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Removal Efficiency, Accumulation and Toxicity of Nickel and Lead using *Ceratophyllumdemersum*

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ABSTRACT: This study involved a laboratory experiments were done for 21st days to investigate the ability of *Ceratophyllumdemersum* to removed, accumulate of nickel and lead from aqueous solution with known concentrations, and study some toxic effects of these metals on it. The results indicate that the *C.demersum*was able to removed, and accumulate Ni more than Pbat the same concentrations and experiments conditions. However, other parameters such as fresh weight, relative growth, total chlorophyll, protein content, tolerance index rate varied in their effects among metal concentrations, metals, and control treatments. The study concluded that *C. demersum* is a good candidate to removed metals from polluted water.

KEYWORDS: Phytoremediation, heavymetals,*Ceratophyllumdemersum*.

I.INTRODUCTION

Pollution by heavy metals poses a threat to the environment and humans through its accumulation in the food chain. One of the promising method for reducing heavy metals from both polluted soil and water is phytoremediation. Phytoremediation is a cheap, cost effective clean-up technique that used various types of plants and associated microorganisms to remove, sequestrate and degrade of pollutants including heavy metals (Pilon-Smits and Freeman, 2006; Stephenson, 2014)). Heavy metals are one of the most dangerous pollutants in the environments, which threaten ecosystem and human health (Danh et al., 2009). Besides the natural sources of heavy metals such as volcanic eruption and geological activity of the earth crust and weathering processes causes transport of large amount of different metals in the environment (Callender, 2003), the anthropogenic sources produce huge amount of heavy metals (Hadia and futekar , 2009). The term heavy metals refers to any metallic element that has the atomic number more than 20 and have relative density more than 4 g/cm3 or 5 times greater than water and are toxic even in low concentration (Hawkes 1997; Sood et al., 2011).These pollutants are common in aquatic ecosystems which is considered as final receptor of them and many traditional technologies have been used to remove and cleanup from aquatic medium, including reverse osmosis,electro dialysis, ion exchange, adsorption(Alyuz and Veli, 2009; Chorom et al., 2012)etc. Which are expensive, need high energy, maintenance and treated specific amount of metals (Mandakini et al., 2016). Phytoremediation is an green emerging technology depends onusing plant root system for treating, translocation, bioaccumulation and degradation wide range of contaminants (Sharma and pandey, 2014). Aquatic macrophytes well known as heavy metals accumulators as they can take up large amounts of metals from contaminated water and soil. Thus, Heavy metals are toxic to plants when exceeding certain thresholds, so plants tolerance to heavy metals must be considered in phytoremediation projects.

Many studies focusing on the capacity of different floating merged and submerged aquatic plant to treated different metals(Rai, 2008) such as submerged, rootless *Cereatophyllumdemersum* L(coontail) which conceded as cosmopolitan species in distribution(Chen et al., 2015). It grows fast in shallow and muddy watersheds and some studies have shown that *C. demersum*is an effective biosorbent for Pb , Cu, and Zn and nickel phytoremediation of industrial wastewater by coontail is applicable.(Chorom et al., 2012;Krems et al., 2013). This plant also acts as biofilter for Cd and Ni (Aravind and Prasad, 2005; Mishra et al., 2006), therefore the present study was performed the metal removal capacity of *Cereatophyllumdemersum* to remediate Nickel and Lead from aqueous solution in laboratory experiments. Mishra et al., (2006)explained that coontail plants exposed to various concentrations of Lead exhibited both phytotoxic and

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tolerance responses. The specific responses were function of concentration and duration. Chen et al., (2015) studied a hydroponic study was conducted to investigate the lead bioaccumulation and tolerance characteristics of *Ceratophyllumdemersum* L. exposed to various lead concentrations.

