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European Journal of Experimental Biology, 2013, 3(6):7-13

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Soil amendments to enhance lead uptake by *Eucalyptus camaldeulensis* cultivated on metal contaminated soil

Khaled Sallami[1], Stephen J. Coupe[2], Jess Rollason[3] and Eshmaiel Ganjian[2]

[1]Faculty of Business Environment and Society, Coventry University, England, United Kingdom
[2]Department of Civil Engineering, Architecture and Building, Faculty of Engineering and Computing, Sir John Laing Building, Coventry University, Coventry, CV1 5FB, England, United Kingdom
[3]Faculty of Health and Life Sciences, Coventry University, England, United Kingdom

ABSTRACT

The use of plant to remove heavy metals from soil (phytoremediation) is expanding due to its cost-effectiveness as compared to conventional methods and it has revealed great possible potential. Since contaminants such as Pb have a limited bioavailability in the soil, methods to facilitate their transport to the shoots and roots of plant are required for successful phytoremediation. The objective of this study was to investigate the effects adding of different rates of EDTA at concentrated (0, 5, 10 and 15 mmol/kg), (0, 5, 10, and 25%) of compost, (0, 25, 50, and 100%) of Hoagland solution, bacterial inoculums, and mixtures of 5% EDTA, 5% compost, 100 Hogaland solution, and bacterial inoculums of an amendment on Pb availability in soil, and plant biomass. The phytoextraction ability was assessed in terms of its metal transfer factors; bioconcentration factor (BF) and translocation factor (TF). Experimental results showed that the higher concentrations of available Pb (12.6 ± 0.47, 17.6 ± 0.68, 13.1 ± 0.27, 15.4 ± 0.66, and 17.2 ± 0.98 mg kg $^{-1}$) were obtained from soil amended with 25% compost, 15 mmol EDTA, 100% Hoagland solution, Bacterial inoculums, and mixed amendments (5% composite, 5 mmol EDTA, 100% Hoagland solution and bacterial inoculums), respectively. Application of mixed amendments increased the fresh and dry weights of shoot and root of E. camaldeulensis cultivated on Pb contaminated soil. The highest accumulation of Pb (585±4.5 and 1073±7.6 mg kg $^{-1}$DW) in plant shoot and root, respectively, were obtained by plant cultivated in soil inoculated with Alcaligenes eutrophus. Based on the BCF and TF values, E. camaldeulensis can be utilized as a good candidate for Pb stabilization in soil.

Keywords: Phytoremediation, Pb-uptake, *E. camaldeulensis*, soil amendments

INTRODUCTION

Heavy metal contamination in soils is one of the world's major environmental problems, posing significant risks to human health as well as to ecosystems. Therefore, the development of a remediation strategy for metal-contaminated soils is necessary for environmental conservation and human health. Phytoremediation, using plants to remove metal pollutants from contaminated soils, is being developed as a new method for the remediation of contaminated land[7 ,12,1]. Heavy metals in soils are generally bound to organic and inorganic soil constituents, or alternatively, present as insoluble precipitates. A large proportion of metal contaminants are unavailable for root uptake by field grown plants. Methods of increasing heavy metal contaminant phytoavailability in soil and its transport to plant roots are vital to the success of phytoremediation[10 ,2,15].

Microbial populations are known to affect trace metal mobility and availability to the plant, through release of chelators, acidification, and redox changes [26, 4]. The presence of rhizosphere bacteria has been reported to increase the concentrations of Zn, Cu, Pb, Cr, or Ni in plants [33, 8, 3]. Improvement of the interactions between plants and beneficial rhizosphere microorganisms can enhance biomass production and tolerance of the plants to heavy metals, and is considered to be an important component of phytoremediation technologies [31, 13, 33, 5].

The use of hyperaccumulator species in a continuous phytoextraction process is limited by the low bioavailability of these pollutants in the process of root uptake [25,24]. . It was demonstrated that the application of mobilizing and, or chelating agents, such as EDTA to the soil is a reliable practice for increasing plant metal bioavailability, uptake and shoot accumulation [19].

