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Phytoremediation technologies for Ni⁺⁺ by water hyacinth

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Phytoremediation of metal contaminated soil and water by *Eichhornia crassipes* (water hyacinth) is promising. The study was conducted to compare the phytoremoval of Ni⁺⁺ from soil and wastewater. For the measurement of phytoremoval from soil, *E. crassipes* was used in a pot experiment. Results showed the removal of (Ni) 24.23 µg/g dry weight of plant and large level calculations show removal of 3449.76 kg/ha of soil, corresponding to 25 µg/g of the added Ni⁺⁺. In the second experiment, Ni⁺⁺ contaminated Hoagland's solution was used for the hydroponic growth of water hyacinth. The result of hydroponic experiment showed the phytoremoval of Ni⁺⁺ from Ni⁺⁺ contaminated wastewater; maximum removal was 1.954 µg/g of dry weight. In third experiment, ash of water hyacinth was used for the adsorption and desorption of Ni⁺⁺. The adsorption capacity was 1.978 µg/g of ash. For the extraction (desorption) of Ni⁺⁺, 3 M HNO₃ was used. Desorption capacity was 3.71 µg/g of ash. The results of comparative study show order of nickel phytoremediation from soil to be greater than that from water by adsorption which was greater than that from water by hydroponic study. For phytoremoval of Ni⁺⁺ from soil and water, water hyacinth plant and its ash showed excellence. The desorbed Ni⁺⁺ can be used in the industries e.g. in Ni plating.

Key words: Nickel, phytoremediation, soil, waste water, water hyacinth, biosorption.

INTRODUCTION

Environmental pollution is a major threat to humanity. It is the result of industrialization, urbanization, and phenomenal growth in population. In the last decade, much information has been obtained on the effect of heavy metal ions on the soil. Elevated levels of nickel (Ni⁺⁺) can pose a major threat to both human health and the environment (Chou, 1989; Shakoori et al., 2003). The International Agency for Research on Cancer (IARC) included Ni⁺⁺ and some of its compounds as probable human carcinogens (Linton, 1993; Goodarzi and Huggins, 2001).

The phytoremediation of heavy metals from soils is emerging as a cost-effective technology (Mahmood et al., 2005a; Chaney et al., 2008; El-Gendy, 2008). Aquatic plants are known to accumulate metals from their environment. The aquatic plants in metallic pollution act as biological filters and biomonitors of environmental metal levels (Sujatha et al., 2001; Liao and Chang, 2004). Heavy metals are ubiquitous environmental contaminants in industrialized societies. Soil pollution by metals differs from air or water pollution, because heavy metals persist in soil much longer than in other compartments of the biosphere (Lasat, 2002; Mahmood et al., 2007b). Several comprehensive studies have been done, summarizing many important aspects of this novel plant based technology (Meagher, 2000; Navari-Izzo and Quartacci, 2001; Lasat, 2002; McGrath et al., 2002; McIntyre, 2003; Singh et al., 2003; Garbisu and Alkorta, 2001; Prasad and Freitas, 2003; Alkorta et al., 2004; Ghosh and Singh, 2005; Pilon-Smits, 2005, Padmavathamma and Li, 2007). These studies give general guidance and recommendations for applying phytoremediation, highlighting

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Abbreviations: BAF, Bioadsorption factor; BDF, biodesorption factor; AAS, atomic absorption spectrophotometer; ANOVA, analysis of variance; DMRT, Duncan's multiple rang test; ICP-ES, inductively coupled plasma emission spectrophotometer.

the processes associated with applications and underlying biological mechanisms.

Water hyacinth a native of South America is also abundantly found in South Asia. Under favorable conditions a growth rate as high as 17.5 metric tons of wet water hyacinth per hectare per day have been reported (Shoeb and Singh, 2000; Mahmood et al., 2009d). Water hyacinth (*Eichhornia crassipes*) is an aquatic plant (El-Gendy, 2008). It is well known for its phytoremediation potential. Zhu et al. (1999) studied the phytoremediation of six trace metals by water hyacinth. Pollutants removal by water hyacinth, grown on the over bank and flood plain soils of the river Yamuna in Delhi, India, was studied by Mehra et al. (2000). They observed that the roots of water hyacinth growing in the over bank soils, are accumulating a number of metals except Co, Al, and Fe. Water hyacinth can be used for the improvement of transparency of water (Chen et al., 2006). Various studies (Zhu et al., 1999; Olivares-Rievmont et al., 2007; El-Gendy, 2008; Mishra et al., 2008) reported the phytoremediation of metals by water hyacinth. The results showed that water hyacinth is a promising candidate for the phytoremediation of wastewater polluted with Ni. After the treatment of wastewater by water hyacinth, it can be used for irrigation purpose (Liao and Chang, 2004; Youngchul et al., 2006). Methods using living wetland plants to remove metals from water appear to be an alternative. Plants that have a high metal bioaccumulation capacity and a good tolerance to high metal concentrations over long periods of time are necessary. Plant's process of metal's removal by binding in soils, precipitation as insoluble salts is described by Rai (2008) who also reported a model for the treatment of industrial effluents, municipal wastewater and ecosustainable utilization of biomass using macrophytes. The aquatic plants like water hyacinth can act as phytoremediator of metals and organic waste from Missa soil series like loamy and calcareous soil of Taxila Pakistan (Chavan et al., 2008; Lone et al., 2008; Mahmood et al., 2009d; Hussain et al., 2010).

