International Journal of Applied Chemical and Biological Sciences (2021), 2(6), 57-67
ISSN 2582-788X

RESEARCH ARTICLE

Article Identifier: https://identifier.visnav.in/1.0001/ijacbs-21k-24007/

Potential of *Pistia stratiotes*, *Eichhornia crassipes* and algae for removing Cobalt (Co) and Nickel (Ni) at various concentrations

Amtul Bari Tabinda, Iqra Azam *, Mehak Shahid, Abdullah Yasar, Rizwan Rasheed

* Sustainable development study center GC University, Lahore, Pakistan

Corresponding Author: iqraazam.ravian@gmail.com

ABSTRACT

Phytoremediation is a new emerging discipline to de-contaminate polluted water. Remediation of pollutants by plants is environmental friendly and cost effective technique. Present study focuses on ability of *Pistia stratiotes, Eichhornia crassipes* and algae for removing nickel (Ni) and cobalt (Co) from aquatic system. Efficiencies of these macrophytes were checked against Ni and Co (2mg/l and 4mg/l concentration for each metal) on lab scale under natural conditions for a period of fifteen days. Samples were collected and analyzed at 0, 5, 10 and 15 days. One-way ANOVA was applied to compare significance level of these plants for removal of these metals. *P. stratiotes, E. crassipes* and algae removed 90.5%, 90.6% and 87.5% at 2mg/l Ni concentration, while 94.55%, 98.1% and 80.25% respectively at 4mg/l Ni from water. Similarly, *P. stratiotes, E. crassipes* and algae removed 95.5%, 98% and 80% Co metal at 2mg/l concentration, but 91.5%, 99% and 79.75% respectively at 4mg/l concentration of Co. Results indicate that E. crassipes is more efficient for removal of both the metals at $p < 0.005$. There was no change in color and biomass of these macrophytes but biomass of algae increased in both treatments (2 and 4mg/l) for both metals during 15 days' period.

Keywords: Phytoremediation, Environmental friendly, *Pistia stratiotes, Eichhornia crassipes*, Algae

1. INTRODUCTION

Pollution of heavy metals is the global concern particularly in water and soil. All the countries become affected by this major problem. Heavy metals contain high molecular weight as compared to other elements and consider as important contaminant in the environment [1].

Heavy metals are the elements that are metallic in nature and their density is much higher than the density of water when we compared both the atomic number of metallic elements are more than 20 heavy metals as a contaminant which are mostly present in our environment are chromium, copper, nickel, zinc, mercury, lead, cobalt [2]. Some metals are important for plant growth in a minute quantity but if their amount increase in the plants then it's not possible for plant to survive [3].

Conventional methods are costly to treat water such as ion exchange and solvent extraction etc. and their results are often in effective and determine high input of energy and then huge amount of money as an operational cost. This method of phytoremediation is much effective and require less energy and less money and operational cost is also very much low [4]. These microorganisms such as bacteria, algae and fungi they also show much high affinity and uptake capacity to accumulate the heavy metals and other metalloids [5].

Different macrophytes has different tendency to absorb heavy metals. Each plant shows the different efficiencies in bioaccumulation of heavy metals [6]. Phytoremediation is the technique in which use the green plants and its connected microorganisms, modification in soil and agronomic strategies to remove the contaminants from the environment. It is an attractive remedial approach due to its cost effectiveness as compared to other remedial techniques [7]. So, choose from appropriate remediation method for certain type of pollutant is necessary. In phytoremediation for specific type of metal specific type of macrophytes becomes selected [8]. Due to higher bioaccumulation of heavy metals in the environment they become more hazardous. The heavy metals bio accumulates in the living organisms and then metabolized and become the part of its biomass or released into the environment [9]. Metal accumulation in the soil and contamination of heavy metals in water leads to reduction in yield, microbial plant growth and then enter into food chain then causes adverse effects on human health and on the environment [10].

