interaction showed that sediment samples had highest Cd content than water and hydrophyte plants (*Pistia*, *Lemna* & *Eichhornia*) and water showed the least values (table 3). Trend of Cd content of analyzed samples can be shown as: Mud> *Eichhornia*>*Pistia*>*Lemna*>Water. Maximum retention of Cd in sediment correlates with its insoluble nature. Water collected from different points in main stream and downstream (S1, S2, S3, S4 and S5) showed comparatively low Cd concentration in water when compared with upstream water (S0). S x C interaction showed that cd content was reduced after passing under vegetation patch of different hydrophytes. But a minor fluctuation/increase in concentration was observed at some sites (S4 or S5) that may be due to the additional waste entering into drain though pipes in between vegetation patch which was under study (table 3).

Concentration of copper (Cu) in water, sediments and hydrophytes: Copper is extremely toxic metal because of its high redox properties. Plant cells need to maintain very low concentration of Cu in their cells. Normal range of Cu required in culture medium is between 0.05 to 0.5 mg/L and average value of Cu content in plant tissues is generally near about 10 mg/kg (Baker and Senft, 1995). Cu contamination of water and soil is continuously being increased due to many anthropogenic activities like mining, smelting, manufacturing, agricultural and waste disposal technologies (Tavker *et al.*, 2021). Data related to cu concentration is presented in Figure 2B. The results obtained after analysis of different samples collected at specified intervals of time from selected sites, showed that Cu contents in water samples was in range of 0.107-5 mg/L while Cu in sediment was in the range of 23.97–57.69 mg/kg while in hydrophytes (*Pistia*, *Lemna* and *Eichhornia*) the Cu concentration showed values with significantly variable ranges as 2.69–37.94 mg/kg in *Pistia*, 2.06–22.0 mg/kg in *Lemna* and 2.81–39.64 mg/kg in *Eichhornia*. All the concentration ranges showed that Cu can be up taken by hydrophytes from water but most of the Cu from water was settled down in bottom sediment. Sample means in C x S x T interaction showed that sediment samples had highest Cu contents then water and hydrophytes (*Pistia*, *Lemna* & *Eichhornia*). Low level of cu in water might be resulted due to hydrophytes Cu uptake (table 4). Trend detected according to results for Cd contents was: Sediment> *Eichhornia*> *Pistia*> *Lemna*> water. All of the samples collected from different sites (S1, S2, S3, S4 and S5) showed low Cu concentrations in water and mud as compared to samples collected from S0. S x C interaction showed that cu content was reduced after passing under vegetation patch of hydrophytes. But a minor increase in concentration was observed at some sites that may be due to the waste extracted from industries and drained though pipes in between vegetation patch which was under study (table 4). Cu uptake observed was more by *Eichhornia* and *Pistia* then *Lemna*.

Concentration of nickel (Ni) in water, sediments and hydrophytes: In the past, Ni was not considered as an essential element for plant growth, but new research has been indicated that it is also required (although in very trace amounts), for normal plant growth (Quartacci *et al.*, 2015). Optimal range for Ni in most of the plant tissues is between 0.05 to 5 mg/kg. Due to its low requirements for plants, it is found in sufficient levels as a contaminant in the soil and water. Although, in woody plants toxicities occur when tissue levels of Ni exceed 80-120 mg/kg. Sensitive plants, such as tomato, could exhibit toxicities even at 10 mg/kg in tissue (Brown *et al.*, 1987). Data related to concentration of Ni is presented in figure 3A. The analysis showed that Ni concentration in water samples was ranged from 0.103–3.00 mg/L whereas in sediment it was ranged from 13.94 to 31.27 mg/kg. While in hydrophytes **t**he concentration of Ni in *Lemna* was significantly different then *Pistia* and *Eichhornia*. Ni contents in *Lemna* were ranged from 3.23 to 37.18 mg/kg. However, in *Pistia* and *Eichhornia* the Ni concentration range was 6.52– 49.02 mg/kg and 6.36–49.13 mg/kg. All the concentration ranges showed that Ni uptake by hydrophytes was higher than its leaching in sediment and in most cases *Lemna* uptake less Ni than *Pistia* and *Eichhornia*. Sample means in C x S x T interaction showed that hydrophtes (*Lemna*, *Pistia* & *Eichhornia*) had accumulated Ni that was up taken from drain`s water polluted by industrial waste (table 5). Some of the Ni was settled down and leached in sediment leaving little amount of Ni in water of drain.