Considerable attention has been focused in recent years upon the field of biosorption for the removal of heavy metal ions from aqueous effluents (Alluri et al., 2007; Mahmood et al., 2010c). The process of heavy metal removal by biological materials is known as biosorption. Biosorption can be defined as "a non-directed physicochemical interaction that may occur between metal/radionuclide species and biomass" (Ahalya et al., 2003). The biosorption process involves a solid phase (sorber or biosorber; usually a biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ion). Due to higher affinity of the sorber for the sorbate species, the latter is attracted and bound with different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution.

Ash of hyacinth contains carbon like charcoal, the negatively charged particles which can adsorb positively charged metal ions (Akorporhonor and Egwaikhide, 2007). As metal ions adsorbed by biomass could be eluted effectively with HNO_3 , while distilled water show negligible metal elution capacity (Alluri et al., 2007). Verma et al. (2008) studied ion exchange during the metal biosorption of Ni^{++} by using dried mass of hyacinth for biosorption because of their smaller size and larger surface area. This feature offers a convenient basis for the production of biosorbent particles suitable for sorption process. They contain many polyfunctional metal-binding sites for both cationic and anionic metal complexes. Potential metal cation-binding sites of algal cell components include carboxyl, amine, imidazole, phosphate, sulphate, sulfhydryl, hydroxyl and chemical functional groups contained in cell proteins and sugars (Alluri et al., 2007; Mahmood et al., 2010c).

This study was conducted to assess the phytoremoval of Ni^{++} from soil and waste water of Taxila. For this purpose, water hyacinth (*E. crassipes*) was used as a phytoremediator of Ni^{++} .

MATERIALS AND METHODS

Pakistan is situated between 24 and 37° N and 61 to 75° E, stretching over 1,600 km from north to south and 885 km from east to west. The tehsil Taxila, district Rawalpindi, Pakistan, is situated at 33° 45' 0" N 72° 48' 36" E the north-western edge of the Punjab Province of Pakistan, about 30 km west-northwest of Islamabad. Taxila's soil belongs to Missa soil series (Typic Ustochrept) (Ali and Higgins, 1967; Mahmood et al., 2009d; Hussain et al., 2010).

For nickel phytoremediation experimental research work is divided in to three parts, that is,

1. Metal's phytoremediation from soil.
2. Metal's phytoremediation from water by hydroponic study.
3. Metal's phytoremediation from water by biosorption, metal recovery by desorption.

Metal's phytoremediation from soil

The soil samples (63) were collected from Wah Cantt area of Taxila district Rawalpindi, Pakistan, by following the standard sampling procedure of Ryan et al. (2001). The samples were air-dried, ground and mixed thoroughly in order to get composite samples. No metallic container was used in order to avoid metallic contamination. These soil samples were added to eighteen labeled plastic containers. Two kilogram (2 kg) soil samples were placed in each labeled plastic container. Calculated amount of $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ (E. Merck Germany) was used for artificial contamination of soil. The given treatments of nickel were from 5 to 25 $\mu\text{g/g}$ of soil. The soil samples were in triplicate. Deionized water (300 ml) was added in all soil samples, and was placed undisturbed for two weeks for wet and dry cycle. Plants were collected from wetlands of Taxila area and washed. These plants were grown hydroponically as described by Zhu et al. (1999) for five weeks. Eighteen plants were weighted and planted in soil present in plastic containers. These plants were watered with deionized water for 10 days. After 10 days, these plants were removed from pots, washed, weighed for fresh and dry

Table 1. Study of fresh weight of water hyacinth in Ni contaminated soil (g).

Treatment of Ni ($\mu\text{g/g}$)	Weight before expt. (g)	Weight after expt. (g)	Difference in fresh weight (g)
0	131.02a	135.57a	+4.55b
5	97.46c	98.57d	+1.11d
10	81.83e	85.46e	+3.63c
15	102.64b	108.11b	+5.47b
20	72.93f	77.34f	+4.41b
25	93.33d	101.79c	+8.46a
LSD (0.05)	0.677	0.483	0.907

Means showing similar letter (s) in a column do not differ significantly at $p < 0.01$.

