Plant treatment of lead-contaminated soil using Dodonnaea viscosa

Sura Shakir Jumaa[1], Mohammed Fadhil Abood[2]

[1,2] Department of Biology, College of Education for Pure Sciences, University of Anbar, Ramadi, Iraq

Email: eps.mohammed.fadhel@uoanbar.edu.iq

Abstract

A field experiment was conducted in Al-Zuhoor Private Arboretum from February to April 2022 to find out the best plant treatment to determine the ability of the Dodonnaea viscosato to absorb the element of lead added to the soil. The field experiment was conducted in 6 kg plastic flowerpots (Capacity 6 kg per flowerpot) according to a full random design and at a rate of 7 treatments and 3 replications for each treatment. Results of the field experiment on soil and shoot total revealed that the treatment with the symbol (NSIP) exceeds natural soil with the plant and the addition of bacterial vaccine and the addition of lead element at a concentration of 750 mg / liter. This has achieved the highest percentage of removal of the lead element amounted to 74.093% in the soil and a percentage of removal in the shoot total 78.513%. Results of the study showed a significant increase in some of the characteristics of the shoot growth in the number of leaves, with the highest number of treatment leaves (SSP) of the sterile soil sterilized with bacterial additive and a concentration of 750 mg/l of lead.

Keywords: pollutants; Dodonnaea viscosa; plants

1. Introduction

Phytoremediation is an important technology in the decontamination resulting from the accumulation of heavy elements in the soil through the use of plants, which have gained considerable attention recent years for several reasons, the most important of which is effective and low-cost and is environmentally friendly. Certain types of plants can accumulate these heavy metals in different concentrations (Tayang & Songachan, 2021).

Many global organizations and research institutions have initiated a long series of experiments and research that resulted in the establishment of permitted concentration limits of chemical agents and organic and mineral compounds found in both Soil, water, air and food (Rehman et al.,2018). Scientists have been interested in examining these elements, their biological effects and their relationship to human health. Thus, they attempted to address the risk of these minerals in the environment. However, these attempts are characterized by the use of traditional treatment techniques, which have posed a difficult challenge to conservationists, especially with the high cost of technological treatments, including soil excavation, landfilling or soil washing and the physical and chemical separation of pollutants, not to mention the negative effects of treatment (Rigoletto et al. 2020).

2. Methods

Preparation of solutions and reagents

Lead nitrate solution

Different concentrations of lead nitrate were prepared by dissolving 1.6 mg / liter of lead nitrate in 1000 ml of distilled water, which is considered the stock solution. The following concentrations were prepared from it (1000, 750, 500, 250 mg / liter) according to the mathematical equation below:

$$Wt(gm) = \frac{pp_m}{1000} \times \frac{M * Wt}{Atomic\ weight \times Atomic\ number} \times \frac{V}{1000}$$

Plant Aspect

10 different types of plants known for their ability to withstand harsh conditions were collected in the wooden canopy of the University of Anbar - Department of Life Sciences to test their ability to withstand the lead element. The lead element was added at a concentration of 750 mg / liter to all plants, and it was noted that the plants tolerated this concentration.

Soil tests

Various tests were conducted for the soil as well as soil texture used in the field experiment, including (EC, PH, TDS, Na, NaCl, K, Ca, Mg, Cl, Co_3, HCO_3, SO_4).

Field experiment Treatments

The field experiment was conducted in plastic flowerpots with a capacity of 6 kg per pot, 1.6 g of lead nitrate was dissolved in 1000 ml of distilled water to obtain a concentration of 1000 mg / L, which is considered the stock s A concentration of 750 mg / L of lead was prepared and added to each treatment and according to the type of treatment.

Bacterial inoculum was prepared in 100 ml of NB liquid agar nutrient medium and added to the treatments that need the vaccine. Table (1) shows the treatments used in the field experiment with 3 replications for each treatment.

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Table (1): Treatments used in the field experiment	
Symbol	Treatment
C1	Dodonaea plant without any treatment (control sample)
Ns1	Natural soil with a lead concentration of 750 mg/L
NSP1	Normal soil with plants and lead concentration of 750 mg/l
SP1	Sterile soil with a lead concentration of 750 mg/L

Complete pictures of the plant were taken at the beginning of the experiment, along with taking the necessary measurements such as stem length, number of branches, and number of leaves.

Watering plants

The plant was watered according to the plant's need for irrigation water, as 500 ml of water was added to all the plant treatments and the growth in the plant was monitored and signs of yellowing were observed on it.

