Phytoremediation of Pb and Cd by native tree species grown in the Kingdom of Saudi Arabia.

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Abstract: Pot culture experiments were conducted to study the remediation potentials of three native tree species *Acacia saligna*, *Eucalyptus rostrata* and *Conocarpus erectus* to remove Pb (0, 500 and 1000 mg kg^{-1} soil) and Cd (0, 5 and 10 mg kg^{-1} soil) from the contaminated soils in order to find out the most effective species that can be used to clean up the soil from heavy metal pollution. Obtained results showed that the highest level of pollution with Pb and Cd caused a significant reduction in vegitative growth parameters and photosynthetic pigments, while proline, Pb and Cd contents in plant organs increased with increasing Pb or Cd level in the soil till certain level. Catalase and Peroxidase activity was stimulated with increasing pollutant levels then tended to decrease at highest level of Pb and Cd. Results indicated that *A. saligna* was the most tolerant species to lead and cadmium pollution followed by *E. rostrara* while *C. erectus* showed low level of tolerance to Pb and Cd pollutants.

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Introduction

Toxic metal pollution in the biosphere has accelerated manifolds due to continuous emission from agricultural industries, mining and other anthropogenic activities such as human foodstuffs by agricultural uses of fertilizers and sewage sludge, as well as by industrial uses of the metal (Fodor, 2002). These heavy metals are causing major environmental and health problems (Hu *at al.*, 2014) and also increasing risk of bone fracture, cancer, kidney disorders and hypertension (Satarug *at al.*, 2011). Some heavy metals such as nickel (Ni), cadmium (Cd), lead (Pb), chromium (Cr) and copper (Cu) found to have adverse effect in plant growth and establishment.

In the Kingdom of Saudi Arabia, many sources of pollution are becoming scarcy because of the industrial development, oil resources, excessive use of chemical fertilizers, huge amounts of vehicles and other activities. All these sources are introducing heavy metals in the environment either in the atmosphere or in the soil and water resources (Al-Homaidan *at al.*, 2011). Recent studies showed that two potentially toxic elements of heavy metals, lead (Pb) and cadmium (Cd), are common in many sites of the country (Adham *at al.*, 2011). These elements were found in vegetables and fruit crops produced from these sites or grown near the tailings where heavy metal concentrations are relatively high (Ayyappan and Ravindran, 2014). Ingestion of toxic metals contaminated food may cause severe health problems (Ang *at al.*; 2010). This has drawn interest to develop a scientifically cost-effective remedial measure to remove heavy metals from contaminated sites. One of the established approaches was through phytoremediation (Aba-Alkhil and Moftah, 2013).

Phytoremediation is a way for cleaning the polluted sites by using trees to extract the heavy metals from the contaminated ground, and accumulate them in roots, stems and branches. Logically, repeating cycles of planting and harvesting of trees accumulated with heavy metals will eventually reduce the concentration of toxic metals in soils to an acceptable level for other uses. Trees have the advantage over grasses and other smaller plants, in that their roots grow deeper; therefore, they are used to reach deeper pollutions in the ground (EPA, 2014). In addition, tree roots are able to produce and release special organic chemicals that create a rhizosphere zone, which is more amenable to microbes that degrade contaminants (Al-Surrayal, 2009).

A green house study shown that *A. mangium* and *A.auriculiformis* accumulated considerable amount of Pb, Cd, As and Hg from sludge treated sand tailings (Ho and Ang, 2003). In this regard, a tree stand was found to have ameliorated the harsh microclimate of polluted site and also have improved its soil properties (Gratao *at al.*, 2005). Using timber species for phytoremediation approach has additional benefit as it also yields timber at the end of its rotation. Many studies in the temperate regions show that tree species were used as bioaccumulators to clean heavy metals such as Ni, Pb, Zn, Cu and Cd (Pulford and Watson, 2003). This implies that tree species have a great potential to remove heavy metals from the contaminated sites. In this regard, Moftah and Al-Ghanim (2009) found that *Eucalyptus rostrata* seedlings were able to accumulate high amounts of Pb when grown in polluted soil of Qassim region in Saudi Arabia. However, this ideal cost-effective approach has some limitations because polluted sites are normally adverse to establishment and growth of plants. Greening polluted sites with tree species is well documented, but unfortunatly no much documentation is available to evaluate the potential and efficiency of the native trees in the practices of phytoremediation. Therefore, this study was conducted to highlight the role of *Acacia saligna*, *Eucalyptus rostrata* and *Conocarpus erectu* tree seedlings, which are locally used in greening and combating desertification, in order to select the best species that can be used in phytoremediation process of heavy metals contaminated soils.

Materials and methods

The present study was carried out during the year of 2013/2014 to determine the ability of some tree species grow in the Kingdom of Saudi Arabia to accumulate Pb and Cd from polluted soil. Three month old seedlings of *A. saligna*, *E. rostrata* and *C. erectu* were transplanted in plastic pots (30 cm×50 cm, diameter and depth) filled with sand and peat moss (2:1 proportion), pH 7.2; one plant in each pot. Before planting, soils were mixed with fine metal powders of $PbCl_2$ or $CdCl_2$ to bring Pb levels of 00, 250, 500 and 1000 mg/kg soil and Cd levels of 00, 25, 50 and 100 mg/kg soil. The levels of $PbCl_2$ and $CdCl_2$ were fixed based on the critical limits as reported by Arriagada *at al.* (2007). Sterilized sand imposed with various heavy metal concentrations was used as a growth medium. The experiment was framed as Completely Randomized Block Design (CRBD) with six replications of one seedling per replication.

To evaluate the efficiency of phytoremediation, it was necessary to homogenize the soil because the concentration of distributed contaminants in the soil plays a fundamental role in such studies. Seedlings were left to grow in a greenhouse under natural light and temperature conditions. Plants of uniform height within species were selected and located in lines with a spacing of 2 m between lines and 1 m between pots to avoid mutual shading.