Table 2. Study of dry weight, removal of Ni per plant, removal of Ni per 100 g of plant dry weight, removal of Ni per g of plant dry weight, removal of Ni per ha of soil.

Concentration of Ni in contaminated soil	Dry wt of plant in g	Removal of Ni from soil per plant (μg)	Removal of Ni in $\mu\text{g}/100$ g of plant dry weight	Removal of Ni per g of dry weight ($\mu\text{g/g}$)	Removal of Ni in kg per hector of soil
0	16.92	0	0	0	0
5	11.09	724.28	6690	6.690	831.35
10	10.40	1453.81	13970	13.97	1628.26
15	12.36	1943.82	15720	15.72	2177.07
20	9.26	1725.59	18630	18.63	1932.66
25	12.71	3080.15	24230	24.23	3449.76

Table 3. Study of pH changes in soil due to growth of water hyacinth.

Treatment of Ni ($\mu\text{g/g}$)	Avg. pH before experiment	Avg. pH after experiment	Avg. pH change
0	8.26	8.30	+0.04
5	8.26	8.26	0.00
10	8.26	8.27	+0.01
15	8.26	8.29	+0.03
20	8.26	8.31	+0.05
25	8.26	8.27	+0.01
LSD (0.05)		0.07956	0.05626

weight. The plants samples were digested in $\text{HClO}_4 / \text{HNO}_3$ (1:2 ratio) mixture and were analyzed for Ni^{++} using atomic absorption spectrophotometer (AAS) and inductively coupled plasma emission spectrophotometer (ICP-ES) (Lab tam 8500) (Mahmood et al., 2010e). Metal content of plants was noted in $\mu\text{g/g}$ of dry weight and kg/ha of soil. The data was statistically analyzed by using analysis of variance (ANOVA) and Duncan's multiple rang test (DMRT) (Badr-uz-Zaman et al., 2002).

This experiment demonstrates the potential of water hyacinth for Ni^{++} phytoremoval from contaminated Missa soil series (Typic Ustochrept) of Wah area of tehsil Taxila, district Rawalpindi, Pakistan. This soil is loamy and calcareous (Ali and Higgins, 1967; Mahmood et al., 2009d; Hussain et al., 2010). The soil pH was measured before and after experiment (Ryan et al., 2001; Hussain et al., 2010). The results are given in Tables 1, 2 and 3.

Metal's phytoremediation from water by hydroponic study

In hydroponic experiment, the main objective was to determine the suitability of water hyacinth for the phytoextraction of Ni^{++} present in wastewater. The ability of water hyacinth to take up and translocate metal was studied under controlled conditions and in specified concentrations. For this purpose Hoagland's solution was used as in previous studies (Hoagland and Arnon, 1950; Zhu et al., 1999; Badr-uz-Zaman et al., 2002). It was diluted to quarter strength. Hoagland's solution containing nickel 5-30 $\mu\text{g/ml}$ was prepared and was diluted to desired concentration (Jeffery et al., 1989). Wildly grown water hyacinth plants were collected from different areas of Taxila. These were washed and tested for metals by using AAS and ICP (Mahmood et al., 2009d).

Some wildly grown water hyacinth plants were collected from

Table 4. Study of changes in fresh weight of water hyacinth grown in Ni contaminated Hoagland's solution.

Concentration of Ni in contaminated Hoagland's solution ($\mu\text{g/ml}$)	Plant's fresh weight in (g) before experiment	Plant's fresh weight in (g) after experiment	Change in plant's fresh weight in (g)
0	64.103d	73.096c	+8.990
5	93.720b	75.320b	-18.400
10	81.140c	76.300a	-4.840
15	56.250f	51.460e	-4.790
20	51.910g	47.830f	-4.080
25	94.180a	71.470d	-22.710
30	57.260e	39.960g	-17.300
LSD (O.05)	0.254	0.242	-

Means showing similar letter (s) in a column do not differ significantly at $p < 0.01$.

Table 5. Study of dry weight, removal of Ni per 100 g of plant dry weight and removal of Ni per g of plant dry weight.

Concentration of Ni in contaminated Hoagland's solution ($\mu\text{g/ml}$)	Plant's dry weight (g)	Removal of Ni in $\mu\text{g}/100\text{g}$ of plant dry weight	Removal of Ni in $\mu\text{g}/\text{g}$ of plant dry weight
0	7.55 c	0	0
5	9.43 b	3.488	0.034
10	9.53 b	6.054	0.060
15	6.39 d	19.546	1.954
20	6.43 d	14.79	1.479
25	10.94 a	5.310	0.0531
30	6.21 d	5.29	0.0529
LSD (0.05)	0.271	-	-

Means showing similar letter (s) in a column do not differ significantly at $p < 0.01$.

different areas of Taxila plants. These were washed and grown hydroponically for five weeks for getting non polluted plants for experiment (Zhu et al., 1999). Twenty one plants were collected from above mentioned samples, washed, weighed and planted in triplicate in known quantity of solutions. For the Hoagland's solution, two types of nutrients were required, that is, macronutrients and micronutrients. The stock solutions of both micro and macronutrients were prepared. These solutions were mixed to prepare one liter solution (Hoagland and Arnon, 1950; Badr-uz-Zaman et al., 2002).

