

Efficiency of *Leucaena leucocephala* and *Pithecellobium dulce* (Roxb.) Plants in Absorbing Heavy Metals Lead and Cadmium and Their Effect on Biochemical Properties

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Abstract. The current study was conducted during the period 10/1/2023 to 6/1/2024 in the fabric shade of the Department of Horticulture and Landscape Engineering / College of Agriculture / University of Basra, to know the efficiency of phytoremediation of Indian almond and Lucinia plants and their ability to absorb heavy metals by adding pollutants to the soil with irrigation water. The concentrations of each were (200,100,0) Pb mg L⁻¹ and (20,10,0) Cd mg L⁻¹. The treatment was done with Puterscine at a concentration of (500,250,0) mg L⁻¹ and the addition was by adding it to the soil. The seedlings were treated with pollutants from 10/1/2024 to 12/1/2024 for a period of three months. After 60 days had passed since the last treatment, the seedlings were treated with Puterscine at a rate of twice during the growing season with a time difference of 30 days between them. The experiment was conducted according to a complete randomized block design and statistical analysis of the experimental data was conducted using the statistical program Genstat and the averages of the treatments were compared using the least significant difference and at the probability level of 0.05. The results indicated the ability of the Indian almond and Lucinia plants to tolerate heavy metal concentrations by measuring the biochemical indicators. The results showed that the treatment with cadmium at a concentration of 20 mg L⁻¹ led to a decrease in the leaf content of total chlorophyll and carbohydrates compared to the control plants, while the addition of the treatment with pyrotresen at a concentration of 500 mg L⁻¹ led to a significant increase in total chlorophyll and treatment with pyrotresen led to a significant increase in carbohydrates. As for the treatment with lead at a concentration of 200 mg L⁻¹, it led to a significant increase in (lead concentration in leaves, lead concentration in roots) compared to the control plants, while the treatment with cadmium at a concentration of 20 mg L⁻¹ led to a significant increase in (cadmium concentration in leaves, cadmium concentration in roots) compared to In control plants, while treatment with Buttercine led to a significant increase in (cadmium concentration in leaves, lead concentration in leaves, cadmium concentration in roots, lead concentration in roots).

Keywords. *leucocephala*, *Pithecellobium*, Roxb, Cadmium.



1. Introduction

Leucaena leucocephala tree is from the fabales family, it is a tropical and subtropical medicinal plant [1], and this tree is characterized by its contribution to improving soil properties, reducing erosion and stabilizing sand [2].

Indian almond (Roxb.) *Pithecellobium dulce* The Indian almond tree belongs to the legume family. It is grown in tropical America, Southeast Asia, India and Mexico. It is a small to medium-sized evergreen thorny tree that reaches a height of 15 meters and has many uses in medical fields [3]. This tree can withstand the harshest environmental conditions, as it can withstand soil salinity and dry land. Indian almond is used as a windbreak and ornamental plant [4]. Pollution is defined as the change in the chemical, physical and biological properties of the environment due to the transfer of pollutants from different sources that cause harm to humans, animals and plants [5]. The spread of pollutants in the soil and air causes many problems at high concentrations and causes harmful effects on ecosystems and on human health and other organisms. They can enter the food chain through contaminated drinking water and agricultural products, which leads to their accumulation in the human body and other organisms [6]. The presence of lead in plant tissues prevents the process of photosynthesis and limits the function of stomata in leaves. High concentrations of lead cause cellular stress in the plant and prevent enzymatic activity, thus causing plant death [7,8]. As for cadmium (cd), it is a highly toxic element. Its appearance at low concentrations is harmful to the plant, as it directly affects the process of photosynthesis, transpiration, and the process of closing and opening stomata, as it moves to the cellular tissues, settles, and causes physiological and morphological changes that slow down the process of plant growth, as well as damage, deterioration, and death [9]. New research has supported the use of green plants as a suitable and low-cost technology [10]. in that they remove pollution by absorbing elements in their parts such as roots and leaves and do not enter the food chain. Buttermine is one of the important biological compounds that are manufactured in plant cell. They are compounds that belong to polyamines that have been classified as plant growth regulators. They are non-nutritive organic compounds that may be naturally formed in the plant or manufactured in the laboratory and that work to stimulate, inhibit or modify one of the physiological processes in the plant when used in low concentrations. Growth regulators play a major role in many growth and development processes. The manufactured compounds have an effectiveness similar to that of plant hormones [11].

The current study aims to:

- Know the extent of the response of the two plant species to pollution with heavy metals and the Buttermine treatment and which parts of the vegetative growth or the root have the highest accumulation of heavy metals.
- The extent of the effect of the pollution treatment, Butyrosin, in raising the efficiency of the two plant species in tolerating pollution by measuring their biochemical indicators.

2. Materials and Methods

The current study was conducted during the period 10/1/2023 to 5/1/2024 in the fabric shade of the Department of Horticulture and Landscape Engineering / College of Agriculture / University of Basra. The experiment was a factorial experiment with three factors. The first factor is the plant type Indian almond and *Lucinia*. The second factor is pollutants (Cd, Pb) at concentrations (200,100.0) mg L⁻¹ Pb and (20,10.0 mg L⁻¹ Cd). The third factor is the pollution treatment with Buttermine (500,250.0) mg L⁻¹ Seedlings were treated with pollutants from 10/1/2023 to 12/1/2023 for a period of three months. After 60 days had passed since the last treatment, the seedlings were treated with Buttermine treatment at a rate of twice during the growing season with a time difference of 30 days between them. The experiment was designed as a factorial experiment with three factors according to the complete randomized block design CRBD with four replications where treatments were compared at Probability low of 0.05 [12].

