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Phytoremediation Potential of *Cynodon dactylon* Pers

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Abstract

Since early 20th century, heavy metal contamination has increased quickly and it has posed a great danger to the environment is treacherous due to its high toxicity. Phytoremediation has gained popularity recently because of its environment-friendly approach. This study was carried out to assess the phytoremediation potential of *Cynodon dactylon* for heavy metals from polluted water. The objective of this research was to grow test plant in nutrient solution with different concentrations (50, 100, 150 and 200 μM) of Lead acetate for 20 days. Fresh and dry biomass of vegetative parts (above and below ground) were determined and the Bio-concentration and Translocation factor was calculated. Results revealed that most of the lead from the solution was absorbed by *Cynodon dactylon*, till the 20th day. Most of the lead was accumulated in the aerial part. The highest lead content was recorded in the above ground part of the plant. This shows that lead has been translocated from the root to the above ground part.

Keywords: *Cynodon dactylon*, Heavy Metals, Phytoremediation

1. Introduction

New challenges are hiked in global development in the field of environmental protection (Bennett *et al.*, 2003). According to Alaerts *et al.* (1996) the biosphere is getting degraded by the release of natural and synthetic substances, which can cause harmful effects on organisms. Heavy metals are easily transported and accumulated in the environment. Anthropogenic activities like agriculture, mining, construction and industrial processes have led to increased heavy metal pollution (Hoseinizadeh *et al.*, 2011). Most of conventional methods have their limitations because they are ineffective, economically expensive and produce large quantities of sludge. Therefore, phytoremediation which is a relatively new, simple and effective technology for the removal of heavy metals from wastewater has gained attention.

As stated by Pilon-Smits (2005) green plants have an enormous ability to uptake pollutants from the environment, and accomplish their detoxification by various mechanisms. Phytoremediation enjoys popularity with the general public as a "green clean" alternative to chemical plants, bulldozers. Today there are many technologies for wastewater treatment and rhizofiltration is one of phytoremediation techniques that is very promising for clean-up of large amount of water with medium and low concentrations of heavy metals. Cule *et al.* (2012) stated that during Rhizofiltration most heavy metals including lead, cadmium, chromium, nickel, arsenic and various radionuclides can be extracted. Removed plants, rich in accumulated pollutants, can safely be processed by drying, burning and composting. Few of the metals can be re-extracted from the ash, which

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further reduces the generation of toxic waste into the environment.

Lead (Pb) is one of the toxic metal pollutants which occurs as a contaminant in all environmental compartments including soil, water, and living organisms It is known to induce a wide range of harmful effects to morphological, physiological and biochemical activities of living organisms (Amin *et al*., 2018).

Cynodon dactylon Pers. (Poaceae) is of interest as an alternative technology for heavy metal removal from polluted water because it has several important features suitable for phytoremediation. The objective of this study, is to investigate the effect of different concentrations of lead on the growth of *Cynodon dactylon.*

2. Materials and Methods

The test plant *Cynodon dactylon* Pers. (Poaceae) was collected from Anthiyur, Erode district, Tamil Nadu, India. According to Pourakbar *et al.* (2007) the plants were surface sterilized with 0.1% $HgCl_2$ with sterilized water, for 10 minutes, and washed 7 times. The seedlings were selected and pruned to have similar height of stem and length of roots. It was planted in peat, in order to get well-cultivated seedlings for one month, which can be later transferred to aqueous solutions.

The one-month-old seedlings were transferred to one litre volume buckets which were half filled with distilled water and with this 0.5 L of 100% modified Hoagland Solution was added (Hoagland and Arnon, 1950). After 15 days, nutrient solution was changed and augmented with four concentrations of Lead acetate (50µM, 100µM, 150µM, 200µM). Experiment lasted for 20 days.

3. Digestion and Analysis of Plant Material

According to Hoenig *et al.* (1998) after 20 days the plants were taken out from the nutrient solution, and separated into underground and above ground parts. After recording the fresh weight of harvested plants after washing, they were dried at 80°C for three days, subsequently the dry weights were determined. Plant biomass was digested by dry ashing. Dried, powdered plant sample in a crucible was placed in a cold muffle furnace, the temperature was progressively elevated to 450°C over two hours and held for four hours. After cooling a drop of distilled water was added. Then 5.0 mL conc. HNO_3 was added to the ash. The sample was slowly heated on a sand bath for 30 minutes, at 120-130°C. To this 5.0 mL of hydrogen peroxide was added with care in small amount to avoid strong foaming. The heating was continued at that temperature, until a clear solution was got. The solution was cooled and its volume was made up to 50.00 mL by adding distilled water. The samples were analysed by AAS, to determine their lead content.

