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BIO-REMEDIATION OF CD-CONTAMINATED SOIL CULTIVATED WITH FABA BEAN VIA APPLICATION OF *ALCALIGENESFAECALIS* RHIZOBACTERIUM

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ABSTRACT:

The present investigation was implemented to search for high efficient bacterial strains in bio-remediating the toxic influence of cadmium sulphate ($CdSO_4.8H_2O$) on faba bean plants grown in sandy soil supplemented with various concentrations of $CdSO_4.8H_2O$. Two isolates of Cd-remediating bacterial strains were obtained from Al-Taif and Al-Madinah Al-Munawrah governorates, KSA from the rhizosphere of cucumber and tomato plants respectively. Regarding efficiency of both isolated strains in remediating $CdSO_4.8H_2O$supplemented nutrient both medium. The absorbed quantities of Cd were 95.66 and 84.00 ppmconsecutively from 277 ppm Cd measured in the control treatment at the rate of 34.53% and 30.33% consequtively.The isolated bacterial strains were genetically identified as *Alcaligenesfaecalisstrain* BAB-1832and *Proteusmirabilis* strain HI 4320. The bio-remediating strains*Alcaligenesfaecalisstrain* BAB-1832 revealed the highest efficiency in absorbing Cd that reached 95.66 ppmfrom 277ppmin the control. Application of *A.faecalissignificantly* increased plant

height of faba bean grown in soil containing 5000 ppm$CdSO_4.8H_2O$, the percentage of increase over the same un-inoculated treatment reached 67.94. Bioremediation of contaminated soil with A. faecalis increased faba bean fresh weight at 5000 and 7500 ppm $CdSO_4.8H_2O$concentrations by 58.18 and 56.53% over the same not bio-remediated treatments. The bio-treatments of *A.faecalisaugmented* faba bean P-content at various concentrations of $CdSO_4.8H_2O$ (5000, 7500 and 10000 ppm). The Bio-remediated treatments of polluted soil with $CdSO_4.8H_2O$ reduced faba bean plant Cd-content in comparison with their un-inoculated treatments. For the nitrogen content of faba bean plants, the differences recorded between the various treatments were not significant.

KEY WORDS: Bioremediation, cadmium sulphate, faba bean, Alcaligenesfaecalis, KSA.

1.INTRODUCTION:

Althoughheavymetalsarenaturallypresentinthesoil,geologicandanthropogenicactivitiesincreasetheconcentrationoftheseelementstoamountsthatareharmfultobothplantsandanimals.Someofthesactivitiesincludeminingandsmeltingofmetals,burningoffossilfuels,useoffertilizersandpesticidesinagriculture,productionofbatteriesandothermetalproductsinindustries,sewagesludge,andmunicipalwastedisposal(Allowa*yet al*., 1990; Raskin*et al*., 1994 and Shen*et al*., 2002). Growth reduction as a result of changes in physiological and biochemical processes in plants growing on heavy metal polluted soils has been recorded (Chatterjee and Chatterjee, 2000 and Oncel*et al*., 2000).

A considerable increase in the discharge of industrial waste to the environment, chiefly soil and water, has led to the accumulation of heavy metals, especially in urban areas. This increase in industrial waste is mainly due to the growth in industry. The release of heavy metals into the soil and water poses a major health concern worldwide, because heavy metals cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. Slow depletion of heavy metals also takes place through leaching, plant uptake, erosion and deflation. Soil properties affect metal availability in diverse ways (Chibuike and Obiora, 2014).

Each heavy metal has unique biofunctions or biotoxicities (Wei *et al*., 2009). Among metals, cadmium is a non-essential element and highly toxic to organisms even at very low dosages. Cadmium damages cells by strong affinity to glutathione and sulphydryl groups in proteins (Cunningham and Lundie, 1993) and displacement of zinc and iron ions from proteins.