High biomass producing plant species have potential for removing large amounts of trace metals by harvesting the aboveground biomass if sufficient metal concentrations in that biomass can be achieved. Addition of fertilizer and compost are the most common method for increasing crop production, and these are also practical options for increasing plant biomass. *Solanium nigrum* is a weed species and its biomass can grow very fast after the addition of fertilizer (Chicken manure and urea) [34, 30].

[9] Reported that the highest concentrations of Pb (548 mg/kg) were found in the shoots of *E. camaldeulensis* grown in Pb contaminated soil. Pb concentrations in the root and shoot of *E. camaldeulensis* grown in contaminated soil were about 11 and 7.5-fold, respectively higher than the Pb concentration in the root and shoot of the same plant grown on the control soil. Therefore, the present study was carried out to assess the effectiveness of *E. camaldeulensis* to uptake and accumulate Pb in their biomass from metal contaminated soil and to evaluate the effect of EDTA, bacteria, nutrient solution, and compost, individual or as a mixture on Pb uptake by *E. camaldeulensis*

MATERIALS AND METHODS

2.1. Soil source, preparation and analysis

Soil samples were collected from site with high Pb contents [9]. Soil samples were mixed in large containers and air-dried at room temperature, then crushed and sieved to remove rocks and un-decomposed organic materials. Soil pH was determined after mixing 1 g of soil in 2.5 mL water for about 5 min [11]. Cation exchange capacity (CEC) was determined by the method of [28]. Total metals in soil were determined by digesting 500mg of soil in a mixture of concentrated 3 HCl / HNO (4:1, v / v) [21]. while extractable metals were measured by shaking 10g (dry wt) moist field soil for 2-h in 20 ml 0.01 M $CaCl_2$ [27].. Sample was filtered and acidified with HNO_3 before analysis. Metal concentrations in acid digest and extract solutions were analyzed by ICP-AES.

2.2. Pot experiment and soil amendments

Subsamples of about 2 kg placed were into plastic pots (18 cm in diameter and 13 cm in depth). Seeds of *Eucalyptus* Sp were sown in the plastic pots which contained metal (Pb) contaminated soil (840 mg/kg dry soil) with four replicates for each treatment. The experiment was carried out in a greenhouse illuminated with natural light. The moisture content of each pot was maintained at 70% WHC by weighing the pots twice a week. After germination, the seedlings were thinned to two plants per pot and grown for eight weeks.

To prepare the bacterial inocula (*Alcaligenes eutrophus*), bacterial cells were grown overnight in 500 ml Erlenmeyer flasks containing 250 ml of sterilised nutrient broth and placed on a shaker at 150 rpm/min at 30^{o}C until late log phase. Bacterial cells were then harvested by centrifugation (4000 g×, 20^{o}C, 10 min), and the pellets were washed twice with sterile saline solution. Bacterial suspensions in the saline solution were then adjusted to an absorbance of 0.5 at 600 nm (e.g. equivalent to approximately 7.4×10^8 c.f.u. ml^{-1}), which were used for soil inoculation [5].

Eucalyptus seeds were sown in plastic pots containing 2 Kg Pb contaminated soils. After 2 weeks, the plants were thinned to 3 plants per pot. 5 ml of the appropriate bacterial suspension was added at a concentration of 10^8 c.f.u ml^{-1} (sterile distilled water) after four weeks from seed germination, whereas for the non-inoculated control plants, the same amount of sterile distilled water was added after plant seedlings (4 weeks). Other pots were amended with three different concentrations of EDTA (ethylenedinitrilotetraacetic acid) i.e. (0, 5, 10, and 15 mmol/kg soil) and control plants were not treated with EDTA. Other experimental pots which contains Pb contaminated soils amended with 5%, 10% and 25% (wt/wt soil : compost). These pot experiments were irrigated with different dilutions of Hoagland's solution (25, 50, and 100%). Mixed amendments *(5% compost + 5 mmol EDTA + 25% hogland solution + 5ml bacterial suspension)* were used in other pots. Plants were grown for three months in a greenhouse illuminated with natural light. The moisture content of each pot was maintained at 70% water holding capacity by weighing the pots twice per week.

