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Phytoremedial Potential of Aquatic Plants for Heavy Metals Contaminated Industrial Effluent

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Abstract:

Research was conducted to assess the potential of 3 native aquatic macrophytes/plant species Water Hyacinth (Eichhorniacrassipes), Water Lettuce (Pistiastratiotes) and Coontail (Ceratophyllumdemersum) by growing on heavy metal contaminated industrial effluent collected from the Fuse shop of Pakistan Ordinance Factory, WahCantt, Rawalpindi. After initiation of the experiment, the plant shoots and associated wastewater samples were collected periodically, after every 6 days interval the experiment was terminated on 24th day. The collected shoots and effluent samples were analyzed for total copper (Cu), chromium (Cr), cadmium (Cd), zinc (Zn), nickel (Ni) and lead (Pb) concentrations. Metal concentration of the effluent was found to be 3.35, 3.81, 3.42 mg, 3.73, 2.66 and 3.33 mg L^{-1} for Cu, Ni, Pb, Cr, Zn and Cd respectively. The concentration of Cu, Ni, Pb, Cr, Zn and Cd observed in Water Hyacinth treated wastewater was reduced to 0.00, 0.20, 0.002, 0.2, 0.02 and 0.01 mg L^{-1} respectively. Water Lettuce reduced the concentration of Cu, Ni, Pb, Cr, Zn and Cd in the wastewater as 0.03, 0.00, 0.002, 0.1, 0.02 and 0.001 mg L^{-1} respectively. Coontail reduced the heavy metal concentration of effluent to 0.05, 0.00, 0.00, 0.13, 0.1, 0.00 mg L^{-1} for Cu, Ni, Pb, Cr, Zn and Cd

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respectively. The total metal concentration observed in the phytoremediation plants was different for different metals. The maximum metal concentration removed by Water Hyacinth was 68.4 g $^{-20L}$, *64.7 g* $^{-20L}$ *for Cr and Cu respectively. The total metal concentration removal by Water Lettuce for Cu was relatively more than Water Hyacinth i.e. 65.1 g* $^{-20L}$. *Coontail efficiently removed highest metal concentration of Cu, Ni, Cr and Cd than both the other aquatic plants which was observed as 73.7 g* $^{-20L}$, *78.1 g* $^{-20L}$, *76.1 g* $^{-20L}$ *and 70.5 g* $^{-20L}$ *respectively. Coontail was found efficient for the removal of heavy metals from the wastewater as compared Water Hyacinth and Water Lettuce.*

Key words: wastewater, macrophytes, industrial effluent, phytoremediation

Novelty Statement:

The study was conducted to analyze the potential of aquatic macrophytes Water Hyacinth (*Eichhorniacrassipes*), Water Lettuce (*Pistiastratiotes*) and Coontail (*Ceratophyllumdemersum*) by growing on heavy metal contaminated industrial effluent collected from the Fuse shop of Pakistan Ordinance Factory, Wah Cantt, Rawalpindi. Results showed that these plant species could be used for managing and decontamination of wastewater with heavy metals.

I- Introduction

There is an increasing concern, for couple of decades, about the rising environmental pollution and its irreparable drastic effects on community health. The polluted water used for growing crops pollutes arable land and also enters into the food chain causing severe health hazards to humans (Aslam, 1998). Effluents contain high amount of heavy metals such as Fe, Cu, Zn, Pb, Cd, Cr and Ni. Industrial and municipal sewage water is considered as a cheap source of irrigation water as it contains some nutritional value and organic matter contents. Continuous uses of such effluents result in heavy metals accumulation in soil and food chain (Malla et al., 2007). Heavy metal ions are either toxic to organisms themselves or induced efficiency in others. Some metals such as Fe, Mg, Co, Cu and Ni are essential nutrients in their low permissible limits. However, if present above the permissible limit these are toxic to biological system (Qadir et al., 1999). The most hazardous metals in the contaminated waste water are Cd, Cr, Cu, Ni, As, Pb and Zn, and are discharged from the chemical intensive industries involved in metal processing. Living organisms absorb heavy metals due to their high solubility in aquatic environment. Toxic substances are released in the environment and also add lethal effects on living organisms in food chain (Dembitsky, 2003) and can cause severe health disorders (Babel and Kurniawan, 2003).

Some heavy metals cause diseases like skin manifestations, intuitive cancers and diseases related to vessels and arteries. Cd can cause injury to kidney, renal disarray and mutations in human cells. Similarly, Cr may cause pain, sickness, diarrhea and queasiness; Cu damages liver causes Wilson disease and insomnia. Further Ni causes dermatitis, nausea, chronic asthma, cough and cancer. Zn leads to depression, lethargy, neurological signs and increased thirst and Pb is a cause of damaging fetal, brain, causes disease of kidneys, circulatory and nervous system (Babel and Kurniawan, 2003). On ranking, Pakistan stands as the 41st in the world in industrial production (IMF WEOD, 2012). The industries comprise of fertilizer, cement, cooking oil, sugar, tobacco, steel, chemicals, machinery and foodstuff processing are the chief cause of contaminating water bodies and urban areas.

Pakistan Ordinance Factory (POF), a largest defense industrial complex under the Ministry of Defense Production located in Wah Cantt, is producing conventional arms and ammunition to international standards and currently POF comprises of fourteen ordinance factories and three commercial subsidiaries. Pakistan Ordinance Factories manufacture commercial explosive, ammunition, hunting and posses' wide-ranging facilities for the manufacturing of brass, aluminum, copper, ingots, extrusion and sections for non-military applications (POF, 2011).

A range of physical, chemical and biological techniques are available to remediate the industrial effluent. Sedimentation, screening, ventilation, filtration, flotation and degasification techniques are the physical methods used to remediate the industrial effluent (Peters et al., 1986). Chemical methods are chlorination, ozonation, neutralization, adsorption and ion exchange (Peters et al., 1986), and biological techniques are activated sludge treatment, trickling filtration, oxidation ponds, anaerobic digestion, septic tanks and lagoons (Foot et al., 1993). Biological treatment techniques include bioremediation, which uses the microbes or plants for the degradation and transformation of pollutants to harmless state within the maximum permissible limits (Muller et al., 1996). Certain species of higher plants have the ability to amass exceptionally high concentrations of metals in their tissues without showing the toxic effect on the plant itself (Klassen et al., 2000; Bennett et al., 2003).

