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Parthenium hysterophorus: Growth Response to Chromium and Nickel Application and Phytoremediation Potential

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Abstract: *Parthenium hysterophorus* L. is an invasive alien species of natural and agroecosystems. It causes biodiversity loss in the former and yield loss in the later. Successful eradication of *P. hysterophorus* is still a distant dream. We argue that the possibilities to exploit this species for material production and services should be explored. Present study evaluates this species for phytoremediation of chromium (Cr) and nickel (Ni). *P. hysterophorus* plants were exposed to four concentrations of chromium (CN10, CN15, CN30 and CN40) and nickel (NN10, NN20, NN40 and NN50). The low concentrations of Cr (CN10) and Ni (NN10 and NN20) enhanced all the growth parameters studied. Shoot accumulation of Ni exceeded the root accumulation (TF<1) while TF>1 was noted for Cr. *P. hysterophorus* accumulates both heavy metals as evident from high biological concentration factor (BCF) and biological accumulation coefficient (BAC). Hence *P. hysterophorus* can act as phytoremediator of Ni and Cr.

Keywords: *P. hysterophorus*, Nickel, Chromium, Biological concentration factor, and Biological accumulation coefficient

INTRODUCTION

Phytoremediation has emerged as a cost effective and eco-friendly technology for cleaning of inorganic and organic contaminants from soil and water [1-6]. Availability of hyper accumulator plant species for target contaminant is basic requirement for successful application of this technology. Metal hyperaccumulator plant species are "*able to grow on metalliferous soils and to accumulate extraordinarily high amounts of heavy metals in the aerial organs, far in excess of the levels found in the majority of species, without suffering phytotoxic effects*" [7].

Heavy metal tolerance and hyper-accumulation are not common traits in plant world. Baker *et al*. [8] estimated the number of metal hyperaccumulators as 417, less than 0.2% of world flora. Van der Ent *et al*. [9] have raised this figure to 579. Mandal *et al*. [10] have listed about 38 taxa in Indian flora suitable for mine spoil sites.

An ideal heavy metal hyper-accumulator should have a high metal accumulation rate and a large biomass. Majority of hyper-accumulators have small biomass because the physiological processes involved in metal accumulation require considerable energy [11]. Therefore, researches are still on to discover efficient metal hyper-accumulators.

Parthenium hysterophorus L. is an invasive alien species of tropical American origin. Contrary to its native range which is limited to twenty countries of South, North and Central America, its introduced range covers thirty four countries [12, 13]. In India this weed is estimated to have infested about 2.0 million hectares of land and causes considerable damage to agricultural and natural ecosystems [12, 14-18]. Until recently *P. hysterophorus* was considered a useless plant species, but recent researches have focussed on exploitation of this abundantly available species as a source of useful products [19-25]. Besides the search for material products, few studies have explored the phytoremediation potential of this species. For instance Ajmal *et al*. [26] studied the adsorption of cadmium (Cd) from wastewater on *P. hysterophorus* detritus; Malik *et al*. [27] studied the levels of lead (Pb), copper (Cu), zinc (Zn), cobalt (Co), nickel (Ni) and chromium (Cr) in plant samples collected from heavy metal contaminated soil; Sanghamitra *et al*. [28] and Nazir *et al*. [22] studied the uptake of Cd; Ahmad and Al-Othman [29] studied the uptake of Cd, Ni, Pb, Zn and iron (Fe) in fly ash amended soil. Hadi and Bano [30] recorded greater biomass production and Pb

accumulation in *P. hysterophorus* plants treated with GA3. Ali and Hadi [31] studied the impact of the application of plant growth regulators and chelating agents on Cd accumulation. It is evident that studies of the heavy metal phytoremediation potential of *P. hysterophorus* are still in a preliminary stage and detailed studies are needed to accept or reject its candidature as a hyper-accumulator of heavy metals in different soil types.

Present study was designed to answer two questions (a) how the biomass production of *P. hysterophorus* is affected by increasing levels of Chromium (Cr) and Nickel (Ni) in soil? and (b) How much Cr and Ni are taken up and transported to shoot in a growth season?