Although the contribution of metals to the aquatic ecosystem sometimes comes from natural sources such as geochemical origin some have, but as some add through the natural system but the one that is most disastrous and toxic to the ecosystem where aquatic species live are from anthropogenic activities most of the discharges are from there and some also include discharges from industries, agriculture and urban land runoff and some of its part come from mining sources. It is the need of time that we restore those places or things which are disturbed by human activities, water should be treated by using macrophytes and this will be helpful in getting clean water and to avoid human from various health risks [11] heavy metals can easily accumulate into the macrophytes like *Pistia stratiotes, Eichhornia crassipes* and algae.

Eichhornia crassipes is the native of South America. It is freshwater plant and mainly present in all the regions in summer season. Due to its flowering appearance this plant used as ornamental plant in all over the world. It is estimated that this plant has ability to absorb the lead, cadmium, nickel, copper, zinc and cobalt [12]. *Pistia stratiotes* is the free-floating aquatic plant that contains hair like roots that extend up to 50 centimeters. This plant contains fleshy leaves arranged in rosette like structure. *Pistia stratiotes* is specie that is used in accumulating the metal it is a hyper accumulator that accumulates the different metals, radio nuclides and other heavy metals also from water. It clears the polluted aquatic system from the metals that are hazardous for human health and fro other species also [13]. *Pistia stratiotes* is considered best plant for the bioremediation. This plant species is also used for the test of different compounds it has some value for quinolone but it is not considering best or it might be less effective for sulphonamide [14].

2. METHOD AND MATERIALS

2.1. Collection of Plants

Eichhornia crassipes, Pistia stratiotes and algae were gathered from Government University Botanical Garden, Lahore, and Jallo Park Lahore Botanical Garden. Plant species were gathered aseptically in plastic containers and brought them to the Laboratory of Sustainable development study center (SDSC), Government College University, Lahore. The plant specimens were carefully washed in the laboratory with the tap water, to remove sludge, dust and other debris from the floor. These two aquatic plants and algae become used to regulate the capacity of two separate concentrations to remove heavy metals.

2.2. Preparation of heavy metals solutions

Cobalt (Co) and Nickel (Ni) solutions were prepared at two distinct volume of 2 mg/l and 4 mg/l levels using their normal solutions.

2.3. Preparation of micro and macro nutrients

Macro and micro nutrients have become very essential to plant food necessities because without them they cannot work properly. For this purpose, each macro and micro nutrient was prepared in 500 ml of water and subsequently infused nutrients using the Clark technique. About, six micro nutrients were prepared. These micro nutrients are: 0.5mM copper sulfate ($CuSO_4.5H_2O$), 7mM Manganese sulfate ($MnSO_4.H_2O$), 2mM Zinc sulfate ($ZnSO_4.7H_2O$), 4mM iron chloride ($FeCl_3.6H_2O$), 0.0086mM ammonium molybedate $(NH_4)6MO_7O_{24}.4H_2O$, 19mM boric acid (H_3BO_3) [15,16].

The macro nutrients were: 1 M potassium nitrate (KNO_3), 1 M ammonium nitrate (NH_4NO_3), 1 M potassium chloride (KCl), 1 M magnesium sulphate ($MgSO_4.7H_2O$) and 1 M calcium nitrate ($Ca(NO_3)_2.4H_2O$), 0.023 M potassium hydrogen phosphate (K_2HPO_4) [16].

2.4. Experimental set-up

About , 18 containers with 15 liters was used for each sample which were filled upto 13 liters of water. 12 macro and micro nutrients were added to baskets (1 ml of each in 1 liter of water). Nutrient quantities are required to satisfy the dietary significance of the plant [16-17]. To each basket, 180 g of each aquatic plant were placed. These plants were kept in containers for 20 days in order to achieve better results. For each concentration, one replicate had also been put in the experimental basket for each plant so that which plant is still working or not running. Two containers were used to regulate plants and water, and water did not contain metals [17].

2.5. Water analysis

The collection of water and plant specimens was done on interval of every 5 days (0, 5, 10, 15) till fifteen day. About 20ml of water sample was collected on every fifth days and then the sample's pH and DO (dissolved oxygen) was analyzed using pH sensor and DO meter. Nitric acid was added to lower the water's pH by less than 2 and then have water samples for heavy metal evaluation [17].

