

Phytoremediation: Plant Synergy - Bacteria for Treatment of Heavy Metals

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Abstract

Soils are polluted with heavy metals and pose a more serious threat to the environment and human health, such as increased toxicity of microorganisms, decreased bioavailability and inhibition of plant growth, as well as leading to an increase in the harmful effect on the physical and chemical properties of the soil, which makes soil treatment more difficult. Biological treatment using microorganisms and plants is a method Effective to treat soils contaminated with heavy metals. It is a widely accepted method that is mostly carried out on site; Hence it is suitable for establishing/replanting crops in treated soil. Four heavy metals were identified in this study, Pb-Ni-Cd-Ar, to be measured in polluted soils, as well as in plants after treatment. The percentage of metals in contaminated soils before treatment was 38.295) for Pb, 18.450 for Cd, and 89.863 for Ni. As for arsenic, it was Less than the device can read it, so it appeared in all treatments in the form of DL) mg/g. Treatment of contaminated plants and soil by the method of inoculation with bacterial suspension outperformed the rest of the treatments, as the removal rates of heavy metals treated with IBMA1 bacteria reached (0.998 for Pb, 1.151 for Cd and 1.021 for Ni) mg /g In IBMA3 bacteria, the removal percentage of heavy metals was (0.984 for Pb, 1.116 for Cd, 1.022 for Ni) mg/g, while for IBMA4 bacteria, the percentage of heavy metals removed was (1.090 for Pb, 1.169 for Cd and 1.044 for Ni) mg/g In all treatments containing plants the percentage of bioaccumulation in the roots was higher than the bioaccumulation in the vegetative total, and the highest bioaccumulation of Ni in the roots of the plant was in the plant only treatment, which amounted to 4.3222 mg/g. processing Plant and bacteria have medium capacity to remove heavy metals from polluted soils.

1. INTRODUCTION

With the development of industrialization and urbanization, the abundance of heavy metals in the environment has tremendously during the past decades, which has raised great concerns around the world [1]. Heavy metal is a group of metals with relatively high chemical densities, atomic weights, and atomic numbers. Common heavy metals/metals include cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), zinc (Zn), copper (Cu), nickel (Ni) and chromium (Cr). These heavy metals / metalloids originate from natural or anthropogenic sources such as water produced from the oil and gas industries [2] noted that the use of phosphate fertilizers in agriculture, mineral mining and smelting, and its use in pesticides, makes it necessary to take remedial measures to prevent heavy metals from entering the terrestrial, air and aquatic environments, and to mitigate land pollution .So far between[3] in his study that there are a variety of heavy metals that are not degradable by any biological or physical process and they are fixed in the soil for a long time, which poses a long-term threat to the environment (and according to their role in biological systems, Heavy metals can be classified according to the study of researchers[4] as essential and non-essential. Essential heavy metals such as copper, iron, manganese, nickel and zinc are essential for physiological and biochemical processes during the plant life cycle however.[5] indicated that they may become toxic when present in excessive quantities. Non-essential heavy metals such as lead, cadmium, and mercury are highly toxic with no known function in plants. Environmental pollution as shown by[6]severely affects a variety of physiological and biochemical processes in plants and crops and reduces agricultural productivity. It can enter the food chain through crops and accumulate in the human body through biomagnification, constituting a major threat to human health. Bioremediation using microorganisms and plants is an effective method for treating soils contaminated with heavy metals. It is a widely accepted method that is mostly carried out on site; Hence it is suitable for establishing/replanting crops in treated soils[7].Phytoremediation is considered one of the best ways to get rid of heavy metals as an alternative to physical and chemical methods for the ease of this technique, its low material cost and the lack of human resource use.[8] and is considered one of the best plant species used in the treatment of heavy metals. [9] indicated the ability of bacteria to promote growth and stimulate growth activity through various mechanisms, such as N₂ fixation, P and solubilization, acidifying Iron, thus helping plants to overcome stress, enhance plant defense to eliminate pathogens, and stimulate the biodegradation process.