Aquatic species that are explored and identified from the polluted water and also tested for the phytoremediation of heavy metals (Pb, Cu, Zn, Cr, Cd, Co, Fe, Hg, As etc) include, *Lemna minor* (Duck Weed), *Eichhornia crassipes* (Water Hyacinth) (Reddy and DeBusk, 1987; So et al., 2003), *Pistia stratiotes* (Water Lettuce) (Sridhar, 2006), *Ceratophyllum demersum* (Coontail) (Rai et al., 1995; Tripathi et al., 1995; Devi and Prasad, 1998; Arvind and Prasad, 2005), *Oenathe javanica* (Water Dropwort), *Polygonum amphibian* (Sharp Dock), *Lepironia articulate* (Calamus) *Hydrocotyle umbellate* (Pennywort) (Prasad and Freitas, 2003), *Elodea canadensis*

(American elodea, Canadian pond-weed) (Pamela et al., 1997), *Phragmites australis* (Reed) (Aslam et al., 2007; Bragato et al., 2006), *Scirpus tabernaemontani* (Zebra rush) (Skinner et al., 2007) and *Ipomoea aquatica* (Water Spinach), *Azolla caroliniana* (Water Ferns) (Rahman and Hasegawa, 2011).
The objectives of the present study were to quantify the metal concentrations in the effluent of the Fuse Shop C-12, Pakistan Ordinance Factory Wah Cantt and to explore the phytoextraction potential of native aquatic plant species for the remediation of effluent.

II- Materials and Methods

i) Industrial Effluent Phytoremediation

This study was conducted to quantify the metal contents in the industrial effluent and to explore the phytoremedial potential of aquatic plants. The experiment was conducted at Bioremediation Site I, at National Institute of Bioremediation, PARC Institute of Advanced Studies in Agriculture, National Agricultural Research Centre (NARC), Islamabad. Industrial effluent was collected from the Fuse Shop C-12, of the Pakistan Ordinance factory (POF), Wah Cantt, Rawalpindi. In the Fuse Shop different ammunition parts are processed for Anodizing (growth of oxide film on certain metals) (Robert, 2002), Passivation (preserving and strengthening the appearance of metallic's) (Brown et al., 2005) and electroplating (depositing a layer of material to give a desired property e.g., corrosion, lubricity etc) (Stelter and Bombach, 2004). The water used in the processes of anodizing, passivation and electroplating of different arms and ammunition was collected separately in three cemented tanks constructed outside the Fuse Shop.

Two composite untreated mixed effluent samples, one for initial characterization and the second sample for phytoremediation experiments, were collected from the outlet of Fuse Shop C-12 in two sequentially washed (tap water, acid and deionized distilled water washing) polypropylene containers. The effluent sample collected for initial characterization was treated with distilled nitric acid to prevent the microbial activities and stored at 4^0C. The effluent samples were then transported to National Agricultural Research Centre, Islamabad.

The collected effluent sample was filtered through 0.42 μm cellulose nitrate filter using vacuum filtration assembly and analyzed for the pH, Hardness, Nitrates, Alkalinity, Electrical Conductivity (EC) and Cations (Na, Ca, K & Mg) using following standard methods. The pH of the effluent was measured by using the pH Micrometer (Tandon, 2005). Total alkalinity of the heavy metal contaminated industrial effluent was measured using titration method using methyl orange indicator as described by APHA (1981). Total Hardness of the effluent was measured by buffer method (APHA, 1981). The Electrical Conductivity of the effluent samples was measured by the potable EC meter (cyber scan series 600) (Tandon, 2005). The determination of Nitrate-Nitrogen of each sample was measured using Hydrazine Reduction Method (Kamphake et al., 1967). The effluent samples were filtered through 0.42 μm pore size cellulose nitrate filter. The filtrate was then analyzed for metals contents Cd, Cr, Pb, Cu, Ni and Zn using Flame Atomic Absorption Spectrometer (PerkinElmer AAnalyst-800).

Three plant species, i.e. Water Hyacinth (*Eichhornia crassipes*), Water Lettuce (*Pistia stratiotes*) and Coontail (*Ceratophyllum demersum*), locally available, were collected from the Bioremediation Plant Nursery of National Institute of Bioremediation, National Agricultural Research Centre, (NARC) Islamabad, Pakistan. The experiment was conducted in the plastic tubs (top diameter was 35" inches and bottom diameter was 25" inches). Twenty liters effluent from bulk was poured into each of the acid washed plastic tubs and the aquatic plants were transferred to the plastic tubs in six replicates, grown for 24 days, and sampled after every 6 days for five times. The samples were withdrawn in 100 mL double distilled washed polyethylene bottles and labeled. Similarly, plant samples were collected after every six days, for five times, and thoroughly washed with running tap water followed by distilled water to avoid any surface contamination. The plant material was cleaned with blotting paper for removing surface moisture and dried at 60 ^{o}C till constant weight. Three aquatic plant species with six replicates were arranged in Randomized Complete Block Design (RCBD). The data was statistically analyzed using two factor (Plant species and time) factorial experiments under RCBD. Analysis of Variance (ANOVA) of the measured parameters was performed using Statistix 8.1 and variable means were compared using Least Significant Difference (LSD).

ii) Analytical Procedure

The collected effluent sample was filtered through 0.42 μm cellulose nitrate filter using vacuum filtration assembly and analyzed for the pH, Hardness, Nitrates, Alkalinity, Electrical Conductivity (EC) and Cations (Na, Ca, K & Mg) using following standard methods. The pH of the effluent was measured by using the pH Micrometer (Tandon, 2005). Total alkalinity of the heavy metal contaminated industrial effluent was measured using titration method using methyl orange indicator as described by APHA (1981). Total Hardness of the effluent was measured by buffer method (APHA, 1981). The Electrical Conductivity of the effluent samples was measured by the potable EC meter (cyber scan series 600) (Tandon, 2005). The determination of Nitrate-Nitrogen of each sample was measured using Hydrazine Reduction Method (Kamphake et al., 1967). The effluent samples were filtered through 0.42 μm pore size cellulose nitrate filter. The filtrate was then analyzed for metals contents Cd, Cr, Pb, Cu, Ni and Zn using Flame Atomic Absorption Spectrometer (PerkinElmer AAnalyst-800).