Two weeks after transplanting, all seedlings were fertilized 3 times, at 30 day intervals, with the complete water-soluble fertilizer “Sangral” compound fertilizer (20N-20P-20K, plus micronutrients) (Sinclair Cmpany, USA) at the rate of 1g/kg soil. All pots were irrigated every 3 days to field capacity. Pots were weighed and supplied with amounts of water equal to evapo-transpiration losses. Plants were harvested 6 months after transplanting. The following parameters were determined:

1- Biomass and water content

At the end of the 6 month experimental period, shoots were harvested and roots were carefully taken out of the soil, washed with tap water and twice with deionized water in order to remove any dust deposits and surface soil, respectively, and their dry weights (DW) were determined after oven drying for 72 h at 70°C and cooled down to room temperature. All dry samples were milled, air dried, and stored until metal content and other determinations were performed. Water content (WC %) was calculated according to the formula:

$$WC\% = (FW - DW)/FW \times 100$$

2- Chlorophyll content

Chlorophyll content was measured at the end of the experiments by the method of Metzner et al (1965). Fresh leaves (0.4 g) were selected randomly from the middle part of each plant, washed with deionized water, and homogenized in a porcelain mortar with 80% acetone solution. The homogenate was centrifuged at 16,000×g for 1 min twice and the measurement of chlorophyll content was done by direct determination of the absorbance at wavelengths 663 and 646 nm at the clear supernatant fraction using spectrophotometer (Shimadzu 1240 UV–Vis).

3- Free proline

Concentrations of free proline were determined according to Bates *at al.* (1973). Leaf samples (approximately 0.2 g) are homogenized in 10 mL of 3% (v/v) aqueous sulfosalicylic acid. The homogenate was filtered through Whatman 41 filter paper. The filtrate, was acidified with glacial acetic acid and ninhydrin (1 mL each) and was heated in water bath at 100 °C for 1 h. The mixture was extracted with 5 mL toluene and the upper (toluene) phase decanted into a glass cuvette and the absorbance was measured at 520 nm. Proline concentrations were calculated using proline standards (0–50 µg/ml) in identical manner as reported by Misra and Saxena (2009).

4- Lead and Cadmium

Lead and cadmium analysis in plant tissues was performed at the end of the experiment by ICP-AES (Leeman Labs PS1000AT) according to the modified method of Pyle and Nocerino (1995). For the sample preparation, 0.5 g of dried ground plant tissue was ashed in the muffle furnace for 16 h at 480°C. Ash was dissolved in 10 ml of 2 N HCl (on a hot plate (~100°C)), solution was filtered, diluted with deionized water to 50 ml, and the cadmium and lead contents were determined.

5- Enzyme activities

a) Peroxidase activity

peroxidase activity was determined as described by Urbanek *et al* (1991) in a reaction mixture (0.2 mL) containing 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 5.0 mM guaiacol, 15 mM H_2O_2 and 50 µL enzyme extract. The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetra-guaiacol formed using its molar extinction coefficient (26.6 $mM^{-1} cm^{-1}$).

b) Catalase activity

Catalase activity was assayed spectrophotometrically by monitoring the decrease in absorbance of H_2O_2 at 240 nm. CAT was measured according to the method of Noctor and Foyer (1998). The enzyme was extracted in 50 mM phosphate buffer (pH=7). The assay solution contained 50 mM phosphate buffer and 10 mM H_2O_2. The reaction was started by addition of enzyme aliquot to the reaction mixture and the change in absorbance was followed 2 min after starting the reaction. Unite activity was taken as the amount of enzyme, that decomposes 1 M of H_2O_2 in one min.

Statistic analysis

The experiment was arranged in a completely randomized design and was analyzed by analysis of variance. All data were statistically analyzed and ANOVA was tested according to Snedecor and Cochran (1980) with the aid of COSTAT computer program for statistics. Differences among treatments were tested with LSD at 5% level of significance.

Results and Discussion

Dry biomass

Data recorded in Table (1) showed that the three tree species were significantly different in total photosynthetically active biomass. This explains why *Acacia* had the highest oven-dry weight, and followed by *C. erectus* and *E. rostrata.* The results indicate that *A. saligna* grown on Pb and Cd contaminated soils produces high yield of timber and thus oven-dry biomass is significantly high.

Results showed also that heavy metal application, particularly at high concentrations, affected the dry weight of shoots and roots of the tree species. Cadmium exhibited maximum inhibitory effect on the shoot and root weights of the species, while the effect of Pb was less than that of Cd. On the other side low levels of Pb be likely to improve shoot and root biomass as compared with control plants. In this regard Pb1 treatment caused an incease in shoot dry weights of *A. saligna*, *E. rostrata* and *C. erectus* by about 15.9%, 13.6% and 8.7%, respectively, as compared with control plants. lead treatments higher that Pb1 treatment caused a gradual decrease in the dry weight of shoots to reach its minimum at Pb3 treatment, at which the biomass dry weight decreased by about 25.8%, 27.4% and 35.8%, respectively, as compared with control plants. The lowest concentration of Cd (Cd1 treatment) didn't show any significant effect on shoot biomass while higher concentrations caused significant reduction in biomass dry weights of the three species. The highest level of Cd (Cd3 treatment) reduced shoot dry weights of *A. saligna*, *E. rostrata* and *C. erectus* seedlings by about 39%, 45.8% and 49.3%, respectively, as compared with control plants.

Table 1. Effect of Pb and Cd pollutants on dry weights of shoots and roots of *A. saligna*, *E. rostrata* and *C.erectus* seedlings (values are the mean of 3 replicates).

Treatment	*A. saligna*		*E.rostrata*		*C. erectus*	
	shoot	root	shoot	root	Shoot	root
Control	30.53±6.2	9.33±2.4	24.42±5.6	8.34±2.5	28.42±5.2	9.25±2.3
Pb1	35.37±7.4	11.25±3.2	27.74±6.8	9.75±3.5	30.90±4.4	9.85±2.1
Pb2	26.45±5.8	10.52±3.5	19.83±4.6	9.11±2.7	25.35±3.5	8.22±3.3
Pb3	22.65±3.2	7.16±2.5	17.72±3.7	6.20±2.5	18.24±4.2	6.78±2.2
Cd1	29.23±4.7	9.54±2.3	25.14±5.6	8.55±3.5	27.63±5.3	9.66±2.5
Cd2	24.37±7.3	8.14±3.3	18.55±4.8	7.85±3.2	22.51±4.2	8.21±2.3
Cd3	18.62±5.2	6.11±2.5	13.24±5.9	5.50±4.9	14.42±3.7	5.82±2.4
LSD (5%)	3.75	1.13	4.12	2.13	3.26	2.03

Roots of the different tree species seemed to be more tolerant to heavy metal pollution than shoots. In this regard, dry weights of roots were not affected significantly with low and medium concentrations of Pb or Cd. Moreover, Pb1 and Pb2 treatments caused an observed increase in dry weight of roots of all tree species as compared with control plants. While Pb3 decreased significantly the dry weight of roots by about 30.3%, 34.5% and 36.6% in *A. saligna*, *E. rostrata* and *C. erectus,* respectively. As for Cd treatments it seems that roots could not tolerate its medium and high concentrations thus the dry weight was decreased significantly, while low level of Cd (Cd1 treatment) slightly increased the root dry weight of all species. The results indicate that each of the three tree species responded differently to the applied metal, but in general the above-ground biomass was strongly reduced in metal treatments by the highest heavy metal concentration.