All treatments were replicated three times in the experiments. The mean values and standard deviations (SD) were calculated.

2.3. Plant harvesting and metal analysis

After two months, whole plants were gently removed from the pots. The plants were washed in deionized water and immersed in a 0.01 M HCl solution to remove any external Pb and rinsed again for washing with deionized water for 1 min. Subsequently, the plants were separated into root and shoot comperents .After that, they were dried at 100°C for 10 min, then at 70°C in an oven until completely dry. The plant was digested with a mixture of HCl/HNO3 (4:1, v/v) [21] and the heavy metals in the digests were determined using ICP-AES.

2.4. Bioconcentration and Translocation factor

The bioconcentration factor (BCF) provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate. It is calculated as the ratio of metal concentration in the roots to that in soil ([Metal] Root/ [Metal] Soil). Translocation factor (TF) was calculated by dividing the concentration of metal concentration in the shoots to the roots ([Metal] Shoot/ [Metal] Root) [18].

RESULTS AND DISCUSSION

3.1. Influence of different soil amendments on Pb availability in soil

The majority of soil metal is commonly found as insoluble compounds not available for the transport into roots, and consequently affects the metal uptake by hyperaccumulating plants. The addition of EDTA, bacterial inoculum and the mixture of EDTA, nutrient solution and compost leads to increase the available Pb in soil. The total nitrogen and phosphorus were increased as a result of the addition of Hoagland solution and compost (Table 1). The addition of composite leads to the slight increase in the available Pb concentration as a result of total Pb concentration in composite. Recently many synthetic chelators have been applied in Pb-contaminated soils to increase the mobility and bioavailability of Pb, thereby increasing the amount of accumulated Pb in the aerial parts of phytoextracting plants [19,29]. Among these chelators, EDTA has been found to be the most efficient in increasing the concentration of water-soluble Pb [32,16]. Soil microorganisms can affect trace metal mobility and availability to the plants, they can produce iron chelators and siderophores for ensuring iron availability, reduce soil pH, and/ or solubilize metal-phosphates [2, 4]. As shown in Table 1. The addition of EDTA, bacterial inoculums and the mixture of EDTA, nutrient solution and compost lead to an increase the available Pb in soil. The total nitrogen and phosphorus were increased as a result of the addition of Hoagland solution and compost. The addition of compost lead to the slightly increase in the available Pb concentration as a result of total Pb concentration in composite and increase in organic matter, nitrogen and phosphorus concentration in soil (Table 1). Stabilization of inorganic contaminants by processes of adsorption, binding or co-precipitation with the additive amendments [17] has been widely researched in the decade [20]. Of the numerous amendments used for *in situ* stabilization of contaminants, organic materials such as biosolids, manures and composts, rich in organic matter, have proved successful at reducing the mobility of contaminants in multi-metal polluted soils [22].

3.2. Effect of soil amendments on plant biomass

The effects of soil amendments on the biomass of *E. camaldeulensis* in terms of fresh and dry weight is presented in Fig. 1 and 2. The effect of application of EDTA on the biomass of *E. camaldeulensis* plants showed a significant decrease in biomass (fresh and dry weight) at highest concentration (15 mmol/kg EDTA) (Fig. 1 and 2). However, there was no significant effect at lower concentrations of EDTA compared with the control. The shoot fresh weight of *Eucalyptus* plants grown on Pb contaminated soils amended with the EDTA (10 and 15 mmol/kg soil) were reduced by 1.1 and 1.2 fold lower than the shoot fresh weight of the same plants grown on the control soil, respectively. The shoot and root fresh weight of *Eucalyptus* plants grown on Pb-contaminated soil amended with compost (5, 10, and 15%), Hoagland solution (25, 50, and 100%), bacterial inoculums, and mixture of the amendments plus EDTA (5%) were increased from 1.1 to 3.9-fold higher compared with control (Fig. 1). In addition, the shoot and root dry weight were also increased from 1.8 to7.5-fold, higher compared with untreated (control) soil (Fig. 2).