2.6. Plant samples analysis

The plant sample was collected at an interval of every five days from the containers and then washed under running tap water. The plant samples were separated them into the leaves and roots and if the stem of any plant available and then store at 0 °C for the chemical analysis also for digestion of plant. Then the plant samples of *Pistia stratiotes, Eichhornia crassipes* and algae from 0, 5, 10 day were stored into petri dish and then place it into oven for 24 hours at 60 °C.

2.7. Plants digestion

For digestion process of plants, nitric acid and per chloric acid (3:1) was used and placed on hot plate for digestion. After digestion of plant material, it was diluted with 50 ml of distilled water. The solution was filtered to get a clear solution into volumetric flask and then 5-7 ml of each sample was stored into airtight bottles. The pH of samples was adjusted (7-8) before testing into the atomic absorption spectrophotometer. Removal of both metals Ni and Co in percentage by using these macrophytes and algae was determined using the Equation (1).

$$\text{Percentage removal} = Ci - Cf \qquad \text{....eq. 1}$$

Where:
Ci = concentration of metal at initial point in water media
Cf = concentration of metal at final point which remains in water media.

2.8. Heavy metals analysis

The collected plant samples were checked on every fifth day. The amount of heavy metal absorbed by plant was analyzed and also which part of plant has the most capability to absorb metals. The calculation of translocation factor (TF) of both metals Ni and Co from roots of macrophyte to the leaves of that macrophyte was done by using the formula:

$$TF = \frac{Cl}{Cr} \qquad \text{...eq. 2}$$

Where:
Cl=metal concentration in leaves of macrophyte
Cr=metal concentration in roots is Cr

The value of Translocation Factor which is less than 1 depict efficient transfer of metals from the roots of macrophytes to the leaves and also in above ground parts of both macrophytes [18]. BAF is the bioaccumulation factor of metal that is determined by using formula:

$$BAF = \frac{Cp}{Cm} \qquad \text{...eq. 3}$$

Where:
Cp= Metal concentration in plant
Cr= metal that is present into the medium

BAF of macrophytes higher than 10 categorized as hyperaccumulator and Bio Accumulation Factor greater than 1 called as bio accumulators [19].

2.9. Parameters to check quality of water

All the samples of water were tested in laboratory of the Sustainable development study center. The BOD and pH of the samples were tested on daily base for checking of temperature by using standard laboratory thermometer and for pH used the pH strips and also used pH meter (pH 300 YSI) for the measurement of pH of samples and to check the DO value of the sample DO EUTECH was used [20].

2.10. Statistical analysis

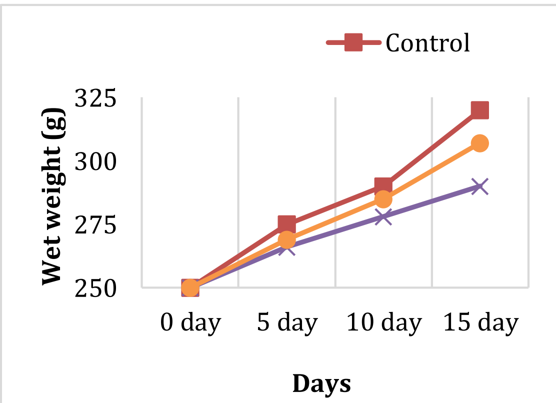
The data was used for statistical analysis that is one-way ANOVA with multiple comparisons between both macrophytes and algae by using SPSS 16.0. In this analysis, 0.05 is taken as a significant value.

3. RESULTS AND DISCUSSION

The wet weight and biomass of the macrophytes and algae were greater than before after a time of fifteen days and also not a huge change seen in the color of macrophytes and algae. Initially weight of *Pistia stratiotes, Eichhornia crassipes* and algae were taken 250g before insert into metal solutions. The wet weight of these plants and algae become increased from 5th day to 15th day by the significant difference. The solution with increase concentration of metal shown more increase in weight of plants and algae. This shows increase in metal concentration becomes increase the wet weight of aquatic plants. Significant increase in wet weight over fifteen days' time period shows in Figure 1.