MATERIAL METHODS

P. hysterophorus seeds were collected from a single population in the AMU campus. The earthen pots of 30 cm diameter were filled with a mixture of garden soil and leaf mold (1:1 w/w). The pots were left for two months and irrigated weekly to exhaust the soil seed bank of weeds. Ten seeds were sown in each pot and finally the plants were thinned out to three per pot. The pots were arranged in a completely randomized block design and placed in a net house under ambient light and temperature. All treatments were replicated thrice and pots were irrigated as per requirement. After 15 days of sowing the treatments of chromium nitrate (10mg, 15mg, 30mg and 40mg/kg of soil herein after abbreviated as CN10, CN15, CN30 and CN40) and Nickel Nitrate (10mg, 20mg, 40mg and 50mg/kg of soil herein after abbreviated as NN10, NN20, NN40 and NN50) were applied to each pot. Control pots were maintained for all treatments and data on reduction/increase on growth parameters are in comparison to control. The three samplings were done at 30, 60 and 90 days after treatment.

Soil analysis

The soil used in this experiment was analyzed for following physico-chemical characteristics following Ghosh et.al. [32] pH, cation exchange capacity, electrical conductivity, total dissolved solids, osmotic pressure, organic carbon, phosphorous, potassium, calcium, magnesium, carbonate, bicarbonate, copper, iron, manganese and zinc (Table1).

Table 1: Physico-chemical properties of the soil used in study

Parameters	Soil
Texture	Sandy loam
pH	7.79
Cation exchange capacity (meq $100g^{-1}$soil)	3.33
Electrical conductivity (µ mhos cm^{-1})	291
Total dissolve solids	789
Osmotic pressure	0.30
Organic Carbon (%)	0.38
Phosphorus (gkg^{-1}soil)	0.110
Potassium (gkg^{-1}soil)	17.5
Calcium (mgl^{-1})	30.1
Magnesium (mgl^{-1})	15.22
Carbonate (mgl^{-1})	19.20
Bicarbonate (mgl^{-1})	198
Cupper (mgl^{-1})	0.67
Iron (mgl^{-1})	4.29
Manganese (mgl^{-1})	3.58
Zinc (mgl^{-1})	7.35

Parameters studied

Root length and shoot length were measured with measuring tape. Fresh root weight, fresh shoot weight, dry root weight and dry shoot weight were taken with an electrical balance. Chlorophyll content (Chlorophyll 'a', Chlorophyll 'b', Total chlorophyll, and Carotenoids) were estimated following Mac Kinney [33].

Heavy metal analysis

Metal levels in soil, root and shoot were determined in90 day old plant material. Acid digests of samples were prepared by USEPA 3051 A (34) method. Soil and plant tissue samples were dried in an oven at 60^0C and ground to 20 meshes using a ceramic grinder. The ground material was digested in nitric acid (69%purity) and hydrochloric acids (28% purity) in (3:1) ratio. The aliquot was diluted to 25ml and analyzed for total metals by (Atomic Absorption Spectrometer 373, Perkin-Elmer, Norwalk, CT, USA). Biological Concentration Factor (BCF), Translocation Factor (TF) and Biological Accumulation Coefficient (BAC) were calculated following Yoon *et al.* [35], Cui *et al.* [36] and Li *et al.* [37].

$BCF = (Metals)_{root}/(Metals)_{soil}$
$TF = (Metals)_{shoot}/(Metals)_{root}$
$BAC = (Metals)_{shoot}/(Metals)_{soil}$

Statistical analysis

The data were analyzed statistically using one way analysis of variance (ANOVA) and pair wise means were compared using Duncan's multiple range test (P = 0.05). The analysis was performed with the software R (38).

RESULTS

Growth and biochemical parameters

30 day stage

Data summarized in Table 2 show a significant increase in root length (12.4%), shoot length (16.4%), root dry weight (0.17%) and shoot dry weight (1.84%) in plants treated with NN20. Photosynthetic pigments except chlorophyll 'b', showed a significant decrease. CN10 caused minor enhancement in plant length (15.76%), fresh root weight (19.23%), dry root weight (33.33%), total chlorophyll content (10.25%), and carotenoid content (19.89%). CN40 caused significant reduction in all parameters (Table 2).

