PHYTOEXTRACTION OF CADMIUM AND LEAD FROM A CONTAMINATED SOIL USING EUCALYPTUS SEEDLINGS

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ABSTRACT

This study aims to estimate critical concentrations of cadmium (Cd) and lead (Pb) in the soil which negatively affect growth of Eucalyptus camaldulensis Dehnh. seedlings, and to estimate some phytoextraction parameters for heavy metals (HM) from the soil to evaluate efficiency of seedlings in their potential use in phytotechnology to improve the environment with phytoremediation. Eucalyptus seedlings were treated with Cd concentrations 0, 25, 55, 85, and 110 mg kg^{-1} dry soil as $CdCl_2$, and Pb concentrations 0, 125, 250, 450, and 550 mg kg^{-1} dry soil as $PbCl_2$, and the experiment was designed using the completely randomized design (CRD) as a two-factor factorial experiments and the data were analyzed using SAS system. Results showed that the highest percentage decrease in dry weight of stems, leaves and roots were 55, 68.6, and 67.2%, respectively, at the interaction (110 Cd and 550 Pb) mg kg^{-1} dry soil compared with control, and Cd concentrations in stems, leaves and roots ranged between (0.375-372.167), (0.417-128.167) and (0.583-162.083) mg kg^{-1}, respectively and Pb concentrations in stems, leaves and roots ranged between (9.583-62.375), (10.042-20.417) and (2.875-73.500) mg kg^{-1}. It was found that values of translocation factor (TF), biological accumulation coefficient (BAC), bioconcentration factor (BCF) and concentration index (CI) for Cd ranged between (0.611-4.239), (1.333-28.790), (0.383- 16.840) and (1-490.812) respectively, and values of TF, BAC, BCF, and CI of Pb ranged between (0.275-5.702), (0.769-4.246), (0.295-7.539) and (1-3.833) respectively, and tolerance index (TI) values ranged between (0.370-1). We concluded that Eucalyptus seedlings are suitable for phytoextraction applications within phytoremediation processes of soils contaminated with Cd and Pb.

Keywords: Phytoremediation, Biomass, Accumulation, Environmental Pollution, Heavy Metals.

الاستخلاص النباتي للكادميوم والرصاص من التربة الملوثة باستخدام شتلات اليوكالبتوس

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المستخلص

تهدف هذه الدراسة الى تقدير التركيزات الحرجة للكادميوم والرصاص في التربة والتي تؤثر سلباً في نمو شتلات اليوكالبتوس (Eucalyptus camaldulensis Dehnh.)، وتقدير بعض مقاييس الاستخلاص النباتي (Phytoextraction) للعناصر الثقيلة (HM) من التربة لتقييم كفاءة الشتلات في إمكانية استخدامها في التقنيات النباتية (Phytotechnology) لتحسين البيئة بتقنية المعالجة النباتية (Phytoremediation). اذ عوملت شتلات اليوكالبتوس بتركيزات الكادميوم صفر و25 و55 و85 و110 ملغم كغم$^{-1}$ تربة جافة على هيئة $CdCl_2$، وتركيزات الرصاص صفر و125 و250 و450 و550 ملغم كغم$^{-1}$ تربة جافة على هيئة $PbCl_2$، وصممت التجربة باستخدام التصميم العشوائي الكامل (CRD) كتجربة عاملية وحلّلت البيانات ببرنامج SAS system. واظهرت النتائج ان اعلى نسبة انخفاض في الوزن الجاف للسيقان والأوراق والجذور بلغت 55 و68.6 و67.2% على التوالي عند التداخل (110 كادميوم و550 رصاص) ملغم كغم$^{-1}$ تربة جافة مقارنة مع الكونترول، وان تركيزات الكادميوم في السيقان والأوراق والجذور تراوحت مدياتها بين (0.375–372.167) و(0.417–128.167) و(0.583–162.083) ملغم كغم$^{-1}$ على التوالي، وان تركيزات الرصاص في السيقان والأوراق والجذور تراوحت بين (9.583–62.375) و(10.042–20.417) و(2.875–73.500) ملغم كغم$^{-1}$ على التوالي، ووجد ان قيم معامل الانتقال (TF) ومعامل التجميع الحيوي (BAC) ومعامل التركيز الحيوي (BCF) ودليل التركيز (CI) للكادميوم تراوحت بين (0.611–4.239) و(1.333–28.790) و(0.383–16.840) و(1–490.812) على التوالي، وان قيم TF وBAC وBCF وCI للرصاص تراوحت بين (0.275–5.702) و(0.769–4.246) و(0.295–7.539) و(1–3.833) على التوالي، وتراوحت قيم دليل التحمل (TI) بين (0.370–1). واستنتجنا ان شتلات اليوكالبتوس صالحة لتطبيقات Phytoextraction ضمن عمليات Phytoremediation للتربة الملوثة بالكادميوم والرصاص.

الكلمات المفتاحية: المعالجة النباتية، الكتلة الحيوية، التراكم، التلوث البيئي، المعادن الثقيلة.