Table 1. Some chemical and physical properties of the used soil.

Properties	Values	Unit
EC	3.53	Dsm ⁻¹

Properties	Values	Unit
pH	8.13	---
Total N	20	Mg.kg ⁻¹
Total P	1.62	Mg.kg ⁻¹
Available K	7.23	Mg.kg ⁻¹
Total Pb	2.14	Mg.kg ⁻¹
Total Cd	0.006	Mg.kg ⁻¹
Sand	75	%
Silt	10	%
Clay	15	%
Soil Texture	Loamy sand	----

Table 2. Some chemical and physical properties of peat moss.

Characteristics	Values
pH	4.5-3.5
Organic matter (%)	97-95%
Total nitrite (%)	1%
Moisture (%)	50%
Density (g.cm)	90-60%

2.1. The Studied Characteristics

2.1.1. Total Chlorophyll Content of Leaves (mg⁻¹.100g⁻¹ fresh weight)

The total chlorophyll pigment in the leaves was estimated by taking a sample from each plant of the experimental unit [13] washed well with water, left to dry in the air, then 0.5 g was taken from each sample and 20 ml of acetone 80% concentration was added to it and the tissue was crushed using a ceramic mortar, then filtered using filter paper and chlorophyll was estimated using a UV-visible spectrophotometer to measure the light absorption of the pigment at two wavelengths (663-645) nm, then the amount of pigment was calculated using the following equation:

$$\text{Total chlorophyll mg/L1} = (663) D \times 8.02 + (645) D \times 20.2$$

Note that:

D663= reading of the device for light absorption at a wavelength of 663 nm.

D645= reading of the device for absorption. The light was at a wavelength of 645 nm.

The results were then converted to the unit (mg/100) g fresh weight.

2.1.2. Estimation of the Content of Leaves of Total Soluble Carbohydrates (mg.gm⁻¹)

The total soluble carbohydrates in the leaves were estimated according to the phenol-sulfuric acid method described by [14] by taking 0.5 g of air-dried leaves and grinding them well and placing them in test tubes with a capacity of (100) ml, then adding 35 ml of distilled water, then closing the tube well, then placing it in a water bath at a temperature of 90 °C for an hour to extract carbohydrates, then the tubes were cooled at room temperature, the extract was filtered using filter paper and 5 ml of the filtrate was taken and 25 ml of distilled water was added to it, then 1 ml of the diluted solution was taken and 1 ml of phenol (5%) was added to it with 5 ml of concentrated sulfuric acid and left to cool at room temperature The room. The light absorbed by the samples was measured at a wavelength of 490nm using a spectrophotometer and carbohydrates were estimated based on a standard curve in which glucose was used according to the following equation:

$$\text{Total soluble carbohydrate (mg.100g}^{-1}\text{)} = (\text{Standard curve carbohydrates} \times \text{Final volume} \times \text{dilatation}) / (\text{Sample weight (g)}) \times 100$$

2.1.3. Digestion and Estimation of Heavy Metals in Leaves and Roots (Cd, Pb)

The method described by [15] was followed, where plant leaf samples were dried using an electric oven at a temperature of 70 °C for 48 hours until the weight was stable, then they were ground well using a ceramic mortar. 0.5 g of plant tissue powder was taken and placed in a 25 ml test tube, and 5

ml of concentrated nitric acid HNO_3 was added to it and left for half an hour. 2.5 ml of perchloric acid HClO_4 62% was added to it and left for 10 minutes, then heated to a temperature of 60°C for a period of 30 minutes. The temperature was raised to 140°C for 60 minutes, then the temperature was raised to 210°C . Heating continued until white steam appeared in the test tube until the volume reached 2 ml, after which the remaining solution was taken and placed in a beaker. My volume is 50 ml and complete with distilled water.

