



Phytoremediation of Nickel, Lead and Manganese in Simulated Waste Water Using Algae, Water Hyacinth and Water Lettuce

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Abstract: The increase in industrial and artisanal mining and mineral processing activities has led to a surge in the quantity of hazardous materials, typically heavy metals that are released into the environment. These hazard materials, when discharge in water bodies, poses serious risk to humans, animals and environment. Phytoremediation is one of the cost effective methods use in the removal of these pollutants from environment. Several plants have been investigated for their phytoremediating potentials. In this paper, the phytoremediation potential of algae, water hyacinth and water lettuce for the removal of Ni, Pb, and Mn was demonstrated. Plants of equal size were grown in aqueous medium and supplemented with different concentration (1.0 mg/dm³, 3.0 mg/dm³ and 5.0 mg/dm³) of multi component metal solution for 15 consecutive days. All the plants revealed a very good accumulation potential, with the accumulation of metals shown to increase with an increase in the initial concentration of the metal solution. At all levels, the plants accumulated the metals more in the root than in shoot except for Mn in water hyacinth. The result showed that water hyacinth was able to accumulate Pb better, while water lettuce showed more preference for Ni and Mn. All the three plants can be used in remediating waste water. Hence, water hyacinth, water lettuce and algae are a promising biomass for phytoremediation.

Keywords: Phytoremediation, Algae, Water Hyacinth, Water Lettuce, Heavy Metals

1. Introduction

Pollution of the aquatic environment by heavy metals is one of the major threats to the water resources of the world today. Heavy metals pollutants are of concern due to the non-degradability hazard they creates (under natural conditions) when discharged into a water body and the serious health complications attributed to them [1-3]. The 1956 Minamata disease caused by mercury contamination on fish is a good example. The disease claimed the lives of nearly 900 people in Japan and more than 2 million were reported to have suffered from different health complications.

Contamination of aquatic environment can therefore be linked to the increase in the anthropogenic activities (ore mining, oil exploration, industrial effluents), which led to generation of tonnes of wastes into aquatic environment, soil, or emitted into the air [4]. The contamination of aquatic ecosystems by heavy metals may also result from weathering of soil and rocks from volcanic eruptions [5], or from natural soils due to the changes in local redox conditions and the corrosion of subsurface engineering structures due to prolonged submergence under acidic groundwater. Okafor et al, (2007) and Mohiuddin, et al, (2010) reported that, trace elements may be immobilised within the stream sediments and thus could be involved in absorption, co-precipitation,

and complex formation [6, 7].

To avert the environmental effect caused by heavy metal pollution, several conventional techniques such as reverse osmosis, ion exchange, chemical precipitation, electrochemical treatment, reverse osmosis, adsorption, electro dialysis etc were reported [8-10]. However, these so called conventional methods are quite costly, energy intensive and metal specific. Therefore, there exists a definite need to develop a low cost, eco-friendly and sustainable technology to serve as substitute to the conventional methods already reported [11].

Thanks to Biotic remediation, it is an eco-friendly, cost effective and promising technology that uses plants to remediate/remove heavy metal from waste water [12]. Rhizofiltration is the most common biotic remediation process that is utilized to absorb and concentrate contaminants from polluted aqueous sources in their roots [13]. The three plants under investigation were reported to use this technique to absorb heavy metal pollutants into their system.

Algae has been recently a plant of interest for biomonitoring eutrophication of organic and inorganic pollutants. Role of algae has been implored in waste water treatment due to its heavy metal cations, based on high negative surface charge. This is an advantage from the viewpoint of tertiary sewage treatment but a disadvantage if the intent is to use waste-grown algae for fish or livestock feeding or composting [14]. Benchakra (2014) reported the use of ten algal spp in Algae Turf Scrubber (ATS) for a period of 21 days for the removal of Zn and Ni. Zn was 99% removed [15]. However, Ni wasn't efficiently removed due to the leaching of air stones used for aeration. The work of Giovanni *et al.*, (2012) used five *Rp palustis* strains (an algal specie) to find out the metal removal capacity of Ru and Ru contaminants in solutions. *Synechocystis Salina* (an algal sp) was also reported to have shown a very good phytoremediating potential for Cr, Fe, Ni and Hg. The plant successfully removed Cr, Fe, Ni, and Hg up to 60%, 66%, 70%, and 77% respectively, in 15 days [5].