Table 2: Effect of various treatments of Cr and Ni on growth and biochemical parameters of *P. hysterophorus* at 30 day stage

Nickel concentrations						Chromium concentrations				
Parameters	control	10	20	40	50	control	10	15	30	40
Root length(cm)	8.20^{c}	10.10^{b}	12.40^{a}	8.90^{bc}	6.00^{d}	8.20^{a}	7.50^{ab}	6.60^{b}	5.20^{c}	5.00^{c}
Shoot length(cm)	15.40^{b}	16.40^{ab}	17.50^{a}	14.80^{bc}	13.50^{c}	15.40^{a}	11.40^{b}	9.60^{c}	8.20^{c}	9.50^{d}
Fresh root weight (gm)	0.50^{bc}	0.61^{ab}	0.72^{a}	0.40^{cd}	0.35^{d}	0.50^{a}	0.31^{b}	0.18^{c}	0.22^{c}	0.03^{d}
Fresh shoot weight (gm)	3.10^{b}	3.90^{a}	4.20^{a}	2.10^{c}	1.90^{c}	3.10^{a}	2.44^{b}	1.80^{c}	0.92^{c}	1.69^{d}
Dry root weight (gm)	0.16^{ab}	0.12^{ab}	0.17^{a}	0.14^{ab}	0.09^{b}	0.16^{a}	0.04^{b}	0.02^{b}	0.01^{b}	0.01^{b}
Dry shoot weight (gm)	1.23^{c}	1.44^{b}	1.84^{a}	0.70^{d}	0.69^{d}	1.23^{a}	0.31^{b}	0.28^{bc}	0.13^{bc}	0.21^{c}
Chlorophyll a (mg/g fw)	0.82^{ab}	0.98^{a}	0.80^{ab}	0.66^{ab}	0.50^{b}	0.82^{a}	0.55^{b}	0.47^{bc}	0.46^{bc}	0.44^{c}
Chlorophyll b (mg/g fw)	0.79^{c}	0.95^{c}	0.77^{c}	1.22^{a}	0.86^{bc}	0.79^{a}	0.45^{b}	$0.33b^{c}$	0.29^{c}	0.25^{c}
Total chlorophyll (mg/g fw)	1.61^{b}	1.93^{a}	1.58^{b}	1.22^{c}	1.20^{c}	1.61^{a}	1.01^{b}	0.80^{c}	0.75^{c}	0.70^{c}
Carotenoid(mg/g fw)	2.02^{b}	2.32^{a}	1.47^{c}	1.50^{c}	1.20^{d}	2.02^{a}	2.17^{a}	1.89^{a}	1.36^{b}	1.05^{b}

60 day stage

Data presented in Table 3 shows enhancement in all parameters such as root length (15.5%), shoot length (43.1%), root dry weight (1.79%), shoot dry weight (7.32%), chlorophyll content (12.77%) and caroteniod content (5.38%) in plants treated with NN20. NN50 caused significant reduction in all parameters. CN10 caused significant decrease in root length, fresh root weight, and total chlorophyll. CN15, CN30, and CN40 treated plants show significant reduction in all parameters except Chlorophyll 'b' in CN15.

Table 3: Effect of various treatments of Cr and Ni on growth and biochemical parameters of *P. hysterophorus* at 60 day stage