Known volume of Hoagland's solution was added in each container of plants. Daily air passage was passed through these containers for three hours after regular interval of time to provide uniform quantity of oxygen to plant roots. In one set of containers, only air was passed through known volume of solution in order to know evaporation of solution. This set was without plants. Daily temperature, percentage humidity and average photoperiod were noted. These plants were grown for 10 days and then were removed. The pre-weighed plants after removal were dried with filter paper sheets and weighed for fresh weights. These were dried at 65°C for 72 h and weighed for dry weight (Badr-uz-Zaman et al., 2002). Changes in fresh weight, dry weight, change in pH of solution and change in concentration of Ni^{2+} were noted. The plants samples were digested by $\text{HClO}_4 / \text{HNO}_3$ (1:2) mixture and were analyzed by Atomic Absorption Spectrophotometer (AAS, GBC 932 Plus). Phytoremoval of Ni^{2+} in $\mu\text{g}/\text{g}$ gram of dry weight was noted and recorded (Mahmood et al., 2010e; Hussain et al., 2010). The phytoremoval of nickel showed removal from liquid media. The

results are given in Tables 4, 5 and 6.

Metal's phytoremediation from water by biosorption, metal recovery by desorption and hydrogen adsorption

Five samples of wildy grown water hyacinth (*E. crassipes*) plants were collected from various locations. These were washed carefully, air-dried, oven dried by procedure already documented (Badr-Uz-Zaman et al., 2002; Shawky et al. 2005) and burnt at 220°C in an electric muffle furnace to obtain ash. Their fresh weight, dry weight and weight of ash were calculated and recorded (Mahmood et al., 2010c). The ash was ground and mixed in order to obtain a composite sample. The pulverized ash had a high surface area. The ash was pretreated with deionized water in order to remove already present soluble metal ions (Alluri et al., 2007; Mahmood et al., 2010c). Stock solution of nickel was prepared by standard procedure. Different concentrations ranging from 5 to 30 μg of metal ml^{-1} were prepared as described by Hasany et al. (1997) and Shawky et al. (2005). The pH value of nickel solution was noted by pH meter (Orion USA made model SA 720) and kept at constant, acidic levels in order to see the effect of biosorbent under same set of conditions. The pH of Ni^{2+} solutions was kept at 6.54. For this purpose, $\text{Na}_2\text{CO}_3/\text{H}_2\text{SO}_4$ was used (Shawky et al. 2005; Mahmood et al., 2010c).

In column method, 10 g ash was used for the adsorption of Ni^{2+} from solutions. At 25°C , 100 ml solutions were used. The triplicate solutions of Ni^{2+} were passed through 10 g ash column. The next

Table 6. Study of change in pH due to water hyacinth grown hydroponically in Ni contaminated Hoagland's solution.

Concentration of Ni in contaminated Hoagland's solution ($\mu\text{g/ml}$)	Average pH before experiment	Average pH after experiment	Change in pH
0	5.12	5.24	+0.12
5	4.29	5.26	+0.97
10	4.18	4.98	+0.80
15	3.8	4.84	+1.07
20	3.86	4.66	+0.80
25	3.89	4.70	+0.81
30	3.96	4.65	+0.69

Table 7. Metal concentration in $\mu\text{g ml}^{-1}$, $\mu\text{g } 100 \text{ ml}^{-1}$ of given solutions and adsorption capacity in $\mu\text{g g}^{-1}$ of plant ash adsorbed Ni in $\mu\text{g g}^{-1}$ of plant ash, Ni desorption in $\mu\text{g g}^{-1}$ of plant ash and biodesorption factor of Ni.

Metal Conc. in $\mu\text{g ml}^{-1}$ solution	Metal Conc. in $\mu\text{g } 100 \text{ ml}^{-1}$ solution	Nickel adsorbed onto ash in $\mu\text{g g}^{-1}$	Nickel biosorption factor of plant ash	Adsorbed nickel onto ash in $\mu\text{g g}^{-1}$	Desorbed nickel in $\mu\text{g g}^{-1}$ of ash	Biodesorption factor of nickel
0	0	0	0	0	0	0
5	500	4.63	0.926	4.63	0.99	0.213
10	1000	9.78	0.978	9.78	2.05	0.209
15	1500	14.70	0.983	14.7	2.49	0.169
20	2000	19.69	0.984	19.69	2.79	0.141
25	2500	24.77	0.990	24.77	3.39	0.136
30	3000	29.79	0.993	29.79	3.71	0.124

Table 8. Study of pH changes during adsorption Ni onto ash of water hyacinth.