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INTRODUCTION

Environmental pollution is the presence in the environment of any pollutant substance in the environment in quantities that directly or indirectly, alone, or by the interaction with others, harms public health or causes the ecosystems to disrupt or stop them from performing their normal role in the ecosystem, and the soil is contaminated by containing a substance or substances in high concentrations that threaten human, animal or plant health or disturb the natural standards of surface and groundwater. Heavy metals are considered the most dangerous pollutants to the environment of living organisms, resulting from the remnants of technologies created to serve humanity such as mining, factories, electric power stations, agricultural pesticides, hospitals, sewage waste, human consumption waste, means of transportation, and the mineral origin of the earth's crust (5, 57). They affect proteins and enzymes, some of which are genotoxic and carcinogenic substances that affect the chemical composition of DNA, as well as inhibit the process of genetic reproduction. Plants possess defenses against heavy metals such as phytochelatins, which can bind the heavy metals to be physiologically inactive or by depositing them in special sites in plant cells (5, 32). It is worth noting that some the heavy metals play a fundamental role in metabolism, while others are trace elements and toxic elements that do not have a biological role, as in Cd and Pb, as they move to humans through the food chain, leaving him with serious diseases such as the programmed killing of his cells, which lead to death (19, 36, 48). Cadmium (Cd) is one of the heavy metals most dangerous to the environment, which is characterized by high toxicity even in its low concentrations as well as its rapid transfer to cellular tissues to settle in it, causing morphological, physiological and biochemical changes as it works to reduce the levels of growth, photosynthesis and cellular processes of the plant, and Cd in its critical concentrations leads to cell damage and plant death (24, 37). As for lead (Pb), which is more prevalent in the environment than other highly toxic heavy metals (23), when it is stabilized in plant tissues, it reduces vegetative growth due to its effect on the structural and physiological properties, as it reduces photosynthesis, impedes the action of stomata in leaves and reduces enzymatic functions in plant tissues, its high concentrations cause cellular stress that leads to plant death (6, 17). In order to get rid of heavy metals, plants and micro-organisms were used to remove them from the environment, as they are the most preserving the environment and living organisms and for the economic and social benefits they generate, phytoremediation has become known as a modern innovation technology that is reflected in the absorption of heavy metals from soil, water and air are then transported and concentrated in their cellular tissues, and phytoextraction, which involves the use of plants to absorb excessively accumulated heavy metals from the surrounding environment, transport and concentrate them in their biomass (3, 5, 16), it is the most common in phytoremediation operations, as it is a very appropriate technique in terms of material cost and as it improves the quality of soil and water satisfactorily within an appropriate time limit, good for extraction, by analyzing and reserving the hazardous contents in the media in which the plants grow, as well as making plans a long-term strategy for making genetic modifications on good plants in the phytoextraction process in order to increase their relatively rapid growth rate as well as to obtain high-yielding biomass and to make them adaptable to different environmental conditions and to demonstrate the characteristics of the different genetic characteristics of the species (45). The accumulation of toxic heavy metals in agricultural areas leads to great concerns about food safety and its impact on the productivity of food crops and thus its negative impact on the food security of human societies (50), and there are types of plants called hyperaccumulators that can absorb and transport large quantities of the heavy metals, and storage in their cellular tissues, and despite their high storage capacity, in cases of soil contamination with large quantities of the heavy metals, plants do not fully perform their function due to their small biomass compared to the size of pollution, so it is preferable to choose plant species with large and fast-growing biomass, that can absorb greater amounts of inorganic pollutants in general and heavy metals in particular (10, 40), therefore, recent studies have encouraged the treatment of polluted soils with heavy metals by selecting forest trees that are distinguished by their lack of contribution to the food chain, and high yield of biomass, and among the forests that are characterized by these characteristics are Eucalyptus, Willow, Poplar and Castor (2, 21). Where a study was conducted on Poplar hybrids in Serbia for Populus deltoides for clone (B-81) and Populus × euramericana for clone (Pannonia) on the possibility of phytoremediation of soils contaminated with Cd using, which were treated with a concentration of Cd 8.14 mg kg^{-1} dry soil, the study showed that the decrease in biomass production was significantly greater in Pannonia than in B-81 (42). And in a study conducted by Lamb et al. (29) in Australia to investigate the effect of Cu, Zn, Cd and Pb on the root elongation for four types of native trees and other natural herbs which treated with concentrations of Cd and Pb 0, 0.1, 1, 10, 100, 500 and 1000 µM, as well as with concentrations of Cu and Zn 0, 0.1, 1, 10, 100, 1000 and 2000 µM, and the results showed that high concentrations of heavy metals reduced the growth rate to 50%, as Cd concentrations of root ranged from 27 µM in Lactuca sativa to 940 µM in Acacia species, and Pb concentrations ranged between 180 µM in L. sativa to 1000 µM in Acacia species, and Cd was found to be more toxic than Pb. As for the study by Ashraf et al. (8) showed an increase in stalk Cd concentrations from (4.20-9.70) and (3.30-8.50) mg kg^{-1} in the seedlings of Conocarpus erectus and Eucalyptus camaldulensis, respectively, when the Cd concentration increased from 5 to 5.15 mg kg^{-1}, and Cd concentrations in the roots increased from (8.20-24) and from (6.10-17.40) mg kg^{-1} in seedlings of C. erectus and E. camaldulensis, respectively, with increasing the soil Cd concentration from (5-5.15) mg kg^{-1}, while the BCF values were greater than one for the two species and for all Cd coefficients, and the BCF values for the two species were higher in C. erectus seedlings than E. camaldulensis, and the TF values were less than one for both species and for all Cd levels, it was higher in E. camaldulensis than C. erectus. Also studied Alkhatib et al. (4) the effects of Pb on growth rate, physiological and biochemical characteristics in Leucaena leucocephala seedlings, as the results showed that the effect of Pb led to a decrease in growth rate of seedlings treated with concentrations 300, 500 and 700 µM, whereas Pb concentrations of 25, 50 and 100 µM did not show any toxic effect on seedlings.

MATERIALS AND METHODS

Field work: On March 14, 2019, ten-month-old were transferred Eucalyptus camaldulensis Dehnh. seedlings from central nursery in the city of Mosul to the wooden shelter in nursery for College of Agriculture and Forestry at University of Mosul at a longitude (43° 09') north and latitude (36° 19') east, and seedlings were planted in pots its internal diameter and depth is 28 cm, then the soil was added to it to bring its dry weight to 13.125 kg pot^{-1} approximately, and seedlings were distributed randomly, and after two weeks of transferring the seedlings to the pots, they were treated with concentrations of Cd and Pb, and the service operations continued, such as watering and weeding, until November 21, 2019, three seedlings from each treatment, randomly selected, and then field measurements were made for them after extracting them from the pots, cleaning them from the soil, separating roots, stems and leaves separately, taking soil samples for each seedling from the area near the roots, and then the plant samples and soil samples were placed in cardboard bags with the number of treatment, refined, and sample type recorded, and (Picture 1) showed the seedlings in the wooden shelter at the start and end of the experiment.

Soil analyzes: The analyzes of the soil sample taken at the start of study (before adding heavy metals) (Table 1), were conducted as indicated by El-Mahrouk et al. (17), which it included measuring the electrical conductivity (EC) according to the ratio (1 soil: 5 distilled water) and was measured using a device EC meter, and the pH was measured using a pH meter and according to the ratio (1 soil: 1 distilled water), and the soil texture was estimated using hydrometer method, and concentrations of Pb and Cd in the soil were estimated at the start of study according to Elmer (18), as well as when removing seedlings from pots (at the end of the experiment).

Measuring dry weight of shoot and root: Stems, leaves and roots were dried at a temperature of 65° C for about three days until the stability of the weight, and dry weights were recorded immediately after they were removed from electric oven using a sensitivity balance of (1) mg.

Picture 1. The seedlings at the start of the experiment (A), and at the end of the experiment (B).

Table 1. Soil properties before treatment.

Parameter	Value with unit
pH	**7.2**
EC	**1.876 dS m^{-1}**
Cd	**0.295 mg kg^{-1}**
Pb	**18.750 mg kg^{-1}**
Sand	**42.55 %**
Silt	**21.2 %**
Clay	**36.25 %**
Texture	**Silty clay**

Determination of Cd and Pb concentrations in plant parts: The wet digestion method was used to prepare plant samples (roots, stems and leaves) and according to the method reported by Elmer (18), the dried plant samples were ground separately and 2 g were taken from each sample and placed in an conical flask, then 10 ml of concentrated nitric acid (HNO3) were added to it, and the samples were left until the next day and then heated on an electric heater until the red fumes from nitrogen dioxide (NO_2) stopped and the samples were left to cool down, and then (2-4) ml of perchloric acid ($HClO_4$) were added to them its concentration is 70%, and the samples were heated again until the end of the white vapors resulting from the perchloric acid, noting that a small volume of the sample remained bright as a result of the digestion process, then the samples were left to cool down and then transferred to a 50 ml beaker and the volume was supplemented with distilled water to the mark became the final volume of the plant samples 50 ml, then the samples were filtered using filter paper, and then the Cd and Pb concentrations of the dried plant samples were estimated by flame method, using an atomic absorption spectrophotometer, SHIMADZU AA7000, Japanese origin, in the Environment and Water Research Department of the former Ministry of Science and Technology in Baghdad.

