PLANTA DANINHA
SBCPD
SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS
<http://www.sbcpd.org>
ISSN 0100-8358 (print)
1806-9681 (online)

Article

EVALUATION OF PHYTOREMEDIATION POTENTIAL OF CASTOR CULTIVARS FOR HEAVY METALS FROM SOIL

Avaliação do Potencial de Cultivares de Mamona na Fitorremediação de Solo Contaminado por Metais Pesados

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ABSTRACT - Phytoremediation is a useful tool to restore heavy metals contaminated soils. This study was carried out to test two castor (*Ricinus communis*) cultivars [Local and DS-30] for phytoextraction of heavy metals from the soil spiked by known concentrations of seven metals (Cu, Cr, Fe, Mn, Ni, Pb and Zn). A pot experiment was laid out by using a completely randomized design. Soil and plant samples were analyzed at 100 days after planting. The data on heavy metal uptake by plant tissues (roots, leaves and shoots) of the two castor cultivars suggested that a considerable amount of metals (Fe = 27.18 mg L^{-1}; Cu = 5.06 mg L^{-1}; Cr = 2.95 mg L^{-1}; Mn = 0.22 mg L^{-1}; Ni = 4.66 mg L^{-1}; Pb = 3.33 mg L^{-1}; Zn = 15.04 mg L^{-1}) was accumulated in the plant biomass. The soil heavy metal content at the end of experiment significantly decreased with both cultivars, resulting in improved soil quality. Therefore, it is concluded that both castor cultivars, Local and DS-30, can be used for phytoremediation of heavy metal-contaminated sites.

Keywords: phytotoxins, soil contaminants, phytoextraction.

RESUMO - A fitorremediação é uma ferramenta útil para recuperar solos contaminados com metais pesados. Este estudo foi realizado para testar dois cultivares de mamona (Ricinus communis) [Local e DS-30] quanto à fitoextração de metais pesados de solo contaminado por concentrações conhecidas de sete metais (Cu, Cr, Fe, Mn, Ni, Pb e Zn). Um ensaio em vasos foi instalado com um delineamento completamente casualizado. Cem dias após o plantio, foram analisadas amostras do solo e das plantas. Os dados referentes à absorção de metais pesados pelos tecidos vegetais (raízes, folhas e parte aérea) dos dois cultivares de mamona sugerem que quantidades consideráveis de metais (Fe = 27,18 mg L^{-1}; Cu = 5,06 mg L^{-1}; Cr = 2,95 mg L^{-1}; Mn = 0,22 mg L^{-1}; Ni = 4,66 mg L^{-1}; Pb = 3,33 mg L^{-1}; Zn = 15,04 mg L^{-1}) foram acumuladas na biomassa da planta. Foi observada redução significativa do teor de metais pesados no solo ao final do experimento com os dois cultivares, resultando em melhor qualidade do solo. Portanto, conclui-se que ambos os cultivares de mamona, Local e DS-30, podem ser utilizados para a fitorremediação de locais contaminados com metais pesados.

Palavras-chave: fitotoxinas, contaminantes do solo, fitoextração.

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Received: June 6, 2017
Approved: January 22, 2018

Planta Daninha 2019; v37:e019180998

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Doi: 10.1590/S0100-83582019370100134

INTRODUCTION

Heavy metal (HM) contamination of terrestrial ecosystems has been accelerated in the past few decades (Atibu et al., 2013). HMs such as Cu, Fe, Se, Ni, Pb etc. are of great concern because these are very persistent, stable and bio-accumulative (Al-Wabel, 2007). Unlike organic pollutants, HMs are non-biodegradable, hence they pose serious public health risks through food chain and loss of biodiversity (Peng et al., 2008). Certain HMs are toxic even at low concentrations and can affect crop production (Yi et al., 2011). Heavy metals are naturally found in soils, and plants use their minute amounts as essential metabolites. In recent years, anthropogenic activities have resulted in enormous increase in both type and concentration of heavy metals in the soil (Mahmood and Malik, 2014). Discharge of contaminated industrial effluents, and atmospheric deposition have been posing a greater threat to ecological integrity of developing countries, e.g., Pakistan. In agricultural soils of Pakistan, presence of HMs has been reported by many workers, and a few sites even show higher HM concentrations than the limits set by the World Health Organization. For instance, Khan et al. (2013) reported that Cd concentration was found to be higher than the limit (0.05 mg kg^{-1}) set by the World Health Organization in agricultural soils of Swat District, northern Pakistan (Khan et al., 2013).