III- Results

i) Heavy metal concentration of effluent after phytoremediation

The Ordinance Factory Effluent was initially analyzed for different physical and chemical parameters (Tables 1 & 2). The effluent was alkaline in reaction having pH of 7.35 that lies in safe limits of effluent disposal standards (pH 6-10). The Electrical Conductivity was 0.53 dS m^{-1}, Carbonates and Bicarbonates were 0.28 and 1.13 mg L^{-1}, respectively, Calcium + Magnesium (Ca + Mg) was 1.68 mg L^{-1} whereas Sodium (Na) and Potassium (K) were 3.45 and 0.78mg L^{-1}, respectively (Table 1). The effluent of the Fuse Shop C-12, Pakistan Ordinance Factory was also initially analyzed for Zn, Cd, Cr, Pb, Cu and Ni concentration (Table. 2). The Cu concentration of the effluent was 3.35 mg L^{-1}, Ni concentration was 3.81 mg L^{-1}, Pb concentration was 3.42 mg L^{-1}, concentration of Cr was 3.73 mg L^{-1}, concentration of Zn was 2.66 mg L^{-1} and that of Cd was 3.33 mg L^{-1}. The observed concentrations of the metals were found higher than their permissible limits for wastewater disposal (Table 2).

An exponential reduction in effluent metals concentrations were observed between six and twelve days after initiation of experiment. Afterwards, the reduction in effluent metal concentration was either very slow or not occurred (Figs. 1-6). The initial Cu concentration of the effluent was 3.35 mg L^{-1} and was significantly ($P < 0.05$) reduced to 0.05 mg L^{-1} on twenty fourth day by Coontail and 0.00 mg L^{-1} by Water Hyacinth and Water Lettuce, Ni concentration was 3.81 mg L^{-1} and reduced to 0.00 mg L^{-1} on twelfth day of the experiment by Water Hyacinth, Water Lettuce and Coontail, Pb concentration was 3.42 mg L^{-1} and reduced to 0.00 mg L^{-1} on eighteenth day by Water Hyacinth and Coontail and 0.00 mg L^{-1} on twenty fourth day by Water Lettuce, concentration of Cr was 3.73 mg L^{-1} decreased to 0.00 mg L^{-1} on eighteenth day by Water

Hyacinth and Coontail and 0.00 mg L^{-1} on twenty fourth day by Water Lettuce, concentration of Zn was 2.66 mg L^{-1}which was reduced to 0.00 mg L^{-1} by Coontail on sixth day and by Water Hyacinth and Water Lettuce on eighteenth day, while the concentration of Cd was 3.33 mg L^{-1} and efficiently reduced to 0.00 mg L^{-1} on eighteenth day by Water Lettuce and Coontail and gradually by Water Hyacinth on twenty fourth day of the experimental period.

A rapid reduction in Cu concentration was observed in effluent samples collected after six days (Fig. 1). Relatively more reduction in metal concentration was observed in effluent treated with Coontail than that of other two aquatic plants and was from 3.5 mg L^{-1} to 2.5 mg L^{-1} during first six days. It was further reduced to 1.68 mg L^{-1} after 12 days, rapid removal of Cu concentration in first six days and then gradual decrease was observed until the concentration reduced to 0.05 mg L^{-1} that was within the safer limits (1.0 mg L^{-1}) on twenty forth day.

Similar metal removal trend was observed for Ni where initially it was removed exponentially by low concentration in effluent sample collected after six days. Relative large reduction in metal concentration was observed in effluent treated with Water Lettuce. While, Water Hyacinth and Coontail aquatic plants decreased the Ni concentration from 3.7 mg L^{-1} to 2.4 mg L^{-1} and 2.06 mgL^{-1} during first six days, respectively (Fig. 2) that was in the safer limits of wastewater disposal (1.0 mg L^{-1}) and was significantly ($P <0.05$) further reduced to 0.01 mg L^{-1} when the sample was collected on twelfth day. It was observed to be 0.00 mg L^{-1} on 24th day.

Linear reduction of Pb concentration in effluent was observed over the experimental period (Fig. 3). Relative faster removal was observed in the effluent treated with Coontail and Water Lettuce than that of Water Hyacinth. After 12 day, Water Lettuce and Coontail decreased the Pb concentration from 3.31 to 1.5 mg L^{-1} and 0.85 mg L^{-1}, respectively. The metal was reduced to safer limits (0.5 mg L^{-1}) of wastewater disposal before the eighteenth day and was further reduced to below detectable limits. Water Hyacinth and Coontail showed almost similar metal removal trend over the experimental period. However, Water Hyacinth metal removal rate was slower and took 24 days to bring effluent Pb concentration below the permissible limits (Fig. 3).

All the three aquatic plants tests showed different Cr removal trend from Cu, Ni, and Pb. Initially, a rapid decrease was observed in Cr concentration of effluent sample collected after six and twelve days. Rather, more rapid reduction in Cr concentration was observed in effluent where Coontail aquatic plants were grown and the concentration was significantly ($P < 0.05$) reduced from 3.81 to 1.12 mg L^{-1} only after six days. Almost all the Cr was removed from effluents by the aquatic plants on eighteenth day of the experiment below detectable limits. After 12 days, a gradual decrease in Cr concentration of effluent was observed. Water Hyacinth and Water Lettuce progressively reduced metal concentration from the effluent from 3.8 mg L^{-1} and 3.7 mg L^{-1} to 2.02 mg L^{-1} and 2.16 mg L^{-1} after six days respectively and reduced the metal concentration to standard level after six day to 0.21 mg L^{-1} and 0.13 mg L^{-1} respectively (Fig. 5).

Zinc extraction by Water Hyacinth was faster than extraction by Water Lettuce and Coontail and the Zn concentration was dropped to <0.03 mg L^{-1} after 6 days before the permissible limits of the wastewater disposal (2.0 mg L^{-1}) (Fig. 6). Further, Zn removal by Water Lettuce dropped from 2.5 to <0.1 mg L^{-1} only after 6 days. After 12 days, a gradual decrease in metal concentration was observed between 6 to 12 days. After 12 days no further reduction in Zn effluent concentrations was observed in all treatments. Cadmium removal behavior by Water Hyacinth, Water Lettuce and Coontail was different than the rest of the metals. However, there was a linear reduction in Cd concentration in the effluent sample till 12 days in all treatments (Fig. 7). Maximum metal reduction was observed in the effluent treated with Coontail and concentration was reduced from 3.33 to 1.71 mg L^{-1} after 6 day. It was further reduced to 0.39 mg L^{-1} after 12 days. Similarly, Water Hyacinth and Water Lettuce reduced metal concentrations from 3.26 to 2.69 mg L^{-1} and from 3.27 to 2.76 mg L^{-1}, respectively. The Cd concentration of effluent was reduced to the safer limits (0.1 mg L^{-1}) by Coontail after 12 days and by Water Hyacinth and Water Lettuce after 18 days, respectively.

ii) Heavy Metal Concentration in Phytoremedial Plants

The metal concentration in Water Hyacinth, grown on the metal contaminated industrial effluent increased exponentially at earlier stages of the experiment. This was obvious from the chemical analysis of Water Hyacinth shoot samples collected after 6 and 12 days after initiation of experiment. Maximum Cu concentration was observed in Water Hyacinth plant samples collected on 24 day (8.44 mg kg^{-1}), and on 6 day the Cu concentration was 5.95 mg kg^{-1}, on twelve day it was 4.12 mg kg^{-1} and on eighteenth day it was 7.23 mg kg^{-1} (Fig. 8a).

