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Phytoremediation of soil contaminated with chromated copper arsenate (CCA) using *Eucalyptus* species

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Abstract

The soil is gradually undergoing contamination with heavy metals and is mainly due to natural processes and human activities. Among various remedial techniques, phytoremediation is known to be effective in mitigation of polluted metals in the soil. The performance of two fast growing tree species, *Eucalyptus citriodora* and *Eucalyptus tereticornis*, was evaluated for their tolerance and accumulation of CCA compounds *viz* Cr, Cu and as in potting conditions. The results showed that seeds of both Eucalyptus species recorded better germination. Similarly, the root and shoot length and biomass of these species were high in saplings grown under lower concentrations and were decreased with increase in the levels of CCA. Surprisingly, the roots of the Eucalyptus species accumulated greater levels of CCA than shoot. Thus, it is concluded that, *E. citriodora* and *E. tereticornis* are potential phytoremediation agents of the soils contaminated with CCA.

Keywords: *Eucalyptus* species**,** chromated- copper- arsenate, tolerance, accumulation

Introduction

Contamination of soil by heavy metals is one of the major environmental hazards worldwide (Garbuio *et al.* 2012) [7]. Heavy metals are generally non-degradable and remain persistent in the soil for a long period by causing long-term effects on the environment (Suman *et al.* 2018) [27]. Chromated Copper Arsenate (CCA) is a wood preservative, used in protection of wood from microbial decay and insect damage. These CCA treated woods are widely used as construction material resulting in increased levels of its components *viz* Cr. Cu and as in the soil by leaching and subsequently causing environmental pollution (Khan *et al.*, 2006) [13]. Accumulation of these heavy metals in agricultural fields initiates concerns on crop productivity and food safety (Saleem *et al*. 2020) [24]. Therefore, decontamination of these heavy metals is pre-requisite for mitigating their negative impact on the environment (Tangahu *et al*, 2011) [28].

Many technologies have been adopted for remediation of soils contaminated with heavy metals (Gu *et al.* 2013) [9]. Comparatively, biological methods (Cesar *et al.* 2012) are considered as promising remediation technologies for their cost-effective and eco-friendly nature than physical (Cappuyns, 2013) [3] and chemical methods (Ali *et al.*, 2013) [1]. Recently, phytoremediation has emerged as green-technology in removing heavy metals from the soil (Ashraf *et al.* 2019) [2]. However, efficiency of this technology is primarily governed by physiological characteristics of plants and nature of heavy metals (Megateli *et al*., 2009) [16]. Many plant species have been used as hyperaccumulators for a wide range of heavy metals in the soil (Saleem *et al*. 2019) [22, 23] and have the ability to take-up large amounts of metals in plant tissues without harmful effects (Ogunkunle *et al.* 2015) [19]. Interestingly, indigenous plant species are proved to be most efficient in accumulation of contaminants in soil than exotic species (Ashraf *et al.* 2019) [2]. Eucalyptus trees show a shallow root system, fast growth, and production of huge biomass (Hazrat *et al*. 2014) [11] in dry and nutrient rich soils. Further, Eucalyptus sp. found high bioaccumulation of metals in its tissues such as Cd, Cu, Pb, Ni, Zn, and Cr (Motesharezadeh *et al.* 2017) [17]. Therefore, studies were conducted to evaluate the efficacy of Eucalyptus *citriodora* and *Eucalyptus tereticornis* in phytoremediation of Chromated Copper Arsenate.

Materials and Methods

Experiments on phytoremediation of soils treated with Chromated Copper Arsenate (CCA) compounds *viz* Cr, Cu and As were conducted in two fast growing Eucalyptus tree species, *Eucalyptus citriodora* and *Eucalyptus tereticornis* in potting conditions. The seeds of *E.* citriodora obtained from CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Bengaluru and *E. tereticornis* from Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore were used in the experiments. The seeds were surface sterilized with Bavastine (0.1%) and sown in the pots of soil supplied with nutrients. Heavy metal tolerance tests in terms of seed germination were conducted by exposing the seeds of both the eucalyptus species to different concentrations of CCA (50 to 5000 ppm) in the germination chamber supplied with constant light and temperature (25±1 °C). Seeds with visible protruded radicals and plumule were considered as germinated. Similarly, the length of shoot and roots of the germinated seedlings were recorded.