Nickel concentrations						Chromium concentrations				
Parameters	control	10	20	40	50	control	10	15	30	40
Root length(cm)	13.50^{bc}	14.70^{ab}	15.50^{a}	11.80^{c}	9.1^{d}	13.50^{a}	9.90^{b}	$8.20b^{c}$	7.90^{c}	7.10^{c}
Shoot length(cm)	40.40^{b}	42.20^{ab}	43.10^{a}	35.10^{c}	29.50^{d}	40.40^{a}	40.90^{a}	31.20^{b}	28.50^{c}	25.20^{d}
Fresh root weight(cm)	5.50^{bc}	6.93^{ab}	7.53^{a}	4.23^{cd}	3.52^{d}	5.50^{a}	3.58^{b}	2.04^{c}	1.88^{c}	1.57^{c}
Fresh shoot weight (gm)	18.30^{b}	19.25^{ab}	20.91^{a}	15.64^{c}	13.98^{c}	18.30^{a}	18.28^{a}	12.20^{b}	10.17^{c}	9.58^{c}
Dry root weight(gm)	1.20^{b}	1.59^{a}	1.79^{a}	0.98^{bc}	0.75^{c}	1.20^{b}	1.75^{a}	0.71^{c}	0.68^{c}	0.55^{c}
Dry shoot weight(gm)	6.13^{ab}	7.18^{a}	7.32^{a}	5.15^{bc}	4.01^{c}	6.13^{a}	5.29^{ab}	5.11^{b}	3.28^{c}	3.11^{c}
Chlorophyll a(mg/g fw)	1.60^{a}	1.69^{a}	1.69^{a}	1.24^{b}	0.86^{c}	1.60^{a}	1.47^{ab}	1.29^{bc}	$1.25b^{c}$	1.10^{c}
Chlorophyll b (mg/g fw)	1.36^{a}	1.55^{a}	1.63^{a}	0.73^{b}	0.62^{b}	1.36^{a}	1.17^{a}	1.15^{a}	0.86^{b}	0.78^{b}
Total chlorophyll (mg/g fw)	2.96^{a}	3.23^{a}	3.34^{a}	1.97^{b}	1.49^{b}	2.96^{a}	2.63^{b}	2.43^{c}	2.10^{d}	1.87^{e}
Carotenoids(mg/g fw)	2.79^{a}	2.84^{a}	2.94^{a}	2.11^{c}	2.34^{b}	2.79^{a}	2.57^{ab}	2.34^{b}	2.69^{a}	2.41^{b}

90 day stage

Table 4 shows the significant enhancement in root length (24.7%), shoot length (67.9%), root dry weight (3.85%), and shoot dry weight (9.47%), total chlorophyll content (30.25%) and caroteniod content (6.02%) by NN20 treatment. NN50 treatment caused significant reduction in all parameters. CN10 caused nonsignificant enhancement for all parameter. The highest reduction was found in plant dry weight (65.5%) at CN40 treatment level.

Table 4: Effect of various treatments of Cr and Ni on growth and biochemical parameters of *P. hysterophorus* at 90 day satge

	Nickel concentrations					Chromium concentrations				
Parameters	control	10	20	40	50	control	10	15	30	40
Root length(cm)	20.40^{bc}	22.40^{b}	24.70^{a}	20.50^{bc}	19.20^{c}	20.40^{d}	28.10^{b}	30.20^{a}	26.10^{c}	24.60^{c}
Shoot length(cm)	60.50^{b}	68.10^{a}	67.90^{a}	42.20^{c}	37.60^{d}	60.50^{b}	78.50^{a}	50.20^{c}	38.20^{d}	32.10^{e}
Fresh root weight(gm)	7.05^{bc}	7.85^{ab}	8.91^{a}	6.27^{cd}	5.12^{d}	7.05^{a}	5.78^{b}	4.10^{c}	3.45^{c}	2.45^{d}
Fresh shoot weight(gm)	25.41^{b}	27.50^{ab}	29.31^{a}	18.56^{c}	16.21^{d}	25.41^{a}	21.25^{b}	17.75^{c}	16.80^{d}	10.52^{e}
Dry root weight(gm)	2.42^{c}	3.01^{b}	3.85^{a}	2.97^{b}	2.01^{d}	2.42^{b}	2.67^{a}	1.95^{c}	1.01^{d}	0.95^{d}
Dry shoot weight(gm)	9.21^{c}	10.45^{a}	9.47^{b}	7.45^{d}	5.62^{e}	9.21^{a}	7.24^{b}	5.41^{c}	4.29^{cd}	3.21^{d}
Chlorophyll a(mg/g fw)	0.99^{ab}	1.07^{a}	1.10^{a}	0.84^{bc}	0.67^{c}	0.99^{b}	1.07^{ab}	1.16^{a}	0.84^{c}	0.81^{c}
Chlorophyll b(mg/g fw)	0.82^{ab}	1.03^{a}	0.76^{bc}	0.74^{bc}	0.59^{c}	0.82^{ab}	0.79^{b}	0.97^{a}	0.72^{b}	0.45^{c}
Total chlorophyll(mg/g fw)	1.81^{b}	2.09^{a}	1.89^{b}	1.58^{c}	1.26^{d}	1.81^{b}	1.86^{b}	2.14^{a}	1.56^{c}	1.26^{d}
Carotenoids (mg/g fw)	2.49^{abc}	2.59^{ab}	2.64^{a}	2.34^{c}	2.42^{bc}	2.49^{a}	2.19^{b}	1.93^{c}	1.77^{d}	1.37^{e}