Copper accumulation by Water Lettuce was very slow at initial stages of experiment. The Cu concentration was increased to 9.77 and to 23.97 mg kg^{-1} on 6 and 12 day of the sample, but highest concentration was observed in Water Lettuce samples collected on 24th day, i.e. 57.84 mg kg^{-1} (Fig. 8b). Coontail aquatic plants grown on contaminated effluent showed incremental Cu accumulation till 18 day, i.e. 2239 mg kg^{-1}, 2626 mg kg^{-1}, and 2785 mg kg^{-1} on 6, 12, and 18 days after transplanting, respectively. However, slight reduction was observed on 24th day (Fig. 8c).

Significantly higher amount of Ni was observed in Water Hyacinth shoot samples collected on 6 day (32.34 mg kg^{-1}) and 12 day (27.33 mg kg^{-1}) of the experiment than that of rest of the sampling (Fig. 9a). Afterwards, reduction in Ni concentration of Water Hyacinth was observed, 13.31 mg kg^{-1} and 5.13 mg kg^{-1} after 18 and 24 days of transplanting, respectively.

The Ni concentration in Water Lettuce shoot samples collected on 6th day was significantly higher than those of the shoot samples collected on 12th, 18th and 24th days. Maximum Ni concentration (81.52 mg kg^{-1}) was observed in Water Lettuce shoot samples collected after six day. Then reduction in Ni concentration was observed in shoot samples collected on twelfth (29.68 mg kg^{-1}), eighteenth (4.43 mg kg^{-1}) and twenty forth day (29.63 mg kg^{-1}) (Fig. 9b).

Initially an increase in Coontail Ni concentration was observed as 304.63 mg kg^{-1} after 6 day and 523.92 mg kg^{-1}. While maximum concentration (523.92 mg kg^{-1}) was observed on 12th day sampling (Fig. 9c). However, significantly lower Ni concentration was observed on 18 day (166.41 mg kg^{-1}) and 24 day (115.66 mg kg^{-1}) than the 6 day and 12 day. Similar trend in the accumulation of Pb by both Water Hyacinth and Water Lettuce was observed (Fig. 10a &10b). The concentration of Pb was observed on the 6th day (37.03 mg kg^{-1}) by the Water Hyacinth plant was significantly higher than those plant samples collected on 12th day (14.94 mg kg^{-1}), 18th day (20.95 mg kg^{-1}) and 24th day (16.79 mg kg^{-1}) (Fig. 10a).

Maximum amount of Pb accumulated (64.31 mg kg^{-1}) by Water Lettuce was observed on the sixth day. Then there was significant reduction in Pb accumulation in following samplings, i.e. 44.32, 22.34, 40.49 mg kg^{-1} on twelve, eighteen and twenty fourth day, respectively (Fig. 10b). Significantly ($P<0.05$) higher concentration of Pb was observed in Coontail samples collected on sixth 399.18 (400 mg kg^{-1}) and twelfth day (400 mg kg^{-1}) than those of collected on eighteenth day (222.24 mg kg^{-1}) and twenty fourth day (174.89 mg kg^{-1}) (Fig. 10c).

Significant ($P<0.05$) increase in the accumulation of Cr was observed in Water Hyacinth shoot samples as compared to other two aquatic plants used in this experiment. The concentration of Cr observed in shoot samples collected after sixth day was 12.68 mg kg^{-1} and on twenty fourth day was 17.89 mg kg^{-1}. Water Hyacinth showed maximum Cr concentration on twenty fourth day with comparison to other aquatic plants during the experimental period (Fig. 11a). Similarly, concentration of Cr in Water Lettuce observed after sixth day was 39.33 mg kg^{-1}, followed by 21.06, 39.01, and 82.11 mg kg^{-1} on twelve, eighteenth, and twenty fourth days, respectively (Fig. 11b). However, highest Cr concentration was observed in Coontail shoot samples collected after six days (586.37 mg kg^{-1}) and a significant increase was seen in the metal concentration as the days passed. The highest concentration was observed on the twenty fourth day i.e. 1931.65 mg kg^{-1} followed by 929.11 mg kg^{-1} and 1454.43 mg kg^{-1} on twelve and eighteenth day of the experiment, respectively (Fig. 11c).

Similar Zn accumulation by Water Hyacinth was observed after 6, 12, 18 and 24 days during the experiment. Concentration of Zn was 34.2 mg kg^{-1} on twelfth day, 34.5 mg kg^{-1} on eighteenth day and 33.4 mg kg^{-1} on twenty forth day (Fig. 12a). Relative higher (but non -significantly ($P<0.05$)) Zn concentration was observed on sixth day in plants samples (40.3 mg kg^{-1}).

Concentration of Zn observed in Water Lettuce on sixth, twelfth and eighteenth day of the experiment was 60.54, 61.08 and 64.45 mg kg^{-1}, respectively. Maximum concentration of the metal was observed on twenty fourth day in the plants samples (Fig. 12b). It was observed that Zn accumulation was statistically similar in Coontail plant samples collected on 6^{th}, 12^{th} and 18^{th} day. While statistically lower Zn accumulation was observed in plant samples collected on 24^{th} day than 6^{th} and 12^{th} day. The Coontail shoot Zn concentration was 750.3 mg kg^{-1} on six day and was 766.93 mg kg^{-1} on twelfth day. Slightly less concentration, i.e. 641.87 mg kg^{-1} and 533.12 mg kg^{-1} was observed on eighteenth and twenty fourth days, respectively (Fig. 12c).

Variation was observed in the concentration of Cd in Water Hyacinth after six days. Cd concentration was observed as 46.74, 20.27, and 33.3 mg kg^{-1} on sixth, twelfth and twenty forth day, respectively. Maximum concentration was observed on eighteenth day (64.2 mg kg^{-1}) as shown in Fig. 13a. Significant ($P<0.05$) increase of Cd concentration was observed in Water Lettuce i.e. 97.43, 191.55 and 292.36 mg kg^{-1} on six, twelve and eighteenth day, respectively. Maximum Cd concentration was observed on the twenty fourth day 768.01 mg kg^{-1} (Fig. 13b).