II. METHODOLOGY

Collection and acclimatization: The plant was collected from East Hammarmarsh, after washed with marsh's water; they put in labelled polyethylene plastic bag and bring to the laboratory, washed several times with tap water to remove any solid particles and organisms. After that, the plants placed in plastic aquarium capacity of 20 litre filled with tap water for one-week acclimatization.
Reagents: A stock solution of lead Pb and Nickel Ni (1000 mg/l) were prepared by dissolved lead Nitrate Pb $(NO_3)_2$ and Nickel chloride (NiCl2).6H2O respectively in deionized water and diluted to get the desired concentration 2, 5, 10, 15, 20, 30 mg/l.
Experimental setup:
After one week of the plant acclimatization, the healthy plant of 25 g were exposed to each concentration 2, 5, 10, 15, 20, 30 mg/l of lead Pb and Nickel Ni, and plant in water without metal concentration served as control. The experiment was performed as triplicate that harvesting each weeks in plastic aquarium containing 2litre of metal concentration in the laboratory condition that 18-22o C, the pH of solution was adjust to 6.5- 7 , the plants were harvested in 7^{th}, 14^{th} and 21^{st} days .

Removal efficiency: The removal efficiency percentage of metal calculated by the equation according to Khellaf and Zerdaoui(2009)

Removal efficiency = $\frac{intialconcentration - Finalconcentraton}{Intialconcentration}$ x 100

Heavy metal concentration in plant tissue: The harvested plant were dried at 80^{o}C for 48hrs until constant weight. 0.5 g of grinded plant digested according to ROPME (1982) with 4:1 HNO3:HClO4, the digested sample were analyse for determined of lead and nickel concentration by using Atomic absorption spectrophotometer.
Bioconcentration factor: this ratio determined by the concentration of metal in plant tissue per initial concentration of external solution as describe in Zayed et al.(1998).
BCF = $\frac{Concentrationofmetalindriedplant}{IntialConcentrationofmetalinsollution}$

Total Chlorophyll content: Chlorophyll content was determined according to Arnon method (1949) , the chlorophyll extract using 0.5 g of fresh weight with 20 ml of 80% acetone , centrifuged at 5000 rpm for 5 minutes for removed remaining particles , the absorbance of extracted solution was determine at 645 and 663 nm by using spectrophotometer .
Total chlorophyll = (12.7 * OD 663) + (16.8 * OD 645)

Protein estimation: the protein content in dried plant samples was determined by estimation of total nitrogen then multiply with factor 6.25 to estimate the protein as percentage value.

Fresh weight and dry weight estimation: harvested plant at 7^{th}, 14^{th} and21^{st} day exposure washed with distilled water then filtered to remove water; the plant weighted using satoriousbalance to determine the fresh weight in gram. The plant dried at 70^{o}C in oven for 48 hrs to constant weight as dry weight (gram).

Tolerance index rate
Tolerance index rate is a tool that measuring the ability of plant to tolerate metal stress that determined by comparing the dry biomass of plants in metal treatment with the control treatment using the following equation described in Wilkins (1978):
Tolerance index = $\frac{biomassintreatedplant}{Biomassofcontrolplant}$ x 100

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III. EXPERIMENTAL RESULTS

Removal efficiency calculation: The result showed (tab.1) that the *C. demersum* was more efficient to remove Ni than Pb.The highest removal efficiency at 2 mg/l for both Ni and Pb, the statistical analysis indicated that there were significant difference at some concentrations ($p < 0.05$).

Tab. 1: mean removal efficiency ±SD of Ni and lead using *C.demersum*

Concentrations mg/l	Ni	Pb
2	79.07 ± 0.62 a	77.00 ± 1.0 b
5	75.14 ± 0.44 a	73.00 ± 2.64 b
10	70.11 ± 0.80 a	70.66 ± 1.15 b
15	66.07 ± 0.17 a	69.00 ± 0.01 b
20	64.92 ± 0.03 a	56.01 ± 0.57 b
30	58.11± 0.45 a	52.66 ±0.58 b

The different letters (between columns) refer to significant difference (p<0.05)

Concentration in the plant tissue: The result showed that the concentration in the plant tissue were increased with increasing period of experiments with significant difference at (p<0.05) as shown in figures1&2 below.

