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ENHANCING PHYTOREMEDIATION OF Pb BY TREATING SOIL WITH CITRIC ACID AND GROWING WHITE JUTE (*Corchorus capsularis*, L.), AND RIVER RED GUM (*Eucalyptus camaldulensis*)

Magdy M. Niazy* and M.E.M. Wahdan

Soils, Water and Environ. Res. Inst., Agric. Res. Cent. (ARC), Egypt

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ABSTRACT: Removal of toxic Pb metals from soil (phytoremediation) was evaluated using white jute (*Corchorus capsularis*, L.) and river red gum, (*Eucalyptus camaldulensis*) in presence of citric acid (CA). Lead was added to soil at 0, 400, 800 and 1200 mg Pb kg⁻¹ soil as lead nitrate. Plant growth (120-day growth) of jute decreased with addition of Pb by 20 to 46% in white jute with no CA and 11 to 33% with CA; 18 to 40% for red gum with no CA and 10 to 26% with CA. Pb uptake increased with increased addition of Pb. River red gum was more efficient as a phyto-remediator than jute. Citric acid enhanced the efficiency of both plants to extract Pb from soil.

Key words: Accumulation, Citric acid, lead, *Corchorus capsularis*, *Eucalyptus camaldulensis*, Phytoextraction.

INTRODUCTION

Lead occurs naturally only in small amounts within the earth's crust. Frequent use in many industrial processes such as mining and smelting activities, paints, mineral oils, explosives sewage sludge are all among sources contributing to environmental pollution with Pb, (Chaney and Ryan, 1994 ; Henry, 2000 ; Abdel-Salam *et al.*, 2015). Low bioavailability and extremely long duration of Pb retention by soil render such contamination being highly serious (Shaw, 1990). With soils and natural waters harbouring various forms of Pb, the element would eventually enter the food chain *via* plants and animals and represent a serious permanent risk to human health. Average contents of Pb in the earth surface range between 10 and 20 mg kg⁻¹, a level which may be rather safe, but when it rises to about 100 mgkg⁻¹ or more it becomes highly serious and indicates severe contamination (Fergusson, 1990). The majority of Pb contents in the environments is immobile, and soils nearby the highways contain considerable concentration of Pb which may be as high as

180 mgkg⁻¹ due to emission of car exhaust fumes (Han *et al.*, 2008). Cleanup technologies such as land filling and chemical treatment have been conducted in order to remediate Pb-contaminated soils, but they are costly and may have adverse effects on soil properties (Pulford and Watson, 2003; Abdel-Salam *et al.*, 2015). Technologies exploiting plants such as phyto-remediation, phyto-extraction, phyto-filtration, phyto-stabilization, phyto-volatization and phyto-degradation may be used for such purposes (Garbisu and Alkorta, 2001). The phytoremediation method depends of using special plants which possess a high capacity for uptake of particular heavy metals (hyper accumulator plants) to be planted in the contaminated soil in order to remove as much possible of the heavy metal (Jing *et al.*, 2007; Shakoor *et al.*, 2013; Abdel-Salam *et al.*, 2015). The plants are ultimately removed and translocated to a far cite and a destroyed, invariable by burning and buried in a very deep location and thus removed from the contaminated soil (Nascimento and Xing, 2006). Another method may be used in combination of

*Corresponding author: Tel. : +201093458689
E-mail address: amagdy16@gmail.com

phytoremediation, is the use of particular chemicals (synthetic or organic) to enhance plant growth the metal uptake (Evangelou *et al.*, 2007; Murakami *et al.*, 2007; Abdel-Salam *et al.*, 2015). This is called chemo-remediation, using chelating chemicals such as citric acid (CA), diethylene-triamine-pentaacetic acid (DTPA) and ethylene-diamine-tetraacetic acid (EDTA) (Sinal *et al.*, 2010; Szczygłowska *et al.*, 2011; Bareen, 2012; Yeh *et al.*, 2012; Chigbo and Batty, 2013). Chelating chemicals help plants to remove more metals (Anwer *et al.*, 2012; Bareen, 2012). As an organic acid, CA has a high biodegradability and less leaching hazard as compared with synthetic chelating agents (Melo *et al.*, 2008; Wuana *et al.*, 2010; Bareen, 2012). Beside increasing metal solubility and uptake by plants, CA could increase the uptake of other nutrients by plants (Turgut *et al.*, 2004; Yeh *et al.*, 2012; Freitas *et al.*, 2013). It proved effective in mobilization and phytoextraction of Cd (Sinal *et al.*, 2010).