Estimation of translocation factor (TF): It was calculated as reported by Rasheed et al. (49) according to the equation (TF = HM_S / HM_R), as HM_S = concentration of heavy metals in the shoot, and HM_R = concentration of heavy metals in the root.

Estimation of biological accumulation coefficient (BAC): It was calculated as reported by Chandra and Hoduck (11) and according to the equation (BAC = HM_{Shoot} / HM_{Soil}), as HM_{Shoot} = concentration of heavy metals in stem and leaves, and HM_{Soil} = concentration of heavy metals in soil.

Estimation of bioconcentration factor (BCF): It was calculated as reported by Chandra and Hoduck (11) and according to the equation (BCF = HM_{Root} / HM_{Soil}), as HM_{Root} = concentration of heavy metals in the root, and HM_{Soil} = concentration of heavy metals in the soil.

Estimation of concentration index (CI): The calculation was done as reported by Chandra and Hoduck (11) and according to the equation (CI = $HM_{treated\ plant}$ / $HM_{normal\ plant}$), as $HM_{treated\ plant}$ = heavy metals concentration in the treated plant, $HM_{normal\ plant}$ = heavy metals concentration in the control plant.

Estimation of tolerance index (TI): Calculated according to Xu et al. (58) and as in the equation (TI = DW_{HM} / $DW_{control}$), as DW_{HM} = total dry weight of the plant treated with heavy metals, and $DW_{control}$ = total dry weight of the control plant.

Statistical Analysis: Used the completely randomized design (CRD) as a two-factor factorial experiments, each factor had five levels with three replicates (5×5×3), and the results were analyzed using SAS system version 9.0 by applying CARDS orders for statistical analyzes required, the Duncan's multiple-range test was used to compare the means values at a probability level 5%.

RESULTS AND DISCUSSION

Effect of Cd and Pb on dry weight of stems: The results of Duncan's multiple range test (Table 2) showed a decrease in dry weight of stems with an increase in concentration of Cd and Pb in the soil, as for effect of interaction between Cd and Pb, it appears that the highest value of dry weight of the stems was 71.067 g at the control which exceeded significantly the smallest value of dry weight of stems was 31.967 g at the interaction (110 Cd and 550 Pb) mg kg^{-1} and dry weight of stems of 40.033 g at the interaction (110 Cd and zero Pb) mg kg^{-1}, and no significant difference was observed between the control and the value 62.933 g, at the interaction (550 Pb and zero Cd) mg kg^{-1}, the results of this study were lower than the results of Shah et al. (52) in their study on Eucalyptus camaldulensis seedlings, which showed that the highest dry weight value of stems reached 166 g at the control and significantly exceeded the smallest value 97 g at the interaction (0.40 Cd and 8 Cr) mg L^{-1}, and the results of this study are close to the values of Bajwa (9) in his study on seedlings of Eucalyptus tereticornis, Leucaena leucocephala, Melia azedarach and Dalbergia sissoo which Malted with Cd concentrations 0, 10, 20, 40, 80 and 120 and Pb concentrations 0, 30, 60, 120, 180 and 240 mg kg $^{-1}$ dry soil, which showed in Eucalyptus seedlings that the highest dry weight of stems reached 91.4 g at the control and dry weight of stems was 55.9 g at the level of 120 Cd, the minimum dry weight of the stems was 45.9 g at a Pb level of 240 mg kg^{-1}. The reason for the decrease in dry weight of stems at high concentrations of Cd and Pb may be attributed to cellular stress that affected the reduction in growth in tissue cells, reduced cell expansion and inhibition, prolongation by inhibiting heavy metals to the action of the proton pump responsible for gradually accumulating protein across the biofilm and thus reducing plant growth (12, 20).

Effect of Cd and Pb on dry weight of leaves: The results of Duncan's multiple range test (Table 3) showed a decrease in dry weight of leaves with an increase in the concentrations of Cd and Pb in the soil, while the effect of the interaction of Cd and Pb shows that dry weight of leaves reached 51.033 g at the control which significantly exceeded the smallest value for leaves dry weight of 16.033 g at the interaction (110 Cd and 550 Pb) mg kg^{-1}, and for a dry weight of leaves of 29.367 g at the interaction (110 Cd and zero Pb) mg kg^{-1}, while no significant difference was observed with the value 50.267 g at the interaction (550 Pb and zero Cd) mg kg^{-1}, and the results of this study are close to the results of Bajwa (9), which showed in Eucalyptus seedlings that the highest value for leaves dry weight was 92.8 g at a concentration of Cd 10 mg kg^{-1} dry soil, and the smallest value was for dry weight for leaves it was 55.9 g at the level of Cd 120 mg kg^{-1}, the smallest value for dry weight of leaves was 37.7 g at the level of Pb 240 mg kg^{-1}, and the highest value for dry weight of leaves was 55.5 g at the control. The decrease in the biomass of plant leaves exposed to heavy metals may be due to poor absorption and transfer of nutrients and water from root to shoot system and between the cells of plant themselves (55).

Table 2. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in dry weight of stems (g).

		Interactions means in dry weight of stems (g) Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Levels	0	25	55	85	110	
	0	71.067 a	60.833 abc	59.700 bcd	64.700 abc	40.033 ef	59.267 a
	125	68.567 ab	58.833 bcd	58.433 bcd	64.167 abc	38.300 f	57.660 ab
	250	66.533 abc	57.533 bcd	57.267 bcd	63.067 abc	36.767 f	56.233 ab
	450	67.667 abc	57.000 cd	57.667 bcd	48.800 de	36.533 f	53.533 bc
	550	62.933 abc	56.400 cd	56.100 cd	42.433 ef	31.967 f	49.967 c
Cd rates		67.353 a	58.120 b	57.833 b	56.633 b	36.720 c	

* Rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Table 3. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in dry weight of leaves (g).