The translocation factor (TF) and bioaccumulation factor (BAF) of both macrophytes and algae are shown in Table 1 and Table 2.

During phytoremediation the metals become



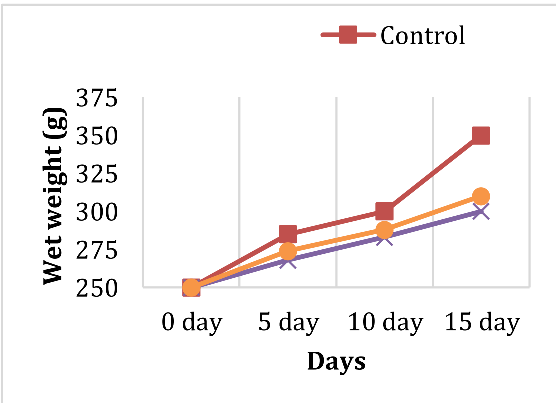
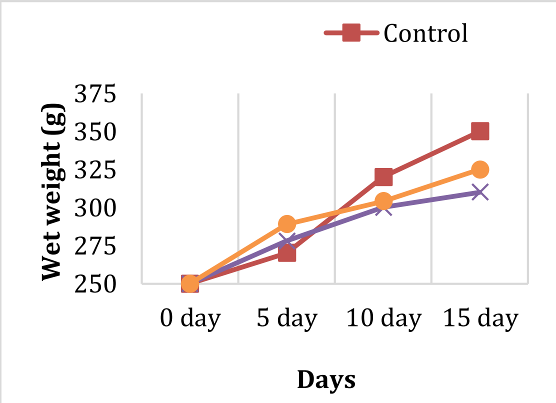


Figure 1. (Left) Wet weight of Eichhornia crassipes at 2 mg/l and 4 mg/l Ni concentration

Figure 2. (Right) Wet weight of Pistia stratiotes at 2 mg/l and 4 mg/l Ni concentration



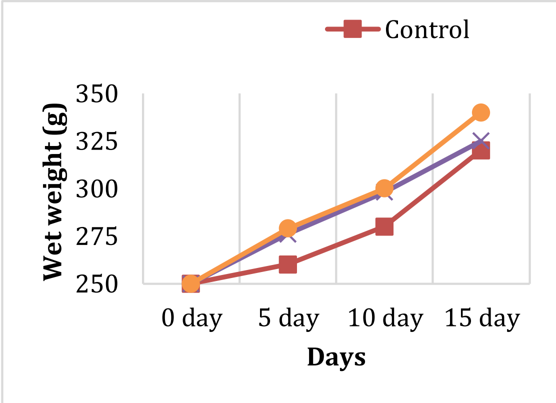
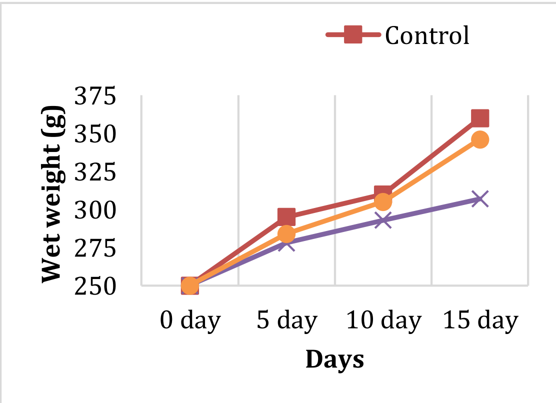


Figure 3. (Left) Wet weight of algae at 2 mg/l and 4 mg/l Ni concentration

Figure 4. (Right) Wet weight of Eichhornia crassipes at 2 mg/l and 4 mg/l Co concentration



