ISSN 2286-4822
www.euacademic.org

EUROPEAN ACADEMIC RESEARCH
Vol. II, Issue 4/ July 2014

Impact Factor: 3.1 (UIF)
DRJI Value: 5.9 (B+)

Screening Potential of Three Native Grass Species for Phytoremediation of Heavy Metals

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Abstract:

Phytoremediation is an emerging, ecofriendly and alternative technology to remove heavy metals from the soil. In the present study three grass species Perotis indica, O., Echinochloa colona, L., and Cyperus rotundus, L. were used for Phytoextraction of lead, cadmium and chromium contaminated soils. The grass species was grown for a period of 60 days in the experimental pots. For every 20 days samples were collected from each pot for estimation of total accumulation of heavy metals in the plant. The bioconcentration factor (BCF) was also calculated. The experimental results showed that among the three grass species, Perotis indica and Echinochloa colona were good accumulators of cadmium and chromium, and Cyperus rotundus was good accumulator of lead, cadmium and chromium.

Key words: Phytoremediation, Lead, Cadmium, Chromium, Bioconcentration factor.

1. Introduction:

Plant processes that promote the removal of contaminants from soil and water are either direct or indirect. Direct processes include plant uptake into roots or shoots and transformation, storage, or transpiration of the contaminant. Indirect plant processing involves the degradation of contaminants by microbial, soil, and root interactions within the rhizosphere (Hutchinson et. al., 2003). Metals like Cadmium (Cd), Lead (Pb), Zinc (Zn), Chromium (Cr), Arsenic (As), Nickel (Ni) and Aluminum (Al) when present in high concentrations in soil exert potential toxic effects on overall growth and metabolism of plants and bioaccumulation of such toxic metals in the plant poses a risk to human and animal health (Agrawal and Sharma, 2006). About 90% of the anthropogenic emissions of heavy metals have occurred since 1900 AD; it is now well recognized that human activities lead to a substantial accumulation of heavy metals in soils on a global scale (e.g. 5.6 – 38 x 106 kg Cd yr-1) (Nriagu, 1996).

Phytoremediation, an emerging cleanup technology for contaminated soils, groundwater and wastewater that is both low-tech and low-cost, is defined as the engineered use of green plants (including grasses, forbs, and woody species) to remove, contain or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds and radioactive compounds in soil or water (Gurbisu and Alkorta, 2003). There is evidence that plants such as *Typha latifolia* and *Cyperus malaccensis* can accumulate heavy metals in their tissues (Yadav and Chandra, 2011) the same phenomena reported in *Sebera acuminate* and *Thlaspi caerulescens* (Cunningham and Ow, 1996) *Sonchus asper* and *Corydalis pterygopetata* grown on lead and zinc mining area in China have been identified as heavy metal hyper accumulators (Yanqun et. al., 2006). There is evidence that plants can

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accumulate heavy metals in their tissues such as *Arabidopsis thaliana (*Delhaize, 1996), *Typha latifolia*, and *Phragmites australis* (Ye, et al., 2011), *T. latifolia* and *P. australis* have been successfully used for phytoremediation of Pb/Zn mine (Ye et. al., 1997a).

However, some authors (Barlow, et. al., 2000; Piechalak et. al., 2002; Sahi, et. al., 2002) suggested that a community of tree, legume and grass based phytoremediation systems would be advantageous. Over 500 plant species comprising of 101 families have been reported, including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae and Euphobiaceae. Metal hyperaccumulation occurs in approximately 0.2% of all angiosperms and is particularly well represented in the Brassicaceae (Kramer, 2010).

2. Materials and methods:

The present study has been carried out during 2012-2013. The grass species selected for the present study included, *Perotis indica, Echinochloa colona,* and *Cyperus rotundus* belonging to Poales. All the plants were affluent and native to the study area i.e. Guntur District. The criteria followed for selection of species their biomass, commonness, tolerance to adverse climatic conditions and these species were fast growing, invasive, have tuberous roots and weedy characters.