		Interactions means in dry weight of leaves (g) Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Levels	0	25	55	85	110	
	0	51.033 a	48.233 a-d	42.400 a-e	39.100 c-f	29.367 fg	42.027 a
	125	48.700 abc	44.567 a-e	40.333 b-e	42.167 a-e	23.633 gh	39.880 ab
	250	50.800 a	45.333 a-e	39.667 cde	39.767 cde	22.700 gh	39.653 ab
	450	52.233 a	38.133 def	38.633 c-f	35.600 ef	18.367 h	36.593 bc
	550	50.267 ab	37.733 ef	37.767 ef	23.200 gh	16.033 h	33.000 c
Cd rates		50.607 a	42.800 b	39.760 bc	35.967 c	22.020 d	

* Rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Effect of Cd and Pb on dry weight of roots: The results of Duncan's multiple range test (Table 4) showed a decrease in dry weight of roots with an increase in the concentration of Cd and Pb in the soil, as for the effect of the interaction between Cd and Pb, it showed that the highest value of dry weight of roots reached 66.033 g at the control it significantly exceeded the smallest value of dry weight of roots of 21.667 g at the interaction (110 Cd and 550 Pb) mg kg^{-1} and the value of 31.467 g at the interaction (110 Cd and zero Pb) mg kg^{-1}, and no significant difference was observed with the value 59.433 g at the interaction (550 Pb and zero Cd) mg kg^{-1}, and the values of this study outperformed the results reported by Nofal et al. (43) in their study on seedlings of Bauhinia purpurea L. treatment with a mixture of Pb, Cd and nickel concentrations 500, 50 and 25 for treatment T1, and 1000, 100 and 50 for T2, 1500, 150 and 75 for T3, and 2000, 200 and 100 for T4 with a unit of mg kg^{-1} dry soil respectively, as well as the control, which showed that the largest value of dry weight of roots was 6.23 g at the control significantly exceeded the smallest value for dry weight of roots 2.68 g at treatment T4. The direct effect of high concentrations of heavy metals caused cellular stress, which resulted in inhibition of root growth that resulted from inhibition of root cell division and reduced nutrient absorption that affected the dry mass of the roots (54).

Table 4. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in dry weight of roots (g).

		Interactions means in dry weight of roots (g) Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	66.033 a	64.100 ab	55.300 b-e	45.900 e-i	31.467 jkl	52.560 a
	125	59.733 abc	56.033 a-d	48.333 d-h	41.467 g-j	28.433 klm	46.800 b
	250	61.467 abc	52.600 c-f	45.300 f-i	39.167 g-j	26.1 lm	44.927 bc
	450	60.433 abc	49.200 d-g	38.900 hij	37.167 ijk	24.567 lm	42.053 c
	550	59.433 abc	34.133 jkl	39.500 g-j	32.033 jkl	21.667 m	37.353 d
Cd rates		61.420 a	51.213 b	45.467 c	39.147 d	26.447 e	

* Rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Effect of Cd and Pb on Cd concentration in stems: The results of Duncan's multiple range test (Table 5) showed an increase in Cd content in stems with an increase in the concentration of Cd and Pb in the soil, and the effect of the interaction between Cd and Pb shows that the highest concentration of Cd in stems was 372.167 mg kg^{-1} at the interaction (110 Cd and 550 Pb) mg kg^{-1}, it was significantly superior to all concentrations of other treatments, and the smallest content of Cd was 0.375 mg kg^{-1} at the control which did not notice a significant difference between it and the concentration of Cd 0.417 mg kg^{-1} at the interaction (550 Pb and zero Cd) mg kg^{-1}, while the concentration of Cd was significantly higher than the concentration of Cd 178.625 mg kg^{-1} at the interaction (110 Cd and zero Pb) mg kg^{-1} at the control, and the study indicated that 19 interactions in which the concentration exceeded the critical limit for Cd (5-10) µg g^{-1} dry weight (44, 51), the values of this study were superior to what was reported by Khamis et al. (27) in their study of Melia azedarach and Populus alba seedlings, which were treated with Cd concentrations 0, 10, 20 and 40 and Pb concentrations 0, 200, 400 and 800 ppm, as they found that the highest concentration of Cd in stems of P. alba seedlings was 11.5 mg kg^{-1} and in stems of M. azedarach seedlings it reached 5.9 mg kg^{-1} in the concentration of Cd 40 mg kg^{-1} dry soil, which was superior to the smallest concentration for Cd whose value approached zero mg kg^{-1} at the control. The reason for the increase in Cd concentration in stems of seedlings at high concentrations of Cd and Pb in the soil may be due to the fact that with the increase in the time of exposure of plant to heavy metals, the activity of the antioxidant system as well as other defenses in plant decreases, which facilitates transfer of heavy metals into the shoot to collect in the cell walls, this is one of the most important sites devoted to heavy metals (22).

Table 5. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in Cd concentration in stems (mg kg^{-1}).

		Interactions means in Cd concentration in stems (mg kg^{-1}) Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Levels	0	25	55	85	110	
	0	0.375 l	4.125 l	13.458 k	55.792 g	178.625 b	50.475 b
	125	0.542 l	12.875 k	17.083 jkJ	69.625 f	91.542 d	38.333 d
	250	0.583 l	14.708 jk	46.083 h	59.792 g	82.625 e	40.758 c
	450	0.375 l	11.542 k	19.500 j	35.417 i	86.542 de	30.675 e
	550	0.417 l	13.375 k	44.750 h	125.125 c	372.167 a	111.167 a
Cd rates		0.458 e	11.325 d	28.175 c	69.15 b	162.300 a	

* Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Effect of Cd and Pb on Cd concentration in leaves: The results of Duncan's multiple range test (Table 6) showed an increase in the concentration of Cd in the leaves with an increase in the concentration of Cd and Pb in the soil, as for the effect of the interaction between Cd and Pb, it was found that the concentration of Cd in the leaves reached 24.333 mg kg^{-1} at the interaction (110 Cd and 550 Pb) mg kg^{-1}, which was significantly superior to the smallest concentration of Cd 0.417 mg kg^{-1} at the control, and it was found that the increase in Cd concentration was not significant for 0.583 mg kg^{-1} at the interaction (550 Pb and zero Cd) mg kg^{-1} at the control, and the Cd concentration was found 15.708 mg kg^{-1} at the interaction (110 Cd and zero Pb) mg kg^{-1} was significantly superior to the control, and the study showed that 15 interactions in which the concentration increased beyond the critical limit of Cd (5-10) µg g^{-1} dry weight, and the results of this study exceeded the values of researchers El-Mahrouk et al. (17) in their study of Salix mucronate seedlings that were treated with Cd concentrations 0, 20, 40, 60 and 80, and Cu concentrations were 0, 50, 100, 150 and 200, and Pb concentrations were 0, 250, 450, 650 and 850 mg kg^{-1} dry soil, which showed that the highest concentration of Cd in leaves approached 4 mg kg^{-1} at the level of Cd 80 mg kg^{-1} soil and significantly superior to the values of other concentrations and the smallest concentration of Cd, which approached zero mg kg^{-1} at the control. The reason for the increase in Cd concentration in seedlings' leaves with the increase in Cd and Pb concentrations in the soil is due to the possibility of plant adaptation to growth in environments contaminated with heavy metals, which enabled it to increase its resistance to some pollutants and to coexist with the new growth nature by developing antioxidants and other defense methods that block minerals, in vacuoles in plant cell walls (14, 46).

Table 6. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in Cd concentration in leaves (mg kg^{-1}).