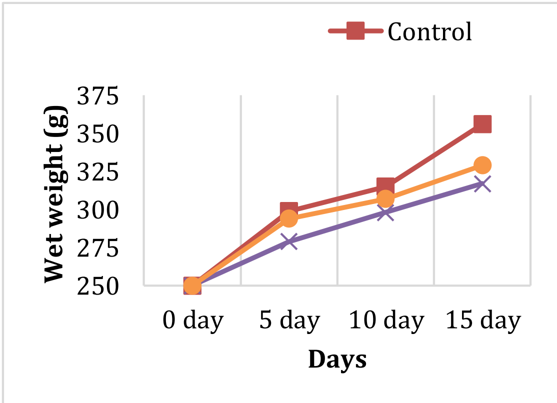


Figure 5. (Left) Wet weight of Pistia stratiotes at 2 mg/l and 4 mg/l Co concentration

Figure 6. (Right) Wet weight of algae at 2 mg/l and 4 mg/l Co concentration

accumulated mostly in roots and less in leaves of Pistia stratiotes and Eichhornia crassipes. Pistia stratiotes absorbed 44.52mg of Ni in its leaves and 48mg in its roots. Eichhornia crassipes absorbed 19.91mg in its leaves, 43.38mg in its roots and 31.05mg in its shoots. Pistia stratiotes accumulated 34.12mg of Co in its leaves and 59.87% in its roots. Eichhornia crassipes accumulated 23.6mg in its leaves and 48mg in its roots and 30.6mg in its shoots. So, in both cases roots absorbed more metals as compared to other aerial parts of aquatic plants (Figure 2). According to Sinha *et al.* (2002) and Suseela *et al.* (2002), the fact behind this reason was slow mobility of heavy metals from roots to other parts of plants [21-22]. Rulangaranga (2003) also used these aquatic plants for removal of heavy metals and reported high concentration was absorbed in roots of plants [23]. Miretzky *et al.* (2004) reported that high concentration of metals accumulated in roots of *Pistia stratiotes* [24]. Odjegba (2007) also reported the metals accumulated in roots of aquatic plants as compared to leaves and shoots [18].

Table 1. TF of both macrophytes and algae

TF	*Pistia stratiotes*		*Eichhornia crassipes*	
	Co	Ni	Co	Ni
	0.59	0.93	0.54	0.45

Table 2. BAF of both macrophytes and algae

BAF	*Pistia stratiotes*		*Eichhornia crassipes*		algae	
	Co	Ni	Co	Ni	Co	Ni
	19.1	13.4	74.1	30.6	3.99	5.53

The amount of Ni removed from *Pistia stratiotes* is almost 92.25%, and from *Eichhornia crassipes* it is almost 94.35% whereas from algae it is 83.87%. Likewise, the amount of metal Co remove from water by *Pistia stratiotes* is up to 94%, from *Eichhornia crassipes* is up to 98% and from algae removal is up to 79.87% (Figure 3).

In *Eichhornia crassipes* absorbance rate of Ni and Co is considerably high ($p<0.05$) in comparison to *Pistia stratiotes* and algae.

In macrophytes no change came in their morphology in fifteen days instead of change macrophytes and algae flourished and gained more weight when experiment accomplished.it is testified that when plant material gained much weight this is showing that it is absorbing metal much faster [13]. *Eichhornia crassipes* having a large biomass and broad leaves that spread on water easily and absorb metal in a good manner that's why consider as a best phytoremediation macrophyte [25]. These macrophytes are classified as bio-accumulator or hyper-accumulator depend on their tendency that how much amount of metal a macrophyte can absorb and also depend on the part of the plant that which part is absorbing more as root