3. Results and Discussion

3.1. Total Chlorophyll Content of Leaves ($\text{mg}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight)

The data in Table (3) show that the treatment with heavy metals has a significant effect, as the *Lucinia* plants significantly outperformed the Indian almond plants in the total chlorophyll trait, as it reached 15.11, $8.72 \text{ mg}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight. As for the effect of pollutants on the total chlorophyll, the control treatment significantly outperformed the rest of the treatments, as it reached 12.37, which did not differ significantly from (cadmium treatment, concentration 10 mg L^{-1} , lead treatment, concentration 200 mg L^{-1}), as the total chlorophyll rate in them reached (12.29, 12.25) $\text{mg}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight compared to the lowest rate recorded by the cadmium treatment, concentration 10 mg L^{-1} . As the data in the table above indicate, the Biotrichin treatment gave a significant difference in the total chlorophyll quality at 500 mg L^{-1} treatment over the rest of the treatments, as it reached $14.25 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight, compared to the lowest rate recorded by the control treatment, as the total chlorophyll rate reached $9.80 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight. As for the effect of the two-way interaction between plant type and pollutants, *Lucinia* plants outperformed in the control treatment, as the total chlorophyll rate in them reached $16.11 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight, which did not differ significantly from (cadmium treatment 10 mg L^{-1} , lead treatment 100 mg L^{-1}), as the total chlorophyll rate in them reached (15.89, 15.06) $\text{mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight, compared to the lowest rate recorded by Indian almond plants in the cadmium treatment 20 mg L^{-1} , as it reached 7.86. The data of the same table also indicate that the interaction between the plant type and the butyricin treatment gave a significant difference in the total chlorophyll property, as the *Lucinia* plants outperformed the other treatments when treated with 500 mg L^{-1} , as it reached $17.80 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight, compared to the lowest rate recorded by the Indian almond plants when treated with the control, as it reached $6.91 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight. While the interaction treatment of pollutants and the biotrichin was superiority, as the cadmium 10 mg L^{-1} and biotrichin 500 mg L^{-1} treatments outperformed the rest of the treatments, reaching $14.97 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight, which did not differ significantly with the control treatment and biotrichin 500 mg L^{-1} , as the total chlorophyll rate in it reached $14.27 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight, compared to the lowest rate recorded by the cadmium 20 mg L^{-1} treatment and the one not treated with biotrichin, reaching $9.28 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight. As for the effect of the triple interaction between plant species, pollutants and biotrichin, *Lucinia* plants significantly outperformed the rest of the treatments when treated with cadmium 10 mg L^{-1} and biotrichin 500 mg L^{-1} , reaching 19.07 compared to the lowest rate recorded by Indian almond plants when treated with cadmium 20 mg L^{-1} , reaching $5.81 \text{ mg L}^{-1} \cdot 100 \text{ g}^{-1}$ fresh weight. It is clear from the results of the table that the total chlorophyll trait was affected by the heavy elements added to the soil, as *Lucinia* plants showed greater resistance to the toxicity of the elements, as the lead element affected the decrease in the percentage of total chlorophyll in the plant, and the reason for this is that the lead element causes inhibition of photosynthesis and increases the effectiveness of the activity of the Chlorophyllase enzyme responsible for the decomposition of chlorophyll [16]. The addition of cadmium contributes to changing many phospholipid compounds in the cell walls that are associated with the process of photosynthesis, because heavy metals cause disruption in the construction and formation of thylakoid membranes. Cadmium also contributes to increasing the activity of the chlorophyllase enzyme [16]. These results are similar to what was reached by [15] in his study on Indian mustard plant, and the reason for this is that biotrichin works to inhibit the decomposition of chlorophyll by the enzyme Chlorophyll dehydrates [17]. Treatment with biotrichin improves the efficiency of the photosynthesis process and maintains the chlorophyll kinase, thus increasing the materials manufactured inside the leaf [18]. These results are similar to what was found by [19] in a study on the response of two leek

plant varieties to mineral fertilization and spraying with biotrichin. These results are similar to what was reached by [20] in his study on the effect of foliar spraying with biotrichin on the ranunculus plant.

Table 3. Effect of the plant species *Lucinia* and Indian almond, concentrations of lead and cadmium pollutants, the pollution treatment Butyrosin, and the interaction between them on total chlorophyll ($\text{mg}^{-1} \cdot 100\text{g}^{-1}$ fresh weight).

Plants	Pollutants ($\text{mg} \cdot \text{L}^{-1}$)	Pollution treatment (Biotecin) $\text{mg} \cdot \text{L}^{-1}$			Average plant	Average pollutants
		0	250	500		
Lucinia	0 con.	13.10	16.45	18.80	15.11	12.37
	100 pb	12.85	15.33	17.01		11.88
	200 pb	11.86	13.65	18.63		12.25
	10 cd	12.92	15.69	19.07		12.29
	20 cd	12.76	13.10	15.51		10.82
Indian almonds	0 con.	7.94	8.01	9.75	8.72	
	100 pb	7.22	8.45	10.44		
	200 pb	6.88	9.94	12.58		
	10 cd	6.71	8.53	10.88		
	20 cd	5.81	7.94	9.83		
L.S.D. $P \leq 0.05$			2.48		1.42	0.47
Average pollution treatment (Biotecin)		9.80	11.70	14.25	Average interaction between plant species and pollutants	
L.S.D. $P \leq 0.05$			1.10			
Average interaction between contaminants and the treatment (biotin)					Lucinia	Indian almonds
	0	10.52	12.23	14.27	16.11	8.56
	100	10.03	11.89	13.72	15.06	8.70
	200	9.37	11.79	15.60	14.71	9.80
	10	9.81	12.11	14.97	15.89	8.70
	20	9.28	10.52	12.67	13.79	7.86
L.S.D. $P \leq 0.05$			1.30			1.02
Average interaction between plant species and pollution treatment (BioTreatse)						
	Lucinia	12.69	14.84	17.80		
	Indian almonds	6.91	8.57	10.69		
L.S.D. $P \leq 0.05$			1.55			

3.2. Estimation of the Content of Total Soluble Carbohydrates in Leaves ($\text{mg} \cdot \text{g}^{-1}$)

The data in Table (4) show that the treatment with heavy metals has a significant effect, as the *Lucinia* plants significantly outperformed the Indian almond plants, as it reached $32.30, 8.92 \text{ mg} \cdot \text{g}^{-1}$. As for the effect of pollutants, the control treatment significantly outperformed the rest of the treatments, as the carbohydrate rate in it reached $19.20 \text{ mg} \cdot \text{g}^{-1}$, compared to the lowest rate in the cadmium treatment at a concentration of $20 \text{ mg} \cdot \text{L}^{-1}$, as the carbohydrate rate reached $13.03 \text{ mg} \cdot \text{g}^{-1}$. The same table also indicates that the Buttercine treatment gave a significant superiority in the total carbohydrate content of the leaves when the Buttercine treatment at a concentration of $250 \text{ mg} \cdot \text{L}^{-1}$, as it reached $17.33 \text{ mg} \cdot \text{g}^{-1}$, compared to the lowest rate recorded in the control treatment, as it reached $12.80 \text{ mg} \cdot \text{g}^{-1}$. While the binary interaction between plant species and pollutants achieved a significant effect, as *Leucaena* plants outperformed the control treatment significantly, reaching $30.58 \text{ mg} \cdot \text{g}^{-1}$ compared to the lowest rate recorded by Indian almond plants in the control treatment, which reached $7.83 \text{ mg} \cdot \text{g}^{-1}$. As for the effect of as for the interaction of plant species and the Buttercine treatment, it was a significant effect, as *Leucaena* plants outperformed the Buttercine $250 \text{ mg} \cdot \text{L}^{-1}$ treatment, as the total carbohydrate content of the leaves reached $24.02 \text{ mg} \cdot \text{g}^{-1}$ compared to the lowest rate recorded by Indian almond plants in the control treatment, which reached $7.19 \text{ mg} \cdot \text{g}^{-1}$, which did not differ significantly with the Buttercine $500 \text{ mg} \cdot \text{L}^{-1}$ treatment, as the carbohydrate rate in it reached $8.94 \text{ mg} \cdot \text{g}^{-1}$. As for the interaction between Buttercine treatment and pollutants, it gave a significant effect, as the control