	Interactions means in Cd concentration in leaves (mg kg^{-1}) Cd concentrations (mg kg^{-1} dry soil)						Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	0.417 k	4.833 jk	5.333 j	34.542 e	15.708 g	12.167 c
	125	0.542 k	5.125 j	4.667 jk	34.958 e	128.167 a	34.692 a
	250	0.417 k	4.583 jk	4.875 jk	37.750 de	13.292 gh	12.183 c
	450	0.583 k	6.917 ij	10.125 hi	39.958 d	123.833 b	36.283 a
	550	0.583 k	4.583 jk	22.125 f	54.833 c	24.333 f	21.292 b
Cd rates		0.508 e	5.208 d	9.425 c	40.408 b	61.067 a	

*** Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).**

Effect of Cd and Pb on Cd concentration in roots: The results of Duncan's multiple range test (Table 7) showed an increase in the Cd concentration in the roots with an increase in the concentration of Cd and Pb in the soil, and the effect of the interaction between Cd and Pb showed that the highest concentration of Cd in the roots was 162.083 mg kg^{-1} at the interaction (110 Cd and 550 Pb) mg kg^{-1}, and it was significantly superior to all concentrations of other treatments, also the smallest concentration of Cd was 0.583 mg kg^{-1} at the control, whereas the concentration of Cd was 30 mg kg^{-1} at the interaction of (110 Cd and zero Pb) mg kg^{-1} was significantly superior to the control, and the study showed that 15 interventions in which the concentration in seedlings exceeded the critical limit of Cd (5-10) µg g^{-1} dry weight, and the results of this study outperformed the results reported by Khamis et al. (27) in their study of Melia azedarach and Populus alba seedlings, who observed that the highest concentration of Cd in roots was found in P. alba and M. azedarach 17.5 mg kg^{-1} at a concentration of Cd 40 mg kg^{-1} dry soil. The reason for the increase in the concentration of Cd in the roots with the increase in the concentration of Cd and Pb in the soil is due to the fact that the roots of plants work to release all factors that help in the chelation and binding of minerals in the area of the soil close to them (28), and that most of the absorption of Cd occurs in The epidermis of the root cap (30), as the root cap lacks a casparian strip that controls the transport of water and salts through the cell wall cavity from the phloem to the xylem, so that Cd is easily transported through cell walls directly to the xylem (27).

Table 7. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in Cd concentration in roots (mg kg^{-1}).

		Interactions means in Cd concentration in roots (mg kg^{-1}) Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	0.583 i	1.833 hi	7.333 h	20.792 f	30.000 e	12.108 d
	125	0.792 i	2.250 hi	13.833 g	25.125 ef	46.542 c	17.708 c
	250	0.750 i	2.542 hi	14.208 g	21.125 f	49.625 c	17.650 c
	450	0.792 i	2.250 hi	12.458 g	29.167 e	69.625 b	22.858 b
	550	0.833 i	2.500 hi	13.833 g	35.167 d	162.083 a	42.883 a
Cd rates		0.750 d	2.275 d	12.333 c	26.275 b	71.575 a	

*** Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).**

Effect of Cd and Pb on Pb concentration in stems: The results of Duncan's multiple range test (Table 8) showed an increase in the concentration of Pb in stems with an increase in the concentrations of Cd and Pb in the soil, while the effect of the interaction between Cd and Pb showed that the highest concentration of Pb in the stems was 62.375 mg kg^{-1} at the interaction (110 Cd and 550 Pb) mg kg^{-1} and this value was significantly superior to all the concentrations of the treatments, and the Pb concentration at the control treatment was 12.417 mg kg^{-1}, and it was found from the results of this study that three values exceeded the critical toxic limit (30 µg g^{-1} dry weight) (47), the results of this study outperformed the results of researchers Mleczek et al. (38) in their study on seedlings of Acer platanoides L., A. pseudoplatanus L., Betula pendula Roth, Quercus robur L., Tilia cordata Miller and Ulmus laevis Pall., which treated with mining sludge containing arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), thallium (TI) and zinc (Zn) at concentrations of 18022, 1030, 4511, 3865, 669 and 1565 mg kg^{-1}, respectively, results showed that the significant superiority of the Pb concentration that approaching 50 mg kg^{-1} in U. laevis seedlings grown in mining sludge on all seedlings. The increase in the concentration of Pb in the stems at high concentrations of Cd and Pb may be due to the possibility of plant accumulation of heavy metals in the shoot due to the productivity of the vegetative biomass that supports the extraction of minerals and their confinement in cell wall vacuoles (7).

Table 8. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in Pb concentration in stems (mg kg^{-1}).

		Interactions means in Pb concentration in stems (mg kg^{-1}) Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	12.417 ijk	11.000 k	11.167 k	12.458 ijk	9.583 k	11.325 e
	125	15.292 hi	11.625 jk	14.708 hij	16.958 gh	12.500 ijk	14.217 d
	250	22.333 def	20.875 ef	16.875 gh	33.625 b	22.458 de	23.233 b
	450	21.125 def	24.458 d	16.250 gh	18.833 fg	18.917 fg	19.917 c
	550	15.125 hij	30.042 c	18.917 fg	24.375 d	62.375 a	30.167 a
Cd rates		17.258 d	19.600 c	15.583 e	21.250 b	25.167 a	

*** Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).**

Effect of Cd and Pb on Pb concentration in leaves: The results of Duncan's multiple range test (Table 9) showed that there are no significant differences with increasing the concentration of Cd and Pb in the soil between the concentration of Pb in the leaves at the control and the interactions between Cd and Pb except for the two interactions (85 Cd and zero Pb) and (85 Cd and 250 Pb) mg kg^{-1} when the concentration of Pb in leaves decreased significantly compared to the control, while the effect of the interaction between Cd and Pb showed that the highest concentration of Pb in the leaves was 20.417 mg kg^{-1} at the interaction (110 Cd and 550 Pb) mg kg^{-1} and did not differ significantly from the control value (18.375 mg kg^{-1}), and the results of this study were lower than the results of researchers Mleczek et al. (38) whose findings showed a clear significant superiority over all types of seedlings for the concentration of Pb whose value approached 50 mg kg^{-1} in the leaves of Ulmus laevis Pall. seedlings growing in mining sludge. The low movement of Pb from soil to leaves was the reason for the absence of significant differences between most of the interactions.

Table 9. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in Pb concentration in leaves (mg kg^{-1}).

	Interactions means in Pb concentration in leaves (mg kg^{-1}) Cd concentrations (mg kg^{-1} dry soil)						Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	18.375 a-d	17.750 a-d	14.500 cde	10.042 f	16.292 a-d	15.392 b
	125	14.625 cde	20.042 ab	14.667 cde	15.833 bcd	18.667 abc	16.767 ab
	250	16.250 a-d	16.625 a-d	18.042 a-d	11.125 ef	17.958 a-d	16.000 b
	450	14.792 cde	14.125 de	18.125 a-d	16.333 a-d	19.542 ab	16.583 ab
	550	14.292 cde	19.917 ab	17.333 a-d	18.792 abc	20.417 a	18.150 a
Cd rates		15.667 cd	17.692 ab	16.533 bc	14.425 d	18.575 a	

* Interactions means that have the same letter are not significantly different at ($P\leq0.01$).

* Rates of Cd that have the same letter are not significantly different at ($P\leq0.001$).

* Rates of Pb that have the same letter are not significantly different at ($P\leq0.05$).

