Effect of heavy elements (cadmium and lead) on the growth of two types of bacteria and their effect on soil properties

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Abstract

The experiment was conducted in the Department of Soil and Water Sciences and the Research Station at the College of Agriculture , University of Kufa in the year 2023-2024 to study the effect The effect of heavy elements (cadmium and lead) on the growth of two types of bacteria On optional culture media for the purpose of studying the effect of cadmium and lead pollution on the growth of two types of bacteria. During this experiment, the minimum inhibitory concentration of the heavy elements used is determined by adding cadmium and lead at four concentrations (0, 25, 50, 50, 75 mM). at the end of the experiment, the number of bacteria is measured after 48 hours of incubation. The second experiment included adding cadmium and lead at four concentrations (0, 25, 50, 50, 75 mM) to soil in small pots. This soil is incubated for 3 months during which the number of bacteria of both types is measured after 3 months of incubation. Heavy elements had a significant effect on the number of bacteria in the soil. Cadmium treatment outperformed lead treatment in reducing the number of bacteria of both types in the soil, as the comparison factor gave the highest average of 46.58 × 106 CFU ml-1, while Bacillus subtilis gave the lowest average of 43.33 × 106 CFU ml-1. Bacterial inoculation, especially inoculation with Bacillus cereus bacteria, also had a significant effect in improving soil properties, including soil reaction degree, electrical conductivity, available nitrogen, and available phosphorus in the soil, as their rates reached 7.59 and 2.29 dS m-1, 10.7 mg kg-1, and 14.55 mg kg-1, respectively .

Key word: heavy elements, cadmium , lead, Bacillus subtilis, Bacillus cereus

Introduction

One of the most important problems in the world in recent years is environmental pollution, as heavy elements come at the forefront of these problems, and these elements are the most toxic because they are not biodegradable, and they remain in the soil for a long time without any chemical change, after which their toxicity is not eliminated and remains in the soil and accumulates environmentally, and therefore it is likely to enter the food chain, and the higher their concentration in the plant exceeds the permissible limit, the more serious their impact on the health of everyone who consumes the plant[18] due to cadmium and lead pollution, toxic symptoms that are harmful to the plant and its growth, as it reduces production and also affects the ionic balance and inhibits the work of enzymes and the absorption of essential elements and disrupts metabolic processes and photosynthesis in plants, while in humans it causes diseases such as kidney failure, high blood pressure and lung damage, and cadmium and lead are considered carcinogenic to humans[9] . There is a possibility of removing heavy elements biologically in the soil by using local isolates of Bacillus bacteria in contaminated soil, as the results of studies showed that the isolates proved their ability to remove heavy elements from the soil[5,14] . Barley Hordeum vulgaris is a crop of the

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Graminaceae family and is an annual winter crop, and one of the crops most adapted to difficult environmental conditions due to its tolerance to cold, salinity and drought, as indicated by [4,7] . Barley ranks fourth after wheat, rice and corn in terms of production and cultivated area, as the global area is 54 million hectares and is cultivated in most Iraqi governorates, as the cultivated area for the year 2018 for the barley crop reached 6101 thousand dunums. Barley production was estimated at 190 thousand tons, as the yield per dunum was 317.1 kg .dunum-1, and Al-Qadisiyah Governorate ranked first. The first in terms of production with a percentage of 33.9% estimated at 65 thousand tons of the total production[17,5] and due to the importance of this topic, the study aimed to. Study the effect of cadmium and lead concentrations on bacterial activities, growth and yield of barley plants.

Materials and methods

Experiment location

The experiment was conducted in the laboratories of the Department of Soil and Water Sciences at the College of Agriculture / University of Kufa, at the research station / University of Kufa using a sandy mixture soil

Table (1) Some chemical and physical properties of the study soil before cultivated

Units	values	traits	
	7.70	Soil pH	
$DS.m^{-1}$	1.9	Electrical conductivity (EC)	
$g.kg^{-1}$	1.21	Organic matter (OM)	
mg.kg-1	5.6	Nitrogen available	
	0.08	Phosphorus available	
	10.3	Potassium available	
$Mmol.mL^{-1}$	147.6	Soluble ions	
	156.3	Sodium	
	65.4	Calcium	
	162.7	Magnesium	
	0.0	Chloride	
	134.2	Carbonates	
	560.0	Bicarbonates	
$mg.L^{-1}$	0.0012	Sulphates	
$mg.L^{-1}$	0.0002	Cadmium Cd	
$Mg.g^{-1}$		Lead Pb	
	1.43	Bulk density	
g.kg-1 soil	3.26	clay	Soil separators
	30.60	silt	
	66.14	sand	
CFU g^{-1} dry soil	200×10^6	total bacteria	