Examples of hyper accumulator plants are Jute (*Corchorus capsularis*) and river red gum (*Eucalyptus camaldulensis*). Jute is a fiber crop, second in the world after cotton in terms of global production, and consumption (Ranjit *et al.*, 2013). It is an annual fiber crop with tall stem and deep penetrating taproot. The plant grows fast and easily in nutrient-poor soil and makes a heavy quantity of valuable biomass. It produces soft, shiny and long fiber for wide usages. It is a completely biodegradable, recyclable and eco-friendly lingo-cellulose fiber. Red river gum is a big with a massive shoot system which should be able to accumulate (Nenman *et al.*, 2012).

The objectives of the current study is to investigate the remediation ability of both plants in remediating soil contaminated with Pb under conditions of adding CA or in its absence.

MATERIALS AND METHODS

During summer season of 2015, the experiment was carried out in a greenhouse illuminated with natural light. In Kafr El-Hamam Agric. Res. Station, El Sharkia Governorate, Egypt. This site is located at 30° -35 N latitude and 30° - 57 E longitudes with an elevation of about 7 meters

above mean sea level. Two plants *i.e.* white jute (*Corchorus capsularis*, L.) and river red gum (*Eucalyptus camaldulensis*) were used in a pot experiment, to measure their capacity to accumulate lead (6.5 kg soil per pot) from a clay soil contaminated with lead. The experimental design was a randomized complete block, with three replicates. There were 12 treatments, 6 receiving lead at 0, 400, 800 and 1200 mg Pb kg⁻¹ soil (as lead nitrate Pb(NO₃)₂), and 6 receiving lead + citric acid at 0.98 g kg⁻¹ soil. Each of the two plants received such treatments. Each pot was seeded with the relevant plant, then after germination, the seedlings were thinned to three plants per pot and grown for 120 days. Pots were watered so as soil moisture was kept at about 70% of water holding capacity. At end of experiment, plants were weighed and samples of soil and plants were taken for analysis. Method of analyses used for soil, plant and water were according to Page *et al.* (1982), Klute (1986) and to Stewart (1989).

Tables 1 and 2 show main properties of soil and water used in the experiment. Extractable lead was extracted from the soil using a mixture of 0.005 M DTPA (diethethylene triamine penta acetic acid) + 0.1 M TEA (triethanolamine) + 0.01 M CaCl₂ at pH 7.3 according to Lindsay and Norvell (1978).

RESULTS AND DISCUSSION

Plant growth

Results in Table 3 show effects of lead addition with or without citric acid on plant dry weight. Lead caused decreases in the different parts of dry weight plant⁻¹. The decreases were greater where Pb was in greater contents. Also it was much lower where citric acid was present. It ranged from about 20 to 46% in white jute where no citric acid as compared with 11 to 33% where citric acid was present. Comparable values for red gum were 18 to 40% in absence of citric acid and 10 to 26% in its presence. Phytotoxic effects of heavy metals including lead induce several physiological and structural disorders in plants (Sharma and Dubey, 2005). Presence of citric acid was of positive effect in increasing the yield of plants. The alleviation effect of citric acid on the toxicity of lead to plant

Table 1. Mechanical and chemical characteristics of the investigated soil before planting

PH	EC dS.m ⁻¹	CaCO ₃ (%)	OM (%)	Particle size distribution (%)			
				Sand	Silt	Clay	Texture grade
7.99	2.3	3.70	2.30	23.64	30.11	46.25	Clay

Table 2. Chemical analysis of water used for irrigation (Tap water)