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Collection interval (C)	Sampling site (S)	Sample type (T)					
		Water	Sediment	*Pistia sp.*	*Lemna sp.*	*Ecchornia sp.*	Analysis means
	S0	0.07	1.960	0.9667	0.2827	0.8300	0.8219 bc
	S1	0.077	1.910	1.7867	0.5770	1.5600	1.1812 a
C1	S2	ND	1.947	0.6907	0.3040	0.8900	0.7663 cd
	S3	0.05	2.190	0.3033	0.2707	0.4800	0.6588 df
	S4	ND	1.663	0.5900	ND	0.6200	0.5747 ef
	S5	0.083	1.243	1.1267	1.1087	1.0940	0.9312 b
	S0	0.23	2.330	0.4700	0.1790	0.5367	0.7491 cd
	S1	ND	1.900	0.2433	0.3013	0.3233	0.5536 ef
C2	S2	0.103	2.047	0.6400	0.2447	0.6800	0.7429 cd
	S3	ND	1.883	ND	0.2557	0.3767	0.5031 f
	S4	0.083	0.977	0.1233	0.1880	0.2100	0.3163 g
	S5	0.073	1.907	0.1400	0.2017	0.2267	0.5097 f
	S0	0.016	1.097	0.9700	0.3933	1.0967	0.7146 cd
	S1	ND	ND	0.8900	0.5867	0.8633	0.4680 f
C3	S2	ND	1.867	0.9867	0.3593	0.9700	0.8365 bc
	S3	ND	1.217	0.1233	0.1893	0.1400	0.3339 g
	S4	ND	1.313	ND	0.1553	ND	0.2937 g
	S5	ND	0.117	ND	0.3823	ND	0.0998 h
Sample means		0.043 d	1.5315 a	0.5584 b	0.3322 c	0.6054 b	

Table 3: Cd concentration interaction (C *S* T) in wastewater stream.

S0= Samples collected from upstream edge, C1=Samples collected in January, S1= Samples collected from mainstream at 150m distance from upstream C2=Samples collected in February, S2= Samples collected from mainstream at 300m distance from upstreamC3=Samples collected in March, S3= Samples collected from mainstream at 450m distance from upstream, S4= Samples collected from mainstream at 600m distance from upstream, S5= Samples collected from downstream summit.

Collection (C)	intervalSampling site (S)	Sample type (T)					
		Water	Sediment	*Pistia sp.*	*Lemna sp.*	*Ecchornia sp.*	Analysis means
	S0	4.800	49.077	16.177	5.493	15.653	18.240 g
	S1	2.267	36.087	5.470	22.090	5.893	14.481 i
C1	S2	2.267	33.977	22.070	18.050	20.280	19.329 f
	S3	3.733	26.397	4.670	4.691	4.763	8.851 o
	S4	2.067	28.150	12.450	6.847	12.630	12.429 k
	S5	5.000	23.923	5.420	12.490	5.297	10.426 n
	S0	3.583	57.690	19.300	2.069	19.100	20.349 d
	S1	1.043	40.317	2.690	4.398	2.807	10.251 n
C2	S2	3.107	52.347	15.800	2.980	16.500	18.147 g
	S3	3.017	46.070	10.323	2.441	12.027	14.776 h
	S4	1.987	39.290	8.020	2.650	7.890	11.967 l
	S5	4.127	30.923	37.947	2.780	39.640	23.083 b
	S0	0.210	45.870	25.530	3.527	25.263	20.080 e
	S1	0.263	34.377	6.957	4.795	6.937	10.666 m
C3	S2	0.187	39.213	29.083	3.692	29.700	20.375 d
	S3	0.107	44.117	31.913	3.484	31.987	22.322 c
	S4	0.110	51.007	36.947	3.750	36.390	25.641 a
	S5	0.177	47.067	10.007	3.960	9.940	14.230 j
Sample means		2.147 d	40.328 a	16.710 b	6.122 c	16.816 b	

Table 4: Cu concentration interaction (C *S* T) in wastewater stream.