Effect of Cd and Pb on Pb concentration in roots: The results of Duncan's multiple range test (Table 10) showed that the concentration of Pb increases with the increasing the concentration of Cd and Pb in the soil, as for the effect of the interaction between Cd and Pb, it appears that the Pb concentration in the roots was 24.958 mg kg^{-1} at the interaction of (110 Cd and 550 Pb) mg kg^{-1}, which It significantly exceeded the smallest value of the Pb concentration (2.875 mg kg^{-1}) at the control, also Pb concentration was 34.417 mg kg^{-1} at the interaction (550 Pb and zero Cd) mg kg^{-1} was significantly superior to the control, concentrations of Pb exceeded 30 µg g^{-1} dry weight, which is the critical toxic limit for most plants, the results of this study were lower than the results of researchers Mleczek et al. (38), whose values showed that the highest concentration of Pb in the roots approached 600 mg kg^{-1} in Acer platanoides seedlings developing in mining sludge, which was not observed significant difference with Pb concentration in roots of Quercus robur and Ulmus laevis grown in mining sludge while it outperformed all other concentrations in seedlings. The reason for the increase in Pb concentration in the roots of seedlings with the increase in the concentration of Cd and Pb in the soil is due to the plant's resistance to the stress of heavy metals through the formation of complexes that work to encapsulate and isolate heavy metals in the root cell vacuoles that have been identified as a detoxification mechanism, as the inner dermis is one of the most important sites for concentrating heavy metals in the roots (53, 33).

Effect of Cd and Pb on TF of Cd: The results of Duncan's multiple range test (Table 11) showed that most of TF values of Cd increased in the seedlings with an increase in the concentration of Cd and Pb in the soil, as for the effect of the interaction between Cd and Pb, it appears that TF value reached 1.224 at the interaction (110 Cd and 550 Pb) mg kg^{-1}, and value of TF was 0.783 at the control, and it was noticed that there was no significant difference between the control and the TF value (0.621) at the interaction between (550 Pb and zero Cd) mg kg^{-1} while value of TF was significantly superior to 3.245 at the interaction between (110 Cd and zero Pb) mg kg^{-1} at the control, the values of this study outperformed the results of El-Mahrouk et al. (17) in their study on Salix mucronate seedlings, as TF for Cd reached 76.91 at the control, and TF value for Cd exceeded 76.68 at the level of Cd 60 mg kg^{-1} soil significantly on TF values for Cd and for value is 69.12 at the level of Cd 80 mg kg^{-1} soil and the lowest value for TF for Cd is 28.08 at the level of Cd 20 mg kg^{-1} soil. The results of this study showed that the seedlings were able to transfer Cd and collect it from the roots to the shoot, as TF value of Cd was (>1) for most seedlings, and this means the ability of the seedlings to accumulate Cd in its shoots, as the transfer of heavy metals from root to shoot depends on the environment and soil that determine the availability of heavy metals, and type of the plant, and the plant considered a hyperaccumulator for HM if the value was (>1) for TF (31).

Table 10. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in Pb concentration in roots (mg kg^{-1}).

		Interactions means in Pb concentration in roots (mg kg^{-1}) Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	2.875 l	7.750 h-k	5.875 jkl	4.458 kl	7.083 i-l	5.608 e
	125	4.917 kl	11.833 gh	7.500 h-k	12.458 g	11.000 ghi	9.542 d
	250	9.625 g-j	11.958 gh	13.958 fg	9.708 g-j	73.500 a	23.750 b
	450	49.583 b	17.750 f	11.667 gh	29.875 d	48.750 b	31.525 a
	550	34.417 c	13.083 g	10.792 ghi	13.500 g	24.958 e	19.350 c
Cd rates		20.283 b	12.475 c	9.958 d	14.000 c	33.058 a	

*** Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).**

Table 11. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in TF of Cd.

		Interactions means in TF of Cd Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	0.783 gh	2.521 bcd	1.406 d-h	2.232 b-f	3.245 ab	2.037 a
	125	0.703 gh	4.197 a	0.814 gh	2.210 b-f	2.371 b-e	2.059 a
	250	0.693 gh	3.919 a	1.800 c-g	2.474 bcd	0.970 gh	1.971 a
	450	0.611 h	4.239 a	1.221 fgh	1.296 e-h	1.515 c-h	1.776 a
	550	0.621 h	3.577 a	2.473 bcd	2.565 bc	1.224 fgh	2.092 a
Cd rates		0.682 d	3.691 a	1.543 c	2.155 b	1.865 bc	

*** Interactions means or rates of Cd that have the same letter are not significantly different at ($P \leq 0.001$).**

Effect of Cd and Pb on BAC of Cd: The results of Duncan's multiple range test (Table 12) showed that most of the BAC values of Cd increased in seedlings with increasing the concentration of Cd and Pb in the soil, while the effect of the interaction between Cd and Pb showed that the highest value of BAC was 28.790 at the interaction (110 Cd and zero Pb) mg kg^{-1} was superior significantly for all BAC values of Cd, and that BAC value of 20.597 at the interaction (110 Cd and 550 Pb) mg kg^{-1} significantly exceeded value 2.106 at the control, while no significant difference was observed between value of 1.333 at the interaction (550 Pb and zero Cd) mg kg^{-1} at the control, and the values of this study outperformed the results of

Ng et al. (41) in their study on the vegetative accumulation of heavy metals in the soil, as they found in Imperata cylindrica plant that BAC value of Cd reached 1.519 at the control did not differ significantly from value 1.343 at a level of Cd 15 mg kg^{-1} soil, while it was found in Pennisetum purpureum that BAC value of Cd was 2.163 at a level of Cd 15 mg kg^{-1} dry soil, and it significantly exceeded value 1.055 at the control. The transport of heavy metals from the soil to the shoot through the roots depends on the environment of the plant and the soil that determines the availability of heavy metals, and the plants with a value (>1) for BAC were considered to be an hyperaccumulator of heavy metals (31), and plant extraction efficiency is related to mineral concentration in plant and dry matter productivity, in order for the plant to be ideal in phytoremediation of a polluted site, it must have high biomass productivity and the ability to withstand and accumulate mineral pollutants (56).

Table 12. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in BAC of Cd.