Subsequent Cd metal concentration was observed in Coontail after six days 4536.53 mg kg^{-1} and slowly the accumulation decreased after eighteen days 4879.86 mg kg^{-1}. Maximum concentration of Cd was observed on twelfth day in sampled plants i.e. 5701.46 mg kg^{-1}. The least metal concentration was observed on twenty fourth day 896.88 mg kg^{-1} as shown in Fig. 13c.

The total metal removed by Water Hyacinth, Water Lettuce and Coontail from the effluent of Fuse Shop C-12, Pakistan Ordinance Factory, Wah Cantt illustrated in Fig. 16. Coontail showed more removal of Ni (78.1 g^{-20L}), Pb (67.1 g^{-20L}), Cr (76.1 g^{-20L}), Zn (50.3 g^{-20L}), Cd (70.5 g $^{-20L}$) and Cu (73.7 g^{-20L}) than Water Hyacinth and Water Lettuce. Whereas, Water Hyacinth removed higher Ni (64.5 g^{-20L}), Pb (63.2 g^{-20L}) and Cr (68.4 g^{-20L}) than Water Lettuce. While Water Lettuce removed more Zn (39.8 g^{-20L}) than Water Hyacinth.

IV- Discussion

An exponential reduction in the heavy metals concentration was observed in effluent samples collected after six days from the plastic tubs where all the aquatic plants were grown. Relatively more reduction in Cu concentration (from 3.5 to 2.5 mg L^{-1}) was observed in effluent where Coontail were grown during first six days. Whereas Cu concentration effluent treated with Coontail was remain fairly constant during the first twelve days, while Coontail treated effluent showed maximum Cu reduction during the first twelve days and effluent Cu concentration was brought within the permissible limits (1.0 mg L^{-1}). Nickel is an essential element for the ecosystems at low concentrations while high concentration is considered as harmful and toxic pollutant. The phytoremediation study indicates that Coontail rapidly removed the Ni during first six days. The reduction (from 3.7 to 2.06 mg L^{-1}) of the Ni concentration in the Coontail treated effluent was observed during first six days. Results showed that Coontail lowered utmost concentration of Ni from the polluted effluent which is in line with the observation of Parneyan et al. (2011). Similar results of Ni concentration reduction in industrial effluent treated with Coontail have also been reported by Mjelde and Faafeng (1997).

Similar to Cu and Ni reduction, decrease in Pb concentration of effluent treated with Coontail was also more than those treated with Water Hyacinth and Water Lettuce. Maximum and rapid removal was observed in Coontail grown effluent which decreased the Pb concentration from 3.31 to 2.14 mg L^{-1} and to 0.85 mg L^{-1} after six and twelve days, respectively. Mishra et al. (2006) reported that when Coontail plants exposed to Pb contaminated effluent for 1 to 7 days, most of the metal was removed only after one day. The results revealed that plants accumulated high amounts of metal. Rapid decrease was observed in Cr concentration in effluent sample collected after six day. The metal concentration was decreased from 3.81 to 1.12 mg L^{-1} by Coontail and Cr concentration increased considerably in Coontail plant. Garg and Chandra (1990) showed a much higher concentration of Cr in Coontail after 48 hours. They observed almost leaner Cr concentration increase in Coontail when grown on effluent of increased Cr concentration, i.e. at a 0.05 ppm effluent concentration it was 117.7 ± 1.20 µg/g, at 0.1 ppm 160.87 ± 0.51 µg/g, and at 1.0 ppm 216.32 ± 0.91 µg/g.

Cadmium pollution of water bodies is alarming, because it is a supplementary constituent and hinders plant development upon accumulation. From the investigated results it was found that rapid removal of Cd concentration from the industrial effluent sample was observed after six days. Relatively more reduction was observed in the effluent by Coontail i.e. 3.33 to 1.71 mg L^{-1} followed by 0.39 mg L^{-1}. The observed results are similar to (Seema, 2008) study conducted for the phyto-remediation of polluted effluent by growing Coontail (*C. demersum).* It was observed that Cd removal was dependent on the plant exposure to concentration. The highest accumulation of Cd, 1293 µg g^{-1} dry weight, was found at 10 microM, after 7 days of time. Plants illustrated noteworthy activities and act as an indicator for the environmental contaminants, for hazardous heavy metals or phytotoxins.

Zinc was removed by Coontail at slower rate and concentration was reduced from 2.52 to 0.45 mg L^{-1} after sixth day. The slow removal of Zn from the effluent may be due to the high concentration of Cd in the effluent that is confirmed by the earlier results reported by Bunluesin et al. (2007). They observed that high concentration of Cd in the effluent decreased the Zn removal. Water Hyacinth was observed to be a relatively better phyto-remedial aquatic plant for Cu, Cd, Zn and Pb polluted industrial effluents. In the first twelve days the metal removal was fairly low and it reduced the Cu (3.55 mg L^{-1}), Cd (3.26 mg L^{-1}), Zn (2.66 mg L^{-1}) and Pb (2.4 mg L^{-1}) concentration to 2.01 mg L^{-1}, 2.15 mg L^{-1}, 0.36 mg L^{-1} and 1.3 mg L^{-1}, respectively, on the twelfth day. Zhu et al. (1999) and Vesk et al. (1999) showed similar results of removing metals from industrial effluent treated with Water Hyacinth. However, in present study for Ni and Cr showed that Water Hyacinth plant removed substantial amount of Ni and Cr from the effluent and reduced the concentration from 3.7 to 2.4 mg L^{-1} and 2.02 mg L^{-1}

in first six days which was relatively at slower rate. Similar results of metal concentration removal from the heavy metal contaminated effluent treated with Water Hyacinth was also reported by Stratford et al., 1984; Yahya, 1990; Abd- Elhamid, 1996.

Water Lettuce was found to have the great ability to remove the Cr, Cu, Fe, Mn, Ni, Pb, and Zn (Lu et al. 2011) and results were in line with the observations made during this study. The reduction observed in Cu (3.55 mg L^{-1}), Pb (3.42 mg L^{-1}), Cr (3.73 mg L^{-1}) and Cd (3.27 mg L^{-1}) concentration to 2.84 mg L^{-1}, 1.91 mg L^{-1}, 2.16 mg L^{-1} and 2.76 mg L^{-1} on twelfth day, respectively. Similar results were reported by Mishra et al. (2008) and Mokhtar et al. (2011) that Water Lettuce has the ability to remove heavy metals (Fe, Zn, Cu, Cr and Cd) from the effluent. The growth of Water Lettuce reduced the Ni and Zn concentration from 3.7 and 2.31 to 1.8 mg L^{-1} and 0.10 mg L^{-1}, respectively, in first six days of the experiment. The removal of Ni was very slow as compared to Zn and gradually reduced to 0.0 mg L^{-1} on the twelfth day of the experiment and was within permissible limits (1.0 mg L^{-1}). These results confirmed the results reported by Ernst et al. (1992) that Water Lettuce when exposed to high Ni concentrations removed large amount of Ni from effluent.