Restoration of our ecosystems from the adverse effects of contaminants is very expensive for the functionality of living systems (Wiszniewska et al., 2016). For example, remediation of contaminated sites in the European Union would cost an estimated amount of €17.3 billion/annum (Bauddh and Singh, 2012). In developing countries such as Pakistan, where population densities are enormous and available resources are limited, a low-cost and ecologically sustainable technology is required to remediate contaminated soil as well as to minimize the associated environmental risks. This in turn also increases the available land resources for agricultural production thus also enhances food security (Back and Rosén, 2006).

Amongst the strategies for reclamation of HM polluted systems, phytoremediation has emerged as one of the least expensive and most reliable technologies in recent years (Cundy et al., 2013). Phytoremediation is a green solution to HM contamination which uses hyperaccumulating green plants to remove, destroy or sequester hazardous substances from the environment (Tangahu et al., 2011). Another important part of phytoremediation is identification and characterization of HM accumulators from natural flora. It is also established that some plant species are endemic to metal contaminated soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds (Schat et al., 2000). Castor bean, a highly adaptive plant, has been identified as a metal tolerant plant under a variety of climatic conditions (Olivares et al., 2013). Moreover, the castor bean plant has received more attention because of its high seed oil yields for synthesis of biofuel in recent years (Berman et al., 2011). For this reason, castor was selected as plant of choice.

The objective of this experiment was to explore the phytoremediation potential of castor bean when grown in soil contaminated with different metals and compare the HM uptake abilities of the selected castor bean cultivars.

MATERIALS AND METHODS

Considering the importance of *R. communis*, the phytoextraction potentials of two cultivars of *R. communis* (Local and DS-30) were tested against these heavy metals. For this purpose, the surface soil (20 cm) from the field currently under wheat–rice cultivation was collected and processed for presence of any roots and debris. Clayey pots of 10 kg soil capacity were filled with soil, and healthy castor seeds were selected based on their appearance from both varieties. Suggested solutions with varying heavy metal concentrations (Table 1) were prepared, labeled and stored for further use in preparation of various the treatments. The control set of pots was filled with normal garden soil while the experimental set of pots was provided with solutions containing seven different HMs (Cu, Cr, Fe, Mn, Ni, Pb and Zn) added to soil, and then each pot was labeled accordingly. Initially, five seeds per pot were sown and once germination was achieved and plantlets were established they were reduced to only one seedling per pot. Each treatment had three replicates along with the control set of pots while a completely random experimental setup was followed. The experiment was carried out at the Department of Soil and Environmental

Science, Gomal University, Dera Ismail Khan under the pot culture in the (31°43'03.12" N 70°50'18.60" E) for 100 days. Composite samples of the soil prior to the experiment were analyzed for physico-chemical characteristics (Table 2). After plant harvest, the soil from each pot was also collected and analyzed for HM content n soil.