Collection interval (C)	Sampling site (S)	Sample type (T)					
		Water	Sediment	*Pistia sp.*	*Lemna sp.*	*Ecchornia sp.*	Analysis means
	S0	1.300	31.057	35.267	13.322	34.817	23.152 c
	S1	2.033	30.177	13.310	20.681	18.323	16.893 g
C1	S2	1.233	23.277	20.650	14.085	20.573	15.964 h
	S3	2.100	25.497	14.070	12.854	16.600	14.224 i
	S4	3.000	27.037	37.260	22.594	37.623	25.503 a
	S5	2.000	13.940	15.227	37.280	15.250	16.739 g
	S0	2.073	25.347	31.610	3.672	31.177	18.776 e
	S1	2.933	25.110	9.400	4.090	9.063	10.119 n
C2	S2	1.467	30.677	27.887	3.680	27.473	18.237 f
	S3	2.000	29.850	15.877	3.233	17.760	13.744 j
	S4	1.990	18.220	49.020	4.777	49.133	24.628 b
	S5	2.097	21.967	33.517	6.689	34.593	23.152 c
	S0	1.103	31.277	7.100	9.039	7.800	11.264 k
	S1	1.073	24.000	29.587	10.391	29.733	18.957 e
C3	S2	0.990	29.257	6.527	9.361	6.360	10.499 m
	S3	0.923	25.920	10.823	8.270	10.563	11.300 k
	S4	1.000	21.653	8.787	8.799	8.670	9.782 o
	S5	0.103	24.867	9.757	9.400	9.873	10.800 l
Sample means		1.634 e	25.504 a	20.871 c	11.234 d	21.504 b	

Table 5: Ni concentration interaction (C *S* T) in wastewater stream

At some points hydrophytes uptake for Ni was more then it leached in sediment but at most sampling sites Ni was detected high in sediment than any hydrophyte. Trend detected after overall calculations according to results for Ni contents was Sediment>*Eichhornia*>*Pistia*>*Lemna*>Water. All of the Sites (S0, S1, S2, S3, S4 and S5) showed no gradual trend of decrease or increase in Ni concentrations whereas high Ni contents was detected at S0, S4 and S5 in *Eichhornia* and *Pistia* as compared to Ni concentration found in mud and water at those sites (table 5). It was revealed that *Lemna* uptake more Ni at S5 and S1 than any other site.

Concentration of manganese (Mn) in water, sediments and hydrophytes: Manganese is an essential micronutrient for plants. So, plants necessarily need it, although in trace amounts, for their normal nutrition and development (Noctor *et al.*, 2007). Mn content in plant tissues differ greatly between species (30-500 mg kg^{-1} dry mass (Millaleo *et al.*, 2010). When present in excessive amounts, Mn is very toxic for most of the plants growth. Toxic level of Mn can cause metabolic alterations, macromolecular damage, disturbed homeostasis and oxidative stress in plants (Polle, 2001; Kim *et al.*, 2015). However, there is a considerable inter- and intra-specific variation for Mn levels and its induced toxicity (Foy, 1988). Data related to concentration of Mn is presented in figure 3B. The result was obtained after analysis of different samples collected at specified time intervals from selected sites. The analysis showed that contents of Mn in water samples was significantly lower than sediment, *Pistia*, *Lemna* and *Eichhornia.* Whereas the concentration of Mn in plants was more than in sediment except in *Lemna* samples collected during C2 and C3 (samples collected in 2nd and 3rd month). In water samples Mn concentration was 0.9 to 6 mg/L, where as in sediment samples it ranged from 207 to 370 mg/kg and in hydrophytes plants *Pistia* and *Eichhornia* and *Lemna* Mn concentration was ranged from 54 to1295 mg/kg, 54 to 1259 mg/kg and 0.8-1296 mg/kg respectively. High Mn concentration was found in first month (C1) and was gradually decreased in hydrophytes. Sample means in C x S x T interaction showed that Plants (*Lemna*, *Pistia* & *Eichhornia*) has accumulated considerable amount of Mn leaving less in water and some was settled down and accumulated in sediment of drain (table 6).