treatment and Buttercine 250 mg L⁻¹ outperformed the rest of the treatments, which reached 21.91 mg.g⁻¹ - which did not differ significantly with the control treatment and Biotrichin 500 mg L⁻¹ as the carbohydrate rate in it reached 19.53 mg.g⁻¹ - compared to the lowest rate recorded by the lead treatment 200 mg L⁻¹ and the non-Biotrichin treatment as it reached 12.63 mg.g⁻¹. While the triple interaction between the plant type, pollutants and Biotrichin treatment achieved a significant effect as the Lucinia plants outperformed significantly in the control treatment and Biotrichin 250 mg L⁻¹ - as it reached 34.84 mg.g⁻¹ compared to the lowest rate recorded by the Indian almond plants in the Cadmium treatment 20 mg L⁻¹ and the non-Biotrichin treatment as it reached 5.81 mg.g⁻¹. This may be due to the negative effects of increasing the cadmium element represented by the small leaf area as in Table (5) and inhibition of chlorophyll synthesis Table (3) and lack of water contributed to the decrease in the total carbohydrate content in the leaves. Increasing the levels of heavy metals leads to inhibition of the activity of the carboxylase enzyme [21].

Adding biotrichin leads to an increase in the effectiveness of the biochemical reactions of the glucose molecule in the metabolic processes of the plant, i.e. the existence of a cooperative relationship between glucose and biotrichin, which results in an increase in the total carbohydrate content.

This result is also similar to the study of [22] in her study on the effect of spraying biotrichin and glutathione on some physical and chemical characteristics of date palms, Halawi variety.

The result of the current study is consistent with what [23] reached in their study on the effect of adding cadmium at two concentrations (30, 10) mg kg⁻¹ in soil planted with seedlings The date palm (Phoenix (dactalifera) date palm variety Deglet Nour is similar to what was found by [15] in his study on Indian mustard plant. The ability of heavy elements to bind to enzymes leads to changes in the activity of enzymes such as inhibiting or stimulating them such as inhibiting the activity of the carboxylase enzyme. It was also found that high levels of lead may lead to inhibition of the activity of enzymes specific to the pentose phosphate production pathway [16]. This may be due to the fact that polyamine compounds such as putrescine are a new class of growth materials and are also anti-stress materials due to their antioxidant properties and their ability to stabilize membranes [24].

Table 4. Effect of the plant species Lucinia and Indian almond, concentrations of lead and cadmium pollutants, the pollution treatment Butyrosin, and the interaction between them on carbohydrates (mg.gm⁻¹).

Plants	Pollutants (mg.L ⁻¹)	Pollution treatment (Biotecin) mg.l ⁻¹			Average plant	Average pollutants
		0	250	500		
Lucinia	0 con.	24.84	34.84	32.08	32.30	19.20
	100 pb	20.31	26.82	23.50		15.74
	200 pb	17.34	19.30	17.65		13.98
	10 cd	13.41	20.27	19.21		14.02
	20 cd	16.16	18.89	17.55		13.03
Indian almonds	0 con.	7.52	8.99	6.99	8.92	
	100 pb	6.71	9.94	7.20		
	200 pb	7.92	11.97	9.74		
	10 cd	8.01	11.88	11.38		
	20 cd	5.81	10.40	9.41		
L.S.D. P≤ 0.05			4.11		1.32	2.08
Average pollution treatment (Biotecin)		12.80	17.33	15.47	Average interaction between plant species and pollutants	
			1.61			
Average interaction between contaminants and the treatment (biotin)					Lucinia	Indian almonds
	0	16.18	21.91	19.53	30.58	7.83
	100	13.51	18.38	15.35	23.54	7.95
	200	12.63	15.63	13.69	18.09	9.89
	10	10.71	16.07	15.29	17.63	10.42
	20	10.98	14.64	13.48	17.53	8.54
			2.61			2.95

Plants	Pollutants (mg.L ⁻¹)	Pollution treatment (Biotecin) mg.l ⁻¹			Average plant	Average pollutants
		0	250	500		
Average interaction between plant species and pollution treatment (BioTreatse)						
Lucinia		18.41	24.02	21.99		
Indian almonds		7.19	10.63	8.94		
			2.28			

3.3. Cadmium Concentration in Leaves (micromols.gm⁻¹)

It is noted from the data in Table (5) that the treatment with heavy metals has a significant effect, as the Indian almond plants significantly outperformed the Lucinia plant, as it reached 0.135, 0.130 micromol.gm⁻¹. The table above also indicates that the effect of pollutants on the cadmium concentration in leaves was significant, as the treatment with a cadmium concentration of 20 mg L⁻¹ significantly outperformed the rest of the treatments, as it reached 0.396 micromol.gm⁻¹, compared to the lowest rate recorded by the control treatment of 0.014 micromol.gm⁻¹.