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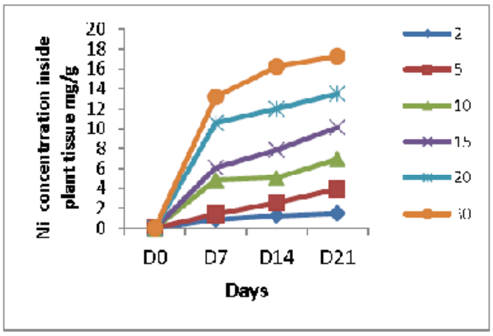

Fig.1: Mean concentration of Ni inside *C. demersum*

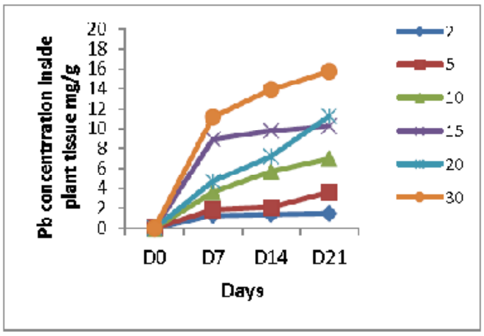

Fig2: Mean concentration of Pb inside *C. demersum*

Bioconcentration factor (BCF): The Bioconcentartion factor is an effective tool to investigate the ability of plants to accumulate the heavy elements in their tissue. The results indicated that the *C. demersum* was more efficient to accumulate Ni than Pbat the same condition and concentrations and the highest BCF was 744 and 738 mg/kg at 2mg/l as shown in Fig3 with significant difference among concentrations.

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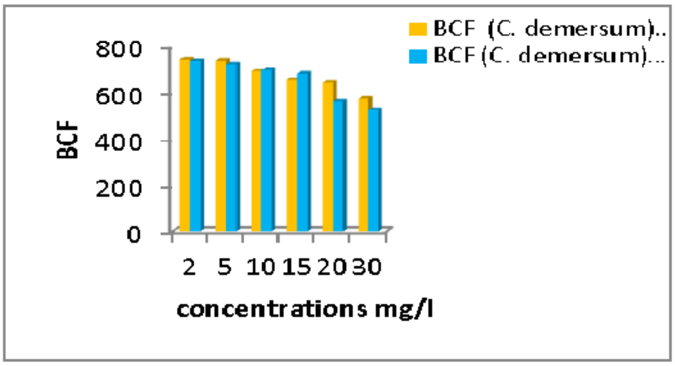

Fig.3: BCF of Ni and Pb in *C. demersum*

Effect on fresh weight and relative growth: Various concentrations of Ni and Pb used in the experiments showed gradual reduction in fresh weight and relative growth with significant difference at ($p<0.05$).The reduction increased with increasing metal concentration, period of experiments and control treatment (stay healthy), while it was observed that the reduction by lead was more than the reduction in Nickel at the same concentration and experiment condition as shown in table2.The reduction of fresh weight and relative growth may be due to many reasons: firstly, the metals effect on the components of plasma membrane causing change in the functional and structural status of it which result inhibition in transportation of liquid between cell and surrounding environment, secondly production of the free radical causing leaching of lipid peroxidation of the membrane and lastly changing in the enzyme activity as a result of metal stress. The plant showed reduction in biomass when grown in contaminated water. As indicated by Razinger et al. (2007) plant, which is considered as a good accumulator, must have a BCF over 1000.