Standard heavy metal solutions (1000 ppm) of Lead, Cadmium and Chromium were purchased from MERCK. These standard solutions were used for preparation of stock solution, from which 5ppm diluted heavy metal working solutions were prepared by taking aliquots of stock solution. These 5ppm solutions were added to the respective sets of each species and heavy metal, on every alternative day. While administering heavy metal laden water, care was taken to prevent leaching from the pot. The alternate day watering schedule was followed to prevent water logging condition in the pots. The plants were grown for a period of two months (60days). Every 20 days the plant samples from each pot were collected and washed thoroughly under running tap water and distilled water so that no soil particles remained. The collected samples were air dried and then placed in a dehydrator for 2-3 days and then dried in an oven at 100^0C. The dried samples of the plant were powdered and stored in polyethylene bags. The powdered samples were subjected to acid digestion. 1gm of the powdered plant material were weighed in separate digestion flasks and digested with HNO_3 and HCl in the ratio of 3:1. A blank sample was prepared applying 5ml of HNO_3 into empty digestion flask. The digestion on hot plate at 110ºc for 3-4 hours or continued till a clean solution was obtained. After cooling, the solution was filtered with Whatman NO.42 filter paper. After filtering the filtrate was analyzed for the metal contents in AAS.

2.1 Description of the grass species for the present study:

1. *Perotis indica, O.*

Photo1. *Perotis indica* Plant and Roots

Scientific Classification:

Kingdom	: Plantae (Angiosperms, Monocots, Commelinids)
Order	: Poales

Family	:	Poaceae
Subfamily	:	Chloridoideae
Genus	:	*Perotis*
Species	:	*indica*
Binomial name	:	*Perotis indica*

Common name of *Perotis indica* is Indian Comet Grass. Native of Africa: also found in west tropical, west-central tropical, east tropical, and western Indian Ocean. China and eastern Asia. India, Indo-China and Malaysia. The species is an annual grass with culms rising to 10–40 cm tall. Leaf-blades are lance shaped, or ovate; 1–3 cm long; 2–7 mm wide. Stalks are oblong, 0.2 mm long. Indian Comet Grass is found growing on river banks and sandy places (Gamble, 2008a).

2. *Echinochloa colona, L.*

Photo 2. *Echinochloa colona* Plant and Roots

Scientific Classification:

Kingdom	:	Plantae (Angiosperms, Monocots, Commelinids)
Order	:	Poales
Family	:	Poaceae
Subfamily	:	Panicoideae
Genus	:	*Echinochloa*
Species	:	*colona*
Binomial Name	:	*Echinochloa colona* (L.)

Echinochloa colona commonly known as Jungle rice has an Indian origin. Now it is widely distributed in tropics and subtropics, including South and Southeast Asia and tropical Africa. Terrestrial, tufted, erect herb, rooting at nodes. Roots fibrous. White or brown. Stems flat, hairy. Nodes glabrous. Stipules absent. Leaves alternate spiral, sessile, linear, more than 2 cm long/wide, apex acute, base clasping, parallel veined. Leaf sheath present (Gamble, 2008b).

3. *Cyperus rotundus, L.*

Photo 3. *Cyperus rotundus* Plant and Roots

Scientific Classification:

Kingdom	:	Plantae (Angiosperms, Monocots, Commelinids)
Order	:	Poales
Family	:	Cyperaceae
Genus	:	Cyperus
Species	:	rotundus
Binomial name	:	*Cyperus rotundus* L.

Cyperus rotundus commonly known as coco-grass, purple nut sedge, red nut sedge, etc., is a species of sedge belongs to Cyperaceae, native to Africa, southern and central Europe and southern Asia (Martin et. al., 2009). The names "nut grass" and "nut sedge" are derived from its tubers, that somewhat resemble nuts, although botanically they have nothing to do with nuts. The root system of a young plant initially forms white, fleshy rhizomes. Some rhizomes grow upward in the soil, and then form a bulb-like structure from which new shoots and roots grow, and from the new roots, new rhizomes grow. Other rhizomes grow horizontally or downward, and form dark reddish-brown tubers or chains of tubers. *Cyperus rotundus* is one of the most invasive weeds known, having spread out to a worldwide distribution in tropical and temperate regions. It has been called "the world's worst weed". The plant has several medicinal uses. The grass is anthelminthic, anti-fungal, anti-parasitic, anti-rheumatic, antispasmodic, aphrodisiac and astringent (Gamble, 2008c).