As for the effect of the butyric acid treatment, it was a significant effect, as the concentration of 500 mg L⁻¹ outperformed the rest of the treatments, as it reached 0.216 micromol.gm⁻¹, compared to the lowest value in the control treatment, as it reached 0.054 micromol.gm⁻¹. The interaction of plant species and pollutants showed significant effect, as the Indian almond plants, when treated with cadmium 20 mg L⁻¹, significantly outperformed the rest of the treatments, reaching 0.430 µmol.g⁻¹, compared to the lowest rate recorded by Indian almond plants when treated with the control 0.014 µmol.g⁻¹. Similarly, the interaction of plant species and the butyricin treatment was also significant, as the Indian almond plants, when treated with butyricin 500 mg L⁻¹, significantly outperformed the rest of the treatments, reaching 0.304 µmol.g⁻¹, compared to the lowest rate recorded by Lucinia plants, reaching 0.053 µmol.g⁻¹. While the binary interaction between pollutants and the biotrichin treatment had a significant effect, as the cadmium treatment of 20 mg L⁻¹ significantly outperformed the rest of the treatments, reaching 0.670 µmol.g⁻¹, compared to the lowest rate recorded by (control treatment and biotrichin 250 mg L⁻¹, control treatment and biotrichin 500 mg L⁻¹), as the cadmium concentration in them reached (0.014, 0.014). µmol.g⁻¹. As for the triple interaction between the plant type, pollutants and biotrichin treatment, it gave a significant difference, as the Indian almond plants outperformed the rest of the treatments when treated with cadmium of 20 µmol.g⁻¹, reaching 0.722 µmol.g⁻¹, compared to the lowest rate recorded by the Lucinia plants when treated with control and biotrichin 500 mg L⁻¹, as the cadmium concentration rate in the leaves reached 0.014 µmol.g⁻¹. This is due to the increase in the concentration of available cadmium in the soil resulting from the addition of high concentrations of the element at the beginning of the experiment, which led to its absorption from the soil and its accumulation in the plant. This is consistent with what [25] found, where they showed that the increase in cadmium content in plants in soil contaminated with cadmium. This is what [26] found, where he showed that increasing the levels of cadmium in contaminated soil led to an increase in its absorbed and accumulated concentration in plant tissues. The presented results show that the addition of butyric acid to the soil had a positive effect, and the reason for this is that the addition of polyamines leads to an increase in the absorption of nutrients, especially potassium, which plays an important vital role in the photosynthesis process by improving growth as well as photosynthetic pigments and absorption of CO₂. These results are similar to the study of [27] on mung bean plants treated with butyric acid to reduce the effect of cadmium pollution. This result is similar to what was reached by [28] in his study on the effect of soil pollution with different levels of heavy elements (Pb, Cd, Ni) on the content of white radish and carrot plants. This result is also similar to what was found by [15] in his study on the phytoremediation of soil polluted with some heavy elements by Indian mustard *Brassic juncea* L. Czern., where the results showed that Indian mustard has the ability to accumulate cadmium in the vegetative group.

Table 5. Effect of the plant species *Lucinia* and Indian almond and the concentrations of lead and cadmium pollutants and the pollution treatment Buttercine and the interaction between them on the concentration of cadmium in the leaves ($\mu\text{mol.g}^{-1}$).

Plants	Pollutants (mg.L ⁻¹)	Pollution treatment (Biotecin) mg.l ⁻¹			Average plant	Average pollutants
		0	250	500		
Lucinia	0 con.	0.016	0.015	0.014	0.130	0.014
	100 pb	0.027	0.032	0.038		0.035
	200 pb	0.041	0.044	0.047		0.041
	10 cd	0.014	0.213	0.360		0.173
	20 cd	0.168	0.302	0.619		0.396
Indian almonds	0 con.	0.014	0.013	0.015	0.135	0.021
	100 pb	0.031	0.038	0.044		
	200 pb	0.046	0.042	0.048		
	10 cd	0.017	0.175	0.260		
	20 cd	0.172	0.396	0.722		
Average pollution treatment (Biotecin)		0.054	0.127	0.216	Average interaction between plant species and pollutants	
Average interaction between contaminants and the treatment (biotin)					Lucinia	Indian almonds
0		0.015	0.014	0.014	0.015	0.014
100		0.029	0.035	0.041	0.032	0.037
200		0.043	0.043	0.047	0.044	0.045
10		0.015	0.194	0.310	0.195	0.150
20		0.170	0.349	0.670	0.363	0.430
				0.036	0.029	
Average interaction between plant species and pollution treatment (BioTreatse)						
Lucinia		0.053	0.121	0.215		
Indian almonds		0.056	0.132	0.304		
				0.023		