		Interactions means in BAC of Cd Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	2.106 nop	1.792 op	2.891 lmn	5.559 h	28.790 a	8.227 b
	125	1.445 p	1.532 p	2.486 mno	7.470 g	13.315 d	5.250 d
	250	2.000 op	3.355 kl	3.997 jk	4.701 ij	7.378 g	4.286 e
	450	1.531 p	2.953 lm	3.591 kl	9.726 f	11.072 e	5.775 c
	550	1.333 p	4.789 i	6.688 g	16.360 c	20.597 b	9.953 a
Cd rates		1.683 e	2.884 d	3.930 c	8.763 b	16.231 a	

* Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Effect of Cd and Pb on BCF of Cd: The results of Duncan's multiple range test (Table 13) showed that most of BCF values increased for Cd in seedlings with an increase in the concentration of Cd and Pb in the soil, as for the effect of the interaction between Cd and Pb, it appears that the highest value of BCF was 16.840 at the interaction (110 Cd and 550 Pb) mg kg^{-1}, It significantly exceeded all BCF values and 3.111 at the control, and BCF value of 8.889 at the interaction (110 Cd and zero Pb) mg kg^{-1} outperformed over the control, while no significant difference was observed between the BCF value of 2.222 at the interaction (550 Pb and zero Cd) mg kg^{-1} at the control, and the values of this study outperformed the results of El-Mahrouk et al. (17) in their study on Salix mucronate seedlings, which showed that BCF value for Cd was 0.27 at the control and the highest value for BCF for Cd was 0.61 when the level of Cd was 80 mg kg^{-1} dry soil, which significantly outperformed all BCF values for Cd. The value of BCF in a plant depends on the type of mineral ion and its concentration in the soil as well as on the type of plant and the surrounding environmental conditions (25). A plant with a BCF value (more than one) is suitable for phytoremediation of the soils contaminated with heavy metals (35, 39).

Effect of Cd and Pb on CI of Cd: The results of Duncan's multiple range test (Table 14) showed that CI values of Cd increased in the seedlings with an increase in the concentration of Cd and Pb in the soil, while the effect of the interaction between Cd and Pb showed that the highest CI value was 490.812 when the interaction (110 Cd and 550 Pb) mg kg^{-1} and this value was superior significantly on all the interference values, and the CI of 184.298 at the interaction (110 Cd and zero Pb) mg kg^{-1} significantly exceeded on the control, and the results of this study surpassed the results of Chandra and Hoduck (11), in their study of the effect of a mixture of Cu, Cd, Cr and Zn at concentrations of 0, 5, 50, 100, 200 and 500 mg L^{-1} on four different poplar hybrids, the results showed that the highest CI value for Cd was 35.69 when interfering 500 mg L^{-1} for Cd, Zn, Cu and Cr in the TD225 hybrid, which significantly outperformed all other the hybrids values.

Table 13. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in BCF of Cd.

		Interactions means in BCF of Cd Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Levels	0	25	55	85	110	
	0	3.111 ef	0.733 i	2.257 fgh	2.559 fg	8.889 b	3.510 c
	125	2.111 fgh	0.383 i	3.162 ef	3.589 e	5.641 d	2.977 d
	250	3.000 efg	0.884 i	2.229 fgh	2.036 gh	7.634 c	3.157 cd
	450	2.533 fg	0.720 i	3.020 efg	7.527 c	7.329 c	4.226 b
	550	2.222 fgh	1.333 hi	2.767 efg	6.394 d	16.840 a	5.911 a
Cd rates		2.596 c	0.811 d	2.687 c	4.421 b	9.267 a	

* Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Table 14. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in CI of Cd.

		Interactions means in CI of Cd Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	1.000 l	7.548 kl	19.763 jk	85.843 fg	184.298 b	59.691 cd
	125	1.360 l	15.226 jk	27.586 ij	102.699 e	188.957 b	67.165 b
	250	1.289 l	16.513 jk	53.356 h	93.602 ef	117.757 d	56.504 d
	450	1.247 l	15.494 jk	32.327 i	75.677 g	195.029 b	63.955 bc
	550	1.322 l	17.098 jk	63.367 h	170.926 c	490.812 a	148.705 a
Cd rates		1.244 e	14.376 d	39.280 c	105.749 b	235.371 a	

* Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Effect of Cd and Pb on TF of Pb: From the results of Duncan's multiple range test (Table 15), it appears that the highest value of TF for Pb was 5.702 at the control which was significantly superior to all other TF values, and the value was 1.675 at the interaction (110 Cd and 550 Pb) mg kg^{-1}, which was significantly higher on the TF value of 0.431 at the interaction (550 Pb and zero Cd) mg kg^{-1}, also the control value significantly outperformed the TF value of 0.431 at the interaction (550 Pb and zero Cd) mg kg^{-1} and the TF value of 1.879 at the interaction (110 Cd and zero Pb) mg kg^{-1}, the values of this study were close to the results of researchers Abbasi et al. (1) in their study on Acer cappadocicum, Fraxinus excelsior and Platycladus orientalis seedlings, the results showed that in F. excelsior seedlings TF value for Pb, which approached 1.8 at the control, exceeded all TF values at Pb levels, except Pb level 200 mg kg^{-1}, whereas the smallest value for TF, which approached 0.8, was at the Pb level 400 mg kg^{-1}. The results of this study indicates the ability of the seedlings to transfer Pb from the root system and collect it in the shoot, as the value of TF (>1) was for most seedlings, and this explains the ability of seedlings to accumulate heavy metals in their shoot, and the reason is due to the existence of an effective mechanism for excessive accumulation on the studied seedlings, which allow the absorption and accumulation of heavy metals from contaminated soil to root and then to shoot (1, 34).

Effect of Cd and Pb on BAC of Pb: The results of Duncan's multiple range test (Table 16) showed that most of BAC values for Pb in seedlings increased with the increasing of Cd and Pb concentration in the soil, as for effect of the interaction between Cd and Pb, it appears that the highest BAC value for Pb was 4.246 at the interaction (110 Cd and 550 Pb) mg kg^{-1}, and significantly exceeded all the interference values, and the BAC value reached 1.579 at the control which significantly exceeded BAC value for Pb of 1.293 at the interaction (550 Pb and zero Cd) mg kg^{-1}, and the value of 0.884 at the interaction (110 Cd and zero Pb) mg kg^{-1}, and the values of this study outperformed the results of Ng et al. (41), which showed in Imperata cylindrica that BAC value for Pb was 0.138 at the control which did not differ significantly from the value 0.359 at the level of Pb 140 mg kg^{-1} dry soil, while the researchers noted in Pennisetum purpureum, BAC value for Pb was 0.436 at a Pb level of 140 mg kg^{-1} dry soil, and it significantly exceeded the value 0.122 at the control. The accumulation of Cd, Cu and Pb in the shoot depends on the level of their concentrations in the root zone, pH in the soil, the type of plant, and the surrounding environment (17).

Table 15. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in TF of Pb.

		Interactions means in TF of Pb Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	**Levels**	**0**	**25**	**55**	**85**	**110**	
	0	**5.702 a**	**1.880 c-f**	**2.252 cde**	**2.614 bc**	**1.879 c-f**	**2.865 a**
	125	**3.363 b**	**1.362 e-i**	**2.018 c-f**	**1.375 e-i**	**1.466 d-h**	**1.917 b**
	250	**2.073 c-f**	**1.599 c-g**	**1.284 e-j**	**2.508 bcd**	**0.275 j**	**1.548 b**
	450	**0.365 ij**	**1.103 f-j**	**1.524 c-g**	**0.594 g-j**	**0.396 hij**	**0.796 c**
	550	**0.431 hij**	**1.934 c-f**	**1.729 c-f**	**1.608 c-g**	**1.675 c-g**	**1.476 b**
Cd rates		**2.387 a**	**1.576 b**	**1.761 b**	**1.740 b**	**1.138 c**	

*** Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).**

Table 16. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in BAC of Pb.