2. MATERIALS AND WORKING METHODS

2.1 THE CORE OF THE TOTOXICITY TEST

The experiment was started on February 17, 2022 in the laboratories of the College of Science, Department of Life Sciences, Tikrit University, which focused on the use of *Festuca arundinacea* and three types of bacteria isolated and identified by NCBI, respectively IBMA1 *Cytobacillus* Sp. IBMA3, IBM4 *Bacillus* SP in the toxicity test for the treatment of oil-contaminated soils. Crude. Ten polyethylene basins of dimensions (40.20.25 cm) length, width and height were used, respectively. 100 kg of contaminated soil were distributed from the same polluted site from which bacteria were isolated, distributed to 10 basins for each basin, 10 kg was measured. The saturation of the contaminated soil was 240 ml of water per kilogram of contaminated soil. The soil was sieved using a mesh with a diameter of 4.75 mm holes to ensure uniformity and removal of coarse parts. As for the plant, the seeds were sterilized before planting to ensure their purity, and the seeds were sterilized using a 1% solution of potassium permanganate for 30 minutes, then rinsed with distilled water.

A method of inoculation with bacteria only. And three other basins filled 10 kg of contaminated soil, and a type of bacteria was added to each basin by the method of pollination by bacteria with the plant and the other three basins were added contaminated soil and bacteria by injecting the roots of the plant with bacteria [10] where small holes were made with a needle. The plant roots were then placed in the bacterial suspension for 24 hours and the plant was then planted in the contaminated soil. As for the last basin of the treatment, only contaminated soil was added with the plant. The bacteria solution was prepared by activating the bacteria on a medium of neutron broth, with a liter density of 0.5 nm. Absorbance was measured under 550 nm with a spectrophotometer and was added at a rate of 240 ml per 2,400.liter of irrigation water to the basin [11] with another basin in which there is only contaminated soil, the time of the experiment was two months.

2.2 SOIL SAMPLE DIGESTION:

Soil digestion was carried out in the Department of Soil and Water Resources, College of Agriculture, Tikrit University, according to what was stated in [12] by (0.4 grams) of air dried soil was weighed and placed in a clean glass flask with a capacity of (250 ml) and (12 ml) was added. of the digestive solution. It consists of (concentrated sulfuric acid, concentrated nitric acid and perchloric acid in a ratio of (1: 1: 3), and the flask is covered with an hour glass to prevent the evaporation of concentrated acids. Samples are then heated on the surface of a heat plate at a temperature of 105 ° C for an hour and a half or until they evaporate and we obtain On dry soil by placing it on the (electric heating plate) and then adding 20 ml of nitric acid to the dry soil sample, after which we transfer the contents of the flask to a volumetric flask of 100 ml after filtering the liquid with filter paper to prevent clogging of the capillary tube of the atomic absorption spectrometer (A.A.S), And we do the dilution process by adding distilled water to (50 ml).

2.3 DIGESTION OF A PLANT SAMPLE:

After the plant sample was collected, dried in an oven at 70 °C for 48 hours and milled, the plant powder was digested by acidic digestion or wet digestion as per [13] where (0.2 g) of the powder of the vegetative part of the plant and (System Root) each part was placed separately in a (Griffin flask) with a volume of 200 ml and then digestion was performed on it after adding sulfuric acid, nitric acid and perchloric acid in proportions 2: 1: 1 for a period ranging from (2-4) hours, taking care to Cover the cups with an hour bottle, then the beaker and hour bottle are washed with distilled water (deionized) and the samples are filtered, then the volume is completed to (50) ml of distilled water, where the sample becomes ready for analysis using A.A.S, and through a standard curve for each mineral, it can be known The concentration of each mineral by which the equation is and expresses the concentration in µg/g dry weight.

2.4 HEAVY METAL IDENTIFICATION:

2.4.1 DETERMINATION OF THE PRESENCE OF HEAVY METALS IN THE SOIL:

The determination of heavy metals for soil samples in the College of Engineering, University of Tikrit, according to what was mentioned in the method of [12] which included the digestion of the soil as in paragraph and after dilution with distilled water, all those who were determined heavy metals in soil samples are absorbed by Atomic absorption device, type (Perkin Elmer) The wavelength and current used for each heavy metal were determined and the absorbance was converted into units of concentration and by reference to the regression equations for the standard curves for the heavy metals under study, and the results were expressed in microgram units of metal and for each grams of dry weight of the soil.