Phytoaccumulation of nickel and chromium

Cr and Ni accumulation by *P. hysterophorus* appears to be dose dependent (Fig. 1 and 2). These two metals, however, differ in their sites of accumulation. Greater proportion of Ni is accumulated in shoot (Fig. 1) while Cr shows greater accumulation in root (Fig. 2). Translocation factor was greater than unity for Ni and less than unity for Cr. Total metal concentration (BCA) for both Ni and Cr was more than 8mg/kg and value of bioaccumulation factor (BAF) is high (Table 5).

Table 5: *P. hysterophorus* with strong endurance and high accumulation of Ni and Cr

Nickel						**Chromium**				
Concentrations	**Control**	**10**	**20**	**40**	**50**	**Control**	**10**	**15**	**30**	**40**
BCF	5.29	9.63	5.95	6.23	5.52	8.22	10.21	7.55	5.41	6.47
TF	1.04	1.01	1.00	0.97	1.02	0.82	0.98	0.91	0.66	0.55
BAC	5.50	9.8	6.00	6.08	5.65	6.77	10.10	6.89	3.58	3.58

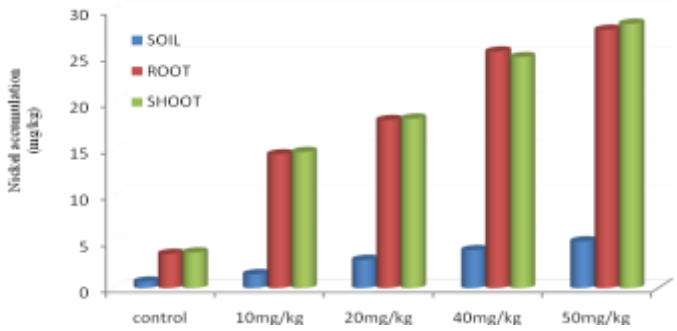

Fig-1: Nickel accumulation (mg/kg) in soil, root and shoot of *P. hysterophorus* plants at 90 day stage

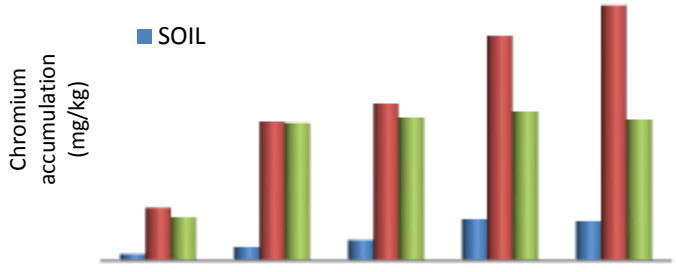

Fig-2: Chromium accumulation (mg/kg) in soil, root and shoot of *P. hysterophorus* plants at 90 day satge

DISCUSSION

All growth parameters and growth related parameters, including biomass production, showed a decline in response to all treatments of Cr and higher levels of Ni (NN40 and NN50). These findings concord with earlier reports on growth suppression by heavy metals. This heavy metal induced biomass reduction is attributable to several underlying anatomical and