		Interactions means in BAC of Pb Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	**Level**	**0**	**25**	**55**	**85**	**110**	
	0	**1.579 efg**	**1.475 ghi**	**1.053 klm**	**0.769 n**	**0.884 mn**	**1.152 c**
	125	**1.228 i-l**	**1.146 kl**	**1.205 jkl**	**0.961 lmn**	**1.598 d-g**	**1.228 c**
	250	**1.583 efg**	**1.923 bc**	**1.433 g-j**	**1.530 fgh**	**2.073 b**	**1.708 b**
	450	**1.842 b-e**	**1.978 bc**	**1.763 c-f**	**1.203 jkl**	**1.820 b-e**	**1.721 b**
	550	**1.293 h-k**	**2.050 b**	**1.859 bcd**	**1.771 c-f**	**4.246 a**	**2.244 a**
Cd rates		**1.505 c**	**1.714 b**	**1.462 c**	**1.247 d**	**2.124 a**	

*** Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).**

Effect of Cd and Pb on BCF of Pb: From the results of Duncan's multiple range test (Table 17) it appears that BCF value for Pb reached 2.560 at the interaction between (110 Cd and 550 Pb) mg kg^{-1}, and significantly decreased from the value 3.026 at the interaction of (550 Pb and zero Cd) mg kg^{-1}, and it was significantly higher than the BCF of 3.026, the values of this study were close to Abbasi et al. (1) in their study on seedlings of Acer cappadocicum, Fraxinus excelsior and Platycladus orientalis, as it showed the significant superiority over all values of BCF for Pb at the control their values approached 3, 2, and 1.6 in P. orientalis, F. excelsior and A. cappadocicum seedlings, respectively. The BCF value in the plant depends on the type and concentration of heavy metals in the soil as well as on the plant type and the surrounding environmental conditions (25), that value of BCF (>1) in the plant is a good indication of phytoremediation of soils contaminated with the heavy metals (35, 39).

Effect of Cd and Pb on CI of Pb: The results of Duncan's multiple range test (Table 18) showed that the CI values of Pb increased with the increasing of Cd and Pb concentration in the soil, as for the effect of the interaction between Cd and Pb, it appears that the highest CI value was 3.833 at the between interaction (110 Cd and 550 Pb) mg kg^{-1}, and it was significantly superior to all CI value, it was noticed that there was no significant difference between the control and the CI of 1.006 at the interaction (110 Cd, zero Pb) mg kg^{-1}, while the CI of 2.013 at the interaction (550 Pb and zero Cd) was significantly higher than the control.

Table 17. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in BCF of Pb.

		Interactions means in BCF of Pb Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Levels	0	25	55	85	110	
	0	0.295 l	0.795 h-k	0.482 jkl	0.305 l	0.484 jkl	0.472 e
	125	0.403 kl	0.857 g-j	0.615 jkl	0.730 ijk	1.128 ghi	0.747 d
	250	0.790 h-k	1.226 g	1.146 gh	0.664 jkl	7.539 a	2.273 b
	450	5.085 b	1.820 f	1.196 gh	2.043 f	4.615 c	2.952 a
	550	3.026 d	1.074 ghi	1.107 ghi	1.108 ghi	2.560 e	1.775 c
Cd rates		1.920 b	1.154 c	0.909 d	0.970 d	3.265 a	

* Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Table 18. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in CI of Pb.

		Interactions means in CI of Pb Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	1.000 ij	1.093 hij	0.958 ij	0.880 j	1.006 ij	0.987 e
	125	1.085 hij	1.314 fgh	1.154 hi	1.431 fg	1.283 gh	1.253 d
	250	1.519 fg	1.554 f	1.516 fg	1.934 de	3.439 b	1.992 c
	450	2.694 c	1.816 e	1.443 fg	2.009 de	2.650 c	2.122 b
	550	2.013 de	2.111 d	1.498 fg	1.817 e	3.833 a	2.254 a
Cd rates		1.662 b	1.578 b	1.314 c	1.614 b	2.442 a	

* Interactions means or rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).

Effect of Cd and Pb on TI: The results of Duncan's multiple range test (Table 19) showed that the TI values decreased in seedlings with an increase in the concentration of Cd and Pb in the soil, as for the effect of the interaction between Cd and Pb, it appears that the smallest value of TI was 0.370 at the interaction (110 Cd and 550 Pb) mg kg^{-1}, and TI value was 0.536 at the interaction (110 Cd, zero Pb) mg kg^{-1}, and the results of this study relatively outperformed the results reported by Kabir et al. (26) in their study on Leucaena leucocephala seedlings treated with concentrations of Pb and Cd 0, 25, 50, 75 and 100 ppm each single and mixed, as they found that the TI value was 0.623 when the interaction (100 Cd and 100 Pb) mg kg^{-1}. The reason for the decrease in TI values with increasing concentrations of Cd and Pb in the soil is due to the stress of heavy metals effect, which leads to inhibit the growth of the plant, which causes a decrease in the biomass, according to the type of plant and type and amount of toxins absorbed in the cellular tissues (13, 15).

CONCLUSIONS: Eucalyptus seedlings have good ability to accumulate and concentrate Cd in their shoot, based on TF values greater than one, as well as for BAC, BCF, and CI which confirmed the ability of seedlings to accumulate and transfer elements from soil to root and shoot, and that seedlings have a good ability to absorb and accumulate Pb in their roots, when increasing the concentration of Pb in the soil, whether alone or in the presence of Cd, as BCF values for Pb increased than one, as well as for TF, BAC, CI and TI, which confirms the seedlings ability to resist high concentrations of Pb. And despite the increase in Cd and Pb concentrations at critical limits in plant parts, seedlings resisted these concentrations even though their growth was negatively affected.

And TF and BAC value greater than one showed that Eucalyptus camaldulensis Dehnh. seedlings were suitable for use in phytoextraction applications of soils contaminated with Cd and Pb within the phytoremediation processes.

Table 19. Duncan's multiple-range test results for effect of Cd and Pb and their interactions in TI.

		Interactions means in TI Cd concentrations (mg kg^{-1} dry soil)					Pb rates
Pb concentrations (mg kg^{-1} dry soil)	Level	0	25	55	85	110	
	0	1.000 a	0.920 a-d	0.837 b-f	0.796 d-g	0.536 ij	0.818 a
	125	0.941 abc	0.848 b-e	0.782 efg	0.786 efg	0.480 jk	0.767 b
	250	0.950 abc	0.826 c-f	0.756 e-h	0.755 e-h	0.455 jk	0.749 bc
	450	0.959 ab	0.767 e-h	0.719 e-h	0.646 hi	0.423 jk	0.703 c
	550	0.918 a-d	0.682 gh	0.709 fgh	0.519 j	0.370 k	0.640 d
Cd rates		0.954 a	0.809 b	0.761 b	0.700 c	0.453 d	

*** Rates of Cd or Pb that have the same letter are not significantly different at ($P \leq 0.001$).**

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