2.4.2 DETERMINATION OF HEAVY METALS:

After the process of digesting the plant samples and adjusting the volume of the solution according to the expected concentration in the samples for a volume of (50 ml), the samples were ready for analysis using the atomic absorption device, where acetylene gas was used and the wavelengths of each metal were determined and the light beam from the cathode lamp was made of metals. whose concentration must be measured, and it is also highlighted by passing it over the flame and burning the mixture at a temperature (2100-2800°C) and during the combustion process where the free atoms reach a stable state, where the atoms are absorbed at a certain wavelength, the bioconcentration factor (BCF) according to the method [14] as in the following equation:

$$BCF = \frac{Cocentration\ Plant}{Concentration\ Soil}$$

3. RESULTS AND DISCUSSION

3.1 HEAVY METALS

The determination of heavy metals for soil samples, according to what was mentioned in the method of [12] where each determination of heavy metals in soil and plant samples is absorbed by the atomic absorption device.

3.1.1 DETERMINATION OF HEAVY METALS BY TREATMENT USING BACTERIA ONLY

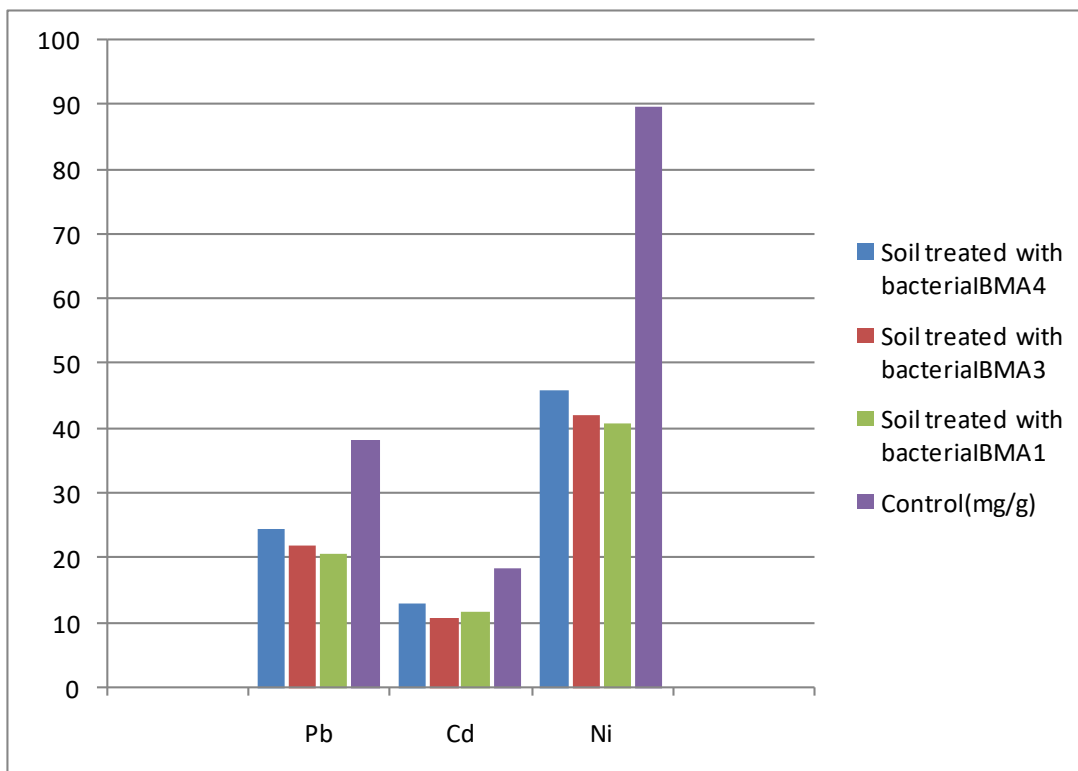
Table (1) and fig (1) show the concentrations of heavy metals (Pb.Cd.Ni.Ar) in soils treated with bacteria only (40.972, 11.934, 20.821, DL) mg/g, respectively. It was shown that the treatment that lasted for two months from 17/2/2022 to 17/4/2022 The ability of medium bacteria to reduce heavy metal concentrations and these ratios were the result of treatment with IBMA1 bacteria. As for the percentages of IBMA3 bacteria, they were as follows: 22.055 for Pb, 10 .775 for Cd and42 .072. As for the Ar, the absorber could not determine the ratio (mg/g) and the percentages of IBMA4 bacteria were (24.509for Pb, 23.013 for Cd and45 .941 for Ni, while the device was unable to determine the percentage of arsenic because it is very low) mg/g. The results show the ability of the different bacteria used in the treatment to remove toxic metals from the soil, but in varying proportions, the reason for this may be due Bacteria use different processes such as sedimentation, biosorption, and enzymatic conversion to break down or reduce heavy metals into harmless or less toxic forms that are more stable, less mobile, or inert. By demonstrated [15]the ability of microorganisms, especially bacteria, to isolate and convert these heavy metals. Now to recommend the most efficient strains for application in biological treatment or phytoremediation strategies with the help of bacteria that may help in improving plant growth in contaminated soils, and this is in agreement with what the researchers[16] and recorded the statistical concentration factor in the following sequence: Pb < Ni < Cd