The experiment was coundected according to the Completely Randomized Design (CRD) with two factors. The first factor included two types of heavy elements (Pb, Cd). The second factor was the use of four concentrations of heavy elements (Pb, Cd) (0, 25, 50, 75) mM.

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The above concentrations of nutrients were added, and then two types of bacteria (Bacillus cereus, Bacillus subtilis) were cultivated. 10 ml/pot of bacteria were added. The experiment continued for 3 months, during which the numbers of bacteria of both types were measured after each month, as well as the degree of soil reaction, the readiness of phosphorus and nitrogen in the soil, and the measurement of cadmium and lead residues in the soil.

Studied traits

Total bacteria count (CFUg-1 dry soil.(

It was estimated by dilution and plate counting method according to [13]. Field soil samples were taken at the end of the experiment and a series of dilutions of the soil suspension were prepared from 1-10 to 6-10. Nutrient Agar was used as a medium, poured into sterile Petri dishes and 1 ml of the 6-10 dilution was added. Then the dishes were placed in the incubator at 28°C for 48 hours. The live bacterial cells were calculated from the product of the number of colonies multiplied by the reciprocal of the dilution.

Soil reaction pH

The soil reaction pH was measured in the saturated paste extract using a pH-Meter as mentioned in [13[

Electrical conductivity EC

The electrical conductivity of soil salts in the saturated paste extract was measured using an EC-Meter as mentioned in [13[

Available nitrogen

Available nitrogen was extracted using potassium chloride KCl and nitrogen was determined using a Kjeldahl apparatus according to the method mentioned in [13[

Available phosphorus

Soil phosphorus was extracted using sodium bicarbonate (NaHCO3) and the extract color was developed using ammonium molybdate and ascorbic acid solution. Phosphorus was determined using a spectrophotometer at a wavelength of 882 nm according to the method mentioned in [13[

Available Potassium

soil potassium was extracted using a flame photometer as mentioned in [13[

Results and Discussion

The effect of heavy elements (cadmium and lead) on the growth of two types of bacteria in the soil :

Number of bacteria

The results of Table (2 and 3) showed that heavy elements had no significant effect on the number of bacteria of both types, while the concentrations had a significant effect on both types of bacteria and the concentration of 50 mM recorded the highest rate of bacterial number, reaching 50 × 106 CFU ml-1 for B. subtilis bacteria. As for the interaction, the cadmium treatment at a concentration of 50 mM was excelled and gave the highest number of bacteria of both types, reaching 50.33 × 106 CFU ml-1 after three months.

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Table (2) Effect of different concentrations of cadmium and lead on the growth of B. cereus (× 106) bacteria in the soil after three months of incubation

Average Elements	Concentration(Mm(				Elements
	75	**50**	**25**	**0**	
45.83	49	50.33	37.33	46.67	**Cd**
45.92	37.67	47.33	52	46.67	**Pb**
	43.33	**48.83**	**44.67**	**46.67**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	3.462	**2.448**	**N.S**		

Table (3) Effect of different concentrations of cadmium and lead on the growth of B. subtilis (× 106) bacteria in the soil after three months of incubation

Average Elements	concentrationmM				Elements
	75	**50**	**25**	**0**	
43.33	39.67	50.33	50.33	37.33	**Cd**
43.00	37.67	47.33	49.67	37.33	**Pb**
	38.67	**46.67**	**50.00**	**37.33**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	N.S	**3.04**	**N.S**		

Soil pH reaction degree-:

The results of Tables (4, 5 and 6) showed that heavy elements have an effect on soil pH, as cadmium was significantly excelled and gave the highest pH of 7.48, while lead gave a pH rate of 7.46, as for the concentrations, the concentration of 50 mM was excelled and recorded a pH rate of 7.51.As for the interaction, the cadmium treatment was excelled at a concentration of 50 mM and gave the highest pH for the medium in which B. subtilis bacteria grew after three months, reaching 7.55 While the growing media with B.cereus bacteria recorded a pH rate, cadmium was significantly excelled and gave the highest pH of 7.549, while the lead treatment gave a pH rate of 7.548. As for the concentrations, the 75 mM concentration was excelled and gave a pH rate of 7.595. As for the interaction, the cadmium treatment at a concentration of 75 mM recorded the highest pH rate of 7.673 after three months. As for the comparison treatment, the lead treatment recorded a pH of 7.61, while the lead treatment at a concentration of 25 mM gave the highest pH rate of 7.71. As for the concentrations, the treatment without adding a pH rate of 7.68 was recorded, while the cadmium interaction treatment at a concentration of 75 mM reached 7.673

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Table (4) Effect of different concentrations of cadmium and lead on the pH of the soil reaction degree after three months of incubation

No bacteria – pH					
Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
7.59	7.67	7.53	7.48	7.68	**Cd**
7.61	7.47	7.58	7.71	7.68	**Pb**
	7.57	**7.55**	**7.59**	**7.68**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.04	**0.03**	**0.02**		

Table (5) Effect of different concentrations of cadmium, lead and inoculation with B. subtilis bacteria on soil pH after three months of incubation

Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
7.48	7.38	7.52	7.55	7.48	**Cd**
7.46	7.43	7.46	7.47	7.48	**Pb**
	7.41	**7.49**	**7.51**	**7.48**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.04	**0.03**	**0.02**		

Table (6) Effect of different concentrations of cadmium, lead and inoculation with B. cereus bacteria on soil pH after three months of incubation

Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
7.549	7.673	7.470	7.527	7.527	**Cd**
7.548	7.517	7.570	7.577	7.527	**Pb**
	7.595	**7.520**	**7.552**	**7.527**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.043	**0.030**	**N.S**		

Electrical conductivity EC-:

The results of Tables (7 and 8) showed that heavy elements have an effect on the EC of the medium in which B. subtilis bacteria grows. Cadmium was significantly excelled and gave the highest EC for the medium,

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reaching 2.97 ds.m-1, while lead gave an EC rate of 2.47 ds.m-1. As for the concentrations, the concentration of 75 mM was excelled and recorded the highest EC rate of 2.96 ds.m-1. As for the interaction, the cadmium treatment was excelled at a concentration of 25 mM. The highest EC rate for the medium in which B. subtilis bacteria grew after three months was 3.06 ds.m-1. As for the growing media with B.cereus bacteria, the lead treatment was significantly excelled and gave the highest EC of 2.8792, while cadmium gave an EC rate of 2.75 ds.m-1, while the concentration of 50 mM was excelled and recorded an EC rate of 2.8583 ds.m-1. As for the B.cereus bacteria interference, the cadmium treatment at a concentration of 75 mM recorded the highest EC rate after three months of 3.46 ds.m-1.

Table (7) Effect of different concentrations of cadmium and lead and inoculation with B. subtilis bacteria on electrical conductivity EC (ds.m-1) after three months of incubation

Average Elements	concentrationmM				Elements
	75	**50**	**25**	**0**	
2.97	3.27	2.97	3.06	2.56	**Cd**
2.47	2.65	2.53	2.11	2.56	**Pb**
	2.96	**2.75**	**2.58**	**2.56**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.04	**0.03**	**0.02**		

Table (8) Effect of different concentrations of cadmium and lead and inoculation with B. cereus bacteria on electrical conductivity EC (ds.m-1) after three months of incubation

Average Elements	concentrationmM				Elements
	75	**50**	**25**	**0**	
2.7575	1.9767	3.2767	2.4767	3.3	**Cd**
2.8792	3.46	2.44	2.3167	3.3	**Pb**
	2.7183	**2.8583**	**2.3967**	**3.3**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.05553	**0.03926**	**0.02776**		

Available nitrogen (mg kg-1 soil:(

The results in Table (9, 10 and 11) indicated that heavy elements have an effect on the available nitrogen of the medium in which B. subtilis bacteria grows. The lead treatment was significantly excelled and gave the highest available nitrogen of 7.42 mg kg-1 soil, while the cadmium treatment gave a rate of available nitrogen of 6.70 mg kg-1 soil. As for the concentrations, they did not have a significant

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effect on the rate of available nitrogen in the soil.