Growth inhibition is a common response to heavy metal stress and is also one of the most important agricultural indices of heavy metal tolerance. Lead is not generally considered to be an essential element for plant growth. The effect of Pb on seedling growth seems to be different with regards to plant species, cultivars, organs and metabolic processes (Sharma and Dubey 2005).

The present results showed that in case of toxic metals with varied concentrations of lead and cadmium, the shoot and root dry weights of all the species reduced with increasing concentration. It was obvious that tree species responded differently to increased Pb and Cd concentrations in the soil. However, growth disorders causing reductions in biomass yields are commonly observed in plants subjected to high metal levels (Panich-Pat *at al.*, 2010). In the present study, *A. saligna* was more tolerant species than the others, thus high Pb and Cd concentrations had less effect on the measured plant biomass than those of the two other species. While, *E. rostrata* and *C. erectus* showed higher reductions in biomass production relative to plants in nontreated soil. Retarded shoot and root growth due to the presence of the root environment with excess of lead and cadmium has been found by Ang *at al.* (2010). The reason for reduction in shoot and roots dry biomass due to heavy metals could be ascribed to the reduction in meristematic cells. Lead and cadmium can also disturb microtubule organization in meristematic cells (Eun *at al.*, 2000) and thus reduce cell enlargement and plant growth. Moreover, the reduction in growth of seedlings by Pb and Cd was attributed to the suppression of the cells elongation which was affected due to an irreversible inhibition exerted by the heavy metal on the proton pump responsible for the process (de Souza *at al.*, 2012).

It should be noted that, based on the growth measures used in the present study, these findings indicate that *A. saligna* was better adapted to contaminated soils. However, The seedling stage experiment demonstrated that the toxicity of a metal, incomparison to another may not be the same at all the stages of plant development. The toxicity of some metals may be so severe that plant growth is reduced before large quantities of the element can be translocate (Umadevi and Avudainayagam, 2013).

It should be mentioned that the absence of visual damage to the seedlings as affected by heavy metals (HM) suggests that *A saligna* and *E. rostrata* plants may have efficient mechanisms to tolerate Pb and Cd metal induced stress under the present experimental conditions. The most noticeable symptoms of Pb and Cd toxicity was in *C. erectus* as the inhibition of plant growth was observed at relatively low concentrations of HM. In addition, plant biomass is a good indicator for characterizing the growth performance of plants in the presence of heavy metals. Similar response to Pb treatment was previously noticed in various plants (Malar *at al.*, 2014).

Plant tolerance to heavy metal stress is estimated based on their root and/or shoot growth inhibition by the metal present in a nutrient solution (Wang and Zhou 2005). Earlier studies on the mechanism of HM toxicity suggested that Pb binds to nucleic acids and causes aggregation and condensation of chromatin, as well as inhibiting the process of replication, transcription and ultimately affecting cell division and plant growth (Patil and Umadevi (2014).

Water content

Data recorded in Table (2) indicated that water content of tree seedlings used in the present study was negatively affected by high levels of Pb and Cd contamination. While, low HM concentrations seemed to have positive effect since WC% increased in shoots and roots of all species at Pb1 and Cd1 treatments. In this regard, low Pb caused an increase in WC% of shoots and roots of *A. saligna* by about 6.6% and 3.8%, respectively, *E. rostrata* by about 6.7% and 2.8%, respectively, and *C. erectus* by about 2.1% and 4.7%, respectively. On the other side, high concentrations of Pb (Pb3 treatment) caused an observed reduction in WC% either in shoots or roots of tree seedlings. In this regard, the most reduction in WC% was observed for *C. erectus* shoots (-18.8%) and roots (-14.1%), while lowest reduction was observed for *A. saligna* shoots (-7.7%) and roots (-13.0%) as compared with control plants.

Table 2. Effect of Pb and Cd pollutants on water content (WC%) of shoots and roots of *A. saligna*, *E. rostrata* and *C.erectus* seedlings (values are the mean of 3 replicates)

Treatment	*A. saligna*		*E.rostrata*		*C. erectus*	
	shoot	root	shoot	root	Shoot	root
Control	75.5±17.2	70.6±12.4	73.6±20.1	72.4±15.2	78.9±17.7	80.4±21.5
Pb1	80.3±21.3	73.3±11.5	78.5±19.4	74.4±14.6	80.5±16.1	84.2±18.2
Pb2	76.8±15.5	68.2±13.4	76.5±13.2	75.8±13.8	75.5±15.6	77.2±15.2
Pb3	70.1±16.5	62.4±15.1	67.7±19.7	63.5±17.6	66.4±14.8	70.5±17.4
Cd1	77.5±18.4	71.3±12.7	75.5±16.2	73.2±15.3	79.7±13.7	81.5±15.2
Cd2	70.8±16.8	70.9±15.2	71.9±15.5	68.4±16.7	70.5±12.9	75.6±18.5
Cd3	65.6±13.8	60.5±14.2	64.3±16.7	64.7±13.9	63.7±15.2	67.5±14.9
LSD (5%)	3.65	4.22	3.12	4.82	5.17	4.75

Data recorded in the same table showed that all Cd treatments resulted in a reduction of WC% in all tree species. The most decrease in WC% was observed at Cd3 treatment, at which WC% of A. saligna, E.rostrata and C. erectus shoots were decreased by about 15.1%, 14.4% and 23.8%, respectively. Similarly, WC% of roots were decreased by about 16.6%, 11.9% and 19.1%, respectively, as compared with control.