Another plant of interest that is used in biotic remediation is Water hyacinth (*Eichhornia crassipes*). It is a free-floating perennial aquatic plant native to tropical and sub-tropical South America, and is now widespread in all tropic climates [16]. *E. crassipes* is well known for its enormous biomass production rate, high resistance to pollution, absorption capacity of heavy-metal and nutrient [17]. These properties qualify it for use in wastewater treatment [17, 18]. A comprehensive overview for Arsenic removal from waste water using *E. crassipes*. Shows that Up to 600 mg of arsenic was removed from the waste water, and 18% of the removed arsenic was recovered under laboratory conditions. The report suggested that, the removal efficiency of *E. crassipes* was due to its high biomass production rate and favourable climatic conditions. Hence, *E. crassipes* represents a reliable alternative for arsenic bioremediation in aquatic system. However, the plant should be used wisely in phytoremediation, since it may result to severe water

management problems due to its huge vegetative reproduction and fast growth rate [19].

Apart from arsenic, the plant can also bio-remediate Cd and Cr, both Cd and Cr where successfully removed to 80.26% and 71.28% efficiency, respectively. The average removal rate of Cr and Cd per day was 0.10mg and 0.2mg respectively [20]. In similar report by Swain *et al.*, (2014), Cu and Cd where successfully removed by *E. crassipes* with over 90% efficiency [21]. The bioconcentration factor of Cu was higher than that of Cd, showing that the plant is a very good accumulator of Cu.

Pistia stratiotes, often called water cabbage or water lettuce is yet another aquatic plant reported for its bioremediation potential [22, 23], metal detoxification, and treatment of urban sewage [24]. Espinosa, (2001) cultured *Pistia stratiotes* in solutions amended with three different Pb levels (2ppm, 4ppm and 8ppm) and reported that the bioconcentration factor (BCF) in the plant tissue increases with increase in Pb level [1]. The report suggested *Pistia stratiotes* might be useful in Pb decontamination process in industrial and domestic wastewaters. Thilakar *et al.*, (2012) compared the use of *Pistia stratiotes* and *Salvinia natans* in remediating Cr and Cu for a period of 10 days in a shaded area [25]. Single metal solution of 25%, 50%, 75% and 100% concentrations of Cr and Cu were respectively used. It was noticed that the maximum concentration of Cr in the 100% solution was 15657ppm and 17066ppm in *Pistia* and *Salvinia* respectively, and that of copper was 74.45ppm and 54.11ppm respectively. These results indicate that both plants are efficient accumulators of Cr and Cu, hence both plant can be effectively use to clean up aquatic ecosystem. Prajapati, (2012) used *Pistia stratiotes* in the removal of Cr and Co. Both Cr and Co were completely removed after 48 hrs of administering the metal solution [12]. However, the removal efficiency of Co in 4 days reduces to 86% when a mixed solution (i.e binary solution of Cr and Co) is administered to the plant. It was concluded that, both Cr and Co can be sustainably removed by *Pistia stratiotes*. Ugya *et al.* (2015) has also given an overview for Hg, Cd, Mn, Ag, Pb and Zn removal using *Pistia stratiotes* in a stream polluted by wastewater from Kaduna refinery and petrochemical company [26].

This current work is done to investigate the bioaccumulative ability of Algae, *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* (water lettuce) in a multi component metal (Ni, Mn and Pb) solution system. The research work would aim on quantification the metal uptake by these plants. Hence, waste biomass handling would be reported else were.

2. Experimental

2.1. Preparation of Blank Solutions

Distilled water (100cm³) was measured in a sample bottle and treated as sample. Calibration standard was prepared from the stock standard, anticipating the expected concentration in the samples by dilution using a serial dilution formula.

$$C_1V_1=C_2V_2$$

$$V_1=C_2V_2/C_1 \quad (1)$$

The amount (V_1) as calculated using equation 1, was measured from the stock prepared in volumetric flask (100cm^3) and diluted to 100cm^3 mark with distilled water. Solutions of 2, 4, 6, 8 and 10ppm were prepared for each metal.