Table (1) Determination of heavy metals by treatment using bacteria only

element	Control(mg/g)	Soil treated with bacteriaIBMA1	Soil treated with bacteriaIBMA3	Soil treated with bacteriaIBMA4

Pb	38.295c	20.821b	22.055d	24.509u
Cd	18.450e	11.934h	10.775j	13.230g
Ni	89.863a	40.972c	42.072d	45.941i
Ar	DL	DL	DL	DL

Fig (1) Determination of heavy metals by treatment using bacteria only



3.1.2 DETERMINATION OF HEAVY METALS USING THE PHYTOREMEDIATION METHOD ONLY

The percentages of removal of heavy metals in the plant and soil are shown in Table (2) and the plant was as follows in the shoot (10,235 in the Pb, 4.470 in the Cd and 26,095 in the Ni. The device could not read the percentage of arsenic, because it is very low). As for the root total, it was the proportions were as follows (15,673 in Pb, 8.934 in Cd and 22,322 in Ni, while in arsenic the percentage was so low that the device did not estimate it), while in soil the percentage of removal of heavy metals was higher than what was in the plant and was (22.539 in Pb and 10 .785 in Cd and 40,843 in Ni. As for arsenic, the device did not determine it because it is low. The above results show the ability of the plant to remove heavy metals at an average rate. This is due to the fact that phytoremediation still suffers from some limitations, as it is a very time-consuming process to clean soil contaminated with heavy metals, especially in medium and highly polluted sites. This may be partly due to the slow growth rate and low biomass production due to heavy metals and it can be concluded that, to some extent, tall fescue may be beneficial in the phytoremediation of heavy metal contaminated sites. More work is needed to enhance phytoremediation methods for heavy metal contaminated soils. These organic materials are consistent with what was stated in[17] It has been shown that the accumulation of heavy metals in the root part is more than its accumulation in the vegetative part, where the main factor that controls the availability of minerals for plants in the soil is the solubility of the mineral, because in order for absorption to occur in the root, soluble species must be present next to the root membrane for a period limited. The form of this soluble species will have a strong influence on the rate and extent of heavy metal absorption. Heavy metals are absorbed by *Festuca arundinacea* accumulating and in its root tissues first appears weak transfer of minerals from roots to shoots. [18] Table (3) Shows BCF results for phytotherapy only the statistical concentration factor was recorded in the following sequence, Pb < Ni < Cd

Table (2) Determination of heavy metals using the Phytoremediation method only

Element	Control(mg/g)	The Phytoremediation method only		Soil treated with plants
		Shoot	root	
Pb	38.295c	10.235jk	15.673u	22.539d
Cd	18.450e	4.470vw	8.934rs	10.785hi
Ni	89.863a	22.095d	24.322u	40.843b
Ar	DL	DL	DL	DL

Table (3) Shows BCF results for phytotherapy only

Element	Plant	Soil	BCF
Pb	25.973	22.539	1.152
Cd	13.404	10.785	1.242
Ni	48.417	40.843	1.185
Ar	DL	DL	DL