Perfus-Barbeoch *at al.* (2002) investigated the effect of Cd on the growth of Vicia faba plants and found that high concentrations of Cd (100 micro m) caused wilting to the plants as a consequence of Cd toxicity. The authors found that Cd reduced stomatal opening via its effect on guard cell regulation. In a recent study, Malar *at al.* (2014) found that relative water content (RWC) in Eichhornia plant increased slightly, up to 400 mg/L Pb concentration, and decreased at higher concentrations compared to the control. Relative water content (RWC) change has been suggested as an indicator of phytotoxicity after heavy metal stress in Indian mustard and Chinese brake fern (Heidari and Sarani, 2011). Relative water content in leaves was slightly higher in lead and cadmium treated plants than in the control at low concentrations. It is most likely that Pb and Cd treatments induced stomatal closure, triggered over the course of the experiment due to the atmospheric carbon fixing activities that were compromised as a consequence (**Chosden** *at al.* 2014).

Chlorophyll content

Data recorded in Table (3) showed clearly that Chl a and b contents of *A. saligna* and *E. rostrata* were stimulated by low (Pb1) and medium (Pb2) concentrations of lead, while Chl a and b content of *C. erectus* were improved only at Pb1. The highest Pb concentration (Pb3 treatment) inhibited the formation of chl pigments. All Cd treatments, on the other side, decreased Chl a and b contents of all tree species except A. saligna which show slight increse in Chl a and b with Cd1 treatment. It is well observed that the destructive effect of Pb and Cd pollution was very high on *C. erectus*, follwed by *E. rostrata* while *A. saligna* was the most tolerant species among all the studied.

Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Zengin and Munzuroglu, 2005). Researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals. In two wheat varieties to which Cd and Pb were applied, total chlorophyll decreased (Oncel *at al.*, 2000). Similarly, reductions in the level of photosynthetic pigments, including Chl- a, b and carotenoids, after exposure to heavy metals has been observed in many plant species (Liu *at al.*, 2014).

Table 3. Effect of Pb and Cd pollutants on chlorophyll a and b (mg/g fwt) of *A. saligna*, *E. rostrata* and *C.erectus* seedlings (values are the mean of 3 replicates).

Treatment	*A. saligna*		*E.rostrata*		*C. erectus*	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
Control	1.62±0.25	0.83±0.15	1.45±0.29	0.72±0.15	2.52±0.43	1.23±0.55
Pb1	2.33±0.45	1.12±0.22	1.73±0.35	0.93±0.20	2.83±0.35	1.52±0.35
Pb2	1.83±0.24	0.92±0.21	1.42±0.32	0.83±0.15	2.21±0.42	1.13±0.23
Pb3	1.22±0.19	0.65±0.19	1.11±0.28	0.64±0.18	2.00±0.55	1.05±0.19
Cd1	1.65±0.21	0.86±0.19	1.34±0.22	0.82±0.21	2.21±0.35	1.12±0.20
Cd2	1.43±0.15	0.54±0.20	1.25±0.19	0.62±0.19	2.00±0.45	0.83±0.26
Cd3	1.14±0.27	0.46±0.22	1.09±0.23	0.41±0.16	1.52±0.30	0.54±0.27
LSD (5%)	0.35	0.23	0.27	0.18	0.32	0.25

It is well known that HMs inhibit metabolic processes by inhibiting the action of enzymes, and this may be the most important cause of inhibition. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cadmium was reported to affect chlorophyll biosynthesis and inhibit proto-chlorophyll reductase and aminolevulinic acid (ALA) synthesis (Gill, 2014). The chlorophyll ratio, which is used as a stress indicator, increased slightly with increasing metal treatments. This was also seen in *Empetrum nigrum* leaves near a copper and nickel smelter in the field (Monni *at al.*, 2001).

In a prvious study, HM found to reduce chlorophyll a and b contents in leaves as well as a reduction of carotenoids, which are photosynthesis accessory pigments with a photo-protective effect that reduces the harmful effects of excess light in the photosynthetic membranes (Uenojo *at al.*, 2007). HM affects chlorophyll degradation, and reductions in chlorophyll content have been observed in plants grown in HM-treated soils (Sharma and Dubey, 2005). Even moderate amounts of Pb affect the content of photosynthetic pigments, which could lead to a reduction in photosynthetic potential and a disruption of plant growth processes (de Souza *at al.*, 2012).

The percent reduction of Chl b in HM treated plants was more than that of overall Chl content comparing with control. This can be associated with the alteration in pigment composition of photosynthetic approach that possesses lower level of light harvesting chlorophyll proteins (LHCPS). The decreased level of LHCPS is an adaptation defense mechanism of leaves and plants, helping them survive under adverse conditions (Gill, 2014). It can be assumed that lead may inhibit chlorophyll biosynthesis by impairing the uptake of essential photosynthetic pigment elements, such as magnesium, potassium, calcium and ion (Iqbal *at al.* 2013). A decreased rate of photosynthetic pigment accumulation in association with Cd and Pb treatment may be the consequence of peroxidation of chloroplast membranes due to increased level of ROS generation (Malar *at al.* (2014).

Proline content

Results in Fig. (1) indicated that proline concentration increased with increasing the level of heavy metals in the soil. This increase in proline with the increase in pollution was more obvious in *A. saligna*, followed by *E. rostrata*. As for *C. erectus*, it didn't show any significant differences in proline content as compared with control plants, except at medium levels of Pb or Cd (Pb2 and Cd2), at which proline concentrations increased by 37.7% and 26.4%, respectively. While in *A. saligna* the percent increases in proline levels were 45.6%, 59.5% and 37.7% at Pb1, Pb2 and Pb3, respectively, while the corresponding increases in *E. rostrata* proline were 32.1%, 44.5% and 15.8%, respectively, as compared with control plants. Moreover, proline content of *A. saligna* increased at Cd2 and Cd3 by about 34.2% and 42.1%, respectively, while the corresponding increase in *E. rostrata* were 31.3% and 24.1%, respectively.