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Table 1. Chemical Parameters of Fuse Shop C-12, Pakistan Ordinance Factory Effluent

Parameters	Unit	Value
pH	--	7.35
Electrical Conductivity	dS m^{-1}	0.53
CO_3	mg L^{-1}	0.28
HCO_3	mg L^{-1}	1.13
Ca + Mg	mg L^{-1}	1.68
Na	mg L^{-1}	3.45
K	mg L^{-1}	0.78

Table 2. Initial Heavy Metal Concentrations of Fuse Shop C-12, Pakistan Ordinance Factory Effluent

Heavy Metals	Observed Concentration * (mg L^{-1})	Permissible Limits of Effluent**
Copper	3.35	1.00
Nickel	3.81	1.00
Lead	3.42	0.50
Chromium	3.73	1.00
Zinc	2.66	2.00
Cadmium	3.33	0.10

* Average of 6 replicates, ** USEPA (1998)

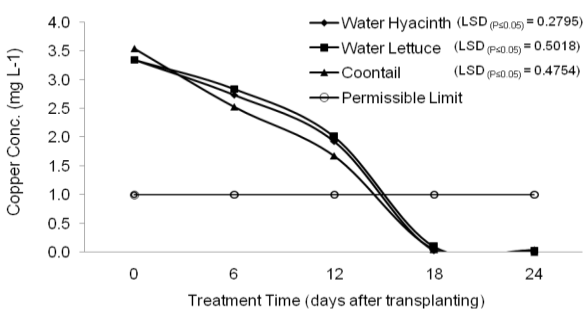

Figure 1. Periodic Reduction in Cu Concentration of Treated Industrial Effluent by Water Hyacinth, Water Lettuce and Coontail

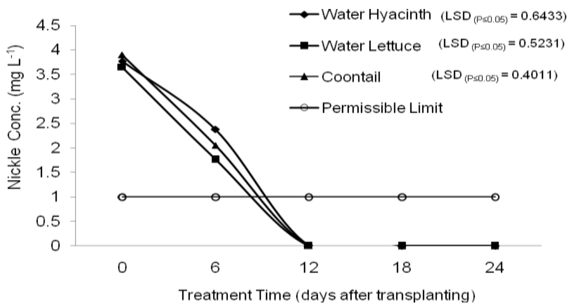

Figure 2. Periodic Reduction in Ni Concentration of Treated Industrial Effluent by Water Hyacinth, Water Lettuce and Coontail

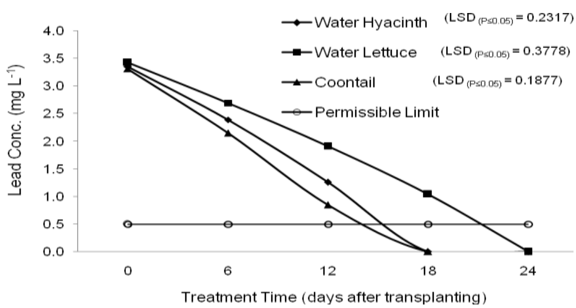

Figure 3. Periodic Reduction in Pb Concentration of Treated Industrial Effluent by Water Hyacinth, Water Lettuce and Coontail

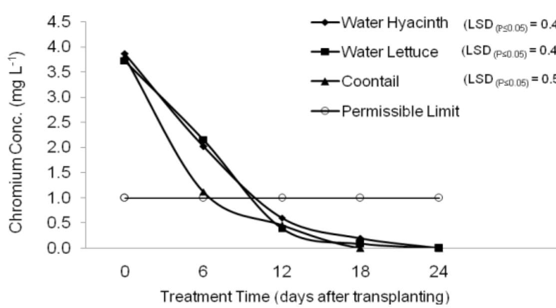

Figure 4. Periodic Reduction in Cr Concentration of Treated Industrial Effluent by Water Hyacinth, Water Lettuce and Coontail

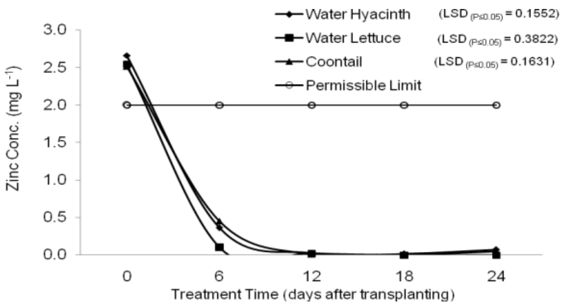

Figure 5. Periodic Reduction in Zn Concentration of Treated Industrial Effluent by Water Hyacinth, Water Lettuce and Coontail

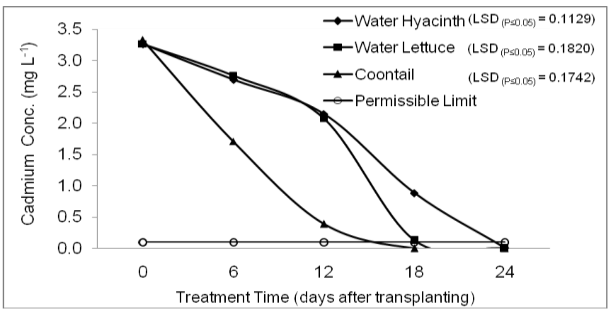

Figure 6. Periodic Reduction in Cd Concentration of Treated Industrial Effluent by Water Hyacinth, Water Lettuce and Coontail