Treatment of Ni ($\mu\text{g/ml}$)	pH before experiment	pH after experiment	Treatment of Ni
0	7.51 a	8.18 d	+0.67
5	6.54 b	8.90 a	+1.55
10	6.54 b	8.29 bc	+1.75
15	6.54 b	8.15 d	+1.61
20	6.54 b	8.30 bc	+1.76
25	6.54 b	8.25 c	+1.71
30	6.54 b	8.32 b	+1.78
LSD (0.05)	0.0553	0.055	-

Means sharing similar letter (s) in a column do not differ significantly at ($p < 0.01$).

day, the metal content of leachate/filtrate was measured by AAS (Atomic Absorption Spectrophotometer) (GBC-932 plus). The quantity of metal already present in ash was measured by AAS using the procedure of Ryan et al. (2001) and Mahmood et al. (2010c).

For desorption studies, 3 M HNO_3 solutions were prepared by the procedure described by Jeffery et al. (1989). The 100 ml HNO_3 solution in triplicate samples was passed through column with metal adsorbent (already metal containing water hyacinth ash). The following day, concentration of metal ions was measured from leachate by AAS as mentioned above. The metal desorption in microgram per gram of ash was computed by the documented procedure (Tan et al., 2007; Mahmood et al., 2010c). The results

are given in Tables 7 and 8.

RESULTS

Increase in fresh weight of water hyacinth when grown in artificially contaminated soil containing 5 to 25 $\mu\text{g/g}$ Ni is shown in Table 1. The fresh weight of these plants was increased as compared to control (with out Ni). Increase in fresh weight shows that Ni favors growth of water

hyacinth.

Table 2 shows that water hyacinth is an excellent phytoremediator of nickel from contaminated soil. Nickel's removal in $\mu\text{g/g}$ of soil is increasing with increase in concentration in soil. Maximum phytoremoval is obtained at $25 \mu\text{g/g}$ of soil. Per hectare removal reveals that on bulk level, this technology is good for copper contaminated soil. Table 3 shows that pH increased as compared to control. This is due to microbial (*Azotobacter*) activities. Increase in pH is also responsible for precipitation of nickel hydroxides and adsorption onto roots.

In Table 4 the average decrease in fresh weight as observed in the present study is shown. This decrease may be due to osmotic potential or due to decrease of moisture content. The fresh weight decreased with increase in concentration of nickel. Table 5 shows that removal of nickel increased with increase in concentration in contaminated water. Maximum phytoremediation of nickel was observed up to $1.954 \mu\text{g/g}$ of plant dry weight. After this, concentration decline was observed.

From Table 6, it is observed that when we grow water hyacinth hydroponically in Hoagland's solution containing various concentrations of nickel due to microbial (*Azotobacter*) activities, pH were increased (Mahmood et al., 2009d). Increase in pH is also responsible for precipitation of nickel hydroxides and adsorption onto roots. Table 7 shows that the adsorption capacity increased with increase in concentration of nickel. The pH was increased due to the formation of hydroxides/oxides of nickel (Mahmood et al., 2010c). Desorption capacity was increased with increase in concentration of nickel. A good recovery of nickel is possible at $30 \mu\text{g/g}$ of ash and may be more beyond this concentration. Table 8 reveals that the pH was increased.

Summary of these results for nickel shows that maximum phytoremoval from soil was $24.23 \mu\text{g/g}$ of dry weight (for $25 \mu\text{g/g}$). Hydroponic experiment showed phytoremoval in $1.954 \mu\text{g/g}$ of dry weight (for $15 \mu\text{g/ml}$). Phytoremoval of nickel by adsorption was $29.79 \mu\text{g/g}$ of ash (when $30 \mu\text{g/ml}$ nickel was given). Desorption capacity shows that $3.71 \mu\text{g/g}$ of ash recovery is possible. The results show that phytoremoval by adsorption onto ash was greater than phytoremoval of nickel from soil which was greater than phytoremoval from hydroponic experiment. Also, when water hyacinth was grown in soil, in hydroponic experiment or in adsorption onto ash, pH was significantly increased.

Ash of hyacinth contains negatively charged carbon particles, and metal ions are positively charged. Heavy metal's particles generally show higher adsorption capacity compared to lighter metals (Alluri et al., 2007). Matai and Bagchi (1980) reported that the ash of water hyacinth contains oxides of Na, K, Ca, and Mg. These oxides are basic or amphoteric. Upon their reaction with water, they produce hydroxides. The pH of ash is increased due to the formation of these hydroxides (Mahmood et al.,

2010c).