3.1.3 DETERMINATION OF HEAVY METALS USING THE METHOD OF INOCULATION BETWEEN PLANTS AND BACTERIA (SYNERGY)

Table (4) show the percentages of heavy metals removal in the method of soil and plant inoculation treatment, where the vegetative sum of the plant was (4.929 for Pb, 2.023 for Cd, 12.965 for Ni and arsenic) was not recorded because it was less than the reading of the device (mg/g and root total) 5.357 for Pb, 3.309 for Cd and 18.012 for Ni and arsenic were not recorded because it was less than the device reading) mg/g and this treatment of plants with IBMA1 bacteria, while the proportions of IBMA3 bacteria treatment for shoots (6,244 for Pb, 3,211 for Cd and 14.860 for Ni and arsenic were not recorded). Because it was less than the device reading (mg/g), and in the root total, the ratios were as follows (7.703 for Pb, 4.583 for Cd, 20,033 for Ni and arsenic were not recorded because it was less than the device reading) mg/g.7 .380 for Pb,4 .841 for Cd, and20 .961 for Ni and arsenic were not recorded because they were lower than the device reading (mg/g), while the radical total ratios were as follows (9.200 for Pb,5 .430 for Cd, and 21.550 for Ni and arsenic were not recorded). It is recorded because it was less than the device reading (mg/g) that the synergistic treatment using plants and bacteria has the advantage in treating soil contaminated with minerals because it is low-cost, harmless, and highly efficient, in addition to being friendly to the ecosystem. This is what we note in the results of our study, where the synergy between plant and bacteria worked to effectively remove heavy metals from the soil. The action of bacteria and plants depended on several mechanisms to treat contaminated soil. The use of plants is the most common strategy for treating mineral-affected areas. Conjugation of plants with bacteria has proven to be an effective approach that ensures cleaning of contaminated soil. The success of this strategy depends largely on the types of plants and bacteria involved in the process. This is in agreement with what was mentioned in it [19-20] While the soil treated with IBMA1 plant and bacteria, the removal rates of heavy metals were good compared to treating the plant only or treated with bacteria only, as follows (10.305 for Pb, 3.632 for Cd and 30.329 for Ni and arsenic) were not recorded because they were less than the device reading) mg/g. The removal ratios for soil treated with plant and bacteria IBMA3 (13.840 for Pb, 5.783 for Cd, 35.136 for Ni and arsenic were not recorded because it was less than the device reading) mg/g. Pb and 6.640 for Cd and 34.921 for Ni and arsenic were not recorded because they were less than the device reading (mg/g). It was found from the results that the removal in the soil is greater than the result of the removal in the root and vegetative group, where the plant transports pollution and separates it like heavy metals. Plants will store many pollutants in biomass that may later be harvested, while microorganisms can convert pollutants such as heavy metals into a stable and/or less virulent type. It will facilitate the uptake

of pollutants such as heavy metals from the roots of plants. By existing microorganisms at intervals, the tissues of aerobic plants will stabilize and/or re-work the pollutants that are translated into less harmful substances and this is consistent with what [21] Table (6) Shows BCF results for phytotherapy inoculation between plants and bacteria, and fig(2) The difference in BCF between plant treatment only and vaccination treatment .The statistical concentration factor was in the following sequence: Pb < Ni < Cd

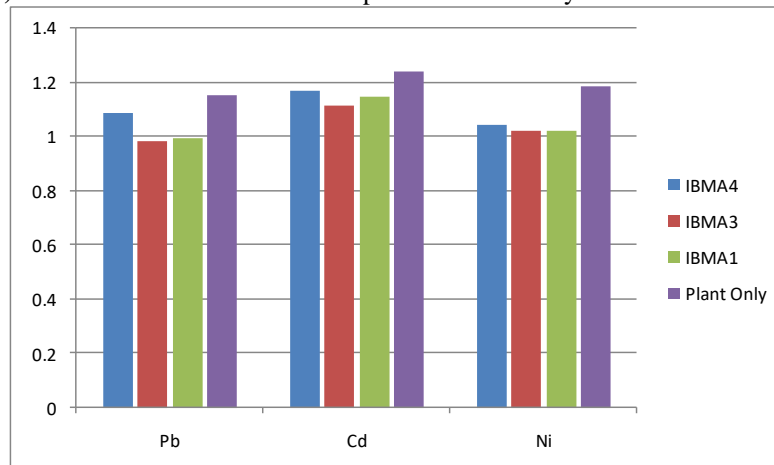
Table (5) Determination of heavy metals using the method of inoculation between plants and bacteria (synergy)