2.2. Sampling

The three aquatic plants [alga (*Spirogyra* sp.), water hyacinth (*Eichhornia crassipes*), and water lettuce (*Pistia stratiotes*)] were obtained from Sokoto metropolis ($13^\circ 3' 5''$ North, $5^\circ 13' 53''$ East), and authenticated by a curator in the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. The plant materials were then Washed, collected in a clean plastic container and kept in biological sciences botanical garden for a period of one week to acclimatize with the environmental conditions of the garden [21].

2.3. Experimental Set up

The experimental sets up for alga and water lettuce were conducted in a transparent container, while water hyacinth placed into a container of 15d m^3 capacity. Metal (Ni, Mn, and Pb) solutions of three different concentrations (1, 3, and 5 mg/dm^3) were prepared, followed by addition of nutrient in the form of Hoagland solution. The Fourth solution (the control) contained only Hoagland solution. To all the solutions, the pH was then adjusted to 6 using acetic acid [16].

The different concentration of the metal solutions were administered to the containers containing the plant materials. The weight of the plant materials was taken before and after administering the heavy metal solution. From each of the containers, 50 cm^3 of water sample was collected after 24 hrs, 5, 10 and 15 days of the metal solution administration. Exactly 5 drops of HNO_3 acid was added to each of the collected water sample (for proper storage) before AAS analyses [16].

The experiment was conducted for a period of 15 days in a shady area according to the technique reported by Banach et al, (2012). At the end of the 15th day, the plants were harvested, washed and then separated into roots and shoot which were then analysed for metal accumulation using standard techniques [10].

2.4. Determination of Heavy Metals

2.4.1. Sample Treatment

The plants were harvested on the 15th day and washed with

distilled water to remove any adhered substance, it was then separated into shoot and root and then shade dried. The dried shoot and root were grinded, sieved and stored for further use.

2.4.2. Digestion

The ashing procedure was done in order to prepare plant samples for elemental analysis [27].

2.4.3. Wet Digestion

Air dried grinded sample (1.00g) was placed in a beaker, followed by the addition of HNO_3 (10 cm^3 , 6M) and HClO_4 (2 cm^3). Each beaker was covered with a watch glass for an hour to allow the initial reaction to subside. The beakers were heated on a hot plate with the temperature not exceeding 90°C for 30 min. The contents were then filtered and transferred into volumetric flask (50cm^3) with subsequent washings and diluted with distilled water to the mark [27].

2.4.4. Aas Analysis

The alpha 4 model of Atomic Absorption Spectrophotometer was set according to the manufacturer's instructions with the wavelength corresponding to that of the metal (Ni, Mn and Pb) under investigation. Standard solutions prepared from the stock solutions and the blank were used to obtain the calibration curves. The absorbance of each sample was measured in triplicate with an automatic calculation of the average concentration in parts per million (ppm).

2.4.5. Statistical Analysis

The analysis was carried out on three independent replicates for every parameter. The results presented in Table 1 are reported as mean \pm standard deviation (SD). Data were analysed by SSPS Anova two ways considering significance at an alpha level of 0.05.

3. Results

The result of heavy metal (Ni, Mn and Pb) analysis in different parts of plants under investigation in a multi component metal solution is summarised in Table 1 and represented in Figures 1 to 3. The bioconcentration and transfer factor of the metals are shown in Tables 2 and 3.

3.1. Nickel Concentration

The variation in nickel (Ni) concentration in different parts of the aquatic plants under investigation is presented in Figure 1. Nickel concentration increases with increase in concentration of working solution from 1- 5 mg/l .

Table 1. Bioaccumulation of Heavy Metal by Different Plant Materials.