3.1 Evaluation of Phytoremediation Efficiency

The Pb concentration in the underground and aerial parts of *Cynodon dactylon* Pers. was calculated (Monni *et al*., 2000).

$$\text{Pb Conc. ppm} = \frac{\text{AAS interpretation}\left(\text{reading}\right) \times \text{dilution factor}}{\text{Weight of plant tissues}}$$

3.2 Calculation of Bio-concentration and Translocation Factors

As stated by Lu *et al.* (2004) and Mun *et al.* (2008) Bio-concentration factor was a useful parameter, to assess the potential of plants for accumulating metals. It was calculated using the formula:

$$\text{BCF} = \frac{\text{Concentration of metal in dried plant tissue}\left(\mu\text{g g}^{-1}\right)}{\text{Initial concentration of metal in external solution}\left(\text{mg L}^{-1}\right)}$$

Padmavathiamma and Li (2007) and Adesodun *et al.* (2010) stated that the Translocation factor (TF) indicates the efficiency of the plant, in translocating the accumulated heavy metals from root to shoot. It is a ratio of the concentration of the heavy metal in shoot to that in its root. It was calculated using the formula:

$$\text{Translocation Factor}\left(\text{TF}\right) = \frac{C_{\text{Shoot}}}{C_{\text{Roots}}}$$

Where, C_{shoot} is concentration of the metal in plant shoot and C_{root} is concentration of the metal in plant root.

3.3 Statistical Analysis

For all experiments, the values are expressed as mean of triplicate analysis of the samples (n=3) ± Standard Deviation (SD).

4. Results and Discussion

Lead is known to be toxic to plants, animals, micro organisms and its effects are generally limited to contaminated areas (European Commission, 2002). According to Cho-Ruk *et al*. (2006) lead exists as an insoluble form in the environment and poses serious human health problems, mainly, brain damage and retardation. "Lead was found to be acute toxic to human beings when present in high amounts. Since Pb^{2+} is non-biodegradable, once soil has become contaminated, it remains a long-term source of lead exposure. Metal pollution has a deleterious effect on biological systems as it does not undergo biodegradation" (Pehlivan *et al*., 2009).

Data presented in Table 1 indicates the lead content in the aerial and underground parts of the test plant on treatment with lead acetate. Lead content in the aerial part increased with increase in lead concentration in the solution. Highest lead content was recorded in the aerial part and it amounted to 30.61±0.31 ppm on treatment with 200μM Pb $(CH_3COO)_2$. When compared to the aerial part the concentration of lead was lowest in the root (23.56 ± 0.15 ppm of lead) at 200μM Pb $(CH_3COO)_2$ concentration. This indicates the high mobility of lead through the plant. The control plants showed the absence of lead in its tissues.

Similar to the present study, Aransiola *et al.* (2013) proved that *Glycine max* absorbed substantial concentrations of Pb in the aboveground biomass compared to concentrations in the root. In contrast to the present study, Cule *et al*. (2012) showed highest lead content in the root of *Canna indica* and lowest in the leaves. According to Ali *et al*. (2012) and Amin *et al.* (2018) Pb concentration was more in root compared to stem and leaves of *Trifolium alexandrinum* and *Cyamopsis tetragonoloba*, respectively.

According to Kopittke *et al*. (2008) the high lead content in the plant tissues is related to the concentration of metal in the surrounding environment. Due to the strong Pb binding with organic and colloidal materials, it is believed that only small amounts of the lead in soil are soluble and available for plant uptake. Begonia *et al.* (2005) stated that the lead accumulation capacity based on their availabilities in the soil varies greatly among different plants species and cultivars.

"Phytoextraction is the ability of plants to absorb, concentrate, and precipitate toxic metals from contaminated soils into the aboveground biomass (ETPMS)" (1997). The plant (*Cynodon dactylon* Pers.) had a very good ability to extract lead from contaminated water. In the present study, the test plant could withstand the presence of lead in the medium. In a period of 20 days, it produced a substantial biomass without using fertilizers.

Table 2 depicts the growth performance of *Cynodon dactylon* in terms of fresh weight. The fresh weight ratio of aerial and underground parts of plant was on an average 40:60. This indicates that when grown in water, *Cynodon*

Table 1. Lead content in the plant parts of *Cynodon dactylon* Pers. in the treatments with, Pb $(CH_3COO)_2 . 3H_2O$ measured on the 20th day of the experiment

Lead Concentration (ppm)	Treatment with Pb $(CH_3COO)_2.3H_2O$				Control
	50μM	100 μM	150 μM	200 μM	
Aerial parts	18.51±0.17	23.64±0.21	25.42±0.28	30.61±0.31	0.00±0.00
Underground part	20.86±0.12	21.80±0.09	22.34±0.17	23.56±0.15	0.00±0.00
Whole plant parts	39.37±0.29	45.44±0.30	47.76±0.45	54.17±0.46	0.00±0.00

Values are mean ± SD (n=3)

Table 2. Fresh Weight of the plant parts of *Cynodon dactylon*

Fresh Weight (g)	Treatment with Pb $(CH_3COO)_2.3H_2O$				Control
	50µM	100 µM	150 µM	200 µM	
Aerial parts	12.64±0.29	12.41±0.37	12.90±0.41	11.90±0.45	5.52±0.19
Underground part	27.32±0.24	21.90±0.29	21.27±0.33	20.65±0.37	8.82±0.22
Whole plant parts	39.96±0.53	34.31±0.35	34.17±0.39	32.55±0.82	14.34±0.41