Table 1 - Concentration of heavy metals applied in the experiment

S. No.	Heavy metal	Concentration (mg kg^{-1})
1	Copper (Cu)	5, 10, 20 and 40
2	Chromium (Cr)	1, 2, 4 and 8
3	Iron (Fe)	10, 20, 40 and 80
4	Manganese (Mn)	10, 20, 40 and 80
5	Nickel (Ni)	5, 10, 20 and 40
6	Lead (Pb)	2, 4, 8 and 16
7	Zinc (Zn)	10, 20, 40 and 80

Table 2 - Physico-chemical characteristics of the soil in use

Propertie	Value
Texture	Loamy sand
pH	7.96
EC (µS cm^{-1})	643
Extractable P (mg kg^{-1})	6.12
Lime (%)	19.33
Organic matter (%)	0.64
Extractable K (mg kg^{-1})	129.5
Extractable Na	92.9
SAR	8.7
Cu (mg kg^{-1})	0.08
Cr (mg kg^{-1})	0.07
Fe (mg kg^{-1})	0.96
Mn (mg kg^{-1})	0.09
Ni (mg kg^{-1})	0.16
Pb (mg kg^{-1})	0.07
Zn (mg kg^{-1})	1.32

Collection and analysis of soil and plant samples for heavy metals

Spiked soil in all pots was analyzed at 100 days (after passing through several wetting and drying stages) after planting castor beans. The samples were placed in plastic bags, air dried, ground with wooden mortar and pestle and then sieved through a 2 mm sieve. The soil samples underwent microwave-assisted aqua regia digestion (8 mL (HCL/HNO_3 3:1) for determination of total heavy metal content. Deionized water was used in the extraction procedure, based on the German Standard Method DIN 38414-S4. The available heavy metal content in soil samples was determined by 0.005 M DTPA extraction (Lindsay and Norvell, 1978). For this purpose, 10 g of soil sample was shaken with 20 mL of the extractant (0.005 mol L^{-1} diethylenetriaminepentaacetic acid + 0.1 mol L^{-1} triethanolamine + 0.01 mol L^{-1} $CaCl_2$, solution at pH 7.3) for two hours, centrifuged and then filtered. Metal contents in the samples were determined by an atomic absorption spectrophotometer (AAS Model GBC SAVANTAA, Australia). HM content was calculated by using the following formula:

$$\text{HM content mg kg}^{-1} = \text{Atm. abs} \times \text{d.f} \times 1000/\text{Vol. of sample in use (g)}$$

Plant leaves, stems, branches and roots were collected at 100 days after sowing, washed with deionized water, oven-dried (70 °C), weighed and ground prior to analysis. All samples were digested in concentrated HNO_3 in the laboratory using a microwave system and left overnight. Oven dried samples were ground and further extracted via wet digestion. After extraction, perchloric acid and H_2SO_4 were added to the samples, which were then digested on a hot plate (120-140 °C) inside the fume hood. The samples were placed in an ice bath, 25 mL distilled water was added, and then the samples were again heated to boil.Final volume of the extract was raised up to 100 mL prior to filtering. Metal contents in the diluted samples were determined by following the methods of Allen et al. (1986).

Statistical Analysis

All measurements were repeated three times, and the data were organized by using Microsoft Excel 2007 and presented as mean standard errors (SEs). Regression analysis for the results of each heavy metal was performed separately as described by Steel and Torrie (1984). The data were analyzed with PROC MIXED and PROC GLM commands for both variety and treatment to test the significant differences ($p<0.05$). The treatments were ranked with Duncan's Multiple Range test. Prior to analysis, data were checked for normality and homogeneity of variances.

RESULTS AND DISCUSSION

Heavy metal concentration of the soil

It was also found that the soil where the local cultivar was grown showed greater Mn content than DS-30. Concentration of Ni, Pb and Zn showed a significant difference in comparison to the variable concentration of the heavy metals applied to the soil. Significant interaction was observed between soil HM content and castor cultivar (Table 3). It is further assumed that castor is a high biomass-producing plant with broad leaves; therefore, it might have accumulated the available heavy metals rapidly, hence a lesser amount was left in the soil.