3.4. Lead Concentration in Leaves ($\mu\text{mol.g}^{-1}$)

Data in Table (6) indicate that treatment with heavy metals has a significant effect, as Indian almond plants significantly outperformed *Lucinia* plants in the characteristic of lead concentration in leaves, as it reached 0.556), 0.311) $\mu\text{mol.g}^{-1}$. As for the effect of pollutants, the lead treatment with a concentration of 200 mg L^{-1} outperformed the rest of the treatments, as it reached 1.565 $\mu\text{mol.g}^{-1}$, compared to the lowest rate recorded by the control treatment, as it reached 0.018 $\mu\text{mol.g}^{-1}$. The data in the table also indicate that the butyrate treatment gave a significant superiority, as the concentration of 500 mg L^{-1} exceeded it, as it reached 0.684 $\mu\text{mol.g}^{-1}$, compared to the lowest rate recorded by the control treatment, 0.232 $\mu\text{mol.g}^{-1}$. Regarding the effect of the binary interaction of plant species and pollutants, the Indian almond plants, when treated with 200 mg L^{-1} , significantly outperformed the rest of the treatments, reaching 1.996 $\mu\text{mol.g}^{-1}$, which did not differ from the *Lucinia* plants when treated with lead with 200 mg L^{-1} , reaching 1.134 $\mu\text{mol.g}^{-1}$, compared to the lowest rate recorded by the *Lucinia* plants in the control treatment, reaching 0.014 $\mu\text{mol.g}^{-1}$. While interaction of plant species and the Buttercine treatment achieved a significant superiority, as the Indian almond plants outperformed when treated with Buttercine with 500 mg L^{-1} , reaching 0.809 $\mu\text{mol.g}^{-1}$, compared to the lowest rate recorded by the *Lucinia* plants in the control treatment, 0.137 $\mu\text{mol.g}^{-1}$. While the binary interaction between pollutants and the butyricin treatment resulted in a significant superiority, as the lead 200 mg L^{-1} and butyricin 500 mg L^{-1} treatments significantly outperformed the rest of the treatments, reaching 2.537 $\mu\text{mol.g}^{-1}$, compared to the lowest rate recorded by the control treatment, which reached 0.016 $\mu\text{mol.g}^{-1}$. As for the effect of the triple interaction between the plant species, pollutants and butyricin treatment, it achieved a significant superiority, as

the Indian almond plants outperformed the rest of the treatments when treated with lead 200 mg L⁻¹ and butyricin 500 mg L⁻¹, significantly, reaching 2.889 $\mu\text{mol.g}^{-1}$, compared to the lowest rate recorded by the Lucinia plants when treated with the control and butyricin 250 mg L⁻¹, which reached 0.013 $\mu\text{mol.g}^{-1}$. The data in the table show that Indian almond plants are more resistant to heavy metal toxicity, and the increase in the accumulation of heavy elements in the vegetative group, especially in the leaves, increases with the increase in the concentration of heavy elements, which indicates the excellent ability of the plant to accumulate these elements. Some plants have the ability to absorb and accumulate large amounts of lead in their living masses, which may reach approximately 2300 mg Pb kg⁻¹ dry matter [29]. The presented results show that adding the biotin treatment to the soil has a positive effect in improving the characteristics of vegetative growth because it contains potassium, which is an element responsible for the transport process in the plant, as it plays an important role in the movement of water, nutrients, and products of the photosynthesis process [30]. This result is similar to what [31] reached, where they showed that the increase in the concentration of heavy elements in the soil was a result of its contamination with toxic heavy elements or waste resulting from sewage, which led to an increase in its availability in the soil, its absorption, and its accumulation in the vegetative group. These results are similar to what was reached by [28] in his study on the effect of soil pollution with different levels of heavy elements (Pb, Cd, Ni) on the content of white radish and carrot plants, where the results showed that white radish plants are superior.

Table 6. Effect of the plant species Lucinia and Indian almond and the concentrations of pollutants lead and cadmium and the pollution treatment Butyricin and the interaction between them on the concentration of lead in the leaves ($\mu\text{mol.g}^{-1}$).

Plants	Pollutants (mg.L ⁻¹)	Pollution treatment (Biotecin) mg.l ⁻¹			Average plant	Average pollutants
		0	250	500		
Lucinia	0 con.	0.015	0.013	0.015	0.311	0.018
	100 pb	0.142	0.384	0.506		0.527
	200 pb	0.464	0.753	2.186		1.565
	10 cd	0.036	0.014	0.045		0.030
	20 cd	0.029	0.025	0.044		0.030
Indian almonds	0 con.	0.017	0.024	0.026	0.556	
	100 pb	0.033	1.029	1.072		
	200 pb	1.534	1.566	2.889		
	10 cd	0.029	0.024	0.027		
	20 cd	0.027	0.025	0.032		
Average pollution treatment (Biotecin)			0.401		0.103	0.141
		0.232	0.385	0.684	Average interaction between plant species and pollutants	
Average interaction between contaminants and the treatment (biotin)			0.126		Lucinia	Indian almonds
	0	0.016	0.018	0.020	0.014	0.022
	100	0.087	0.706	0.789	0.344	0.711
	200	0.999	1.159	2.537	1.134	1.996
	10	0.032	0.024	0.036	0.031	0.026
	20	0.028	0.025	0.038	0.032	0.028
			0.283			0.231
Average interaction between plant species and pollution treatment (BioTreatse)						
	Lucinia	0.137	0.237	0.559		
	Indian almonds	0.328	0.533	0.809		
			0.179			