PH	EC dS.m ⁻¹	Pb mg.l ⁻¹
7.38	0.52	0.018

Table 3. Effect of Pb added to soil with or without citric acid in irrigation water on plant dry weight (shoots + roots) of 120-day old white jute (*Corchorus capsularis*), and river red gum (*Eucalyptus camaldulensis*)

Added Pb (mg kg ⁻¹)	White jute				Red gum			
	Without citric acid		With citric acid		Without citric acid		With citric acid	
	g plant ⁻¹	Decrease (%)						
0	8.95	--	9.15	-	13.65	-	14.53	-
400	7.15	20.1	8.19	10.5	11.23	17.7	13.15	9.5
800	5.17	42.2	7.89	13.8	9.15	33.0	12.57	13.5
1200	4.75	46.9	6.18	32.5	8.17	40.1	10.79	25.7
LSD 0.05	1.51	2.7	1.63	5.0	1.74	3.8	1.64	5.6

is shown in the low negative toxic effect on plant. Citric acid exhibits a chelation to soluble lead (Sinha *et al.*, 2010; Szczygłowska *et al.*, 2011; Ehsan *et al.*, 2014), thus reducing its retarding effect of plant growth.

Uptake of Pb by Plant

Results in Table 4 show the uptake values of lead by plants (roots + shoot). Addition of lead increased Pb uptake. The increases were greater with the increase in Pb addition. Also the uptake was much greater in presence of CA. The increase in uptake ranged from about 10 folds to

16 folds in the white jute plant where no citric acid as compared with.. 13 folds to 20 folds in presence of CA. Comparable values for red gum were 15 folds 19 folds in absence of CA and 19 folds to 31 folds in its presence. Increased uptake of Pb with increasing addition of Pb is a direct consequence of added Pb. The increases caused by citric acid indicates the chelation effect of the acid on the added Pb as well as its enhancement effect on plant growth. Citric acid addition was reported to increase the uptake of heavy metal due to its chelating effect (Hocking *et al.*, 1997).

Table 4. Effect of Pb added to soil with or without citric acid in irrigation water on Pb uptake (in roots + shoots) of 120-day old white jute (*Corchorus capsularis*), and river red gum (*Eucalyptus camaldulensis*)

Added Pb (mg kg ⁻¹)	White Jute		River red gum	
	Without citric	With citric	Without citric	With citric
	µg plant ⁻¹	µg plant ⁻¹	µg plant ⁻¹	µg plant ⁻¹
0	192	294	358	521
400	2113	4175	5581	10167
800	2750	5512	6211	12435
1200	3433	6296	7070	16507
LSD 0.05	24	29	31	32

Lead in Soil at End of Plant Growth

Data in Table 5 show the lead contents in soil at end of plant growth. Contents of total Pb were about 19 mg kg⁻¹ in soil of the white jute, and 15 mg kg⁻¹ in soil of the river red gum increased up to 35 folds by increased addition of Pb. The DTPA-extractable contents amounted to a very small fraction of the total (from 2% to 5% of total Pb). Contents in soils treated with CA were lower, demonstrating the chelation effect of CA.

Bio-concentration Factor (BCF)