Element	Control (mg/g)	inoculation between plants and bacteria								
		Soil treated with plants and bacteria IBMA1	Soil treated with plants and bacteria IBMA3	Soil treated with plants and bacteria IBMA4	IBMA1 Bacterial treated plant		IBMA3 Bacterial treated plant		IBMA4 Bacterial treated plant	
					Shoot	root	shoot	root	Shoot	Root
Pb	38.295c	10.305q	13.840j	15.210f	4.929w	5.357r	6.244v	7.703m	7.380m	9.200s
Cd	18.450e	4.632x	6.983v	9.640q	2.023z	3.309y	3.211y	4.583x	3.841y	5.430r
Ni	89.863a	30.329d	35.136e	35.921e	12.965m	18.012j	14.860o	20.033d	15.961f	21.550f
Ar	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL

Table (6) Shows BCF results for phytotherapy inoculation between plants and bacteria

Element	bacteria IBMA1			IBMA3 bacteria			IBMA4 bacteria		
	Plant	Soil	BCF	plant	Soil	BCF	Plant	Soil	BCF
Pb	10.286	10.305	0.998	13.624	13.840	0.984	16.58	15.210	1.090
Cd	5.332	4.632	1.151	7.794	6.983	1.116	11.271	9.640	1.169
Ni	30.977	30.329	1.021	34.893	34.136	1.022	37.511	35.921	1.044
Ar	DL	DL	DL	DL	DL	DL	DL	DL	DL

Fig (2) The difference in BCF between plant treatment only and vaccination treatment



3.1.4 DETERMINATION OF HEAVY METALS BY ROOT INJECTION METHOD

Table (7) show the percentages of removal of heavy metals in the method of treatment by root injection to the soil and the plant, where in the plant the vegetative total was (5.352 for Pb, 2.722 for Cd, 14.116 for Ni and arsenic) it was not recorded because it was less than the device reading) mg/g and the total Roots (8.577 for Pb, 4.681 for Cd and 22.521 for Ni and arsenic were not recorded because it was less than the device reading) mg/g and this treatment of the plant with IBMA1 bacteria, while the percentages of IBMA3 bacteria treatment for shoots were (7.992 for Pb, 3.033 for Cd and 15.490 for Ni and arsenic). It was recorded because it was less than the device reading (mg/g, and in the root total the ratios were as follows (10.023 for Pb, 5.424 for Cd and 23.210 for Ni and arsenic) it was not recorded because it was less than the device reading (mg/g). 8.487 for Pb, 4.902 for Cd, and 18.730 for Ni and arsenic were not recorded because it was less than the device reading) mg/g, while the ratios of the radical total were as follows (11.080 for Pb, 6.040 for Cd, and 21.020 for Ni and arsenic were not recorded because they It was less than the device reading) mg/g .Moreover, these beneficial microorganisms (bacteria) are able to enhance the bioavailability of minerals in the roots of plants. Increased plant growth and mineral uptake increases the effectiveness of phytoremediation processes combined with bioremediation. As stated in a study by [22] .The results of removal in soil treated with plant and IBMA1 bacteria are as follows (12.923 for Pb and 5.236 for Cd and 33.029 for Ni and arsenic were not recorded because it was less than the device reading) mg/g and the removal percentages for soil treated with plant and IBMA3 bacteria (15.253 for Pb and 7.681 for Cd and 35.933 for Ni and arsenic were not recorded because it was less than the device reading) mg/g. It was found that the removal rates for soils treated with plants and bacteria IBMA4 were as follows (17.032 for Pb and 9.132 for Cd and 38.103 for Ni and arsenic were not recorded). Because it was less than the device reading) mg/g

The treatment with plants and bacteria (root injection), and the removal rates of heavy metals were close to the method of pollination treatment, although the root injection method targeted the roots only and the bacteria did not contact the soil [23].table(8) Shows BCF results for phytotherapy inject plant roots with bacteria and fig(3) shows The difference in BCF between plant treatment only and inject plant roots with bacteria tretment