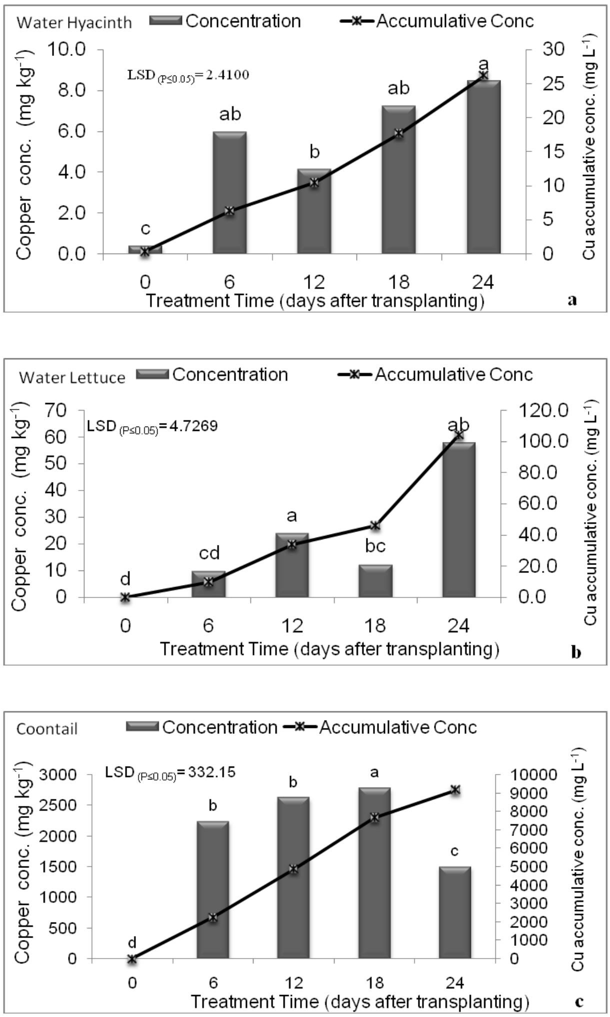

Figure 8. Copper Concentration in Aquatic Plants Grown on Effluent of Pakistan Ordinance Factory, Wah Cantt: (a) Water Hyacinth, (b) Water Lettuce and (c) Coontail.

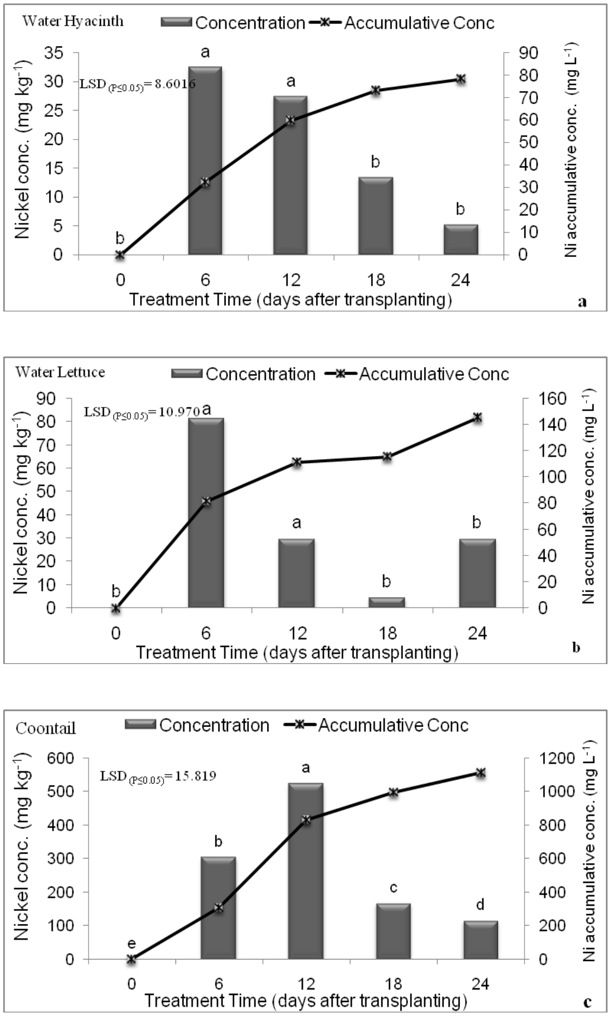

Figure 9. Nickel Concentration in Aquatic Plants Grown on Effluent of Pakistan Ordinance Factory, Wah Cantt: (A) Water Hyacinth, (B) Water Lettuce and (C) Coontail

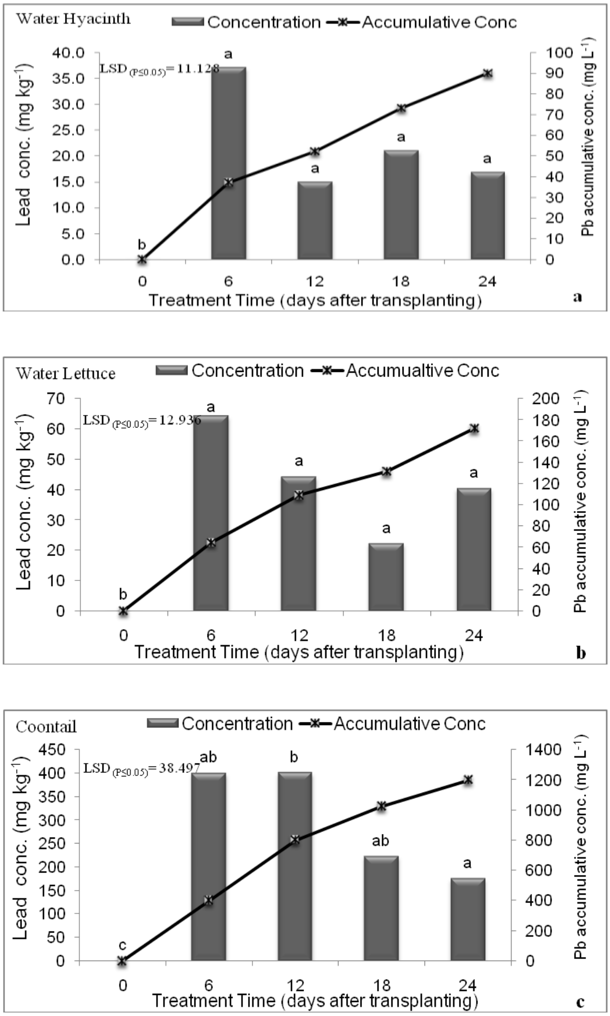

Figure 10. Lead Concentration in Aquatic Plants Grown on Effluent of Pakistan Ordinance Factory, Wah Cantt: (a) Water Hyacinth, (b) Water Lettuce and (c) Coontail.