2.2 Calculation of Bioconcentration factor (BCF):

The concentration, transfer and accumulation of metals from soil to roots and shoots was evaluated in terms of Biological Concentration Factor (BCF) and Translocation Factor (TF). Biological Concentration Factor (BCF) was calculated as metal concentration ratio of plant roots to soil (Yoon et. al., 2006). The Bioconcentration Factor (BCF) of metals was used to determine the quantity of heavy metal absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005).

3. Results and Discussion:

Accumulation of heavy metals in three grass species:

Grass species, *Perotis indica, Cyperus rotundus and Echinochloa colona* were selected for the present study. These three grasses were highly resistant species that can survive hardships of soil and climate. The accumulation and absorption of lead, cadmium and chromium were studied. The total accumulations were estimated in these three grass species viz. *Perotis indica, Cyperus rotundus* and *Echinochloa colona.*

3.1 *Perotis Indica:*

Accumulation of Lead (mg/kg):

Lead concentration in the control was 23.59 mg/kg. The absorption of lead was marginal up to 20 days. Thereafter from 40-60 days the increase of lead concentrations was only 1.25 mg/kg (Table 1). The total accumulation in the whole plant by 60th day was only 1.77 mg/kg. In Australia, vetiver grass was used to stabilize landfill and industrial waste sites contaminated with heavy metals such as Cd, As, Ni, Cu, Pb and Hg (Truong and Baker, 1998). In China, vetiver grass was planted in a large scale for pollution control and mine tail stabilization (Chen, 2000). Vetiver grass (*Vetiveria zizanioides,* L.) was used for Cadmium (Cd) and Lead (Pb) removal and reported that the accumulation rates for lead was very high (9.72-88.14%), for cadmium the accumulation rates was fairly low. Therefore, vetiver grass use to Phytoextraction of lead and phytostabilization of cadmium contaminated soils (Minh and Khoa, 2009).

Accumulation of Cadmium (mg/kg):

The initial concentration of cadmium was 0.83 mg/kg which has reached to 2.78 in first 20 days. The total accumulation of cadmium in 60 days was 8.52 mg/kg (Table 1). The increase of concentration was highest from 20-40 days (2.78 to 7.2 mg/kg) and from then onwards accumulation was slow from 40-60 days. *Chenopodium album L.*, halophytic weed, was a good candidate for Phytoextraction of cadmium from the contaminated soil (Mazhari and Bahare, 2012). Several species, such as hemp dogbane (*Apocynum cannabinum*), common ragweed (*Ambrosia artemisiifolia*), nodding thistle (*Carduus nutans*), and Asiatic dayflower (*Commelina communis*), were shown to have superior Pb-accumulating properties (Berti and Cunningham, 1993).

Accumulation of Chromium (mg/kg):

The total accumulation of chromium in the plant in 60 days was 65.63 mg/kg. The initial concentration was 9.61. *Perotis indica* has accumulated 30.83 mg/kg of chromium in first 20 days (Table 1). The concentration of chromium continued to increase from 20th to 40th day (30.83 to 40.5 mg/kg). However, a substantial accumulation was observed from 40th day to 60th day (40.5 to 75.24 mg/kg). The results revealed that *Perotis indica* was a good accumulator of chromium.

Table 1: Accumulation of Lead, Cadmium and Chromium (mg/kg biomass) in *Perotis indica* during the experimental period.