3.5. Cadmium Concentration in Roots ($\mu\text{mol.g}^{-1}$)

Data in Table (7) show that treatment with heavy metals has a significant effect, as Indian almond plants significantly outperformed Lucinia plants in the characteristic of cadmium concentration in

roots, as it reached 0.765), 0.436) micromol.g⁻¹. As for the effect of pollutants, the cadmium treatment at a concentration of 20 mg L⁻¹ significantly outperformed the rest of the treatments, as the average cadmium concentration in the roots reached 1.849 micromol.g⁻¹, compared to the lowest rate recorded by the control treatment, as it reached 0.040 micromol.g⁻¹. The data in the table above also indicate that the biotrichin treatment gave a significant superiority, as the biotrichin treatment with a concentration of 500 mg L⁻¹ significantly outperformed the remaining concentrations, reaching 1.200 micromol.g⁻¹, compared to the lowest rate recorded by the control treatment, as the cadmium concentration rate in the roots reached 0.078 micromol.g⁻¹. Regarding the effect of the tow factors interaction of the plant species and pollutants, the Indian almond plants, when treated with cadmium 20 mg L⁻¹, significantly outperformed the rest of the treatments, as it reached 2.268 mg L⁻¹, compared to the lowest rate recorded by the Lucinia plants in (control treatment, lead treatment 100 mg L⁻¹), as the cadmium concentration rate in the roots in them reached 0.030 mg L⁻¹. The results also showed the interaction of the Indian almond plants significantly outperformed when treated with Buttercine 500 mg L⁻¹, as it reached 1.500 micromol.g⁻¹, compared to the lowest rate recorded by the Lucinia plants in the control treatment, as it reached 0.066 micromol.g⁻¹. A significant effect was also observed in the interaction of pollutants and the butyricin treatment, it was significant, as the cadmium treatment (20 mg L⁻¹) significantly outperformed the rest of the treatments, reaching 3.929 micromol.g⁻¹, compared to the lowest rate recorded by the lead treatment (100 mg L⁻¹) which reached 0.033 micromol.g⁻¹. While the triple interaction between the plant type, pollutants and butyricin treatment achieved a significant effect, as the Indian almond plants outperformed the rest of the treatments when treated with cadmium (20 mg L⁻¹) and butyricin (500 mg L⁻¹) significantly, reaching 4.734 micromol.g⁻¹, compared to the lowest rate of cadmium concentration in the roots at (control treatment, butyricin (500 mg L⁻¹), lead treatment (100 mg L⁻¹) where the cadmium concentration in them reached 0.027 micromol.g⁻¹. The results of the table show that the characteristic of cadmium concentration in the roots with the addition of heavy elements to the soil, as almond plants showed greater resistance to cadmium toxicity, that the increase in cadmium absorbed from the soil is due to its increased availability in the soil, then its absorption by the plant and its accumulation in the root system, and that the reason for the increase in cadmium concentration in the roots of the Indian almond plant is due to its possession of a taproot, a large green group and a high surface area compared to the roots of the Lucinia plant, which works to absorb cadmium and accumulate it in the root system, and this is consistent with what [32] reached, who indicated that some vegetable crops have a large root system and a high surface area, which leads to an increase in root secretions, which helps to reduce the degree of soil reaction and increase the availability of heavy elements and their absorption and then their accumulation in the roots of plants. The data in the table indicate that adding the Biotrichin treatment to the soil has a positive effect on the concentration of cadmium in the roots. This is due to the fact that it contributes to many vital processes in the plant, including the process of gene expression, protein and DNA synthesis, and cell division. In addition, spraying with Biotrichin increases the internal content of growth regulators such as indole acetic acid, gibberellins, and cytokinins, and reduces growth inhibitors such as abasic acid [33]. This result is similar to what was found by [28] in his study on carrot and white radish plants. These results are similar to the study of [27] on the mung bean plant treated with Biotrichin to reduce the effect of cadmium pollution.

Table 7. Effect of the plant species Lucinia and Indian almond, concentrations of lead and cadmium pollutants, Biotrichin pollution treatment, and their interaction on the concentration of cadmium in the roots (micromols.gm⁻¹).

Plants	Pollutants (mg.L ⁻¹)	Pollution treatment (Biotecin) mg.l ⁻¹			Average plant	Average pollutants
		0	250	500		
Lucinia	0 con.	0.053	0.053	0.043	0.436	0.040
	100 pb	0.039	0.040	0.057		0.037
	200 pb	0.044	0.039	0.042		0.038
	10 cd	0.163	0.434	1.241		1.038
	20 cd	0.031	1.137	3.124		1.849
Indian	0 con.	0.031	0.034	0.027	0.765	

Plants	Pollutants (mg.L ⁻¹)	Pollution treatment (Biotecin) mg.l ⁻¹			Average plant	Average pollutants
		0	250	500		
almonds	100 pb	0.027	0.034	0.029	0.064	0.101
	200 pb	0.033	0.037	0.036		
	10 cd	0.175	1.540	2.676		
	20 cd	0.193	1.878	4.734		
			0.247			
Average pollution treatment (Biotecin)		0.078	0.518	1.200	Average interaction between plant species and pollutants	
Average interaction between contaminants and the treatment (biotin)			0.068		Lucinia	Indian almonds
	0	0.042	0.043	0.035	0.049	0.030
	100	0.033	0.037	0.043	0.045	0.030
	200	0.038	0.038	0.039	0.041	0.035
	10	0.169	0.987	1.958	0.612	1.463
	20	0.112	1.507	3.929	1.430	2.268
			0.175			0.143
Average interaction between plant species and pollution treatment (BioTreatse)						
	Lucinia	0.066	0.340	0.901		
	Indian almonds	0.091	0.704	1.500		
			0.110			