Further, the seedlings of 3 to 5 cm length were transplanted into a potting medium filled with sufficient quantity of air dried soil supplied with necessary nutrients. The commercially available CCA having a proportion of Cr (K2Cr2O7-50%), Cu (CuSo4, 5H2-37.5%), and As (As2O5, 2H2- 12.5%) was used in the study. The potting medium was supplied with each concentration (250 to 2500 ppm) of CCA separately. Later, the seedlings of one month old were transplanted into the earthen pots of 2000cc capacity. Suitable management practices, including sufficient irrigation and weed control were performed during the experiments. After six months, the shoot (stem and leaves) and roots of each test tree species were harvested. The stem, leaves and roots were dried and estimated the biomass.

Similarly, the roots were separated from soil by means of screens and washed out with running water. About 1g each of leaves, stem and roots of both the tree species in each earthen pot were sampled and digested in a heated di-acid mixture of concentrated HNO3 and HCl (3:1, v/v). The concentration of Cr, Cu and as in the samples were measured with flame atomic absorption spectrometer (GBC, Germany). The experimental data were analyzed using SPPS 20 and the mean values of each treatment in four replications are expressed in mean± standard error. The differences between the treatments were tested statistically at 0.05% and probability by one way ANOVA.

Results

Germination of Seeds and Growth of Eucalyptus Seedlings

The germination of seeds and also the growth of seedlings of Eucalyptus species in respect of shoot length and root length were recorded at different concentrations of CCA (Table.1). The observations revealed that the seeds of *E. citriodora* species showed better germination than *E. teresticornis* in different concentrations of CCA.

The rates of germination of seeds of *E. teretocornis* and *E. citriodora* recorded in 50ppm CCA concentration were 67.67 % to 90.00% respectively. However, the percent seed germination was decreased with increase in the concentrations of CCA. The *E. citriodora* recorded 70.13% germination in contrast to 31.67% germination in *E. tereticornis* in 5000 ppm CCA concentration. Similarly, the shoot and root lengths of seedlings of Eucalyptus species also varied through different concentrations of CCA. The greater shoot lengths of 10.33 cm and 2.77 cm and root lengths of 5.83 cm and 1.53 cm were recorded in *E. citriodora* and *E. tereticornis* respectively in control pots without application of CCA. However, shortest shoot and root lengths of 2.03 cm and 0.30, and 0.40cm and 0.10 cm was recorded in the seedlings of *E. citriodora* and *E. tereticornis* in 5000 ppm CCA concentration respectively.

Table 1: Effect of Chromated copper Arsenate (CCA) on seed germination and growth of *Eucalyptus citriodora* and *Eucalyptus tereticornis* seedlings

Concentration of CCA (ppm)	***Eucalyptus citriodora***			***Eucalyptus tereticornis***		
	Germination (%)	**Shoot length (cm)**	**Root length (cm)**	**Germination (%)**	**Shoot length(cm)**	**Root length (cm)**
Control	90.00a	10.33a	5.83a	67.67a	2.77a	1.53a
50	90.00a	9.33b	5.20a	65.67a	2.47ab	1.00b
100	88.33a	8.00c	4.63b	56.33b	2.00bc	0.77bc
500	86.66b	6.00d	2.60c	50.67c	1.55c	0.47cd
1000	84.10b	4.00e	2.10c	46.00d	0.77d	0.27cd
5000	70.13c	2.03f	0.40d	31.67e	0.30de	0.10d
SE$_{(0.05)}$	6.32	0.56	0.44	3.616	0.3401	0.318
CD$_{(0.05)}$	11.87	0.98	0.77	6.365	0.5986	0.5611
p-value	2.86E-10	1.56E-12	1.14E-10	3.29E-12	4.97E-08	8.90E-05

Data scored on completion of 21 days after keeping for seed germination.