Heavy metal	Control	20th day	40th day	60th day	Total accumulation
Lead	23.59±0.13	23.99±0.08	24.11±0.15	25.36±0.07	1.77
Cadmium	0.83±0.13	2.78±0.08	7.2±0.15	9.35±0.09	8.52
Chromium	9.61±0.12	30.83±0.08	40.5±0.15	75.24±0.09	65.63

3.2 *Echinochloa Colona:*

Accumulation of Lead (mg/kg):

The total accumulation of lead in *Echinochloa colona* was 8.82 mg/kg in 60 experimental days. The initial concentration of lead was 20.13 mg/kg which increased to 28.95 mg/kg by the end of the experimental period. The accumulation was 5.18 mg/kg in the first 20 days (Table 2). By 40th day the concentration of lead reached to 28.84 mg/kg in an increase of 3.53mg/kg from 20 to 40 days. Thereafter the accumulation stabilized. By 60th day the accumulation was 28.95 mg/kg showing an increase of only 0.11 mg/kg from 40th day to 60th day. *S. nigrum* was designated as hyper-accumulator of Cd and the BAC of *C. dactylon* was very high for Pb (BAC = 4.6) and based on Pb tolerance this species was recommended for phytoremediation processes (Wei, 2004). *B. reptans*, *S. nigrum P. oleracea* and *X. stromarium* had very high BCF values and could be useful for Phytostabilization of soils contaminated with Cu and Pb. *Canna indica* effectively translocate lead and chromium to aerial parts while the roots retain high quantities of cadmium, nickel and zinc. The total absorption was high for zinc followed by chromium (Subhashini et. al., 2013). The chlorophyll levels decreased after dosing with Cu, Zn and Pb in *Cladonia rangiformis* (Qadir et. al., 2004).

Accumulation of Cadmium (mg/kg):

The back ground concentration of cadmium was 0.21 mg/kg in the beginning of the experiment which increased to 13.11 mg/kg in 60 days, showing a total accumulation of 12.9 mg/kg (Table 2). In the first 20 days of experimental period the accumulation of cadmium was slow and later increased to 8.05 mg/kg to 13.11 by 40^{th} and 60^{th} days, respectively. The accumulation was maximum between 20 and 40 days. *Brassica juncea* has been studied extensively for its tolerance to heavy metals in general and Cd in particular and has been suggested as a good bioremediator of the metal contaminated soil (Nouairi and Ammar, 2009); (Soleimani et al., 2009).

Accumulation of Chromium (mg/kg):

The total accumulation of chromium in *Echinochloa colona* in the 60 experimental days was 47.73 mg/kg (Table 2). The back ground concentration of chromium in the beginning of the experiment was 9.51 mg/kg. The accumulation in the plant continued up to 60^{th} day. By 20^{th} day the accumulation reached to 19.03 which further increased to 39.41 and 57.24 by 40^{th} and 60^{th} days, respectively. The accumulation was highest between 20 and 40 days and lowest in first 20 days.

Table 2: Accumulation of Lead, Cadmium and Chromium (mg/kg biomass) in *Echinochloa colona* during the experimental period.

Heavy metal	Control	20th day	40th day	60th day	Total accumulation
Lead	20.13±0.19	25.31±0.11	28.84±0.18	28.95±0.1	8.82
Cadmium	0.21±0.18	1.83±0.19	8.05±0.13	13.11±0.16	12.9
Chromium	9.51±0.19	19.03±0.16	39.41±0.19	57.24±0.13	47.73

3.3 *Cyperus Rotundus:*

Accumulation of Lead (mg/kg):

The initial concentration of lead was 8.99 mg/kg in the beginning of the experiment. The concentration of lead subsequently increased to 36.17 mg/kg by 40th day, there after the accumulation slowed down from 20-40-60 days to 36.17-36.66-37.35, respectively (Table 3). The total accumulation in 60 days was 28.36 mg/kg. This reveals that *Cyperus rotundus* is a good accumulator of lead.

In present study the *Cyperus rotundus* was ascertained as a good accumulator of lead based on the total accumulation of lead. This species being native, wild and produce considerably less biomass could be a potential species for Phytoextraction of lead from contaminated soils. Pb concentration was more in roots compared to both stem and leaves. The amounts of lead in roots were higher in Bermuda grass (*Cynodon dactylon*) compared to shoot[31]. The present study reports that the cadmium, lead and chromium were absorbed in higher quantities. Studies on cabbage (*Brassica rapa* L.) and wheat (*Triticum aestivum* L.) grown in Pb-contaminated soils, revealed a significantly higher concentrations of Pb in the shoots and roots (Shen et. al., 2002). While for *Buddleja scordioides* (Buddlejaceae) and *Cerdia congestiflora* (Caryophilaceae) the Pb bioconcentration factors were 1.31 and 1.05, respectively, which indicated that they are lead-tolerant species. *Brassica juncea* (Indian mustard) a high-biomass plant could accumulate Pb, Cr (VI), Cd, Cu, Ni and Zn (Nanda Kumar et. al., 1995; Raskin et. al., 1994).