3.6. Lead Concentration in Roots (micromols.gm⁻¹)

Data in Table (8) show that treatment with heavy metals has a significant effect, as Indian almond plants significantly outperformed Lucinia plants in the characteristic of cadmium concentration in roots, as it reached (1.849, 1.345) micromol.gm⁻¹. The table above also indicates that the effect of pollutants gave a significant difference, as the lead treatment of 200 mg L⁻¹ outperformed the rest of the treatments significantly and gave the highest concentration of lead in the roots, as it reached 4.298 micromol.gm⁻¹, compared to the lowest rate recorded by the cadmium treatment of 10 mg L⁻¹, as it reached 0.419 micromol.gm⁻¹. As for the effect of the biotrichin treatment, it had a significant effect, as the biotrichin treatment with a concentration of 500 mg L⁻¹ outperformed the rest of the treatments, reaching 2.493 micromol.g⁻¹, compared to the lowest rate recorded by the control treatment, which reached 0.433 micromol.g⁻¹. While the binary interaction between the plant type and the pollutants achieved a significant effect, as the Indian almond plants outperformed the rest of the treatments when treated with lead (200 mg L⁻¹), significantly, as the concentration of lead in the roots reached 5.098 micromol.g⁻¹, compared to the lowest rate recorded by the Lucinia plants when treated with cadmium (20 mg L⁻¹), which reached 0.411 micromol/g. The effect of the binary interaction between the plant species and the biotrichin treatment was significant, as the Indian almond plants outperformed the biotrichin 500 mg L⁻¹ treatment significantly and gave the highest concentration of lead in the roots, reaching 2.804 µmol.g⁻¹, compared to the lowest rate recorded by the Lucinia plants in the control treatment, reaching 0.422 µmol.g⁻¹. The same table also showed significant interaction effect of the pollutants and the biotrichin treatment gave a significant difference, as the lead 200 mg L⁻¹ and biotrichin 500 mg L⁻¹ treatments outperformed and gave the highest concentration of lead in the roots, reaching 6.964 µmol.g⁻¹, compared to the lowest rate recorded by the cadmium 10 mg L⁻¹ treatment, reaching 0.364 µmol.g⁻¹. As for the effect of the triple interaction between the plant type, pollutants and the biotrichin treatment, it gave a significant effect, as the Indian almond plants outperformed the lead treatment of 200 mg L⁻¹ and biotrichin 500 mg L⁻¹ significantly and gave the highest concentration of lead in the roots, reaching 7.926 micromol.g⁻¹, compared to the lowest rate recorded by the cadmium treatment of 10 mg L⁻¹ 0.342 micromol.g⁻¹. This may be due to the increased concentration of absorbed and accumulated lead within the root system of the plants and the amount of lead added to the soil. Lead is absorbed by the plant in the form of Pb+2, which is found in the acidic soil solution, during which carbon dioxide CO₂ is released, which is not absorbed by the plant. The

plant roots absorb lead and it is transferred to the rest of the plant parts and reduces the chlorophyll pigment and also increases oxidative stress, which leads to the death of the plant. This result is similar to what was found by [28] in his study on the carrot and white radish plants, and this result is similar to what [15] reached in his study on the Indian mustard plant, where the study concluded that the Indian mustard plant is one of the plants that accumulate heavy elements. The result of the study is also similar to what was reached by [34], that the specific increase in lead in the roots is directly proportional to its available concentration in the soil, which leads to its accumulation in the roots of plants. The reason for the deposition of lead in the root system more than the vegetative system is its lack of movement and transfer to the leaves, and that lead ions have the ability to bind to root cells, which contributes to restricting its movement inside the roots of plants. The addition of the pollution treatment Butyrosin has a significant effect in improving soil properties and reducing the negative impact of pollutants, which leads to increased water absorption, which has an important role in the development and growth of roots and maintaining the water content of cells compared to plants growing in polluted conditions. These results are similar to what was reached by [35] in his study on the effect of butyric acid and p-coumaric acid on date palm seedlings, where the study showed that the butyric acid pollution treatment reduced the effect of lead pollution.

Table 8. Effect of the plant species *Lucinia* and Indian almond and the concentrations of lead and cadmium pollutants and the butyric acid pollution treatment and the interaction between them on the concentration of lead in the roots ($\mu\text{mol} \cdot \text{g}^{-1}$).

Plants	Pollutants (mg.L ⁻¹)	Pollution treatment (Biotechin) mg.l ⁻¹			Average plant	Average pollutants
		0	250	500		
Lucinia	0 con.	0.425	0.436	0.477	1.345	0.437
	100 pb	0.441	1.900	3.539		2.389
	200 pb	0.467	4.027	6.002		4.298
	10 cd	0.386	0.428	0.424		0.419
	20 cd	0.393	0.374	0.466		0.443
Indian almonds	0 con.	0.480	0.438	0.367	1.849	0.217
	100 pb	0.446	3.175	4.836		
	200 pb	0.453	6.915	7.926		
	10 cd	0.342	0.478	0.456		
	20 cd	0.498	0.497	0.437		
Average pollution treatment (Biotechin)		0.433	1.866	2.493	Average interaction between plant species and pollutants	
Average interaction between contaminants and the treatment (biotin)			0.168		Lucinia	Indian almonds
	0	0.452	0.437	0.422	0.446	0.428
	100	0.443	2.537	4.187	1.960	2.819
	200	0.460	5.471	6.964	3.498	5.098
	10	0.364	0.453	0.440	0.412	0.425
	20	0.445	0.435	0.451	0.411	0.477
Average interaction between plant species and pollution treatment (BioTreatse)			0.375		0.306	
	Lucinia	0.422	1.433	2.181		
	Indian almonds	0.443	2.300	2.804		
			0.237			

Conclusion

We conclude from the current study that the Indian almond plant is more resistant than the *Lucinia* plant in terms of damage caused by lead and cadmium pollutants, and that high concentrations of them negatively affected all biochemical characteristics, and that the pollution treatment with Buttercine at a

concentration of 500 mg L⁻¹ is more effective than a concentration of 250 mg L⁻¹ in reducing the damage caused by different concentrations of pollutants Cd, Pb.

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