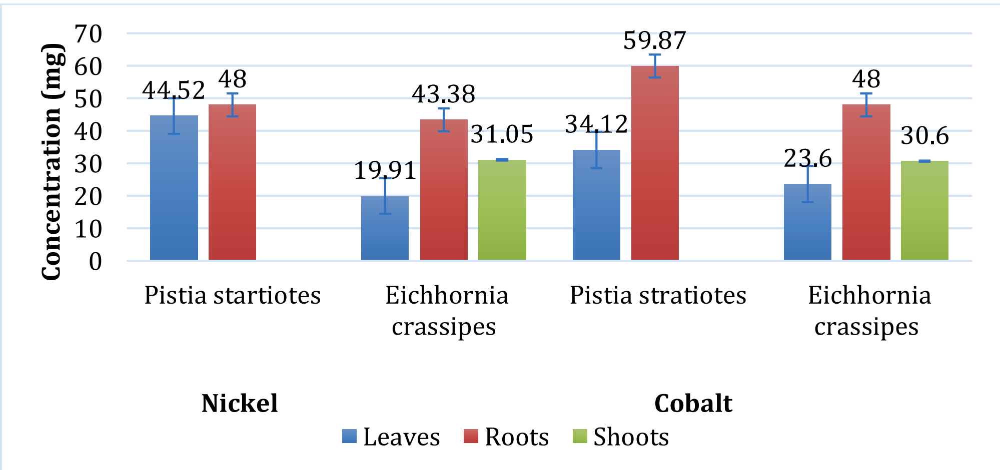


Figure 7. Mean concentration of Ni and Co accumulated in leaves, roots and shoots of Pistia stratiotes and Eichhornia crassipes

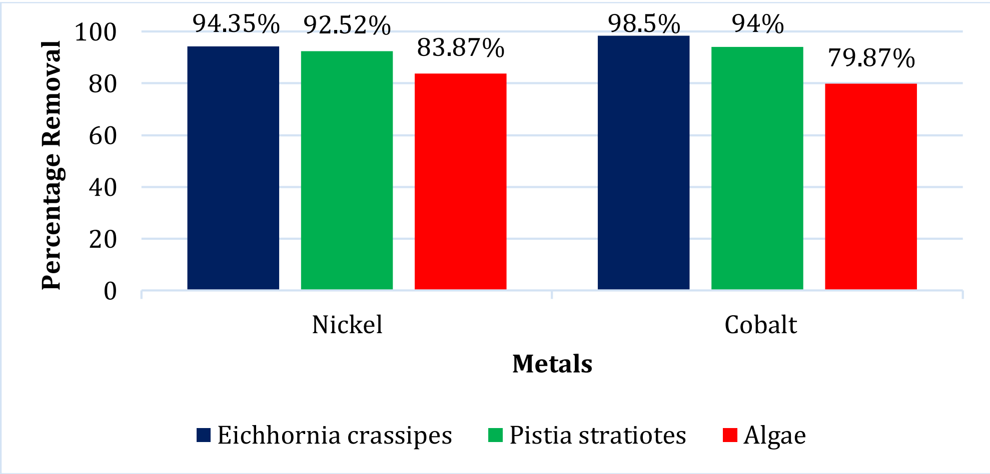


Figure 8. Percentage removal of Ni and Co by Pistia stratiotes, Eichhornia crassipes and algae

is mostly consider as hyper-accumulator because it absorbs more metal than the other parts of the macrophytes [24]. *Eichhornia crassipes* is consider as a best macrophyte it can absorb maximum amount of metal also it has large biomass as well as broaden leaves.

Bio-accumulation factor of both macrophyte and algae was greater than 1 which is showing that they have the capability to accumulate the metals in them some are simply bio-accumulators where as some parts are hyper-accumulators as well translocation factors of both macrophytes also calculated which shows that these plants roots have capability to translocate that metal into the upper part of macrophyte.

One-way ANOVA was also used for the data which shows the potential of macrophytes and algae among them potential of *Eichhornia crassipes* ($p<0.05$) considerably high [26] than the *Pistia stratiotes* and algae for metal Co. Whereas *Pistia stratiotes* also shows significant result for the metal Co but its more efficiency seen for the metal Ni .This shows that these macrophytes and algae has the capability to absorb these metal very well although algae can absorb less amount than macrophytes but it can also has the capacity to absorb metals. More than 90% of the heavy metals were absorbed by these macrophytes [27]. These macrophytes can be used in more than one cycle depending on their condition. As these macrophytes are showing good results we can use them into the aquatic environment where a large amount of metals is present and that are harmful for the species of aquatic habitat [28]. These macrophytes also used as bio monitors [29] and rhizofiltration [30] can also be done through them.