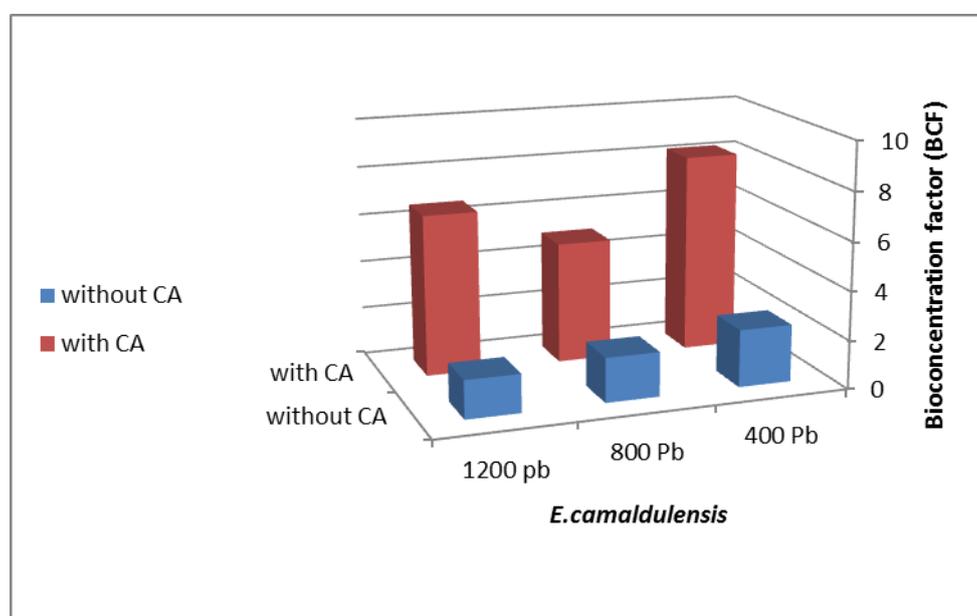
The bio-concentration factor (BCF) is the ratio of metal content in the plant roots to that in soil (Malik *et al.*, 2010). Values of BCF for lead in soil under jute varied by 0.92 to 2.93 (Fig. 1). The lowest was by the treatment receiving the low rate Pb with added CA and the highest was given by the treatment receiving the low rate Pb with highest addition of Pb. Values for the soil under the red gum plant ranged from 0.51 to 7.92 with a pattern of change rather similar to that of the soil under the jute plant. Arifin *et al.* (2012) noted that BCF for the heavy metal of Cd decreased with increase of the metal in soil indicating a restriction in soil-root transfer where there is a high rate of the metal in the soil. Fitz and Wenzel (2002) concluded that plants of BCF values less than one not suitable as phyto-remediators of heavy metals. According to Yoon *et al.* (2016), plants which are suitable as phyto-remediators include wiregrass gentian (*Gentiana pennelliana*) which is capable of accumulating heavy metal in the roots.

Overall Assessment and General Conclusions

Lead showed an extreme phytotoxicity to plants, most probably through its severe physiological disorders in plants (Sharma and Dubey, 2005). Reduction in protein synthesis (Stiborova *et al.*, 1987) and photo-synthesis, and damage to cell and sub-cellular organelles (Hauck *et al.*, 2003) are among the negative effect caused by the metal. The decrease in plant growth exhibited in the current study reflects the retarding effects in plant physiology and metabolism (Alia and Saradhi, 1991; Baker and Walker, 1990). The toxicity increased with increases in the metal in the root zone (Gao *et al.*, 2010). Presence of citric acid alleviated the toxic effect of the metal. The acid must have caused positive metabolic processes in plants including mobilization of weakly soluble essential nutrients (Strom *et al.*, 2001). Gao *et al.* (2010) observed that citric acid increased plant growth of black nightshade berries (*Solanum nigrum*). Najeeb *et al.* (2011) noted a promoting effect of citric acid to the growth for common rush (or soft rush) herbaceous flowering plant (*Juncus effuses*) under high contents of Mn in the root zone Ehsan *et al.* (2014) noted alleviating effect of citric acid to Rapeseed (*Brassica napus*), under Cd high contents in the root zone. High contents of Pb around plant roots causes disruption to plant chloroplasts, protein synthesis, and photosynthesis (Ali *et al.*, 2013b, 2013a and Vassilev *et al.*, 1995). Disruption of chloroplasts was attributed to a rise in the activity of the chlorophyllase enzyme (Hegedus *et al.*, 2001).