MATERIALS AND METHODS

Isolation and efficiency of Cd-remediating bacterial strains:

A number of rhizosphere soil samples were collected from different locations of Saudi Arabia governorates representing different environmental conditions, i.e. Al-Riyadh, Al-Taif, Al-Baha and Al-Madinah where the standing crops were alfaalfa, cucumber, fig and tomato respectively. The serial dilutions and plate count method using nutrient agar medium supplemented with different concentrations of $CdSO_4.8H_2O$ (350, 450 and 550 ppm) was used for isolation of Cd tolerant isolates. The growing bacterial colonies on the medium containing the highest concentration of cadmium (550 mg/l $CdSO_4.8H_2O$) were picked up and purified then kept on the same nutrient agar medium supplemented with the highest concentration of Cd.

The bacterial isolates that picked up from the highest concentration of cadmium (550 mg/l $CdSO_4.8H_2O$) were selected for evaluating their abilities in absorbing cadmium from the liquid medium. Flasks contain sterile 100 ml of nutrient broth medium supplemented with cadmium sulphate (550 mg) was inoculated with a loop of fresh culture of the respective isolate. The inoculated flasks were

incubated at 30°C ±2 for 7 days in a rotary shaker at 100 rpm. After the incubation period, 25 ml of each culture representing the various isolates was transferred into digestive tubes; a digestive mixture composed of nitric acid and perchloric acid in ratio 1:6 v/v was added to each tube then heated on sand bath till the samples became clear. The digested samples separately filtered and cadmium was determined in the filtrate using spectrophotometer (ICP-DES-Oplmia 8000) (Odokuma and Akponah, 2010).

Identification of the Cd-remediating bacterial strains:

The various isolated microbial strains were identified by Macrogen Company (10F, 254 Beotkkot − roGeuncheon − qu, Seoul, Rep. of Korea) using 16s ribosomal RNA gene.

The pot experiment:

A pot experiment was carried out in the green house of the biological science department, faculty of science, King Abdulaziz University to study the bioremediation process of Cd heavy metal in sandy soil cultivated with faba bean. Plastic pots of 25 ml diameter were filled with 8 kg per each sandy soil of pH 7.8 and E.C. 1.1dSm-1. The soil in each pot was artificially contaminated with cadmium sulphate ($CdSO_4.8H_2O$) by 5000 and7500 or 10000ppm according to the proposed treatments. Three pre-germinated faba bean seeds were cultivated in each pot and immediately inoculated with the respective metals, bio remediating heavy metals isolated strains where each pot received 30 ml inoculum of the respective strains and irrigated with tap water to the field capacity (without excess water leaking outside the pot), each treatment was triplicate. Irrigation was carried out according to the plant needs with half strength of modified, where both compounds $Ca(NO_3)_2.4H_2O$ and NH_4NO_3 were eliminated) Hoagland nutrient solution (Hoagland and Arnon, 1950) and tap water interchangeably. As a leguminous plant, the whole treatments of faba bean were inoculated with a liquid culture of Rhizobiumleguminosarum previously isolated from faba bean root nodules.

The following scheme of work was applied:

- Control
- 5000 ppm Cd
- 5000 ppm Cd + Alcaligenesfaecalis
- 7500 ppm Cd
- 7500 ppm Cd + Alcaligenesfaecalis
- 10000 ppm Cd
- 10000 ppm Cd + Alcaligenesfaecalis

STATISTICAL ANALYSIS:

The obtained results were statistically analyzed using the computer program SPSS 17(2007). ANOVA test (one way) was applied to differentiate between the various means of treatments. The experimental design was complete randomized block design. Differences among means were compared using the least significant difference (LSD) at $p < 0.05$.

RESULTS AND DISCUSSION

Isolation and efficiency of cadmium-remediating bacterial strains:

A number of isolates were obtained from different Saudi governorates; the isolated strains were tested for their ability in absorbing cadmium from the liquid nutrient medium supplemented with

cadmium sulphate (550 mg/l $CdSO_4.8H_2O$).Two isolates obtained from Al-Taif(T) and Al-Madinah Al-Munawarah (M) governorates from the rhizosphere soil of cucumber and tomato showed high efficiency in cadmium remediation.Plate (1) is an overview of the cadmium-remediating isolates and the apparent changes of the inoculated flasks (T & M) in comparison with the un-inoculated medium as control (C).It is clear that the inoculated medium is clearer than the control due to the absorption of Cd by the tested strains.