Dodonaea viscosa preparation for laboratory measurements

The Dodonaea plant was taken and separated from the soil it was planted in, then the shoot system was separated from the root system, and it was completely cleaned of plankton and dirt. The wet weight of each of the shoot and root system was measured, then the shoot system, the root system and the soil of the plant were dried using an oven at a temperature of 60 °C until the weight became constant, then the dry weight of both the shoot system and the root system was measured.
The plant was separated from the soil and was then collected in polyethylene bags. Then, samples were taken from the treated soil and the information was recorded on it, dried in an oven at a temperature of 60 °C for 48 hours and kept in sterile plastic cans. The root system was separated from the shoot system of all treatments and the wet weight of each of them was measured using a sensitive balance and then dried using oven at a temperature of 60 ° C for 48 hours as well and the dry weight of all samples was measured.

Digestion and analysis of soil and plant samples

Soil samples, root system and shoot total were prepared to perform digestion treatments on them using the Kjeldahl digestion apparatus. The weight of 1 g of the sample was placed in a 250 ml test tube (Kjeldahl Tube), and 10 ml of concentrated sulfuric acid and 3 ml of perchloric acid were added to it and left for half an hour, then transferred to the heating device. It was heated gradually (300,200,120) °C until it became a clear solution and diluted to 50 ml with distilled water.

Estimation of lead in the study samples

The lead element was estimated in all samples using an atomic absorption spectrophotometer (AAS) before the start of the experiment and after the end of the experiment. The standard curve was prepared according to the method described by (Mandels et al., 1974) and as shown in Table (2), as the standard curve was drawn in Figure (1) to estimate lead depending on the absorbance and lead concentration.

Table (2) Preparation of the standard curve for lead		
Absorbance (nm)	Standard lead concentration (ml)	Tube Number
0.01	1	1
0.027	2	2
0.065	4	3
0.11	6	4

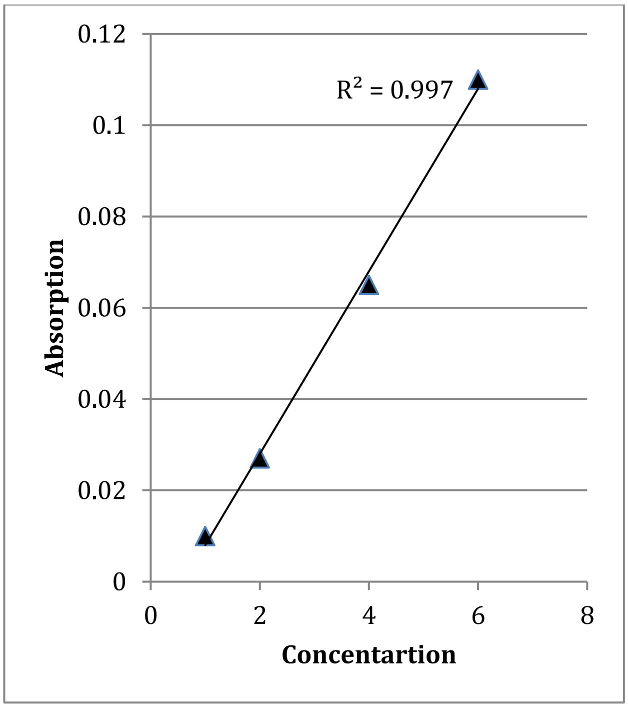

Figure (1) The standard curve for the determination of lead

3. Statistical Analysis

The results were analyzed statistically and the averages were compared using the least significant difference (LSD) at a significance level of 0.05 using the SSPS statistical program.

4. Results and Discussion

Shoot Aspect

A group of plants was selected and treated with a concentration of 1000 mg/L of lead to find out the most tolerant species, as shown in Table (3).

Table (3): the ability of selected plants to absorb lead at a concentration of 1000 mg/l	
Vegitative type	**The scientific name**
Iraqi clove	*Iraqi Dianthus*
Sesban	*Sesbania cecban*
Bird-of-paradise	*Caesalpinia gilliesii*
Sticky hop bush	*Dodonaea viscosa*
China pinks	*Dianthus chiaensis*
Iranian Cape periwinkle	*Vinca rosea*
Button mangrove	*Conocarpus erectus*
Lanceleaf Buttonwood	*Conocarpus lancifoliun*
Iraqi Cape periwinkle	*Vinca rosea*
Jumbay, pearl wattle	*Leucaena leucocephala*

Preliminary results showed the superiority of the Dodonaea viscosa plant over the rest of the plants in its ability to absorb the concentration of 1000 mg / liter of lead element and added in the form of lead nitrate salt. The plant did not show any signs of wilting and was growing naturally. Sesbania cecban came in second place followed by Caesalpinia gilliesii. This was followed by the rest of the species, and thus the Dodonaea plant was chosen as the best types of plants tolerant of high concentrations of lead.