4. CONCLUSION

The aquatic plants and algae indicated high efficacy for Co and Ni removal from different metal solutions. Extreme of both of metals Ni and Co was indicated into the roots of macrophytes other than leaves and shoots. Translocation factor for these macrophytes is also less than 1 which showing their higher efficiency. BAF of both macrophytes and algae is higher than 10 that show they act as hyper-accumulator. The comparison between both plants and algae showed that accumulation rate for the metal Co and Ni was greater in *Eichhornia crassipes* and then in *Pistia stratiotes* and then in algae without any physical change in color and increase in wet weight. Thus these aquatic macrophytes and algae become used for the treatment of metal contaminated sites, waste water and industrial effluents that contain metals.

5. ACKNOWLEDGEMENT

NA

6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

7. SOURCE/S OF FUNDING

NA

8. REFERENCES

1. Wang, W., Dei R. (2011). Metal stoichiometry in predicting Cd and Cu toxicity to a freshwater green alga Chlamydomonas reinhardtii. *Environmental Pollutant*. **142 *(2)*:**303-312.
2. Prajapati S., Meravi N., Singh S. (2012). Phytoremediation of chromium and cobalt using Pistia stratiotes: A sustainable approach. *Proceedings of the International Academy of Ecology and Environmental Sciences*. **2 *(2)***: 136-138.
3. Newete S., Byrne M. (2016). The capacity of aquatic macrophytes for phytoremediation and their disposal with specific reference to water hyacinth. *Environment Science and Pollution Research*. **23**:10630-10643.
4. Gupta, K., Sriwastav, A., Ansari, F.A., Nasr, M., Nema, A.K. (2017). Phytoremediation: an eco-friendly algal technology for bioremediation and bioenergy production. In: Bauddh, Singh, Korstad (Eds.), *Phytoremediation Potential of Bioenergy Plants*. 431–456.
5. Singh D., Tiwari A., Gupta R. (2012). Phytoremediation of lead from wastewater using aquatic plants. *Journal of Agricultural Sciences and Technology*. **8**:01–11.
6. Souri Z., Karimi N., de Oliveira L. (2017). Antioxidant enzymes responses in shoots of arsenic hyper accumulator, Isatis cappadocica Desv. under interaction of arsenate and phosphate. Environment Technology.
7. Njagi J., Akunga D., Njagi M., Ngugi M., Njagi E. (2016). Heavy metal pollution of the environment by dumpsites: a case of Kadhodeki Dumpsite. International *Journal of Life Sciences and Scientific Research*. **2*(2)***: 191-197.
8. Putra R., Cahyana F., Novarita, D. (2015). Removal of lead and copper from contaminated water using EAPR system and uptake by water lettuce (Pistia stratiotes). *Procedia Chemistry*. 381-386.
9. Valipour A., Raman V., Ahn Y. (2015). Effectiveness of domestic waste water treatment using a bio-hedge water hyacinth wetland system. *Water Research*. **7**:329-347.
10. Zhou G., Peng F., Zhang, L., Ying G. (2012). Biosorption of zinc and copper from

aqueous solutions by two freshwater green microalgae Chlorella pyrenoidosa and Scenedesmus obliquus. *Environmental Sciences and Pollution Research*. **19 *(7)***:2918–2929.