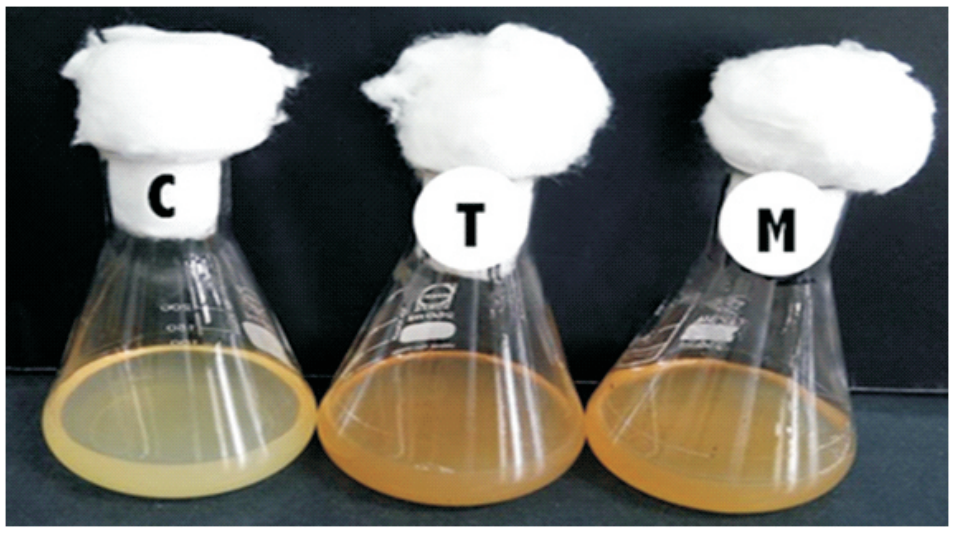

Plate 1: The apparent changes in nutrient broth medium supplemented with $CdSO_4.8H_2O$ due to inoculation with Cd-remediating strains (T & M) in comparison with the control (C).

Quantitatively, the absorbed amounts of Cd by the two isolated strains T and M were 95.66 and 84.00 ppm respectively, with no significant difference between them, while the total amount of Cd measured in the control was 277 ppm Cd. This finding is in agreement with Mohammad *et al.* (2013) who found that Bacillus thuringiensis strain OSM29 was able to remove Cd and Pb from soil contaminated with these heavy metals at a biosorption capacity of 87% for Cd and 90% for Pb with an optimum contact time of 30 minutes. Furthermore, Amoozegar (2012) found a bacterium belonging to genus Halomonas in saline environment in Iran was able to uptake more than 50% and 90% Cd and Pb respectively. Chen *et al*. (2008) reported that soil microbes play significant roles in the process of Cd and Pb bioremediation either through absorption, transformation or degradation.

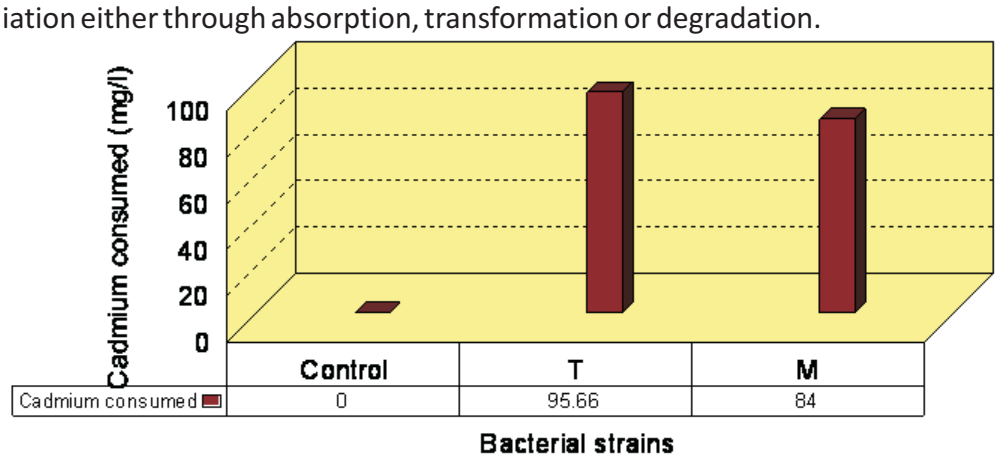

Fig. 1: Efficiency of both isolated bacterial strains in cadmium absorption from the liquid nutrient medium.