3.3. Effect of soil amendments on Pb accumulation by *E. camaldeulensis*

Application of EDTA leads to increase Pb concentration in plant shoot from 544±4 mg kg^{-1} dwt in control plant to 552±3, 567±4.3, 572±2.6 mg kg^{-1} dwt in plant cultivated in soil amended with 5%, 10%, and 15% EDTA, respectively (Fig. 3). Application of EDTA was shown to promote Pb accumulation significantly in *B. juncea* when grown in artificially contaminated soil [16]. The accumulation of Pb in shoots was increased with increasing the concentrations of EDTA compared with plant roots. The highest accumulation of Pb (371.4 mg kg^{-1}dwt) was observed with the highest does of EDTA (10 mmol EDTA kg^{-1}) and Pb (500 mg Pb kg^{-1}) in shoots [16]. Bacterial inoculations led to increases in the Pb accumulation in shoots by 1.3-fold higher than the control (Fig. 3). Rhizosphere microorganisms may interact symbiotically with roots to enhance the potential for metal uptake [6, 14].

[5] reported that the concentration of extractable Ni was increased from a high Ni soil of 2.2 to 2.6 mg kg^{-1} when the soil was inoculated with *Microbacterium arabinogalactanolyticum* AY509224.

3.4. Effect of soil amendments on accumulation and translocation of Pb

The mobility of the heavy metals from the polluted substrate into the roots of the plants and the ability to translocate the metals from roots to the harvestable aerial part were evaluated, respectively, by means of the bioconcentration factor (BCF) and the translocation factor (TF). The ability of plants to tolerate and accumulate heavy metals is useful for phytoextraction and phytostabilization purpose [35]. . Plants with bioconcentration factor greater than one (BCF> 1) and translocation factor less than one (TF< 1) have the potential for phytostabilization [35]. As shown in Table 2 the BCF in plant with different amendments is greater than 1 and this indicate that the plant used in this experiment can used for Pb stabilization in soil and prevent leaching into the groundwater.

Table 1: Physico-chemical characteristics of Pb unplanted contaminated soil with different amendment after one month in pot experiment

Amendments	pH	CEC	EC	OM	N	P	Available Pb
			mmhos cm^{-1}	(%)	mg kg^{-1}		mg kg^{-1}
Composite (C)							
0%	7.71	8.09	1.60	0.09	0.14	0.70	8.8 ± 0.1
5%	7.81	8.21	2.12	1.15	0.52	0.75	9.6 ± 0.15
10%	8.10	8.28	2.98	2.35	0.92	0.81	10.2 ± 0.25
25%	8.20	8.33	3.15	4.54	2.31	0.98	12.6 ± 0.47
EDTA (B)							
0 mmol	7.71	8.09	1.60	0.09	0.14	0.70	8.8 ± 0.1
5 mmol	7.68	8.11	2.05	0.09	0.13	0.72	14.2 ± 0.86
10 mmol	7.64	8.15	2.51	0.11	0.13	0.81	16.8 ± 0.30
15 mmol	7.60	8.25	2.98	0.12	0.15	0.83	17.6 ± 0.68
Hoagland solution (H)							
0%	7.71	8.09	1.60	0.09	0.14	0.70	8.8 ± 0.1
25%	6.54	8.25	1.82	0.16	0.19	0.81	9.5 ± 0.20
50%	6.40	8.51	2.34	0.25	0.25	0.93	11.6 ± 0.74
100%	6.05	8.95	2.87	0.37	0.48	1.52	13.1 ± 0.27
Bacterial inoculum's (B)	6.6	8.3	1.68	0.15	0.14	0.90	15.4 ± 0.66
Mixed amendments (5%C+ 5 mmol E+ 100% H+B)	6.9	9.05	2.35	0.20	0.55	2.25	17.2 ± 0.98