Values are mean ± SD (n=3)

Table 3. Dry Weight of the plant parts of *Cynodon dactylon*

Dry Weight (g)	Treatment with Pb $(CH_3COO)_2.3H_2O$				Control
	50µM	100 µM	150 µM	200 µM	
Aerial parts	5.32±0.08	4.22±0.07	4.84±0.11	3.91±0.05	1.60±0.09
Underground part	5.32±0.21	4.96±0.34	4.99±0.14	4.45±0.29	1.64±0.15
Whole plant parts	10.64±0.29	9.18±0.41	9.83±0.25	8.36±0.34	3.24±0.24

Values are mean ± SD (n=3)

dactylon tends to grow good root system and rhizome. At lower concentration (50µM Pb concentration), the fresh weight of both the aerial and underground parts increased considerably. Maximum fresh weight was recorded in the root (27.32±0.24g) at a concentration of 50µM lead acetate. The fresh weight of the test plant decreased as the Pb concentration increased. Overall, there was an increase in fresh biomass of test plant, as compared to control, which indicates its high tolerance to the presence of lead.

The ratio of the aerial and underground parts on a dry weight basis was on an average of 50:50. Compared to control plants, the test plant showed increase in dry weight. Lead stress at 200µM exhibited reduced root dry weight (4.45±0.29g) and shoot dry weight (3.91±0.05g) in the test plant when compared to 50,100 and 150µM concentrations of lead acetate (Table 3).

Likewise, Ignatius *et al*. (2014) proved that lower concentrations of lead increased the plant biomass of *Plectranthus amboinicus* slightly. Likewise, Cule *et al.* (2012) showed that *Canna indica* plants were highly tolerant to the presence of lead and showed very good plant growth.

Fitz and Wenzel, (2002) stated that the bioconcentration factor (BCF) and translocation factor (TF) values help to identify the suitability of plants for phytoremediation (i.e., phytoextraction or phytostabilization) by explaining the accumulation characteristics and translocation behaviours of metals in plants. Plants with BCF and TF values > 1 are considered promising phytoextractor, suitable for phytoextraction, while those with BCF and TF < 1 are not suitable for phytoextraction/phytostabilization. According to Mendez and Maier (2008) plants with BCF > 1 and TF < 1 are considered potential phytostabilizers suitable for phytostabilization.

As seen in Table 4 the highest BCF (6.8±0.15), was recorded in plant growing in 50µM lead acetate concentration while the lowest BCF (2.4±0.38) was

Table 4. Bioconcentration Factor and Translocation Factor of Lead, in *Cynodon dactylon*

BCF and TF	Treatment with Pb $(CH_3COO)_2.3H_2O$				Control
	50µM	100 µM	150 µM	200 µM	
BCF Values	6.8±0.15	4.0±0.21	2.7±0.35	2.4±0.38	0.00±0.00
TF Values	0.8±0.23	1.1±0.14	1.2±0.06	1.3±0.19	0.00±0.00

Values are mean ± SD (n=3)

recorded in 200µM lead acetate concentration. Our results showed that, *Cynodon dactylon* had BCF values > 1 at 50,100,150 and 200µM concentrations. Similarly, Thayaparan *et al*. (2013) revealed that BCF of *Azolla pinnata* increased with increased lead concentration. Amin *et al*. (2018) in *Cyamopsis tetragonoloba* and *Sesamum indicum* showed BCF values > 1 at all Pb treatments.

As stated by Usman *et al*. (2012) the success of the phytoextraction process depends on heavy metal removal by the shoots. It is therefore suggested that, the plants having higher metal concentration in its shoot than in its root can be considered as accumulator for phytoremediation. Since, *Cynodon dactylon* also showed BCF values > 1, it could be used as an "accumulator" in phytoremediation processes.

Ma *et al*., (2001) and Yoon *et al*. (2006) stated the Translocation Factor (TF) is defined as the ratio of the metal concentration in the shoots to that in the roots. Plants with TF values > 1 are classified as high-efficiency plants for metal translocation from the roots to shoots. In this study, all concentrations of lead acetate (except 50µM) showed TF value > 1. The highest TF (1.3±0.19) was recorded in 200µM lead acetate concentration.

In contrast, Amin *et al*. (2018) revealed that *Cyamopsis tetragonoloba* and *Sesamum indicum* plants showed TF values < 1 at all Pb treatments. Similar trend was noticed by Ignatius *et al*. (2014) in *Plectranthus amboinicus* where the TF value of all treatments was below1, showing poor translocation of Pb from the root to the shoot.

So, this study signifies the importance of the monocot weed *Cynodon dactylon* Pers. in bioremediation. *Cynodon dactylon* are ubiquitous, cosmopolitan and vegetative and have relatively high biomass. Therefore, *Cynodon dactylon* could be recommended for phytoremediation of lead.

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