DISCUSSION

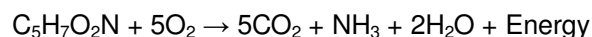
Water hyacinth was grown in artificially contaminated soil containing Ni $5 - 25 \mu\text{g/g}$ of soil. The fresh weight of these plants was increased as compared to control (with out Ni). Increase in fresh weight shows that Ni favors growth of water hyacinth (Qian et al., 1999). Table 1 shows the change in fresh weight. The increase in fresh weight was reported by Lu et al. (2004). Table 3 shows that pH of soil increases. This increase in pH shows that mechanisms other than acidification, such as ion exchange and roots exudation, may be responsible for the increased heavy metal uptake in plants (Kashim and Singh, 2002). Nitrogen containing organic compounds due to microbial activities change into NH_3 (Sooknah, 2001; Mahmood et al., 2009d), this ammonia may increase the pH. The pH changes are related to Ni removal (Kashim and Singh, 2001). Table 2 shows dry weight, phytoremoval of Ni $\mu\text{g/g}$ of dry weight and per hectare removal from soil. Removal of metals from soil has being shown by Kashim and Singh (1999).

Water hyacinth was grown in Hoagland's solution containing Ni $5 - 30 \mu\text{g/ml}$. The fresh weight of these plants was decreased as compared to control (with out Ni) (Table 4). Table 6 shows that pH was increased with the growth of plants and with increase in concentration of Ni. This may be due to oxides or hydroxides of alkali metals like K^+ or Na^+ . Soil extractable K^+ and Na^+ concentrations less than 60 and $44.4 \mu\text{g/g}$, respectively, are below the critical level (Khan et al., 2004). Kashim and Singh (2002) reported that due to increase of pH, there was increase in the uptake of metals. This increase in pH by water hyacinth has also being verified by Santos and Lenzi (2000). Nitrogen containing organic compounds due to microbial activities is converted to NH_3 (Sooknah, 2001); this ammonia could increase pH. Soil pH is considered to be one of the most important chemical factors controlling the availability of heavy metals in soil. The pH of soil and soil solutions increase when metals are given to plants. The growth of water hyacinth at the pH of 8.5 is verified by Del-Mar-Delgado et al. (1994) and Mahmood et al. (2009d).

The pH changes are related to Ni phytoremoval (Kashim and Singh, 2001). In a similar study, Akcin et al. (1994) reported that the uptake of Pb by water hyacinth was increased between the alkaline pH. Mehra et al. (2000) showed that the increase of pH of soil was due to the growth of water hyacinth on the bank of river Yamuna of India. Malik (2007) studied the growth of water hyacinth at a pH up to 8 .

Increase of K^+ and Na^+ in soil is may be due to decay/decomposition of water hyacinth plant material as described by Gupta et al. (1996) who also studied the order of release of various elements from decaying leaves of hyacinth and gave it as $\text{K} > \text{C} > \text{Na} > \text{N}$.

The phyto-removal of Ni from Hoagland's solution (Zhu et al., 1999) is shown in Table 5. The phyto-removal was 1.954 µg/g dry weight, when given treatment of 15 µg/ml Ni. Nickel accumulation by hyacinth was also referred by Zayed et al. (1998). The sudden decrease in phyto-removal in µg/g of dry weight after 15 µg/ml could be due to increase in dry weight of plants. In Hoagland's solution, acidic pH was used (Qian et al., 1999). Water hyacinth provides support to attach bacterial biofilm. These bacteria solubilize metals and actively degrade organic matter. A summary of the multiple biochemical steps is shown below



Where, $C_5H_7O_2N$ is a generalized formula of bacterial biomass obtained from experimental studies. This ammonia production is the cause of increase of pH. In this oxidation, oxygen is provided by roots of water hyacinth, from atmospheric diffusion, or from inorganic molecules such as nitrates and sulphates (Sooknah, 2000). Del-Mar-Delgado et al. (1994) and Mahmood et al. (2009d) verified this increase in pH. The use of water hyacinth for the removal of Zn, Cd, and Hg from waste water approves the authenticity of phyto-removal of Ni^{++} (Hasan et al., 2007; Skinner et al., 2007).

Kong et al. (1999) studied the hyper accumulation of P and N compounds by hyacinth. The compounds of N like ammonia and ammonium ions are responsible for the increase of pH, because like P, lone pair of electrons are also present in N. Lone pair donors are basic in nature.