Identification of the isolated cadmium-remediating bacterial strains:

Regarding the identification of both isolated Cd-remediating bacterial strains, they were identified as Alcaligenesfaecalis strain BAB-1832 (T) and Broteus mirabilis strain HI 4320 (M).

Bio-remediation of Cd in a pot culture of faba bean experiment

The highest efficient bacterial strain in Cd absorption (*Alcaligenesfaecalis*)-that absorbed95.66 ppm from 277 ppm Cdmeasured in the control treatment-was used in bio-remediating Cd that present in different concentrations, i.e. 5000, 7500 and 10000 ppm as $CdSO_4.8H_2O$ in the soil cultivated with faba bean. A pot experiment was carried out to investigate the impact of *biofertilization* of faba bean with Cd-remediation *bacteriumAlcaligenesfaecalis* on certain growth (plant height, fresh weight and dry weight) and some plant nutrient contents (nitrogen, phosphorus and potassium) parameters. Moreover, the heavy metal Cdwas assessed to evaluate its level in faba bean plants and to how extent they affected by inoculation with *Alcaligenesfaecalis.*

Regarding the tested faba bean growth parameters, the data presented in Table (1) show that the control treatment (0 $CdSO_4.8H_2O$) recorded the highest significant value for plant height. In addition, application of the bio-remediating*Alcaligenesfaecalis* significantly increased plant height at Cd concentration 5000 ppm in comparison with the same un-inoculated treatment, the percentage of increase reached 67.94. The same finding was revealed with Cd concentration 7500 ppm where the significant increase reached 60.01%. For faba bean plant fresh weight, inoculation with Alcaligenesfaecalis significantly augmented fresh weight at both Cd concentrations 5000 and 7500 ppm when compared with the un-inoculated similar treatments. The percentages of increases reached 58.18 and 56.53 respectively. With regard to the highest concentration of CdSO4.8H2O (10000 ppm), the *bioremediation* treatment of *Alcaligenesfaecalis* induced insignificant increase over the similar un-inoculated treatment, the percentage of increase reached 10.76.Two rhizobacterial strains of Pseudomonas sp. obtained from heavy metal polluted soil by Franco-Hernandez (2010) favored the radical growth of Lens esculentum seedlings that were exposed to higher concentration of Pb. Application of PGPR to soil contaminated with heavy metals significantly reduced the toxicity of metals and improved the growth and yield of chickpea (Gupta *et al*., 2004), green gram (Vignaradiata) (Faisal and Hasnain, 2006), tomato, Indian mustard and canola (Burd*et al*., 2000). Watanabe (2001) found that the bacteria Ralstoniaeutropha significantly reduced the toxicity of soil polluted with Cd and decreased its effect on growth of tobacco plants.Moreover, Pishchik*et al*. (2002) found that the PGPR strain Khuyveraascorbata immobilized Cd and promoted growth of barley plants in the presence of toxic Cd concentration.

As to thefaba bean dry weight, Table (1) also shows that the differences recorded between various treatments are not significant. The recorded dry weight of faba bean plants ranged from 1.61 to 2.75 g/plant being the highest with the control treatment and the lowest with the un-inoculated treatment of 7500 ppm Cd concentration.Nevertheless, application of *A. faecalis* improved the faba bean plants dry weight at 5000 and 7500 ppm Cd concentrations in comparison with the same not bio-treated concentrations. These results are on the same line with those obtained byGomaa *et al.* (2012) who studied the influence of both non-symbiotic *Azospirillum lipoferum* and *symbiotic Rhizobium leguminosarumbv. trifolii* on bioremediation of both heavy metals Cd and Zn in a polluted soil cultivated with wheat plants. They found that the combined application of *Azospirillum* and Rhizobium in the presence of 300ppm Cd induced highly significant increase in wheat shoot dry weight in comparison with the positive control.