Accumulation of Cadmium (mg/kg):

Cyperus rotundus has accumulated 16.35 mg/kg of cadmium in 60 days. The initial concentration was 0.52 mg/kg. Cyperus has accumulated 3.09, 13.11 and 16.87 mg/kg by 20th, 40th and 60th days, respectively (Table 3). *Cyperus rotundus* was a good accumulator of cadmium. Cd was predominantly accumulated in the roots of both *Brassica juncea* and *Brassica napus* (Nouairi et. al., 2006) and the studies on *Elsholtzia splendens* also revealed the same (Peng and Yang, 2005).

Accumulation of Chromium (mg/kg):

Chromium concentration was 6.28 mg/kg at the beginning of the experiment. There was a substantial accumulation in 40 days (64.4 mg/kg). The accumulation increased to 25.53 mg/kg in first 20 days and later to 64.4 mg/kg by 40th day (Table 3). The increase of accumulation was less from 40th day to 60th day (only 1.98 mg/kg i.e. from 64.4 to 66.38 mg/kg). The total accumulation of chromium in 60 days was 60.1 mg/kg which revealed that *Cyperus* was a good accumulator of chromium. Similar results were obtained on evaluating the potential of *Pongamia pinnata* (Leguminosae- Papillionoideae) for Phytoremediation of Chromium (Cr), Copper (Cu) and Nickel (Ni) (Shirbhate and Malode, 2012).

Table 3: Accumulation of Lead Cadmium and Chromium (mg/kg) in *Cyperus rotundus* during the experimental period.

Heavy metal	Control	20th day	40th day	60th day	Total accumulation
Lead	8.99±0.15	36.17±0.19	36.66±0.08	37.35±0.19	28.36
Cadmium	0.52±0.15	3.09±0.19	13.11±0.08	16.87±0.19	16.35
Chromium	6.28±0.15	25.53±0.19	64.4±0.08	66.38±0.18	60.1

Table 4: Lead, Cadmium and Chromium Bioconcentration factor table

Name of the species	Lead	Cadmium	Chromium
Perotis indica	0.19	23.28	4.83
Echinochloa colona	0.93	35.26	3.51
Cyperus rotundus	2.99	44.67	4.42

In *Perotis indica* lead bioconcentration factor was 0.19, cadmium bioconcentration factor was 23.28 and chromium bioconcentration factor was 4.83. This indicates that the *Perotis indica* can tolerate high levels of cadmium and chromium, and hence it can be recommended for removal of cadmium and chromium from contaminated soils. The BCF for lead in *Echinochloa colona* was 0.93, cadmium bioconcentration factor was 35.26 and chromium bioconcentration factor was 3.51. The bioconcentration factor was >1, the species could be used for Phytoextraction of cadmium and chromium contaminated soils. The BCF for lead in *Cyperus rotundus* was 2.99, cadmium bioconcentration factor was 44.67 and chromium bioconcentration factor was 4.42. The BCF values for the heavy metals in the present study ascertain that it is a potential species for removal of lead, cadmium and chromium from contaminated soils.

4. Conclusions:

Perotis indica was good accumulator of cadmium and chromium. The species can be recommended for the Phytoextraction of cadmium and chromium contaminated soils. *Echinochloa colona* was good accumulator of cadmium and chromium. The species can be recommended for the Phytoextraction of cadmium and chromium contaminated soils. *Cyperus rotundus* was good accumulator of lead, cadmium and chromium. The species can be recommended for the Phytoextraction of lead, cadmium and chromium contaminated soils. Among the experimental species *Cyperus rotundus* the potential species for the removal of Pb, Cd and Cr while *Perotis indica* and *Echinochloa colona* are the effective species for the phytoextraction of cadmium and chromium contaminated soils.

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