Plant (mg/kg)	Concentration (mg/l)	Heavy Metal		
		Ni	Pb	Mn
Algae (control)		ND	ND	305.62 \pm 0.01
Water hyacinth (control)	Shoot	ND	ND	710.54 \pm 0.01
	Root	ND	ND	964.17 \pm 0.01
Water Lettuce (control)	Shoot	ND	ND	2.39 \pm 0.02
	Root	ND	ND	8.21 \pm 0.76

Plant (mg/kg)	Concentration (mg/l)	Heavy Metal		
		Ni	Pb	Mn
Algae	1	10.96±4.74	250.12±2.94	195.79±0.02
	3	79.18±4.74 ^c	292.37±6.1	388.39±0.01 ^b
	5	112.76±2.28 ^b	539.46±17.06 ^c	567.30±0.1 ^a
Water hyacinth Shoot	1	12.11±0.18	90.09±0.99	37.51±1.13
	3	25.44±0.83	102.89±11.41	68.37±1.4
	5	28.04±0.76	413.99±1.62	109.94±2.76
Water hyacinth Root	1	28.04±0.54	413.99±7.71	0.29±0.11
	3	32.67±1.45	606.03±7.34 ^b	9.7±1.41
	5	113.89±3.52 ^c	737.90±10.5 ^a	32.47±0.62
Water Lettuce Shoot	1	5.05±0.02	0.49±0.12	62.89±0.2
	3	35.69±0.01	7.98±2.78	85.42±0.02
	5	27.60±0.01	15.08±1.43	119.86±0.3
Water Lettuce Root	1	31.35±0.01	398.20±0.1	183.40±0.02 ^c
	3	98.65±0.2 ^d	474.91±0.01 ^c	213.72±0.1 ^d
	5	115.21±0.1 ^a	513.94±1.12 ^d	227.30±0.01 ^c

Results are expressed as mean±SD.

Values with superscript ^(a-e) are the first five best bioaccumulating plant/plant part at alpha 0.05.

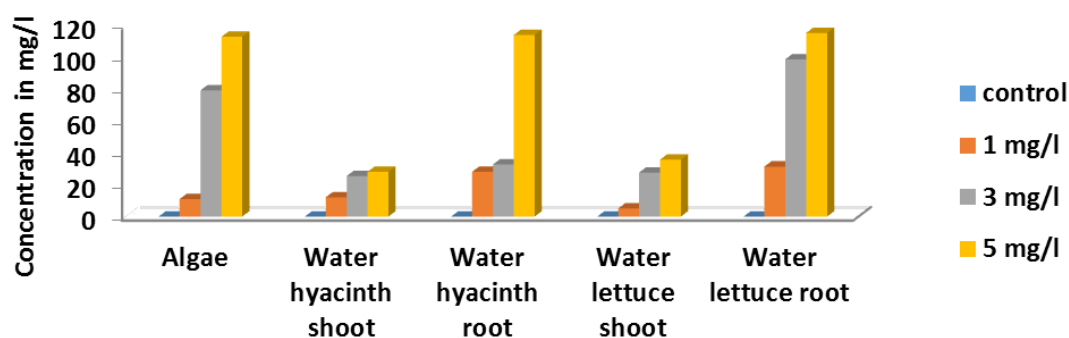


Figure 1. Nickel Concentration in Different Part of Plant Samples.

3.2. Lead Concentration

Lead (Pb) concentration increase in the plant sample with an increase in the concentration of the working solution (Figure 2). No lead (Pb) was detected in the control solution.

All the plants were able to accumulate fair amount of the lead (Pb) with water hyacinth root at 5 mg/l concentration exhibiting the highest accumulation.

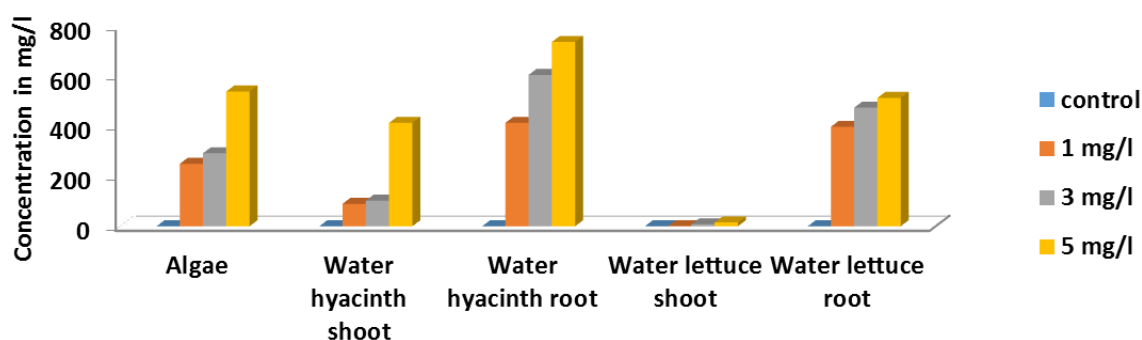


Figure 2. Lead Concentration in Different Part of Plant Samples.