Table 1: Impact of various treatments of cadmium on some growth parameters of faba bean plants.

Treatments \ Parameters		Plant height (cm)	Fresh weight (g/plant)	Dry weight (g/plant)
Cadmium concentrations($CdSO_4.8H_2O$ ppm)	Control	43.16 a	35.84 a	2.75 a
	5000	22.33 cd	14.49 c	1.70 a
	7500	18.33 d	12.95 c	1.61 a
	10000	25.00 cd	12.83 c	1.99 a
Cadmium concentration (ppm) + *A. faecalis*	5000	37.50 ab	22.92 b	2.09 a
	7500	29.33 bc	20.27 bc	1.98 a
	10000	21.66 cd	14.21 c	1.94 a

Values of the same letters are not statistically significant; values are the means of three replicates.

Table (2) illustrates the effect of various Cd concentrations in the presence or absence of the bio-remediating A. faecalis on certain nutrients content of faba bean plants. Regarding nitrogen content, the recorded differences among the various treatments were not statistically significant. The nitrogen content of faba bean plants ranged between 13.69 and 29.53 mg/g dry weight. The highest value was found due to the application of *A. faecalis* with the highest level of Cd (10000 ppm). These obtained results are in agreement with those obtained by Gomaa *et a*l. (2012) where they found that the nitrogen content of wheat plant roots treated with heavy metals Cd or Zn in the presence of *Azospirillum* and/or *Rhizobiumdid notdiffered* significantly between the different treatments.

Table 2: Influence of various treatments of cadmium on some nutrients content and Cd in faba bean plants.

Treatments \ Parameters		N-content (mg/g)	P-content (mg/g)	K-content (mg/g)	Cd-content (ppm)
Cadmium concentrations ($CdSO_4.8H_2O$ ppm)	Control	13.69 a	0.20 de	9.36b	ND e
	5000	25.53 a	0.12 e	10.98 a	0.7bc
	7500	17.56 a	0.27 d	5.91d	1.1ab
	10000	29.26 a	0.40 c	0.59d	1.6a
Cadmium concentration (ppm) + *A. faecalis*	5000	17.64 a	0.53 b	7.75c	0.6c
	7500	15.71 a	0.64ab	7.73c	0.4 cd
	10000	29.53 a	0.71 a	0.83bc	1.4 a

Values of the same letters are not statistically significant; values are the means of three replicates; ND, not detected.

As to the phosphorus content of faba bean plants, Table (2) also indicates that the bio-treatments of Alcaligenesfaecalis in presence of the various concentrations of Cd significantly surpassed their corresponding not bio-treated treatments. The bio-treatments induced results of faba bean P-content ranged from 0.53 to 0.71 mg/g dry weight being the highest with the bio-treatment of *A. faecalis* + 10000 ppm $CdSO_4.8H_2O$ and the lowest with A. faecalis + 5000 ppm $CdSO_4.8H_2O$. Further, the un-inoculated treatments resulted in faba bean P-content ranged between 0.12 and 0.40 mg/g dry

weight while the control treatment recorded 0.20 mg/g dry weight. In general, it was observed that P-content of faba bean plants increased with increasing $CdSO_4.8H_2O$ concentration. These results are on the same line with those obtained byPishchik*et al*. (2002) who demonstrated that various N fixing and auxin-producing PGPR immobilized Cd and increased nutrient uptake by barley plants in the presence of toxic Cd concentrations.

As for the Cd-content of faba bean plants, Table (2) shows that the bio-treatments of A. faecalisreduced the Cd-content in comparison with their corresponding treatments that do not receive the bio-remediating *A. faecalis*. Generally, Cd-content ranged from not detected amount in the control treatment to 1.6 ppm in the un-inoculated treatment and received 10000 ppm $CdSO_4.8H_2O$.Damodaranet al. (2011) attained bio-sorption of about 73.79% of Cd within 30 days by Saccharomyces cerevisiaefrom contaminated soil with 100 and 300 ppm of Cd.

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