As for the interaction, the lead treatment was excelled at a concentration of 50 mM and gave the highest available nitrogen of the medium in which B. subtilis bacteria grows after three months, amounting to 7.87 mg kg-1 soil. As for B.cereus bacteria, cadmium was significantly excelled and gave the highest available nitrogen, reaching 10.7 mg kg-1 soil, while cadmium gave a rate of available nitrogen of 7.0 mg kg-1 soil. As for the concentrations, they exceeded the 50 mM concentration and recorded a rate of available nitrogen of 10.2 mg kg-1 soil. As for the interaction of B.cereus bacteria, the cadmium treatment at a concentration of 50 mM recorded the highest rate of available nitrogen after three months, reaching 14.1 mg kg-1 soil. As for the comparison media, the lead treatment recorded available nitrogen of 6.97 mg kg-1 soil, while the 75 mM concentration treatment gave a available nitrogen amount of 7.35 mg kg-1 soil. As for the interaction, the lead treatment with a concentration of 25 mM recorded a available nitrogen rate after three months of 7.71 mg kg-1 soil .

Table (9) Effect of different concentrations of cadmium and lead on available nitrogen (mg kg-1) after three months of incubation

No bacteria – N					
Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
6.74	7.59	6.90	6.30	6.18	**Cd**
6.97	7.10	6.91	7.71	6.18	**Pb**
	7.35	**6.90**	**7.01**	**6.18**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.20	**0.14**	**0.10**		

Table (10) Effect of different concentrations of cadmium and lead and inoculation with B. subtilis bacteria on available nitrogen (mg kg-1) after three months of incubation

Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
6.70	4.90	7.70	7.70	7.80	**Cd**
7.42	7.10	7.87	6.90	7.80	**Pb**
	6.00	**7.13**	**7.30**	**7.80**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	N.S	**N.S**	**N.S**		

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Table (11) Effect of different concentrations of cadmium and lead and inoculation with B. cereus bacteria on available nitrogen (mg kg-1) after three months of incubation

Average Elements	concentrationmM				Elements
	75	50	25	0	
10.7	6.3	14.1	14.0	8.4	**Cd**
7.0	7.7	6.3	5.6	8.4	**Pb**
	7.0	**10.2**	**9.8**	**8.4**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.033	**0.023**	**0.017**		

Available phosphorus (mg kg-1 soil-:(

The results in Tables (12, 13 and 4) showed that cadmium and lead had an effect on the available phosphorus of the medium in which B. subtilis bacteria grows after three months. Lead was significantly excelled and gave the highest available phosphorus of 26.86 mg kg-1 soil, while the cadmium treatment gave a rate of available phosphorus of 23.26 mg kg-1 soil, while The concentration of 75 mM gave a available nitrogen rate of 28.23 mg kg-1 soil. As for the interaction, the lead treatment with a concentration of 75 mM was excelled and gave the highest available phosphorus to the medium in which B. subtilis bacteria grew after three months, amounting to 39.12 mg kg-1 soil. As for B. cereus bacteria, cadmium was excelled significantly and gave the highest available phosphorus amounting to 21.68 mg . kg-1 soil, while lead gave a available phosphorus rate of 16.04 mg kg-1 soil. As for the concentrations, the concentration of 50 mM was excelled and recorded a available phosphorus rate of 19.07 mg kg-1 soil. As for the interaction of B. cereus bacteria, the cadmium treatment with a concentration of 50 mM recorded the highest available phosphorus rate after three months, amounting to 26.81 mg kg-1 soil. As for the comparison media, the lead treatment recorded a available phosphorus rate of 19.35 mg. kg-1 soil, while the concentration of 75 mM recorded a available phosphorus amount of 22.67 mg kg-1 soil. As for the interaction, the lead treatment with a concentration of 75 mM recorded a available phosphorus rate after three months of 29.10 mg kg-1 soil

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Table (12) Effect of different concentrations of cadmium and lead on available phosphorus (mg kg-1) after three months of incubation