Ni solutions of various concentrations (5 – 30 µg/ml) were passed through column filled with fixed weight of ash of water hyacinth. By using difference in concentration of Ni in original solution and in lecheate, the adsorption was measured. Table 8 shows that pH was increased as compared to control. Table 7 shows phyto-removal of Ni. Adsorption of Ni (µg/g of ash), increases with increase in concentrations of Ni. Maximum removal of Ni was 29.79 µg/g of ash, when given concentration was 30 µg/ml. Adsorption of metals depends upon pH, shaking time and amount of adsorbent (Rauf et al., 2003). During adsorption of metals pH is increased (Qadeer and Saleem, 1997; Webster et al., 1998). Many authors stated that adsorption range of pH is 5-8.5 (Schroth and Sposito, 1998; Mahmood et al., 2010c).

Table 7 shows that when 3 M HNO_3 was used, desorption of Ni µg/g of ash is increased with the increase in concentration of Ni. Maximum desorption of Ni was 3.71 µg/g of ash. These results show highest significance of this technology for making Ni reusable but when it is present in low concentration. Its removal percentage is decreased with increase in concentration but is reasonable for reuse. All these results are verified by Kashim and Singh (1999, 2001, 2002), Das, (2004), Yan and Viraraghavan (2003) and Mahmood et al. (2010c).

Phytochelatin is a small glutathione derived metal-binding peptides which are a part of the plant metal

detoxification system (Clemens, 2001). In phyto-removal of metals these may be responsible. Fungi like *Aspergillus niger* grow on roots of water hyacinth and can grow on an alkaline media (pH greater than 7) that can adsorb metals. The adsorption of metals onto the roots of water hyacinth is also possible due to *A. niger* (Tabassum, 2003). The enzyme system of water hyacinth is evidence as a water purifier (Mishima et al., 2006). Similar study shows the phyto-removal of metals from Hoagland's solution (Zhu et al., 1999). Biosorption of metals by plant's roots from aqueous solutions showed that Na, K, Mg and Ca ions are simultaneously released into bulk. This shows the involvement of ion-exchange mechanism in metal uptake and passive sorption (Nancharaiiah et al., 2006). Metal's accumulation by hyacinth was also shown by Zayed et al. (1998). Water hyacinth is an absorber of metals (Zhu et al., 1999; Qian et al., 1999). Mohamed et al. (2009d) showed same type of results while studying a number of elements including six metals.

This technique also offers several advantages including cost effectiveness, high efficiency, and minimization of chemical/biological sludge. In countries, with the rush for rapid industrial development coupled with lack of awareness about metal toxicity, there is an urgent need for developing an economical and eco-friendly technology which satisfies these demands when other conventional methods fail (Mahmood et al., 2010c).

Ash contains negatively charged carbon particles, and metal ions are positively charged. Heavy metal's particles generally show higher adsorption capacity compared to lighter metals (Alluri et al., 2007). Matai and Bagchi (1980) reported that the ash of water hyacinth contains oxides of Na, K, Ca, and Mg. These oxides are basic or amphoteric. Upon their reaction with water, they produce hydroxides (Mahmood et al., 2010c). The pH of ash is increased due to the formation of hydroxides. Insoluble hydroxides are formed: Ni^{2+} due to low K_{sp} (constant for solubility product) these are precipitated. Precipitation or co-precipitation is also cause of adsorption onto ash of these metals.

Based on the spontaneous nature of the sorption process, it is possible that mechanism of sorption may be ion exchange in nature. The most important components in the plant's ash are oxides/hydroxides of Ca, Mg, K, Na, Al, Fe, and Si. These undermine phenomena such as adhesion, precipitation, co-precipitation, sequential precipitation, surface precipitation, and adsorption (Vengris et al., 2001; Gupta and Bhattacharyya, 2008). Van der Waals forces may be responsible for physical adsorption, while precipitation and ion-exchange may be responsible for chemi-sorption (Alluri et al., 2007). The pretreatment of ash with deionized water and adsorption results have also been verified by recent studies (Zhang et al., 2009). The deionized water may remove soluble ions and create sites for attachment of metal ions.

The bioadsorption factor (BAF) and biodesorption factor

(BDF) are very important terms in metal sorption and desorption studies (Tables 7). We calculated both factors for water hyacinth's ash, as described by Sanchez-Galvan et al. (2008). The BAF increased for Ni^{2+} but decreased for Cd^{2+} . The BDF decreased for Ni^{2+} . The increases in BAF may be due to low K_{sp} , while the decrease in BAF could be due to high K_{sp} of oxides and hydroxides. The increase in BDF may be due to high K_{sp} while decrease in BDF may be due to low K_{sp} of metal nitrates.

Results show that due to adsorption, pH values were increased. This increase is due to oxides/hydroxides in ash of various elements (Matai and Bagchi, 1980). The Ni^{2+} reacts with water to produce hydroxides. The hydroxides and oxides are responsible for this increase in adsorption, as described by previous studies (Park and Regalbutto, 1995; Tamura et al., 1996).