3.3. Manganese Concentration

There was considerably large amount of manganese detected in control sample, and pretty good amount was accumulated by all the three plants under investigation. From

Figure 3 below, it can be observed that algae showed the highest accumulation of the metal. Manganese concentration followed similar trend like the previous metals with highest concentration in roots than shoots.

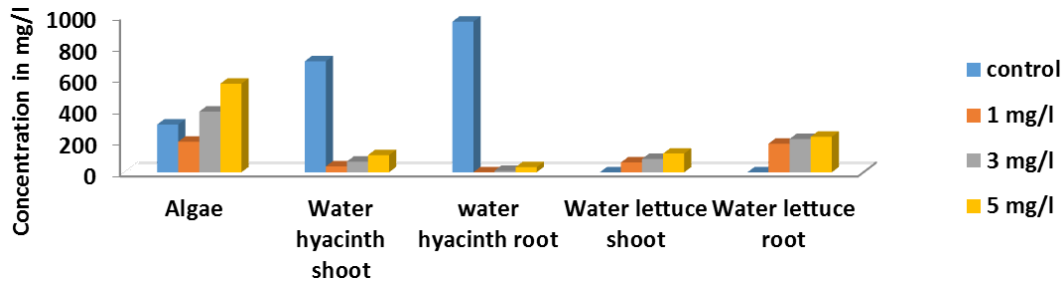


Figure 3. Manganese concentration in different part of plant samples.

3.4. Bioconcentration Factor

Bioconcentration factor is a useful parameter for assessing the potential of trace metal accumulation. When metal concentration in water increases, the amount of metal accumulated in plant increases and thus the bioconcentration factor decreases. Bioconcentration factor also provides index of the ability of plant to accumulate metal with respect to the concentration of that metal in the substrate [21]. Results in Table 1 showed that water hyacinth was able to concentrate the Ni, and Mn relative to Pb, while water lettuce was able to accumulate Pb. Larger value of the bioconcentration factor implies better accumulation capability [16].

Table 2. Bioconcentration factor of Algae, Water hyacinth and Water lettuce.

Plant	Concentration (mg/l)	Heavy metals (mg/kg)		
		Ni	Pb	Mn
Algae	1	10.96	250.1	19.79
	3	26.39	97.46	129.46
	5	22.55	107.89	113.46
Water hyacinth shoot	1	12.11	90.09	37.51
	3	8.48	34.30	22.79
	5	5.61	82.80	21.99
Water hyacinth root	1	28.04	413.99	0.29
	3	10.89	202.01	3.23
	5	22.79	147.58	6.49
Water lettuce shoot	1	5.05	0.49	62.89
	3	11.90	2.66	28.47
	5	5.52	3.02	23.97
Water lettuce root	1	31.35	398.2	183.4
	3	32.88	158.30	99.71
	5	23.04	102.79	45.46

Bioconcentration factor= a/b , where a =metal concentration in plant and b =metal concentration in water [21&26].

3.5. Transfer Factor

The Transfer factor (TF) expresses the capacity of a plant to store the metals in its upper part. This is defined as the ratio of metal concentration in the upper part to that in the roots. If $TF > 1$ it indicates that the plant translocates metals effectively from roots to the shoot [28]. Out the three metals on Mn was effectively translocated. Water hyacinth was able to translocate Mn according to results in (Table 3). Biotransfer factor shows the ability of the plant to transport metal ion in to the shoot tissues [16].

Table 3. Transfer factor.