No bacteria – P					
Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
17.99	**16.24**	**18.78**	**20.96**	**15.97**	**Cd**
19.35	**29.10**	**16.16**	**16.17**	**15.97**	**Pb**
	22.67	**17.47**	**18.57**	**15.97**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.34	**0.24**	**0.17**		

Table (13) Effect of different concentrations of cadmium and lead and inoculation with B. subtilis bacteria on available phosphorus (mg kg-1) after three months of incubation

Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
23.26	17.33	22.92	21.57	31.22	**Cd**
26.86	39.12	18.98	18.13	31.22	**Pb**
	28.23	**20.95**	**19.85**	**31.22**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.22	**0.16**	**0.11**		

Table (14) Effect of different concentrations of cadmium and lead and inoculation with B. cereus bacteria on available phosphorus (mg kg-1) after three months of incubation

Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
21.68	20.41	26.81	17.35	22.14	**Cd**
16.04	15.50	11.32	15.19	22.14	**Pb**
	17.96	**19.07**	**16.27**	**22.14**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.32	**0.23**	**0.16**		

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Available potassium (mg kg-1 soil-:(

The results in Table (15, 16 and 17) showed that the elements cadmium and lead have an effect on the available potassium of the medium in which the bacteria grow. The lead treatment in the growing media with B. subtilis bacteria was excelled after three months, significantly, and gave the highest rate of available potassium, which reached 102.26 mg kg-1 soil.While the cadmium treatment gave a rate of available potassium, which reached 95.68 mg kg-1 soil. As for the concentrations, the concentration 0 was excelled and recorded a rate of available potassium, which reached 118.47 mg kg-1 soil. As for the interaction between the elements and concentrations, the lead treatment was excelled at a concentration of 0 and gave the highest rate of available potassium for the growing media with B. subtilis bacteria after three months, which reached 118.47 mg kg-1 soil. As for the growing media with B.cereus bacteria, cadmium was significantly excelled and gave the highest rate of available potassium, which reached 95.00 mg kg-1 soil, while the lead treatment gave a rate of available potassium, which reached 88.08 mg kg-1 soil. As for the concentrations, the 50 mM concentration was excelled and recorded a rate of available potassium, which reached 102.45 mg kg-1 soil. As for the interaction of B.cereus bacteria, the cadmium treatment at a concentration of 50 mM recorded the highest rate of available potassium, which reached 124.08 mg kg-1 soil, after three months. While the comparison media recorded cadmium rate for available potassium amounted to 76.85 mg kg-1 soil, while the concentration of 25 mM recorded a available potassium amount of 85.10 mg kg-1 soil. As for the interaction, the lead treatment with a concentration of 25 mM recorded a available potassium rate after three months amounting to 85.07 mg kg-1 soil.

Table (15) Effect of different concentrations of cadmium and lead on available potassium (mg kg-1) after three months of incubation

No bacteria – K					
Average Elements	**concentrationmM**				**Elements**
	75	**50**	**25**	**0**	
76.85	82.79	75.18	85.14	64.29	**Cd**
72.00	70.78	67.86	85.07	64.29	**Pb**
	76.79	**71.52**	**85.10**	**64.29**	**Average concentration**
	interaction	**concentration**	**Element**		**LSD 0.05**
	0.77	**0.54**	**0.39**		

Table (16) Effect of different concentrations of cadmium and lead and inoculation with B. subtilis bacteria on available potassium after three months of incubation

Average Elements	concentrationmM				Elements
	75	**50**	**25**	**0**	
95.68	72.44	101.70	101.70	118.47	**Cd**
102.26	97.11	100.18	93.27	118.47	**Pb**
	84.78	**95.14**	**97.48**	**118.47**	**Average concentrati on**
	interacti on	**concentr ation**	**Element**		**LSD 0.05**
	3.29	**2.33**	**1.64**		

Table (17) Effect of different concentrations of cadmium and lead and inoculation with B. cereus bacteria on available potassium after three months of incubation

Average Elements	concentrationmM				Elements
	75	**50**	**25**	**0**	
95.00	73.83	124.08	98.66	83.41	**Cd**
88.08	104	80.82	84.09	83.41	**Pb**
	88.91	**102.45**	**91.38**	**83.41**	**Average concentratio n**
	interaction	**concent ration**	**Element**		**LSD 0.05**
	0.849	**0.6**	**0.424**		

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