Table 3 **-** Comparison of Ni, Pb and Zn content (mg kg^{-1}) left in the soil

Cultivar	Ni Content (mg L^{-1})				Mean	Pb Content (mg L^{-1})				Mean	Zn Content (mg kg^{-1})				Mean
	5	10	20	40		5	10	20	40		5	10	20	40	
Local	0.39 b	0.86 b	1.76 b	4.11 a	1.78 ^{NS}	0.41 b	0.89 b	1.35 b	3.46 a	1.52 ^{NS}	2.66 e	6.05 de	10.0 b	13.91 a	8.15^{NS}
DS - 30	0.46 b	0.75 b	1.18 b	5.21 a	1.90	0.40 b	0.85 b	1.21 b	3.20 a	1.41^{NS}	3.11 de	6.48 cd	9.90 bc	16.18 a	8.91^{NS}
Mean	0.42 b	0.80 b	1.47 b	4.66 a		0.40 b	0.87 b	1.28 b	3.33 a		2.88 d	6.26 c	9.95 b	15.04 a	
LSD Conc.			1.10					0.89					2.60		
LSD Cult.			NS					NS					NS		
LSD Conc.×Cult.			1.56					1.26					3.69		

Means with similar letters do not differ significantly at $p<0.05$.

This decreasing trend was the same in soil samples collected from the pots of both cultivars (Tables 4 and 5). There was maximum decrease in the soil spiked with the highest concentration of metals; i.e., Cu 40 mg kg^{-1}, Cr 8 mg kg^{-1}, Fe 80 mg kg^{-1}, Mn 80 mg kg^{-1}, Ni 40 mg kg^{-1}, Pb 16 mg kg^{-1} and Zn 80 mg kg^{-1}, which proved that the castor plants accumulated a greater amount of metals in their biomass where the metals were available in excess amount. This also establishes the suitability of the plant as an effective accumulator of HMs and therfor castor cultivars can be successfully used for restoration of the HM affected soils. Very slight and non-significant differences were found in the comparison of soil samples analyzed for both cultivars, which shows that phytoremediation ability mainly depends upon the biomass of the plant rather than genotype. Based on these instant findings, it can be concluded that castor cultivars have maximum heavy metal uptake potential and are potential phytoremediators. Scientists have recently found that Castor oil plants can be used as phytoremediators of the contaminated soils because they are highly tolerant to high concentrations of HMs, e.g., Cu, Fe, Mn and Zn (Abreu et al., 2012). Previous studies have also shown a decrease in Zn and Cd concentrations in soil as a result of plants uptake with passing more number of days (Uena et al., 2004). Puschenreiter et al. (2003) also found similar results in their study on heavy metal-contaminated soils.

Uptake and accumulation of heavy metals in roots

Results are indicative of a linear relationship between dosage of HM and their subsequent uptake by the plant's roots. Thus, maximum heavy metals were accumulated in the pots with the highest metal concentrations. Both cultivars took up an equal amount of all these metals, and there were no significant differences in uptake by the two genotypes. These findings are in line with the previous findings of Kozanecka et al., (2002), who also found similar trends of HM uptake in different forest plant species of *Cladonia clavatum* (wolf's claw), *Dryopteris filix-mas* (shield fern), *Convalaria maialis* (convallaria), and *Vaccinium myrtillus* (bilberry) in Poland. The instant results are slightly different from the previous findings of Knezevic et al., (2009). in which the level of heavy metals (Pb, Ni, Fe, Zn and Mn) was compared in two species of plants, *P. fortune* and *P. elongate*, and *P. elongate* proved to be a hyperaccumulator of Fe and Ni as compared to *P. fortune*. Zhi-Xin et al. (2007) also reported slightly different results from their experiments: sunflower, *Ricinus*, alfalfa and mustard were used to test the bioaccumulation of Pb and Cd in hydroponic cultures. These slight differences in metal accumulation might be attributed to the different experimental conditions. Researchers also found that because of the well-known

Table 4 **-** Comparison of Fe and Mn content (mg kg^{-1}) left in the soil

Cultivar	Fe Content (mg L^{-1})				Mean	Mn Content (mg L^{-1})				Mean
	10	20	40	80		10	20	40	80	
Local	3.45 d	6.11 cd	11.91 b	26.21 a	11.92^{NS}	0.11 b	0.14 b	0.15 b	0.16 a	0.14 b
DS - 30	3.91 d	7.05 b	10.65 b	28.16 b	12.44	0.18 b	0.22 ab	0.25 ab	0.28 a	0.23 a
Mean	3.68 d	6.58 c	11.28 b	27.18 a		0.14	0.18	0.20	0.22	
LSD Conc.			1.2577					NS		
LSD Cult.			NS					0.0434		
LSD Conc.×Cult			2.9491					0.1523		

Mean with similar letters do not differ significantly at $p<0.05$.