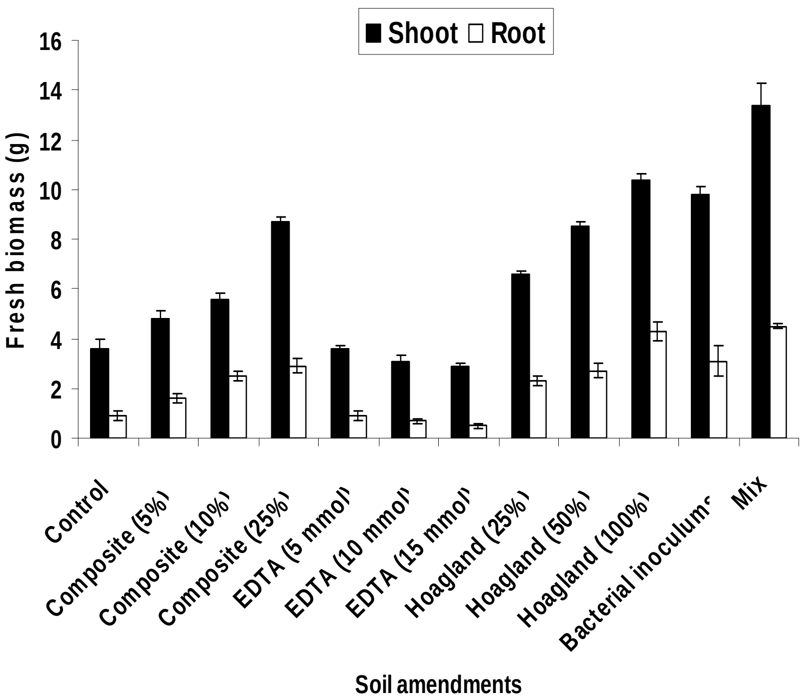

Fig. 1. Effect of different soil amendments on fresh biomass of *E. camaldeulensis*

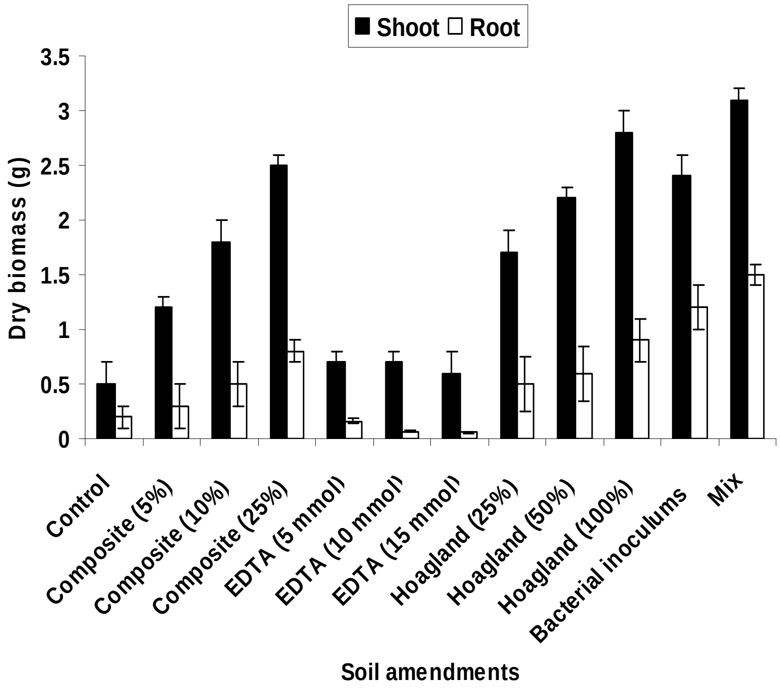

Fig. 2. Effect of different soil amendments on dry biomass of *E. camaldeulensis*