Early studies showed that proline accumulated in plant tissues as a response of exposing plants to environmental stress (Bačkor *at al.*, 2004). It is well known that abiotic stresses, including heavy metal stress, result in producing high amounts of reactive oxygen species (ROS) in plant cell leading to toxicity of plant tissues (Malar, 2014). Many workers reported that ROS is one the initial responses of plants to stress (Bačkor *at al.*, 2004; Bhardwaj *at al.*, 2009). Generation of free radicals and reactive oxygen species is stimulated in the presence of metals, and this can seriously disrupt normal metabolism through oxidative damage to cellular components (Ang *at al.* 2010). To mitigate and repair the damage initiated by active oxygen, plants have developed a complex antioxidant system. Antioxidants like systeine, proline, ascorbic acid and nonprotein thiol (sulfhydryl) play an important role in chelation and detoxification of toxic metal ions (Singh and Sinha, 2005).

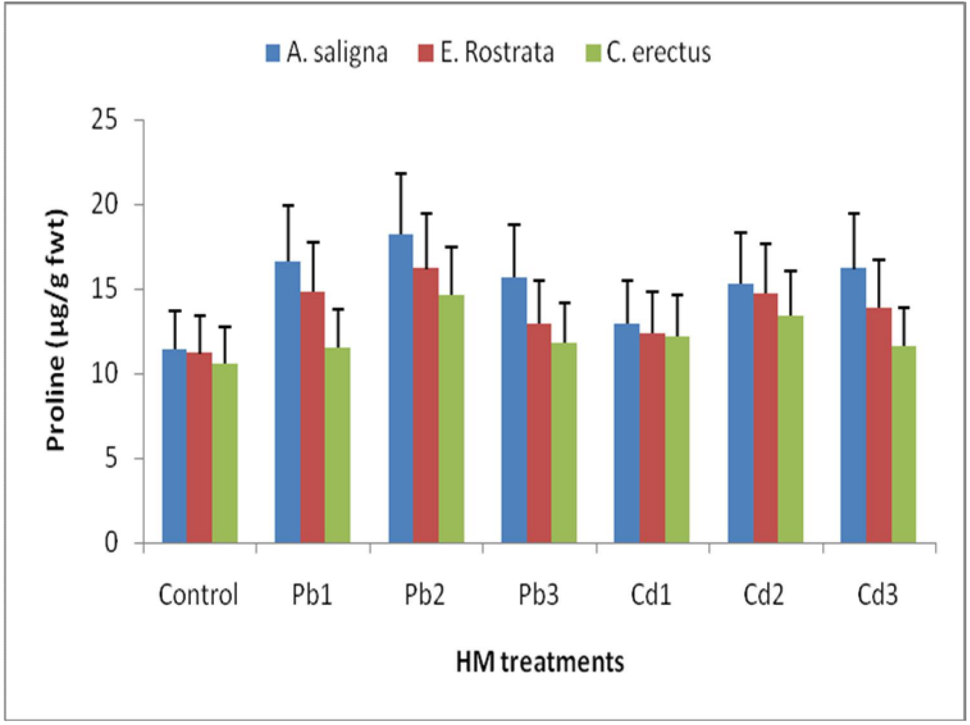

Figure 1. Effect of Pb and Cd pollutants on proline concentrations in *A. saligna*, *E. rostrata* and *C. erectus* seedlings. (vertical bars represent the SD values).

Under stress conditions, proline considers one of the most important materials that protect plant cells against these ROS (Ashraf and Fooad, 2007). The present study showed that proline concentration increased significantly in *A. saligna* and *E. rostrata* but was slightly improved in *C. erectus* under same treatments of Pb and Cd as compared with control plants except when pollutant concentration increased. In this respect, Dinakar *at al.,*(2008) found that proline levels increased in *Arachis hypogaea* L. seedlings after 25 days with increasing heavy metal concentrations in the growth media. Szalai *at al.* (2002) reported that proline concentration increased under high levels of heavy metal to decrease the water potential under these unfavorable conditions, thus, proline may accumulate to regulate water balance inside plants.

Heavy metal concentrations

The results in Figs (2 and 3) indicate that both Pb and Cd accumulated in the roots of *A. saligna* and *E. rostrata* in amounts higher than those accumulated in their shoots. The reverse was true for *C. erectus* as both metals accumulated in aboveground parts in levels higher than those accumulated in the roots. The highest accumulation of heavy metals was recorded in *A. saligna* followed by *E. rostrata* and finally *C. erectus* seedlings which absorb the lowest amount of heavy metals.

Maximum accumulation of Pb and Cd content in *A. saligna* was about 625 and 62 mg.kg-1 dwt, respectively, in roots followed by shoots (about 258 and 32 mg.kg-1dwt, respectively). In *E. rostrata* the maximum value of Pb and Cd in roots was 552 and 52 mg.kg-1dwt, respectively, followed by shoots (316 and 43 mg.kg-1dwt, respectively), while in *C. erectus* the maximum accumulation of Pb and Cd in roots was 165 and 35 mg.kg-1dwt, respectively, and in shoots was 285and 56 mg.kg-1dwt, respectively. Therefore, the translocation factor value for Pb and Cd was found to be less than 1 for *A. saligna* (0.41 and 0.52) and *E. rostrata* (0.57 and 0.84). Though, Pb was largely stored in plant roots exposed to 1000 mg/L Pb and 100 mg/L Cd treatments, only lesser amounts of Pb were translocated to aerial parts of the plants. While in *C. erects*, the translocation factor value was found to be more than 1 (1.7 for Pb and 1.8 for Cd). This result clearly indicate that the large amount of metal content was accumulated in roots, but only lower levels of Pb and Cd content were translocated into shoots of *A. saligna* and *E. rostrata* seedlings. While the reverse was true for *C. erectus*.

Clearly there were significant differences between selected species in their absorption of Pb and Cd, either on the bases of roots or shoots. It is clear also that the tolerant species such as *A. saligna* has the ability to keep the heavy metals in their root system and do not move them to the aboveground parts. These results support those obtained by Gratao *at al.* (2005) who found a positive relationship between the accumulation of Pb or Cd and the ability of plants to tolerate these metals. Therefore some species have a mechanism to tolerate heavy metal toxicity through prevention of the translocation of Pb and Cd from roots to shoots as reported by El-Gamal and Tantawy (2010) who found that plants differ in their ability to accumulate the heavy metals from the growth media. They indicated that some species accumulate heavy metals in their roots while others transfer them to the shoots. In the present study, it seems that *A. saligna* and *E. rostrata* are more tolerant species to heavy metal toxicity than *C. erectus*.