Desorption results show the desorption capacity of metal and BDF. The metal desorption by HNO_3 was low as compared to metal adsorption. This desorption was sufficient to recover these metals for reuse. These results show that this technique is very useful for the recovery of these metals when present in the range of 5-30 $\mu\text{g ml}^{-1}$ in metal-polluted water.

In the process of desorption these metals reacted with 3 M HNO_3 forming nitrates which are soluble in acidic aqueous media. So these are desorbed and leached from the substrate surfaces. The differences in desorption capacity and BDF are due to variation in K_{sp} of nitrates (Fridenberg, 1974).

Conclusions

From the present investigations, it was shown that water hyacinth showed excellent removal of Ni^{++} from Missa soil of Taxila. The developed technologies used can be applied for phytoremediation of Ni^{++} contaminated soils. The study also reveals that the major advantages of biosorption over conventional treatment methods include: Low cost; high efficiency; minimization of chemical and low biological sludge. This technology requires no additional nutrient requirement, has the cheapest regeneration of biosorbent and high possibility of metal recovery. Moreover, the raw material is locally available. Thus water hyacinth showed efficient removal of Ni^{++} from waste water.

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APPENDICES

Soil data

Appendix 1. Analysis of variance for fresh weight before experiment (nickel).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	5	755.542	151.108	195.294	0.0000
Within	12	9.285	0.774		
Total	17	764.827			

Coefficient of variation = 1.56%.

Appendix 2. Analysis of variance for fresh weight after experiment (nickel).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	5	358.377	71.675	1313.144	0.0000
Within	12	0.655	0.055		
Total	17	359.032			

Coefficient of variation = 0.38%.

Appendix 3. Analysis of variance for difference in fresh weight (nickel).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	5	224.405	44.881	61.152	0.0000
Within	12	8.807	0.734		
Total	17	233.212			

Coefficient of variation = 14.78%.

Appendix 4. Analysis of variance for pH before experiment (nickel).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	5	0.003	0.001	0.255	0.006
Within	12	0.027	0.002		
Total	17	0.030			

Coefficient of variation = 0.58%.

Appendix 5. Analysis of variance for pH after experiment (nickel).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	5	0.075	0.015	11.261	0.0003
Within	12	0.016	0.001		
Total	17	0.091			

Coefficient of variation = 0.45%.

Appendix 6. Analysis of variance for change in pH (nickel).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	5	0.051	0.010	4.351	0.0172
Within	12	0.028	0.002		
Total	17	0.079			

Coefficient of variation = 55.38%.

Appendix 7. Analysis of variance for dry weight (g) (nickel).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	5	12.675	2.535	34.664	0.0000
Within	12	0.878	0.073		
Total	17	13.553			

Coefficient of variation = 4.82%.

Appendix 8. Analysis of variance for phytoremoval (nickel).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	5	416866.266	83373.253	48.305	0.0000
Within	12	20711.901	1725.992		
Total	17	437578.167			

Coefficient of variation = 18.78%.

Hydroponic data**Appendix 1.** Analysis of variance for fresh weight before experiment (Ni)

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	6	5922.970	987.162	45934.433	0.0000
Within	14	0.301	0.021		
Total	20	5923.271			

Coefficient of variation = 0.21%.

Appendix 2. Analysis of variance for fresh weight after experiment (Ni).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	6	3832.826	638.804	34256.690	0.0000
Within	14	0.261	0.019		
Total	20	3833.087			

Coefficient of variation = 0.23%.

Appendix 3. Analysis of variance for difference in fresh weight (Ni).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	6	1072.740	178.790	3308.005	0.0000
Within	14	0.757	0.054		
Total	20	1073.496			

Coefficient of variation = 2.01%.

Appendix 4. Analysis of variance for dry weight (Ni).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	6	64.279	10.713	447.361	0.0000
Within	14	0.335	0.024		
Total	20	64.615			

Coefficient of variation = 1.92%.

Adsorption and desorption data**Appendix 1.** Analysis of variance for pH after experiment (Ni).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	prob
Between	6	1.164	0.194	222.558	0.0000
Within	14	0.012	0.001		
Total	20	1.176			

Coefficient of variation = 0.35%.

Appendix 2. Analysis of variance for concentration (Ni).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	6	0.258	0.045	223.000	0.0000
Within	14	0.003	0.000		
Total	20	0.270			

Coefficient of variation = 5.66%.

Appendix 3. Analysis of variance for desorption (Ni).

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob
Between	6	37.311	6.219	113.555	0.0000
Within	14	0.767	0.055		
Total	20	38.078			

Coefficient of variation = 10.97%.