Three plants of Dodonaea were selected and separated from the soil growing in them, and the root system was separated from the shoot system, and each was grinded separately and digested using the AAS device to find out the concentrations of lead present in them before treatment. mg / L and for soil 298.1 mg / L as shown in Table (4).

Table (4): the natural lead concentrations in the roots, shoot system and soil of Dodonaea viscosa before treatment.	
Lead concentration (mg/L)	**the sample**
251.6	root total
304	shoot total
298.1	the soil

A sample of the soil was prepared and on which a set of analyses were conducted. These included the pH, which reached 7.34, and the electrical conductivity (EC) 1250 micro Siemens / cm. The total dissolved salts (TDS) was estimated, which amounted to 669 parts per million. The percentage of sodium chloride, NaCl, amounted to 2.5%. Potassium, calcium, magnesium, chloride, carbonic acid, and sulfate were (58.2, 2.6, 35.2, 19, 19.3, 143, 211, 186) parts per million, respectively. The soil analysis showed that the soil texture is sandy and mixed, as shown in the Table (5).

Table (5): soil analyses cultivated with Dodonaea viscosa before treatment with lead		
Concentration	**Measuring unit**	**Characteristic**
7.34	------	pH
1250	micro siemens/cm	electrical conductivity EC
669	parts per million ppm	Total dissolved salts (TDS).
2.5	percent%	Sodium chloride NaCl
58.2	parts per million ppm	Sodium Na
2.6	parts per million ppm	K potassium
35.2	parts per million ppm	Calcium Ca
19.3	parts per million ppm	Magnesium Mg
143	parts per million ppm	Cl- chloride
211	parts per million ppm	HCO3 carbonic acid
Nil	parts per million ppm	carbonate CO3
186	parts per million ppm	SO4 sulfates
	Mixed sand	

Characteristics of shoot growth of Dodonaea viscosa

Measurements of some shoot growth characteristics of Dodonaea viscosa plant were taken at the beginning and end of the experiment, which was represented in the rates of stem length, number of branches and number of leaves.

Table (6) shows the rate of measuring the length of the stem (cm) at the beginning and end of the experiment and for all the studied treatments. It is noted from the aforementioned percentages that there is an increase in the length of the stem of the plant treatment (NSP) by 6.3 cm, compared to the control sample, in which the increase rate was 5.66 cm. .

According to the current study, the stem length of the control sample was 39 cm at the beginning of the experiment and increased to 44.66 cm at the end of the experiment. The experiment showed that with the presence of natural soil with a lead concentration of 750 mg/L, the stem length at the beginning of the experiment was 40 cm and at the end, it increased to 46.3 cm.

The current study agreed with another previous study, which discussed the effect of saprophytic fungi on the growth, uptake, transport and fixation of lead in Dodonaea Viscosa. The study showed that the stem length of the non-inoculated seedlings increased particularly in the presence of lead at the end of the experiment, reaching 10 and 26% of the longest stem, respectively, compared to the controls grown without lead. On the contrary, root elongation showed a significant decrease (2-17%) as an effect of the fungus, compared to non-inoculated seedlings at the end of the experiment. (Rojas-Loria et al. 2012).

The current study differed from a previous study on Dodonaea viscosaas a plant medium for heavy metal contaminated soils in abandoned mines. The latter study confirmed that during the first 3 months of exposure (beginning of the experiment), the plant stem height was not affected by any treatment; However, after 6 months of exposure (the end of the experiment), the control plants showed a significantly higher stem length increase compared to the plants growing on the residue bed (Castañeda-Espinoza et al., 2022).

The current study differed with another study conducted in 2017 to extract cadmium and its transport in saline soils by Hemerocallis fulva plants and Dodonaea viscosa. The study showed that Dodonaea fiscosa showed a significant decrease in stem length under different cadmium concentrations (50, 100, 150 ppm). This decrease was highly significant at the highest concentration of cadmium (150 ppm) when compared without cadmium and plants treated with cadmium (C1, C2, C3). Lower concentrations of cadmium were not statistically significant compared to control group C. Similarly, the lowest concentration of cadmium (50 ppm) showed a significant increase in stem length compared to control group C (Ahmad et al., 2017).