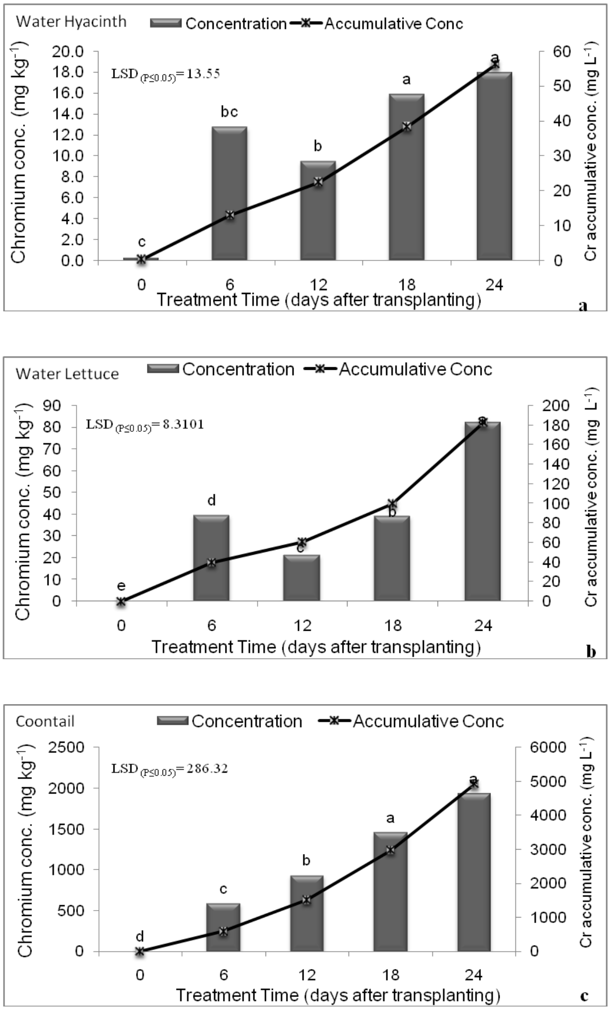

Figure 11. Chromium Concentration in Aquatic Plants Grown on Effluent of Pakistan Ordinance Factory, Wah Cantt: (a) Water Hyacinth, (b) Water Lettuce and (c) Coontail.

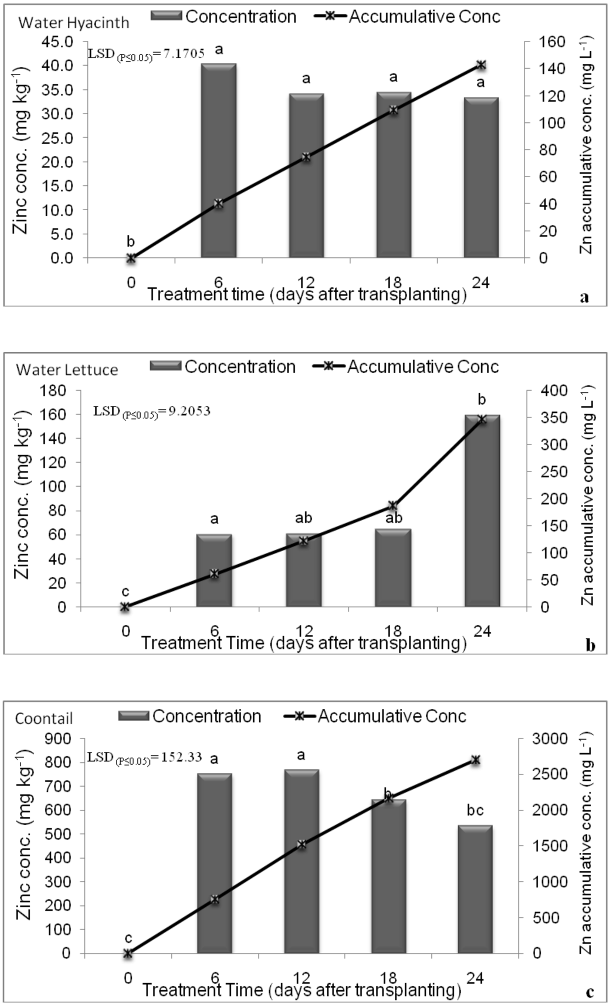

Figure 12. Zinc Concentration in Aquatic Plants Grown on Effluent of Pakistan Ordinance Factory, Wah Cantt: (a) Water Hyacinth, (b) Water Lettuce and (c) Coontail.

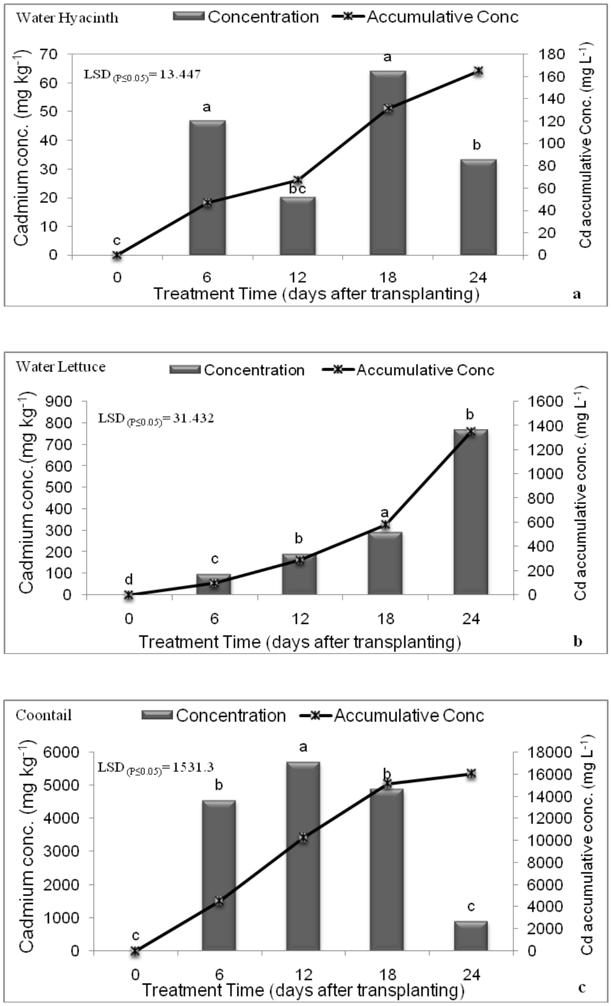

Figure 13. Cadmium Concentration in Aquatic Plants Grown on Effluent of Pakistan Ordinance Factory, Wah Cantt: (a) Water Hyacinth, (b) Water Lettuce and (c) Coontail

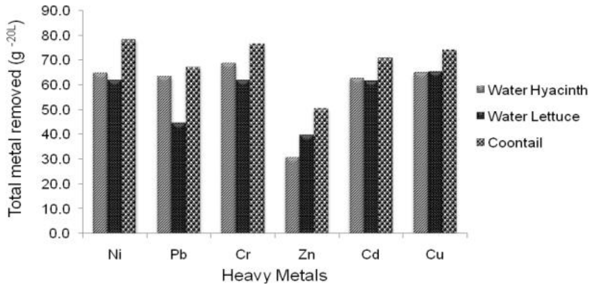

Figure 16. Total Metals Removed by Water Hyacinth, Water Lettuce and Coontail from the Heavy Metal Contaminated Effluent of Fuse Shop C-12, Pakistan Ordinance Factory, Wah Cantt