11. Rao M., Acharya Y., Bala A., Paramben S., Sowmya K., and Raj B. (2017). Study of heavy metals in abnormal growth and development using an alternate animal model: Heterometrus fulvipes. *International Journal of Life Sciences and Scientific Research*. **3*(1)***:800-807.
12. Qin H., Zhang Z., Liu M., Liu H., Wang Y., Wen X., Zhang Y., Yan S. (2015). Analysis of the death causes of water hyacinth planted in large-scale enclosures in Dianchi Lake. *Environmental Research. Yangtze*. **24 *(4)***: 594–602.
13. Smolyakov S. (2016). Uptake of Zn, Cu, Pb, and Cd by water hyacinth in the initial stage of water system remediation. *Applied Geochemistry*. **27 *(6)***: 1214-1219.
14. Ojoawo S., Udayakumar G., Naik P. (2015). Phytoremediation of phosphorus and nitrogen with Canna x generalis reeds in domestic wastewater through NMAMIT Constructed wetland. *Aquatic Procedia*. **4**:349–356.
15. Victor K., Seka Y., Norbert K. (2016). Phytoremediation of wastewater toxicity using water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes). International *Journal of Phytoremediation*. **18*(10)***:949-955
16. Tabinda A.B., Rabia I., AbdullahY., Anum I., & Adeel M. (2018). Phytoremediation potential of Pistia stratiotes and Eichhornia crassipes to remove chromium and copper. *Environmental Technology*.
17. Mokhtar H., Morad N., Fizri F. (2011). Hyperaccumulation of copper by two species of aquatic plants. *International Conference on Environment Science and Engineering*. **8**:115-118.
18. Odjegba V., Fasidi M. (2007). Changes in antioxidant enzyme activities in Eichhornia crassipes (Pontederiaceae) and Pistia stratiotes (Araceae) under heavy metal stress. *Journal of Biological Research*. **55**: 815-823.
19. Baker AJM, Brooks RR (1989). Terrestrial higher plants which hyper accumulate metallic elements-a review of their distribution, ecology and phytochemistry. *Biorecovery*. **1**:81–126.
20. Ma, L. Q., Komar, K. M., Tu, C., Zhang, W., Cai, Y., & Kennelley, E. D. (2001). A fern that hyperaccumulates arsenic. *Nature*, **409*(6820)***, 579-579.
21. Sinha S, Saxena R, Singh S (2002). Comparative studies on accumulation of Cr from metal solution and tannery effluent under repeated metal exposure by aquatic plants: its toxic effects. *Environ Monitoring Assessment*. **80*(1)***:17–31.
22. Suseela, M. R., Sinha, S., Singh, S., & Saxena, R. (2002). Accumulation of chromium and scanning electron microscopic studies in Scirpus lacustris L. treated with metal and tannery effluent. *Bulletin of environmental contamination and toxicology*, **68*(4)***, 540-548.
23. Rulangaranga ZK, Mugasha AL (2003). Effects of chromium uptake on the growth characteristics of Eichhornia crassipes

(mart.) solms. *Tanzania Journal of Science*. **29*(1)***:109–125.

24. Miretzky, P., Saralegui, A., & Cirelli, A. F. (2004). Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina). *Chemosphere*, **57*(8)***, 997-1005.
25. Odjegba VJ, Fasidi IO (2004). Accumulation of trace elements by Pistia stratiotes: implications for phytoremediation. *Ecotoxicology*. **13*(7)***:637–646.
26. Li, Q., Zhan, J., Chen, B., Meng, X., & Pan, X. (2016). Removal of Pb, Zn, Cu, and Cd by two types of Eichhornia crassipes. *Environmental Engineering Science*, **33*(2)***, 88-97.
27. Mishra, V. K., & Tripathi, B. D. (2009). Accumulation of chromium and zinc from aqueous solutions using water hyacinth (Eichhornia crassipes). *Journal of Hazardous Materials*, **164*(2-3)***, 1059-1063.
28. Upadhyay, A. R., Mishra, V. K., Pandey, S. K., & Tripathi, B. D. (2007). Biofiltration of secondary treated municipal wastewater in a tropical city. *Ecological engineering*, **30*(1)***, 9-15.
29. Pajevic, S. P., Kevresan, Z. S., Radulovic, S. B., Radnovic, D. V., Vuckovic, M. S., & Matavulj, M. N. (2003). The role of macrophytes in monitoring the impact of heavy metal effluents on the aquatic environment. *Central European Journal of Occupational and Environmental Medicine Hungary*.
30. Agunbiade, F. O., Olu-Owolabi, B. I., & Adebowale, K. O. (2009). Phytoremediation potential of Eichornia crassipes in metal-contaminated coastal water. *Bioresource Technology*, **100*(19)***, 4521-4526.