The results showed also that both Pb (Fig 2) and Cd (Fig 3) increased in plant tissues with increasing both metals in the growth media and reached the highest levels at Pb3 and Cd3 treatments. The present results support those found by Piechalak *at al.* (2002). It is well noticed that the amounts of Pb that accumulated in the selected species were much higher than that of Cd either in plant roots or plant shoots.

Although higher concentrations of both metals were found in roots of plants as in this study, significantly high concentrations were also measured in the above-ground tissues and together with the fact that there was no reduction of biomass suggested that *A. saligna* and *E. rostrata* could be a possible Cd and Pb hyperaccumulators. An efficient HM accumulation mechanism in seedling roots could represent a new and interesting phenomenon for establishment of phytoremediation strategies, in which higher levels of the contaminant remain tightly attached to plant tissues (Brunet *at al.* 2008). Recent reports show also that HM accumulation was found to be higher in the roots than in the shoots of *Brassica rapa* (Cenkci *at al.* 2010). The ability of the plant in accumulation and tolerance to HM ions indicates that this plant species may have an efficient hyperaccumulation mechanism for removal of heavy metal ions from contaminated sites and water bodies (Malar *at al.* 2014).

The ideal plant species to remediate a heavy metal contaminated soil would be a high biomass-producing crop that can both tolerate and accumulate the contaminant of interest (Pulford and Watson 2003), yet hyperaccumulator plants are usually small with a shallow root system and low biomass production and the technology for their large-scale cultivation is not fully developed; therefore, their use is rather limited (Saifullah *at al.* 2009). In contrast, plants with good growth usually exhibit low metal accumulation or the metals are accumulated mainly in the roots besides having a low tolerance to heavy metals. Therefore, if such a combination is not possible, a trade-off. between hyperaccumulation and lower biomass must be made (Pulford and Watson 2003). The concentrations of metals achieved in plant tissues of *A. saligna* considered together with its high biomass production and its metal tolerance suggest that it could be used for phytoextraction research. Although roots of *A. saligna* accumulated higher concentrations of Pb and Cd than shoots, average Pb and Cd accumulation in shoots was found to be high in relation to the total accumulated cadmium by the whole plant (Figs 2 and 3) due to the high above-ground biomass production by the plant, an important feature for phytoextraction applications Ang, et al (2010). In this concern, Pulford and Watson (2003) described that the ability of tree species to retain the majority of absorbed heavy metals in the roots minimizes transport to the shoots and represents a tolerance mechanism important for phytoremediation processes.

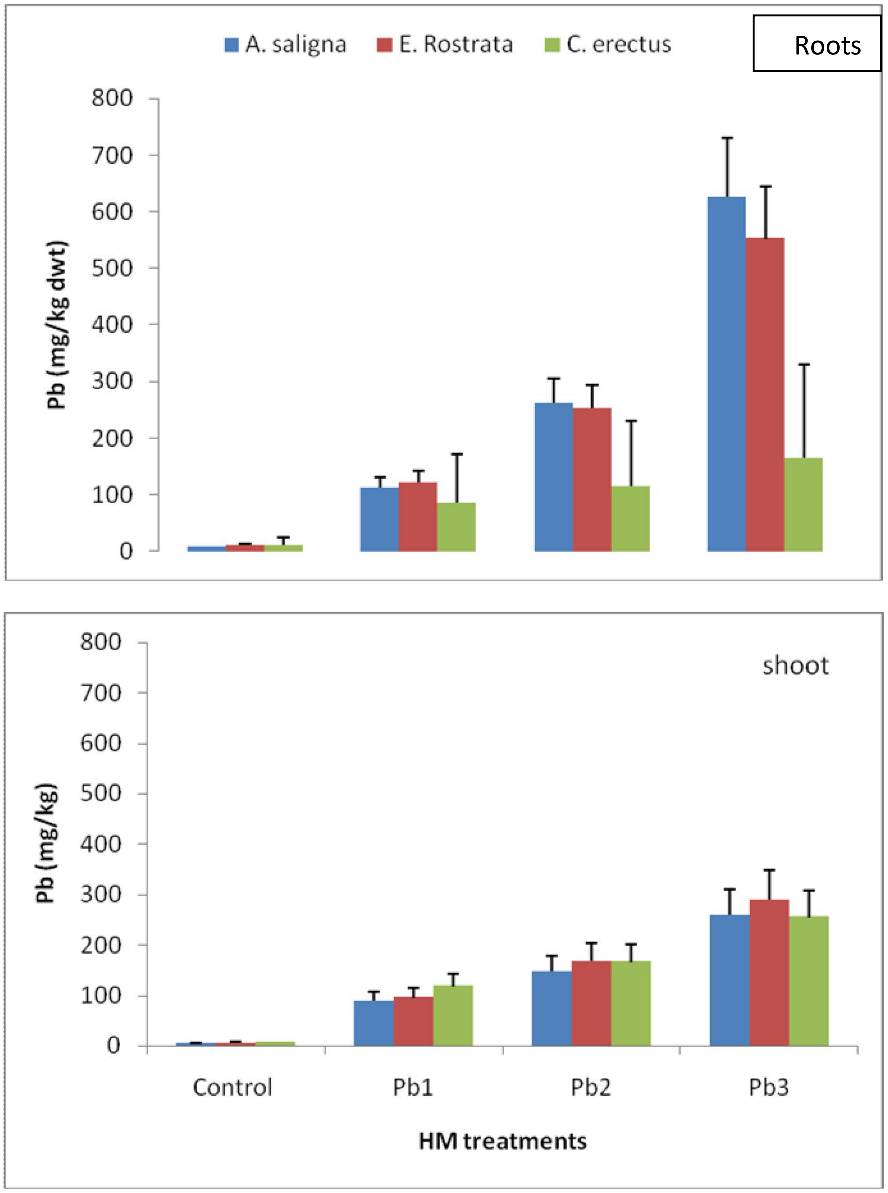

Figure 2. Effect of HM pollutants on Pb accumulation in roors and shoots of *A. saligna*, *E. rostrata* and *C. erectus* seedlings. (vertical bars represent the SD values).