The results of the statistical analysis showed that there were significant differences between the averages of stem length before and after treatment with lead at the significance level ($P<0.05$) for all treatments. The smallest significant difference was 3.335.

Table (6): the average stem length (cm) of Dodonaea viscosa before and after the completion of the experiment.

Average stem length at the end of the experiment (cm)	Average stem length at the beginning of the experiment (cm)	Type of treatment
44.6	39	C control sample
46.3	40	NSP Normal soil with plants and lead concentration of 750 mg/l
LSD P<0.05=3.335		

As for the number of branches shown in Table (7), there were no significant differences at the beginning of the experiment compared to the end of the experiment. The current results showed an increase in the number of branches in the control sample (C) compared to the rest of the treatments, as the number of branches reached 8 branches/plant at the beginning of the experiment and 8.3 branches/plant at the end of the experiment. As for the presence of natural soil with a lead concentration of 750 mg/L.NSP, the number of branches did not record any improvement at the beginning of the experiment over its end. Thus, the number of branches at the beginning and end of the experiment was 5.6 branch/plant.

The current study is consistent with the study by (Espinoza et al., 2022) on Dodonaea viscosaas a plant medium for soil contaminated with heavy metals in abandoned mines, which confirmed a significant and consistent increase in the number of branches in control plants throughout the exposure period (before and after the experiment). This stood in contrast to plants growing on the substrate that did not show changes in the number of branches.

The results of the current study differed from the study by (Khudhair & Zaki, 2007), which used the phytoremediation of lead metal in the contaminated soil of some sites in the city of Baghdad, using the Dodonaea viscosa plant. The results of the study showed a small number of branches in the control group before and after the experiment compared to the number of branches in the rest of the treatments in the presence of lead.

The results of the statistical analysis showed that there were no significant differences between the average number of branches before and after treatment with lead at the significance level ($P<0.05$) for all treatments.

Table (7): the average number of branches (branch / plant) of *Dodonaea viscosa* before and after the completion of the experiment.

Average number of branches at the end of the experiment	Average number of branches at the beginning of the experiment	Type of treatment
8.3	8	C
5.6	5.6	NSP
LSD P<0.05=1.186		

Results of the current study, shown in Table (8), showed that there was a significant increase in the average number of leaves in the control sample compared to the rest of the soil types after lead intervention, as the average number of leaves at the beginning of the experiment was 213.3 leaves / plant and at the end of the experiment 323.3 leaves / plant.

With the presence of natural soil with a lead concentration of 750 mg/L, the average number of leaves at the beginning of the experiment was 150 leaves/plant. This increased to 356 leaves/plant at the end of the experiment.
The current study is consistent with a previous study to evaluate heavy metal pollution in plants planted next to electricity generators in the city of Ramadi using Dodonaea viscosa plant. The results confirmed an increase in the average number of leaves in the control sample in the presence of one or all of the heavy metals (cadmium, cobalt, lead, zinc, nickel, chromium, copper and manganese) (Al-Heety et al., 2021).
The current study differ in its results from another previous study of the alteration of mercury to the root bacterial community in a Brazilian wetland and how it was biologically manipulated by combining bacteria with Dodonaea viscosa. It was shown that there was a slight increase in the average number of leaves per plant in soil contaminated with element mercury before and after the experiment compared to the average number of leaves in the control group (Mariano et al., 2020).
The results of the statistical analysis showed that there were significant differences between the average number of leaves before and after treatment with lead at the level of significance (P < 0.05) and for all treatments. This was evidenced by a significant increase in the number of leaves at the beginning of the experiment and the end of the experiment, compared to an increase in the number of leaves in the control sample.

Table (8): the average number of leaves of *Dodonaea viscosa* before and after the completion of the experiment

Average number of leaves at the end of the experiment (leaf / plant)	Average number of leaves at the beginning of the experiment (leaf / plant)	Type of treatment
323.3	213.3	C
356	150	NSP
LSD P<0.05=89.626		

Estimating lead percentages in samples at the end of the experiment and the removal percentage

The results of the census showed the percentage of lead in plant treatments, and the percentage of removal was measured as follows:

Percentage of lead removal in the soil

Through experiments with plant treatments, the results shown in Table (9) showed that the average concentration of the total lead element in the control sample amounted to 566 mg / L and the average concentration of the remaining lead element was 325.1 mg / L, while the percentage of removal was 42.56% in the control sample, while the average concentration of lead was 42.56% in the control sample. The total lead concentration in the NS sample (natural soil with a lead element concentration of 750 mg/L is 1316 mg/L and the average remaining lead concentration is 632.13 mg/L. The percentage of removal was 51.96%, NSP treatment (natural soil with plants and a lead concentration of 750 mg/L). The mean total lead concentration was 1316 mg/L, the remaining lead concentration was 686.43 mg/L, and the percentage removal percentage was 47.83%. The treatment of (SP sterile soil with a lead concentration of 750 mg/L) was 1316 mg/L. liters and the average concentration of the remaining lead element was 788.53 mg / liter while the removal rate was 40.08%.
The results of the statistical analysis showed that there were significant differences between the rates of residual lead concentrations in the soil at the significance level (P<0.05) for all treatments.

Table (9): the total and residual lead concentration rate and the percentage of removal in the soil used in agriculture

Removal percentage (%)	Total lead concentration (mg/L)	Residual Lead Concentration (mg/L)	Type of treatment
42.56	566	325.1	C control sample
51.96	1316	632.13	N.S Normal soil with a lead concentration of 750 mg/l
47.83	1316	686.43	NSP Normal soil with plant and lead concentration 750 mg/L
40.08	1316	788.53	SP Sterile soil with a lead concentration of 750 mg/L
LSD P<0.05=59.124			

Table (10) shows the total and residual lead concentration rate and the percentage of removal in the shoot system of Dodonaea viscosa plant. The results showed that the control sample treated the lowest removal percentage of 8.43% compared to the natural soil (NSP) treatment with the plant. The lead concentration was 750 mg/L and a removal rate of 67.88%.
The results of the statistical analysis showed that there were significant differences between the rates

of residual lead concentrations in shoot system at the significance level (P<0.05).

Table (10) showing the average concentration of total and residual lead and the percentage of its removal in the shoot system of *Dodonaea viscosa*			
% of removal	Total lead concentration (mg/L)	Residual lead concentration (mg/L)	Type of treatment
8.43	304	278.36	C
67,888	1045	335.56	NSP
LSD P<0.05=29.699			

Table (11) shows the results of the current study represented by the total and remaining lead concentration in the root system of Dodonaea viscosa plant with the percentage of removal. A glance at these results shown in the table shows that the control sample (C), which also recorded the lowest percentage of removal, 1.577%, while the treatments (NSP) showed a removal percentage of (58.41%).

The results of the statistical analysis showed that there were significant differences between the rates of residual lead concentrations in the root system between the (NSP) treatment compared to the control sample, and the results of the analysis showed at the significance level (P<0.05).

Table (11): the total and residual lead concentration rate and the removal percentage in the root system of Dodonaea viscosa plant			
% removal	Total lead concentration (mg/L)	Residual lead concentration (mg/L)	Type of treatment
1.57	251.6	247.63	C
58.41	1001.6	416.56	NSP
LSD P<0.05=10.171			

Table (12) shows the average dry weight of the root and shoot plant Dodonaea viscosa. It can be noticed from the table, we find that the average dry weight in the control sample (C) was 3.105 g. As for the natural soil treatment with plants and a lead concentration of 750 mg / L (NSP). The average dry weight of the root system was 3.327 g, which is higher than the control sample. As for the average dry weight for the shoot system of Dodonaea viscosa. The control sample treatment (C) recorded the highest average dry weight of the shoot total at 15.972 gm/plant, while the dry weight decreased in the NSP treatment to record an average dry weight of 14.596 gm/plant.

The decrease in dry weight in some treatments in the root and shoot system may be due to the negative effects of the accumulation of lead in high concentrations and its effect on division and cellular differentiation and damage to the peripheral cells of the roots. This leads to a change in the regularity of the tissues specialized in transport, as the high concentrations of heavy elements have toxic effects that, in turn, causes a significant reduction in the growth of leaves and roots, and shows a significant reduction in the shoot characteristics of the plant (Athar & Ahmad, 2002).

The results of the statistical analysis showed that there were no significant differences between the average dry weight of the root total and all treatments at the significance level (P<0.05).

Table (12): The average dry weight of the root and shoot of Dodonaea viscosa		
Shoot dry weight (g/plant)	Root dry weight (g/plant)	Type of treatment
15,972	3,105	C
14,596	3,327	NSP
LSD P<0.05=2.684		P<0.05 = 8,347 LSD

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