Table 5. Effect of Pb added to soil with or without citric acid in irrigation water on total and DTPA-extractable Pb (mg kg^{-1}) remaining in soil after growth of 120-day old white jute (*Corchorus capsularis*), and river red gum (*Eucalyptus camaldulensis*)

Treatment Pb mg kg^{-1}	White jute				River red gum			
	Without citric acid		With citric acid		Without citric acid		With citric acid	
	Total Extractable	DTPA Extractable	Total Extractable	DTPA Extractable	Total Extractable	DTPA Extractable	Total Extractable	DTPA Extractable
0	18.7	0.9	12.1	0.55	15.5	0.3	9.1	0.3
400	319.8	6.8	173.8	10.1	210.0	4.4	93.9	5.8
800	464.5	31.8	270.4	40.3	370.7	25.7	197.6	33.8
1200	664.7	55.7	458.0	70.2	549.0	39.8	230.3	42.9
LSD 0.05	17.2	4.8	16.1	5.4	14.	3.2	12.6	3.9

**Fig. 1.** Bioconcentration factor as influenced by different lead concentrations including (lead addition, 400, 800 and 1200 mg lead kg^{-1}) with or without citric acid in *Corchorus capsularis*, L. and *Eucalyptus camaldulensis*

Disruption of gas exchange and plant stomatal conductance, which would lead to reduce the rate of chlorophyll synthesis, may occur under stress of high heavy metal contents (Balakhnina *et al.*, 2005).

The alleviating effect of citric acid may be due to increases in chlorophyll contents and gas exchange rate (Wang *et al.*, 2004). The increased uptake of elements from soils caused by citric acid was reported by Lambers *et al.* (2006). Citric acid and other low molecular

organic acids produce protons and electrons which remove metals in the rhizosphere (Jones and Brassington, 1998). Williams *et al.* (2006) concluded that citric acid increased the uptake of Cd in Indian mustard (*Brassica juncea*) plants. Processes causing metal accumulation in plant include mobilization and uptake compartmentation, sequestration within plant roots, increased efficiency of xylem loading and storage in leaf cells. (Kabata-Pendias and Pendias, 1989, Marschner, 1995, Williams, 2000 and Clemens, 2001).

The capacity of phyto-remediator plants in acquiring heavy metals is a function of the contents of metal in soil, soil physical and chemical properties, physiological state of the plants, specificity of the metal and the mechanism of acquisition (Raskin *et al.*, 1994). Plants respond to heavy metal toxicity in different ways including immobilization, exclusion, compartmentalization and synthesis of metallothioneins (cysteine-rich proteins which binds heavy metals) (Sanita di Toppi and Gabbriellini, 1999)

The current study shows that red river gum (*Eucalyptus camaldulensis*) is more efficient as a phytoremediation plant than jute (*Corchorus capsularis*), and that citric acid can enhance such remediation.

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حامض الستريك لتعزيز المعالجة النباتية للرصاص باستخدام الجوت الأبيض والكافور البلدي

مجدي محمد نيازي - مصطفى عيسى وهدان

معهد بحوث الأراضي والمياه والبيئة - مركز البحوث الزراعية- مصر

استخدام النباتات لإزالة المعادن الثقيلة والسامة من التربة (المعالجة النباتية) كبديل فعال واقل تكلفة من الأساليب التقليدية تم اختيار نبات الجوت الأبيض والكافور البلدي لركم الرصاص داخل أنسجة تلك النباتات عندما تزرع في التربة الملوثة وفي هذه الدراسة تم استخدام حامض الستريك لزيادة امتصاص الرصاص والحد من تأثير الرصاص السلبي على النباتات وكذلك الستريك أيضاً يؤدي إلى تسريع عملية المعالجة النباتية للرصاص من خلال النباتات مفرطة التراكم للرصاص مثل الجوت الأبيض والكافور البلدي وأظهرت النتائج أيضاً أن متوسط الوزن الجاف للجوت الأبيض والكافور البلدي ينخفض في التركيزات المرتفعة من الرصاص وان الكافور أكثر قدرة من الجوت الأبيض في إزالة الرصاص في التربة، وأن تركيز الرصاص في النباتات المعاملة بحامض الستريك كان أعلى من تركيز الرصاص في النباتات غير المعاملة.

المحكمون :

١- أ.د. علي أحمد عبدالسلام
٢- أ.د. السيد عوض محمد عوض

أستاذ الأراضي - كلية الزراعة بمشهر - جامعة بنها.
أستاذ الأراضي المتفرغ - كلية الزراعة - جامعة الزقازيق.