Effects of CCA on Biomass of Eucalyptus Seedlings

The biomass of Eucalyptus seedlings in respect of fresh and dry weights was recorded in different concentrations of CCA (Table. 2). The observations showed a significant fluctuation in fresh and dry weights of *E. citriodora* and *E. tereticornis* seedlings in different heavy metal concentrations. Comparatively, *E. tereticornis* showed higher biomass than *E. Citriodora* species. Similarly, the maximum fresh and dry weights of *E. tereticornis* and *E. citriodora* recorded were 25 and 11.88g and 23.85 and 10.37g respectively in 250 ppm CCA concentration. However, the biomass of seedlings decreased with increase in concentrations of CCA. The minimum fresh and dry weights of biomass recorded in *E. tereticornis* and *E. citriodora* were 11.43 and 5.79 g and 10.04 and 5.31g respectively in 2500 ppm CCA concentrations.

Table 2: Effect of Chromated copper Arsenate (CCA) on accumulation of biomass of seedlings of *Eucalyptus citriodora* And *Eucalyptus tereticornis* seedlings

Concentration of CCA (ppm)	***E. citriodora***		***E. tereticornis***	
	Fresh weight (g)	**Dry weight (g)**	**Fresh weight**	**Dry weight (g)**
Control	24.04a*	10.41a	24.5a	10.840ab
250	23.85a	10.37ab	25a	11.880a
500	22.67b	9.78ab	23.13b	10.150bc
750	18.33c	9.70b	19.24c	10.030bcd
1000	15.91d	8.90c	16.25d	9.370cd
1250	15.31de	8.28cd	15.51de	8.740de
1500	14.35e	7.68d	14.76e	7.960ef
2000	12.95f	6.88e	13.6f	7.000fg
2500	10.04g	5.31f	11.43g	5.790g
SE$_{(0.05)}$	0.34	0.136	0.29	0.512
CD$_{(0.05)}$	1.10	0.694	1.019	1.347
p-value	1.98E-16	9.8E-12	6.02E-17	9.8E-08

*Different letters in the column indicate significant differences at 5% probability level

Accumulation of CCA Compounds in Eucalyptus Saplings

Accumulation of CCA in the form of its components *viz* Cr, Cu and as were recorded in root and shoot of *E. citriodora* saplings (Table. 3). Accumulation of test compounds was significantly increased with increase in concentration of CCA in soil in roots and shoot of both tree species. Comparatively, roots accumulated greater levels of CCA compounds than stem and leaves. The maximum and minimum concentrations of Cr deposited in roots were 5.197 and 0.576ppm respectively. Similarly, the higher and lower levels of Cu and as accumulated in roots were 3.810 and 0.200ppm, and 0.518 and 0.029ppm respectively.

Furthermore, the Cr, Cu and as were well accumulated in shoot parts *viz* stem and leaves of *E. citriodora* on exposure to different CCA concentrations. The maximum and minimum quantities of Cr found in stem and leaves were 2.393 and 0.093ppm, and 3.463 and 0.096ppm, respectively. Similarly, the higher and lower levels of Cu and as accumulated in stem and leaves were 1.596 and 0.077ppm, and 2.447 and 0.086ppm, and 0.251 and 0.019ppm, and 0.320 and 0.025ppm respectively.

Accumulation of Cr, Cu and as, the components of CCA were recorded in root and shoot of *Eucalyptus tereticornis* saplings (Table. 4). The results revealed that the CCA compounds were found accumulated in roots and shoots. The levels of heavy metals were high in 2500 ppm and low in 250 ppm CCA concentrations. The maximum and minimum levels of Cr found in roots were 5.923 and 0.853 ppm. Similarly, higher and lower levels of Cu and as accumulated in roots were 3.960 and 0.210ppm, and 0.507 and 0.033ppm respectively.