Table 2 : mean ± SD of fresh weight and relative growth after 21st of exposure of *C. demersum* for Ni and Pb

Concentration mg/l	Fresh weight (g)		Relative Growth	
	Ni	Pb	Ni	Pb
Control	24.24 ±0.01 **a**	24.24± 0.01 **a**	0.96± 0.01 **a**	0.96±0.015 **a**
2	22.42 ± 0.21 **b**	19.68± 0.09 **b**	0.89± 0.008 **b**	0.78±0.03 **b**
5	22.17 ± 0.19 **b**	19.00± 0.01 **c**	0.88± 0.009 **b**	0.75± 0.01 **c**
10	21.68 ± 0.09 **c**	17.77± 0.08 **d**	0.86±0.003 **c**	0.70± 0.03 **d**
15	20.98± 0.01 **d**	15.02± 0.009 **e**	0.83± 0.05 **d**	0.59± 0.005 **e**
20	19.54 ± 0.46 **e**	14.20± 0.08 **f**	0.78±0.18 **e**	0.56±0.002 **f**
30	19.02 ± 0.14 **f**	13.30± 0.19 **g**	0.75±0.05 **f**	0.53± 0.08 **g**

The different letter between if rows refers to significant difference ($p<0.05$)

Total chlorophyll: The decrease in chlorophyll content considered one of the toxic symptoms because of metal stress to the plant after exposure to metal concentrations. The result showed (table 3) that the total chlorophyll content was

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inhibited in all concentrations comparing with control, and the reduction increased with increasing concentrations. This reduction may be due to effect on the biosynthesis of protein and also the metal tightly joined with protein causing reduction of accumulation of lipo- protein and may be due to the inhibition of enzyme activity that are responsible for chlorophyll and photosynthesis.

Table 3 : mean ± SD of total chlorophyll content after 21st of exposure of *C. demersum* for Ni and Pb

Concentration	Ni	Pb
Control	7.30± 0.10 a	7.30± 0.10 a
2	7.07± 0.01 b	6.98± 0.02 b
5	6.85± 0.005 c	6.30± 0.001 c
10	5.64± 0.1 d	6.12± 0.1 d
15	5.59± 0.1 d	5.67± 0.32 e
20	5.11± 0.21 e	5.16± 0.05 f
30	4.54± 0.21 f	4.10± 0.08 g

The difference letter (between rows) refer to significant difference ($p<0.05$)

Protein content: The results showed in table 4, that the protein content declined with increasing the metal concentrations comparing with control treatment at significant difference ($p<0.05$) . This reduction may be due to degradation by proteases enzyme because of metal stress, (Romero-Puertas et al., 2002), or may be due to the metal stress which effects on inhibit a large number of enzymes having functional sulfhydryl groups (-SH group). It results in a deleterious effect on the normal protein by disrupting pathways and protein synthesis.

Table 4: mean ± SD of protein % after 21st of exposure of *C. demersum* for Ni and Pb

Concentration	Ni	Pb
Control	34± 1.0 a	33± 0.20 a
2	32± 1.12 a	31± 1.0 b
5	30± 1.02 a	28.2± 0.1 c
10	26± 1.0 a	24.4± 1.0 d
15	24± 1.01 a	21.1± 0.1 e
20	20± 1.0 a	17.13± 0.25 f
30	18± 1.03 a	13.30± 0.20 g

The difference in letter (between rows) refer to significant difference ($p<0.05$)

Tolerance Index Rate (TIR):The result showed (table 5) that the *C. demersum* was tolerance to both metals, but it is more tolerance to nickel than lead , and this may be due to the lead is more toxic to plants than nickel even though in low concentration.

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Table 5: Tolerance of *C. demersum* for both Ni and Pb

Concentration	Ni	Pb
2	97.76± 0.1 a	96.20± 0.1 a
5	96.80± 0.1 b	94.59± 0.2 b
10	94.37± 0.20 c	85.00± 0.1 c
15	91.42± 0.1 d	72.85± 0.01 d
20	84.816± 0.12 e	67.52± 0.1 e
30	82.647± 0.21 f	64.04± 0.21 f

The difference in letter (between rows) refer to significant difference ($p<0.05$)

IV. CONCLUSION

The results obtained indicated that the *Ceratophyllumdemersum* has able to tolerate both metals with different efficiencies, so it can consider as a good candidate in Phytoremediation technology of heavy metals , and it can be classified as a moderate accumulator due to the value of BCF that is less than 1000 mg/kg.

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