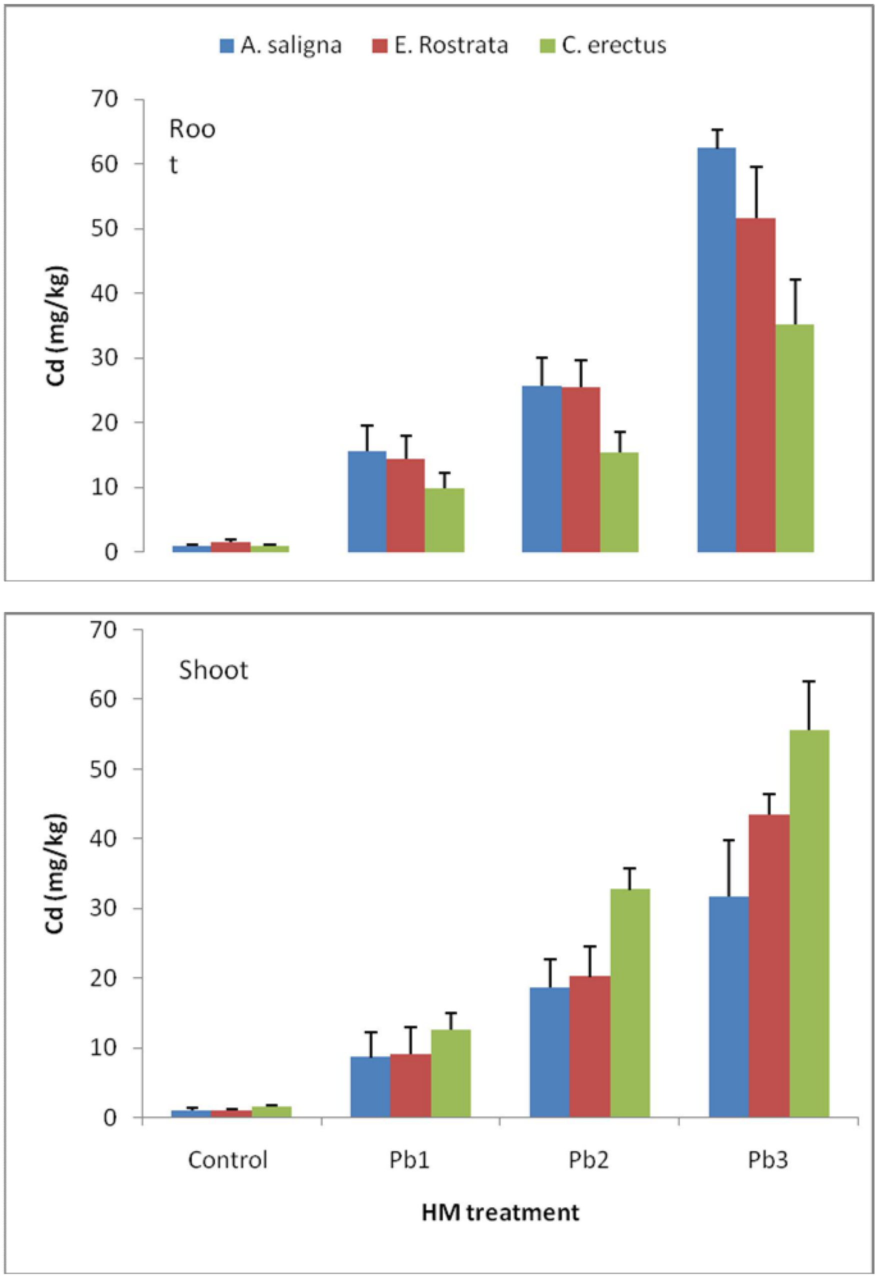

Figure 3. Effect of HM pollutants on Cd accumulation in roors and shoots of *A. saligna*, *E. rostrata* and *C. erectus* seedlings. (vertical bars represent the SD values).

Activity of antioxidant enzymes

a- Catalase

It was obvious that catalase activity increased at Pb1 and Pb2 treatments in all the plant species, then dercreased at Pb3 treatment as compared with control (Fig.4). The increase in catalase activity under Pb1 and Pb2 treatments was more obvious in *A saligna* followed by *C. erectus* and finally *E. rostrata*. In this regard, the increase in catalase activity at Pb2 treatment was about 60% in *A. saligna*, 42% in *E. rostrata* and 30% in *C. erectus*. Pb3 treatment, however, Pb3 caused a decrease in catalase activity in all the plant species as compared with control. Most decrease was observed in *C. erectus*. Data showed also that catalase activity in *A. saligna* and *E. rostrata* increased at Cd1 and Cd2 treatments while in *C. erectus* catalase activity increased only at Cd1 then decreased in all species at higher levels. The increase on catalase activity at Cd2 was about 27% in *A. saligna* and 17% in *E. rostrata*.

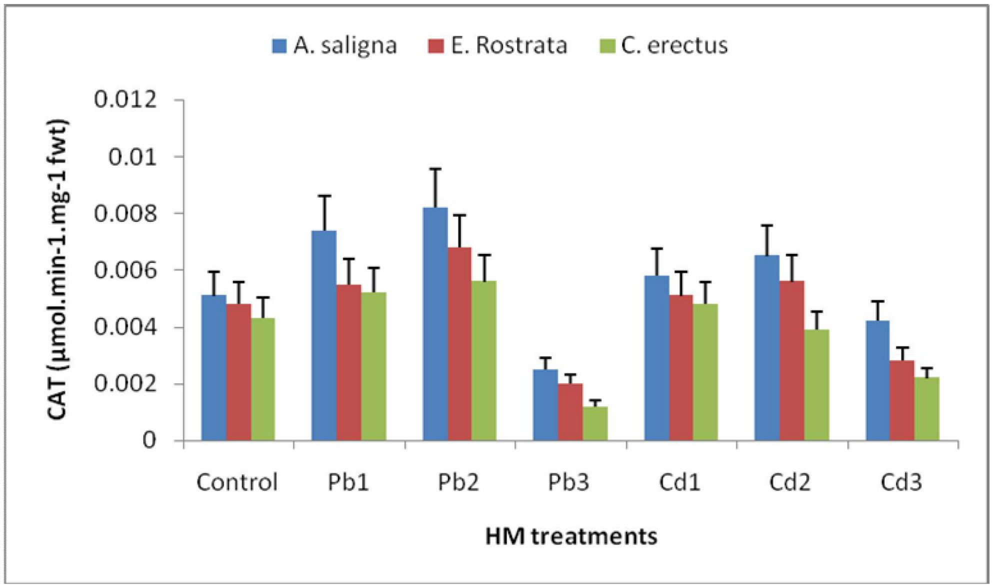

Figure 4. Effect of HM pollutants on Catalase activity in *A. saligna*, *E. rostrata* and *C. erectus* seedlings. (vertical bars represent the SD values).