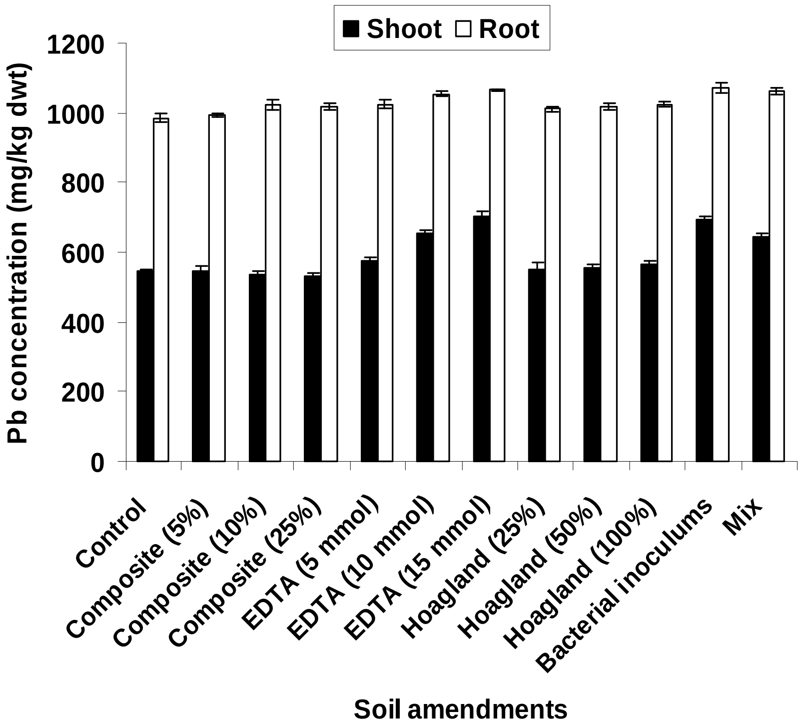

Fig. 3. Effect of different soil amendments on Pb concentration in *E. camaldeulensis* biomass

Table 2: Accumulation and translocation of Pb in plant grown in Pb contaminated soil mixed with different amendments

Amendment	Bio-concentration factor (BCF)	Translocation factor (TF)
Control	1.17	0.55
Composite (5%)	1.18	0.55
Composite (10%)	1.03	0.52
Composite (25%)	1.21	0.52
EDTA (5 mmol)	1.22	0.56
EDTA (10 mmol)	1.25	0.62
EDTA (15 mmol)	1.26	0.66
Hoagland (25%)	1.2	0.54
Hoagland (50%)	1.21	0.54
Hoagland (100%)	1.22	0.55
Bacterial inoculums	1.27	0.64
Mix	1.26	0.6

CONCLUSION

Phytoremediation is still in its research and development phase, with many technical issues needing to be addressed. The results, though encouraging, suggest that further development is needed. Phytoremediation is an interdisciplinary technology that can benefit from many different approaches. Results already obtained have indicated that *Eucalyptus camadulensis* can be effective in metal remediation, the processes that affect metal availability, metal uptake, translocation, chelation were investigated. As a result, it can be concluded that some amendment can be used successfully to increase the metal bioavableilty. The study indicated that, without amendment, the availability of heavy metals in soil is relatively low compared with amendment additives. Comparison between applied soil amendments that the metal bioavabletly were increase with increasing the concentration EDTA of from 5 mmol to 15 mmol also with bacterial inoculums (*Alcaligenes eutrophus*) and Mixed amendments to enhanced lead phytoremediation. The results of present pot experiment showed that Eucalyptus *camadulensis* can tolerate a wide range of Pb concentrations, and accumulate high concentrations of Pb in their above-ground parts (shoots) with adding some amendments such as EDTA and Alcaligenes eutrophus bacteri EDTA, and also has a great ability to dissolve the metal in the soil and also to enhance the accumulation of Pb in shoots of *Eucalyptus camadulensis* with the 15 $mmol.kg^{-1}$ EDTA and *Alcaligenes eutrophus* bacteria. Therefore, the *Eucalyptus camadulensis* can be a suitable plant for phytoremeadtion, especially for Pb contaminated soils and the application of EDTA and *Alcaligenes eutrophus* bacteria, which can significantly increase the metal concentration in harvestable above ground parts of plant.

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