Similar tendency was also recorded in shoot parts such as stem and leaves. The maximum and minimum concentrations of Cr found in stem and leaves were 2.683 and 0.090ppm and 4.570 and 0.100 ppm. The maximum and minimum levels of Cu deposited in stem and leaves were 1.910 and 0.093ppm/g and 3.203 and 0.096ppm respectively. The high and low levels of as found in stem and leaves were 0.251 and 0.019ppm and 0.392 and 0.028ppm respectively.

Table 3: Effect of Chromated copper Arsenate (CCA) on accumulation of Chromium, Copper and Arsenic in root and shoot of *Eucalyptus citriodora* seedlings of six months age.

Concentration of CCA (ppm)	Chromium (ppm)			Copper (ppm)			Arsenic (ppm)		
	Root	Shoot		Root	Shoot		Root	Shoot	
		Stem	Leaves		Stem	Leaves		Stem	Leaves
250	0.576h	0.093fg	0.096g	0.200g*	0.077f	0.086g	0.029fg	0.019cd	0.025ef
500	0.973g	0.136efg	0.213g	0.265g	0.102e	0.206f	0.061efg	0.032cd	0.032ef
750	1.380f	0.276ef	0.590f	0.590f	0.206de	0.290e	0.073ef	0.043cd	0.050e
1000	2.030e	0.346de	1.077e	1.130e	0.303de	0.336e	0.111de	0.073cd	0.099d
1250	2.630d	0.543d	1.930d	1.803d	0.490d	0.56cd	0.141cd	0.119bc	0.127d
1500	3.437c	1.187c	2.337c	2.553c	0.620c	0.953c	0.197c	0.177ab	0.182c
2000	4.4633b	1.690b	2.833b	3.407b	0.736b	1.843b	0.280b	0.239a	0.267d
2500	5.197a	2.393a	3.463a	3.810a	1.596a	2.447a	0.518a	0.251a	0.320a
SE±	0.126	0.1324	0.502	0.1866	0.042	0.2444	0.037	0.064	0.029
CD$_{(0.05)}$	0.217	0.229	0.869	0.322	0.073	0.4228	0.065	0.110	0.0515
p-value	1.79E-21	4.29E-15	4.88E-17	1.18E-16	1.35E-19	8.63E-11	4.42E-12	6.67E	3.02E-11

*Different letters in the column indicate significant differences at 5% probability level

Table 4: Effect of Chromated copper Arsenate (CCA) on accumulation of Chromium, Copper and Arsenic in root and shoot of *Eucalyptus teretocornis* seedlings of six months age.

Concentration of CCA (ppm)	Chromium (ppm)			Copper (ppm)			Arsenic (ppm)		
	Root	Shoot		Root	Shoot		Root	Shoot	
		Stem	Leaves		Stem	Leaves		Stem	Leaves
250	0.853f	0.090c	0.100fg	0.210d*	0.093f	0.096d	0.033ef	0.019cd	0.028ef
500	1.120ef	0. 183c	0.156fg	0.393d	0.206ef	0.273d	0.061ef	0.032cd	0.047ef
750	1.663de	0.290c	0.236ef	0.633cd	0.320e	0.403d	0.081def	0.043cd	0.068def
1000	2.183d	0.420c	0.396e	1.177cd	0.643d	0.620cd	0.114de	0.73cd	0.099de
1250	3.657c	0.586c	1.877d	1.880bc	0.813b	1.173bc	0.191cd	0.119bc	0.138cd
1500	4.807b	1.443b	2.483c	2.603ab	1.217c	1.800b	0.260bc	0.177ab	0.210c
2000	5.817a	2.556a	3.723b	3.500a	1.530b	2.610a	0.359b	0.239a	0.296b
2500	5.923a	2.683a	4.570a	3.960a	1.910a	3.203a	0.507a	0.251a	0.392a
SE±	0.458	0.453	0.114	0.841	0.137	0.416	0.067	0.063	0.049
CD$_{(0.05)}$	0.793	0.784	0.198	1.455	0.237	0.719	0.116	0.109	0.085
p-value	4.16E-13	9.86E-08	5.21 E-22	7.37E-06	3.27E-13	5.15 E-09	3.54E-08	6.67E-05	1.05 E-08