Plant	Concentration (mg/l)	Heavy metals (mg/kg)		
		Ni	Pb	Mn
Water hyacinth	1	0.53	0.22	129.34
	3	0.78	0.17	7.43
	5	0.25	0.56	3.39
Water lettuce	1	0.16	0.001	0.34
	3	0.28	0.02	0.40
	5	0.27	0.03	0.53

Transfer factor= c/d , where c =metal concentration in shoot and d =metal concentration in root (Liao and Chang, 2004; [26].

4. Discussion

Heavy metal accumulation in plants

As seen in Figure 1 and Table 1, water hyacinth root, water lettuce root and algae shows a very good potential to be used in Nickel decontamination, although it's an essential micro nutrient [20]. The results also revealed that, the plant root has higher affinity for Nickel ion than shoot (Table 1 and Figure 1). This could probably be as a result of some physiological barriers against metal transport i.e due to the inability of the plants to translocate Ni from the root to the shoot (Table 3). In all the plants, the bioaccumulation of Nickel increases with the increase in the metal concentration (Table 1 and Figure 1). The trend of Nickel accumulation in the different plants can be seen in the following order; water lettuce root > Algae > water hyacinth root > water lettuce shoot > water hyacinth shoot.

Although Lead concentration was observed to be high in all the plants, water hyacinth root showed the highest accumulation; 737.90 mg/l (Table 1). Like in the case of Ni, the metal accumulation in root is higher compared the shoot (Figure 1). This could be attributed to the avoidance of metal toxicity by the plants, or as a result of greater surface area, hence more adsorption capacity on the roots compared to the shoots [29, 30]. This could also be as a result of poor translocation factor of the metal from the root to the shoot (Table 3). Algae in 5 mg/dm³ multi metal solution emerged second best accumulator of Pb, and the least accumulation was seen in water lettuce shoot (Figure 2). Therefore, judging from the results obtained in Table 1, water hyacinth root has the highest potential to remove lead (Pb) from waste water followed by Algae. The values obtained are in accordance with the previously reported work, although a

bit higher [14].

Moderate to low concentration of Manganese was observed in all the plants under investigation. However, a noticeably large amount was recorded in control sample, this could be as a result of its need in physiological activities since it is an essential micro nutrient for plants [20&31]. Algae recorded highest concentration having 567.30 mg/l, followed by water lettuce with 227.30 mg/l, and the least accumulation in water hyacinth root; 15.08 mg/l. The values obtained for Manganese are in good correlation with previously reported work of Al-Homaidan *et al.*, (2011) [32].

In all the plants, there exists no heavy metal in control sample except manganese (Table 1). Additionally, the heavy metal concentration increases with an increase in the concentration of the multi component metal solution (5mg/l > 3mg/l > 1mg/l). From the statistical analysis, values with different superscript in Table 1 are significantly different from one another, while those with the same superscript are not significantly different down the table for each metal respectively.

Of the metals, Lead has the highest accumulation in the following decreasing order W. H root 5ppm> W. H root 3ppm> Algae 5ppm, followed by Manganese with highest accumulation in algae 5ppm> algae 3ppm> W. L 5ppm, and Nickel is the least accumulated, with highest accumulation in Algae 5ppm>Algae 3ppm>W. L (W. H=water hyacinth, W. L=water lettuce, ppm=mg/l). In summary, the accumulation of metals under investigation decreases in the following order; Pb > Mn > Ni. Considering the ionic radius and electronegativity of Pb, the priority for its accumulation could be supported, since these factors are believed to influence metal absorption in certain ways.

There was high absorption level in all the three plants for all the metals, this signifies that the plants under investigation can serve as good remediating tool. A surplus advantage that increases their value in phytoremediation, is their ability to accumulate more than one metal at a time.

5. Conclusion

In conclusion, studying the phytoremediating potentials of algae, water hyacinth and water lettuce revealed that all the plants were able to accumulate fair amount of lead (Pb) and nickel with water hyacinth root exhibiting the highest accumulation. Algae accumulated highest amount of manganese. Hence, the plants under investigation has proven to be good accumulators of Ni, Pb and Mn, and therefore, providing a sustainable and environmentally friendly way of Ni, Pb and Mn removal from waste water. The highest metal accumulation properties shown by water hyacinth and water lettuce is attributed to their large body mass and their rooted nature, which favoured their rhizopheric activity and enhances metal uptake.

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