Table 5 **-** Comparison of Cu and Cr content (mg kg^{-1}) left in the soil

Cultivar	Cu Content (mg L^{-1})				Mean	Cr Content (mg L^{-1})				Mean
	5	10	20	40		5	10	20	40	
Local	0.600 b	1.160 b	2.060 c	4.980 a	2.20^{NS}	0.240 c	0.470 c	0.860 c	2.370 a	0.985^{NS}
DS - 30	0.660 b	1.010 b	2.060 b	5.150 a	2.22	0.510 c	1.027 bc	1.043 bc	2.220 ab	1.200
Mean	0.630 c	1.085 bc	2.060 b	5.065 a		0.375 c	0.748 bc	0.952 b	2.295 a	
LSD Conc.			1.2438					0.4944		
LSD Cult.			NS					NS		
LSD Conc.×Cult			2.2842					1.2239		

Means with similar letters do not differ significantly at $p<0.05$.

agronomic characteristics of cultivation and the high biomass production of switch grass, it is practicable to use switch grass for in situ phytoextraction of heavy metals (Chen et al, 2012). Other researchers also reported increased uptake of heavy metals by roots and leaves of vegetables during their experiments (Perveen, et al., 2011).

Uptake of heavy metals by plant parts

Plant biomass analysis of heavy metal uptake from the spiked soil by two castor cultivars (Local and DS-30). Heavy metal uptake by both cultivars sgowed a significant linear increase with increasing concentrations of heavy metals. Maximum uptake of HMs occurred in those plants which were treated with higher concentrations compared to the pots with lower heavy metal concentrations. Similarly, maximum linear increase of the study heavy metals was found in the plant samples collected after an interval of 100 days. Uptake performance of both Castor cultivars (Local and DS-30) was statistically at par with each other at each comparative concentration, which shows that the uptake ability of castor is not dependent upon genotype.

Based on these findings, it can be safely estimated that castor plants have accumulated a substantial amount of heavy metals in their biomass and can remediate heavy metal-contaminated soils. The instant findings are supported by the results of Kozanecka et al., (2002), who reported that heavy metal accumulation increased in plants with increasing intervals. Gregory and Crowder (1983) also found increased accumulation of metals (Cu, Ni, Zn, Fe, Mn and Mg) in tissues of *Typha latifolia* collected from the contaminated soils. Zeittouni et al., (2007) also studied Castor as phytoremediator and found similar findings for Zn uptake. Homer et al. (1991) and Niu et al. (2007) also found similar results for accumulation of HM (Fe, Ni, Cr, Cu, Pb and Cd) in different plant species. Various other studies also revealed that metals are accumulated in plant parts during their growth on contaminated soils (Kumar et al., 1985; Zacchini et al., 2009).

The foregoing results indicate that castor plants have performed well in terms of heavy metal accumulation in their biomass. A substantial amount of metals was extracted from the soil and was accumulated in plant parts (roots, leaves, stem). This has proved castor plants to be good phytoremediators of heavy metals from contaminated soils. The use of castor plants for phytoremediation of contaminated soils has another advantage: these plants can be widely used only for this purpose because they are not an edible crop. Because of its wide leaves and greater amount of biomass, the castor beans can perform better as compared to other plants. After metal uptake by plants was compared, non-significant differences were found between both the cultivars.

ACKNOWLEDGEMENTS

The study was carried out with the support of Pakistan Science Foundation (PSF), Islamabad, Pakistan.

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