*Different letters in the column indicate significant differences at 5% probability level

Discussion

The characteristics of plants such as roots, shoot and biomass play an effective role in assessing phytoremediation of heavy metal contaminated soils. The rates of seed germination, root and shoot lengths and biomass of *E. citriodora* and *E. tereticornis* were decreased with the increase in CCA concentrations. The results also prove that Eucalyptus species have very good tolerance against CCA compounds as evident by their seed germination and size of biomass (Yan *et al.* 2020). Although eucalyptus species are not hyper-accumulators, they produce a large biomass and remove large quantities of CCA compounds. It is apparent that heavy metals are taken up by tree roots from the soil and subsequently transported to shoots. In the present study, both Eucalyptus species accumulated significant amounts of Cr, followed by Cu and as. Similarly, the roots accumulated greater quantities of heavy metals than the shoot. Khan (2001) [12] found a higher amount of Cr accumulation in the root of *Dalbergia sisso* followed by stem and leaf with mycorrhizal colonization. However, a greater amount of Cr was found in both the root and stem (Manikandan *et al.* 2016) [15].

Similarly, accumulation of Cu was found in greater amounts in root, followed by leaf and stem. Pahalawattaarachchi *et al.* (2009) [20], recorded greater amounts of copper in roots than aerial parts, and is a strategy to protect the sensitive parts of the plant from metal toxicity by acting as a barrier for metal translocation. Similarly, Fernandez and Henrique (1991) [6] found that Cu tolerant plants prevent copper from reaching stem and leaves by keeping it in their roots. Our results are also supported by Sela *et al*., (1989) [25], who found that the accumulation of metals in roots and shoots varies with metal concentrations.

The heavy metal, as was found in greater amounts in root compared to shoot as it is not readily translocated to the shoot (Rahman *et al*. 2007) [21]. The findings in the present study reveal that metal ions taken up by plant species from the contaminated soil are retained primarily in the roots and only a small proportion was translocated to above ground mass. The metal-tolerant species have barrier mechanisms against the toxicity caused by the heavy-metal initiated pressure, but the duration and magnitude of exposure and other natural conditions add to the effect of heavy metals (Nagajyoti *et al*., 2010) [18].

The greater levels of metals accumulation in Eucalyptus species is attributed to the well-developed detoxification mechanisms (Cui *et al*. 2007, Ghosh and Singh, 2005) [5, 8]. Shanab *et al*. (2007) [26] found that the plants grown in metal enriched substrata take up metal ions and uptake is influenced by the viability metals in the soil. The differential accumulation of heavy metals in plants is also determined by mobility and solubility of metals (Guilizzoni, 1991) [10]. Similarly, the woody species produce a very high amount of biomass which facilitates the accumulation of high levels of heavy metals in their shoot system (Luo *et al*. 2016). Similarly, these tree species have a deep root system, which can effectively reduce soil erosion and prevent the dispersal of contaminated soil to the surrounding environment (Suman *et al*. 2018) [27].

The present study substantiates efficiency of *E. citriodora* and *E. tereticornis* in phytoremediation of Cr. Cu and as through tolerance and accumulation in their roots and shoot. However, growth of Eucalyptus saplings and their performance may be restricted to experimental conditions including limited soil in the earthen pots and short- duration of growth period. Based on the results of the study, it is concluded that, Eucalyptus species are promising tree species in phytoremediation of Cr, Cu and As contaminated soil through better germination together with production of huge biomass, and accumulation of greater levels of CCA compounds. Further, the heavy metals accumulated by the trees do not get into the food chain since the tree is mainly used as a source of timber.

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