Table (7) Determination of heavy metals by root injection method

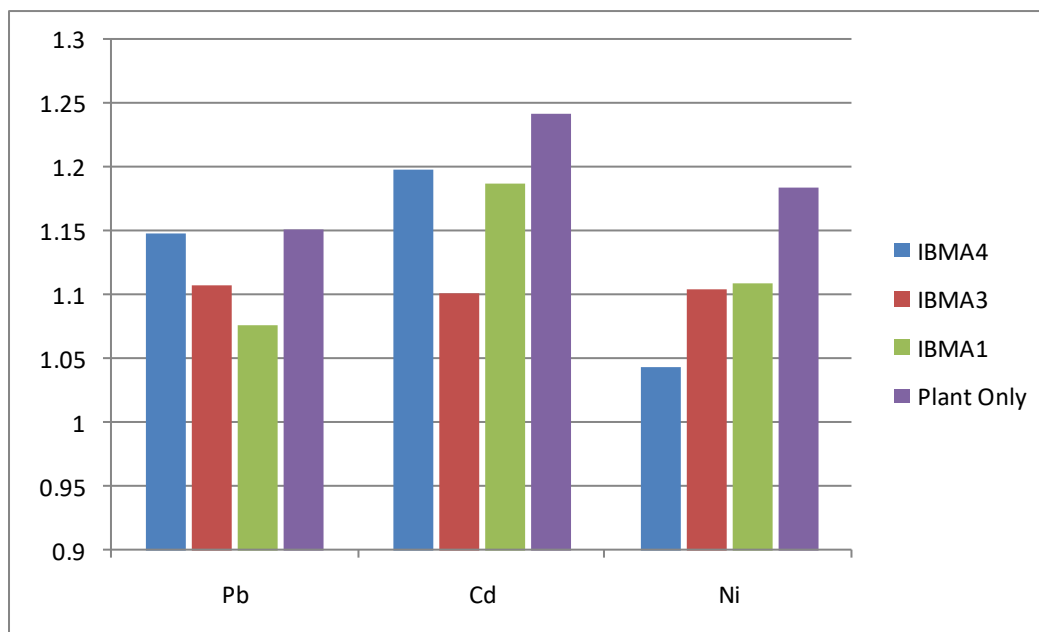
Element	Control (mg/g)	inject plant roots with bacteria								
		Soil treated with plants and bacteria IBMA1	Soil treated with plants and bacteria IBMA3	Soil treated with plants and bacteria IBMA4	IBMA1 Bacterial treated plant		IBMA3 Bacterial treated plant		IBMA4 Bacterial treated plant	
					shoot	root	shoot	Root	shoot	Root
Pb	38.295c	12.923e	16.253j	17.032g	5.352u	.8577o	7.992k	10.023e	8.487o	11.080j
Cd	18.450e	6.236w	7.681q	9.132r	2.722z	4.681s	3.033t	5.424u	4.902s	6.040w
Ni	89.863a	33.029c	35.933b	38.103a	14.116f	22.521d	15.490j	23.210k	18.730u	21.020d
Ar	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL

Table (8) Shows BCF results for phytotherapy inject plant roots with bacteria

Element	IBMA1 bacteria			IBMA3 bacteria			bacteria IBMA4		
	Plant	soil	BCF	Plant	soil	BCF	plant	Soil	BCF
Pb	13.929	12.923	1.077	18.015	16.253	1.108	19.567	17.032	1.148

Cd	7.403	6.236	1.187	8.457	7.681	1.101	10.942	9.132	1.198
Ni	36.637	33.029	1.109	39.71	35.933	1.105	39.751	38.103	1.043
Ar	DL	DL	DL	DL	DL	DL	DL	DL	DL

Fig (3) The difference in BCF between plant treatment only and inject plant roots with bacteria treatment



CONCLUSIONS

Phytoremediation is a treatment technique for cleaning pollutants from the environment using green plants. Phytoremediation can be an alternative solution as a green solution. A technology to treat areas contaminated with heavy metals. Many plants have high potentials such as bio-reactivity. The ability of plants is also used to extract, decompose, stabilize and volatilize heavy metals. The applied thermal, mechanical and chemical methods can be used to treat these pollutants, but they are expensive. Environmentally destructive and energy consuming. Bioremediation was developed to be an environmentally friendly and cost effective cleaning technology. Phytoremediation, a collaborative association between plants and microbes, is an emerging and cost-effective on-site technology that provides a viable option in treating such polluted environments. Current research focuses on plant-microbe interactions during phytoremediation and how these beneficial interactions lead to improved plant growth and a pollution-free environment.

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