b- peroxidase

Data in Fig. (5) showed that peroxidase activity increased as Pb or Cd concentrations increased in the soil up to certain level then started to decrease with any further increase in the level of heavy metals. In this concern, Pb1 and Pb2 caused an increase in peroxidase activity by about 34% and 69%, respectively, in *A. saligna,* and by about 53% and 79%, respectively, in *E. rostrata*, as compared with control plants. The corresponding increase of peroxidase activity in *C. erectus* was about 23% and 58%, respectively, as compared with control. On the other side, Pb3 caused a decrease in enzyme activity by about 14%, 24% and 23% in *A. saligna*, *E. rostrata* and *C. erectus* seedlings, respectively as compared with control treatment. Similarly, Cd1 and Cd2 caused an increase in peroxidase activity in all plant species as compared with control. While Cd3 decreased the activity of the enzyme in all the species.

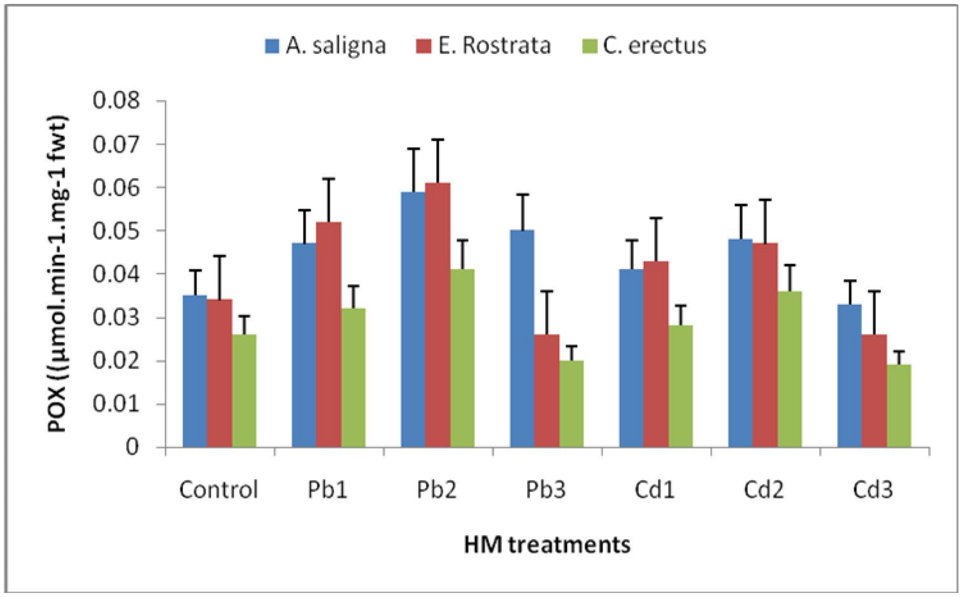

Figure 5. Effect of HM pollutants on peroxidase activity in *A. saligna*, *E. rostrata* and *C. erectus* seedlings. (vertical bars represent the SD values).

Registered results showed that the activity of antioxidant enzymes were elevated with increased Cd and Pb concentration. These results are similar to those reported by Heidari and Sarani (2011) and Malar *at al.* (2014) who found that Pb and Cd treatments caused an increase in the activity of antioxidant enzymes such as peroxidase and catalase in Indian mustard. They found that the increase in peroxidase activity was about 63% when plants were treated with Pb and was 51% when treated with Cd as compared with control treatment. An early study by Metwally *at al.* (2003) showed that heavy metals form reactive oxygen species (ROS) which cause an oxidative stress and that causes a physiological damage to plants. The higher antioxidative ability was observed in plants, indicating that the increased antioxidative activity might reflect a damage response to stress factors, which was in agreement with the report of Sharma and Dubey (2005), who presumed that high lipid peroxidation and anti-oxidative ability were parts of a damage response to salinity in rice cultivars.

Development of oxidative stress in plants exposed to heavy metals (Metwally *at al.*, 2003) is largely ascribed to heavy metal induced disbalance between the generations of toxic oxygen radicals and their scavenging through the anti-oxidative defense mechanism. The latter provides an efficient system for detoxification and scavenging of the toxic oxygen species through an adaptive mechanism involving upregulation of anti-oxidative enzymes such as peroxidase, catalase and others (Heidari and Sarani, 2011) these reactions also down regulate the conversion of the super oxide ions to the highly reactive and genotoxic hydroxyl (OH) ions (Bhardwaj *at al.*, 2009).

In the present study, it seems that plants tried to protect themselves via the activation of the anti-oxidant enzymes system which include catalase and peroxidase (Figs 4 and 5). These enzymes used to convert ROS to water and oxygen useful for plants (Heidari and Sarani, 2011). With the increase in heavy metal concentration in the soil or with increasing the time of plant exposure to heavy metals, the activity of the antioxidant system decreases (Heidari and Sarani, 2011). In the present study it was clear that the highest concentrations of Pb and Cd cause a significant decrease in the activity of peroxidase and catalase (Figs 4 and 5). These results support the earlier findings of Fornazier *at al.* (2002) and Shim *at al.* (2003). As we have compared between both the metals, Cd was found to be more toxic than Pb because the lower concentration of Cd have almost the same effect on the enzyme activities as those effects resulted from the higher concentration of Pb.

Conclusion

To the best of our knowledge, the present work is considered one of the first studies focusing on the use of native tree species in phytoremediation and rehabilitation of Pb and Cd contaminated soils. phytoremediation. The results suggested that *A. saligna* was the species that was most tolerant to high Pb and Cd concentrations in soil, while *E. rostrata* showed moderate tolerance. Of the three Saudian native species studied, C. erectus was found to have the lowest tolerance to high concentrations of Pb and Cd.

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