Collection interval (C)	Sampling site (S)	Sample type (T)					
		Water	Sediment	*Pistia sp.*	*Lemna sp.*	*Ecchornia sp.*	Analysis means
	S0	2.733	255.82	834.1	834.1	935.7	572.49 d
	S1	6.000	258.07	798.3	1296.2	789.4	629.58 c
	S2	4.200	207.17	1295.2	1259.2	1259.8	805.10 a
C1	S3	3.033	226.09	1265.2	846.9	1245.5	717.49 b
	S4	4.933	274.93	973.5	837.0	967.3	611.53 c
	S5	3.933	356.94	743.8	973.7	767.3	569.14 d
	S0	2.567	284.19	913.5	1.0	914.6	423.27 g
	S1	2.027	370.32	393.3	16.7	392.7	235.02 i
	S2	1.143	290.13	91.4	12.8	93.2	97.72 k
C2	S3	3.033	250.35	1089.4	0.8	1084.8	485.69 e
	S4	1.940	289.19	971.3	2.8	972.1	447.49 f
	S5	1.960	301.65	389.5	3.0	398.a2	218.86 i
	S0	1.283	350.72	617.4	5.4	466.8	288.32 h
	S1	1.247	290.31	203.3	7.4	202.6	140.97 j
	S2	1.907	310.91	95.0	4.3	94.9	101.39 k
C3	S3	1.120	325.12	111.1	1.4	111.0	109.93 k
	S4	0.950	317.97	109.0	2.7	108.0	107.73 k
	S5	1.020	340.84	54.8	4.9	54.7	91.25 k
Sample means		2.50 d	294.55 c	608.28 a	339.46 b	603.25 a	

Table 6: Mn concentration interaction (C *S* T) in wastewater stream.

It was observed by the analysis that during 1st and 2nd months of sampling (C1 and C2) plant showed more concentration of Mn at all the points than in sediment and water (table 6). Whereas after 3rd month of sampling (C3) low Mn concentration in hydrophytes was noted than sediment. According to overall means trend detected for Mn contents was as *Pistia>Eichhornia>Lemna*>sediment>water. All of the sites (S0, S1, S2, S3, S4 and S5) showed no gradual trend of decrease or increase in Mn concentration whereas there was an increase in Mn content detected after S1 in hydrophyte samples.

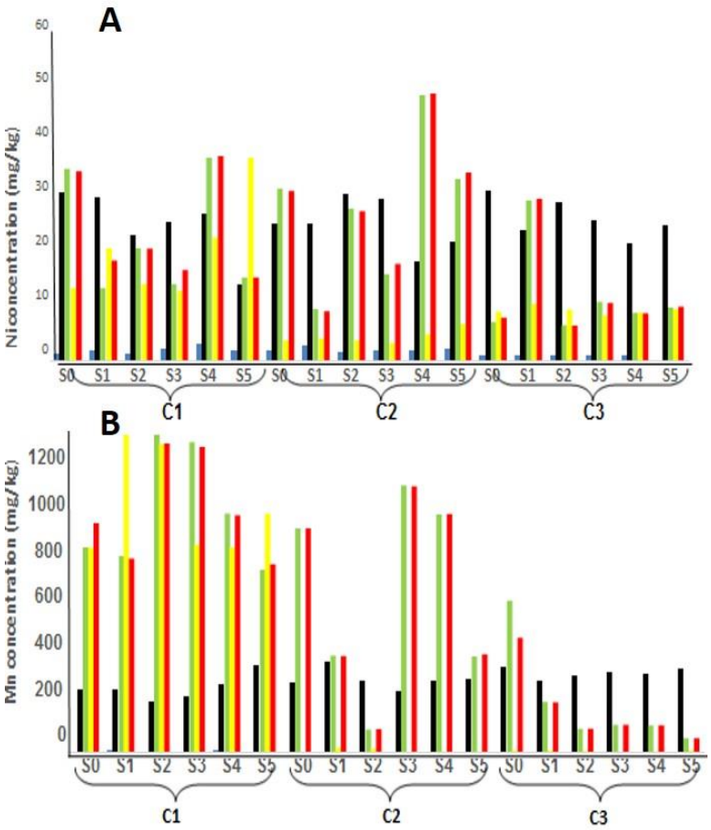

Figure 3: (A) Concentration of Nickel (Ni), and (B) Manganese (Mn) in water (blue bar), sediment (black bar), *Pistia* (green bar), *Lemna* (yellow bar), *Eichhornia* (red bar) collected from wastewater stream.

A decrease in Mn concentration in hydrophytes was also noted at S4 and S5 which might be because of disturbance in health of plants or any unfavorable conditions.

CONCLUSION: In conclusion sediment samples showed highest Cd, Cu concentration compared to water and hydrophytes. The order of HMs concentration in hydrophytes was Mn>Ni>Cu>Cd. In addition Ni concentration was highest in sediment samples. Mn concentration was detected highest in hydrophytes compared to water and sediment samples. The presence of HMs in sediment was of critical concern for those people who pleasingly use sediment as an additive to improve their soil fertility. Current results showed that drain Same nullah water contained HMs and therefore unfit for irrigation purpose. In addition results confirmed that hydrophytes had dynamic phytoremediation potential for above mentioned HMs

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