physiological factors. Rucin´ska-Sobkowiak [39] concluded that decreased root elongation, impaired secondary growth, slowing down of long distance water transport due to decreased hydraulic conductivity in the root, stem and leaf midrib, reduced vessel size and partial blockage of xylem elements may create 'internal drought' in plants. Inhibition of photosynthesis by heavy metals is an extensively and intensively studied phenomenon [40-46]. All steps of photosynthesis are known to be adversely affected by heavy metals. Impaired photosynthesis logically leads to a diminished gross production and reduced biomass production. Nickel is an essential element [47] and Aligarh soil has low level of this metal. Therefore gain in biomass accumulation and other growth parameters due to lower doses of Ni (NN10 and NN20) may be due to increased soil level of this essential element. Chromium is a toxic metal even at low level and plays no role in physiological processes; hence it causes significant reduction in growth parameters even at low levels. Slight increase in some growth parameters caused by low Cr levels may be ascribed to the phenomenon of hormesis which is defined as *'a dose response relationship phenomenon characterized by low-dose stimulation and high-dose inhibition*' [48].

Three ratios, bioconcentration factor (BCF), translocation factor (TF), and biological accumulation coefficient (BAC) are used to quantify the phytoremediation potential of a plant species. BCF is the ratio of metal concentration in root to metal concentration in soil. TF is an estimation of the ability of a species to translocate heavy metals from root to shoot. BAC is the ratio of metal concentration in soil to metal concentration in shoots. High BCF values obtained in this study indicate that *P. hysterophorus* is capable of removing both Cr and Ni from the soil (Table 5 and Fig. 1 and 2). Estimation of Cr and Ni accumulation in root and shoot of *P. hysterophorus* (Fig. 1 and 2) and translocation factor (TF) values (Table 5) clearly show that *P. hysterophorus* transports Ni to shoots more efficiently (TF= 0.97-1.02) than chromium (TF=0.55-0.98). While Ni is nearly equally partitioned between root and shoot, the amount of Cr transported to shoot is nearly 50% of the amount accumulated in roots. Malik et al. [27] and Ahmad and Al-Othman [29] reported much higher TF_{Ni} values (2.6 and 4.3 respectively). Does it mean that different populations of *P. hysterophorus* differ in their ability to transport Ni from roots to shoots? Different populations of *P. hysterophorus* need to be evaluated under identical conditions following identical experimental protocol to answer this question. It may lead to discovery of line(s) of *P. hysterophorus* with high TF values.

I haves been suggested that species with high BCF and TF values are ideal for phytoextraction of heavy metals. They remove large quantity of heavy metals from soil and greater portion of the metal taken up is translocated to harvestable aerial parts. The species with high BCF but low TF values are suitable for phytostabilization [49]. Such species remove metals from the soil and retain their greater portion in below ground parts. Thus, they reduce the amount of metals available for downward movement to ground water.

BCF and BAC values show that *P. hysterophorus* absorbs and accumulates Ni and Cr more efficiently at low, 10mg/kg, soil level (Table 5). Gain in total dry weight (16%) at this level of nickel may be an additional advantage for utilization of this species in phytoremediation of nickel. Absorption of Cr at CN40 (BCF= 6.47) is better than that of Ni at NN40 and NN50 (BCF= 6.23 and 5.52 respectively). At these concentrations Cr the TF values (0.55) are much lower than TF values for Ni (0.97 and 1.02) at NN40 and NN50. Therefore *P. hysterophorus* can be used for phytostabilization of Cr.

We must accept that *P. hysterophorus* is now an integral part of flora of India and, at least presently, its effective control or eradication is not an achievable proposition. Both chemical and bio-control methods are fraught with the danger of doing more harm than good. The best option appears to exploit this species for products and services including phytoremediation. At least three traits of *P. hysterophorus* as documented in this study, make it a good candidate for further research. Firstly, it gained biomass at low nickel level and showed a smaller loss of biomass (15%) at low chromium level. Secondly, this species grows nearly round the year. It can germinate any time when moisture is available. A light irrigation can provide required moisture level. Thirdly, it needs no inputs for its growth.

CONCLUSION

Low Ni doses (NN10 and NN20) had promotive effect on growth. Higher doses of Ni and all doses of Cr caused significant decrease in growth. Lower doses of Cr caused a small loss in biomass. Both Cr and Ni are absorbed with nearly equal efficiency. Major part of Ni is transported to shoots but greater portion of Cr remains in roots. Data suggested that *P. hysterophorus* can be used for phytoextraction of Ni from soil with low Ni contamination and phytostabilization of Cr.

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