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TOXICITY OF Pb AND CD TO SYMBIOSES BETWEEN FABA BEAN PLANT AND RHIZOBIA AS WELL AS BIOREMEDIATION USING MICROSYMBIONT

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ABSTRACT

Laboratory and green house experiments were conducted to investigate the effect of pollution with Pb and Cd on microsymbiont (*Rhizobium leguminosarum* biovar *Viceae*) and its macrosymbiont (faba bean plant) as well as the role of inoculation with heavy metals tolerant rhizobia on amelioration of heavy metals toxicity effects. Many rhizobial isolates (*R. leguminosarum* bv. *Viceae*) were isolated from faba bean fields irrigated with polluted sewage water (El-Gabal El-Asfar and El-Hamoul locations) which had different degrees of heavy metals in addition to control site irrigated with normal water (El-Reyad location).

The highest concentration of Pb and Cd in soil and plant were found in El-Gabal El-Asfar followed by El-Hamoul then El-Reyad.

Rhizobial isolates which isolated from the above study areas subjected to different concentrations of Pb and Cd in YM medium, the rhizobial number of isolates differentially decreased by increasing heavy metals concentrations. Isolates of RH_6 and RH_8, which isolated from polluted sites were the most tolerantn to heavy metals. On the other hand, isolates of RH_1 and RH_2 (isolates from normal site) showed a high sensitivity to heavy metals.

The increases of Pb and Cd concentrations significantly decreased plant dry weight (g/plant), nodules dry weight (g/plant) and N%. Inoculation of faba bean plants with different rhizobial isolates increased plant parameters (as compared to) N-fertilized un-inoculated plants under normal or pollution conditions. Inoculated plant with isolates RH_6 and RH_8 attained good tolerance to heavy metals toxicity and gave highest records of dry weight of plant and nodules as well as N% even at high concentrations of Pb or Cd.

Consequently, it is to recommend that inoculate of faba bean plants grown in heavy metals cultures or in polluted sites using tolerant and efficient rhizobial strains to ameliorate and reduce toxicity effect of heavy metals.

INTRODUCTION

Over the last decade, the consumption of metals and chemicals in the process of industries has increased dramatically. Industrial uses of metals such as metal plating, industrial processes utilizing metal as catalysts, have generated large amount of aqueous effluents that contain high level of heavy metals. The most harmful heavy metals are Cd and Pb (Kasan and Baecker, 1989). A number of sites contaminated by heavy metals around the world are associated with human activities such as discharge of wastes into natural waterways, various metallurgical industries, accidental spills or mining (Schmidt and Schlegel, 1994).

The harmful effect of heavy metals pollution on soil microorganisms have been extensively studied under field and laboratory conditions and that toxicity is due to disruption of essential functions, competition for sites with essential metabolites, replacement of essential ions, reaction with SH-group; with the phosphate groups of ADP or ATP, damage to cell membranes and denaturation of proteins (Alloway and Ayres, 1997). On the other hand, many of heavy metals are toxic to plants as well as animals, tend to be mobile in the food chain, which means they can be bioconcentrated in animals, including humans, who are at the top of the food chain. The toxicity of some heavy metals such as lead and cadmium on humans were accumulates in the kidneys and the liver. Acute Cd and Pb poisoning may cause severe nausea, salivation, vomiting, abdominal pains, anemia and diarrhoea (Flick *et al.,* 1971 and Nordberg, 1978).

In addition, the effects of heavy metals on nitrogen fixation by legumes found little evidence that symbiotic N_2-fixation was sensitive to heavy metals toxicity. The study of Rother *et al.* (1983) examined nodulation and N_2-fixation by white clover growing on mine spoils with up to 216 mg Cd/kg^{-1} and 30,000 mg Pb/kg^{-1} and found only slight decreases at the most contaminated sites. On the other hand, some reports have shown that heavy metals negatively affect N_2-fixation and nodulation of leguminous plant (Baath, 1989).

Chaudri *et al.* (1992) found no decline occurred in rhizoibal numbers in experimental trials after two months exposure to heavy metals. After 18 months, the number of rhizobia in the control soils had declined by 90%.

Tolerance and adaptation of microorganisms to heavy metals are common phenomena and the presence of tolerant plants and microorganisms in polluted environments has frequently been observed (Zumbroich *et al.,* 1994). Angle *et al*. (1993) examined several strains and species of *Rhizobium* and *Bradyrhizobium* for their response to the presence of heavy metals on agar growth media. They found that *Bradyrhizobium japonicum* was the most metal-tolerant of the microorganisms examined. Nour El-Din (1997) indicated that inoculation of lead polluted soybean plants with *Bradyrhizobim japonicum* decreased plant absorbed Pb by about 50%.

The aim of the study is the investigation of harmful effect induced by Pb and Cd soil pollution on symbiotic relationship between faba bean plants and *Rhizobium legumnosarum* biovar *Viceae* and the bioremediation effect of rhizobial inoculation on amelioration of their toxicity on faba bean plant.

MATERIALS AND METHODS

Prevalence of heavy metals tolerant bacteria were prepared as following:

a. Isolation of root nodule bacteria from faba bean plants grown in different polluted regions in Egypt.
b. The isolates were evaluated for growth under stress of different concentrations of heavy metals salts namely lead acetate and cadmium chloride using synthetic media (*In vitro* evaluation).
c. Effect of heavy metals on plant dry weight, nodules dry weight and N% of inoculated faba bean plants in relation to un-inoculated one after 50 days of growth (*in vivo* evaluation) as pot experiment in greenhouse at Sakha Agricultural Research Station, Microbiology Lab. through winter season of 2007.

Media used:

a. Yeast extract mannitol (YEM), (Vincent, 1970):

This medium was used to isolate and study the growth of *Rhizobium* isolates as affected by different concentrations of heavy metals. The composition is as followingL (g/L) K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.2, NaCl 0.1, Mannitol 10.0, yeast extract 1.0 adjusted to pH 6.8 and autoclaved at 121ºC for 20 min. to prepare a solid medium 15-20 g agar L^{-1} were added. In case of bromothymol blue yeast extract mannitol agar, 5 ml per litre medium was added and in congored yeast extract mannitol agar medium was prepared by addition 10 ml of 1/400 aqueous solution of Congored to each litre of the previously described yeast mannitol agar medium.

b. Nutrient solution (Skrdleta *et al.,* 1984):

Pots were surface irrigated once or twice weekly according to the prevailing climatic conditions using a nutrient solution of the macroelements with the following composition (g/L): K_2HPO_4 0.2, NH_4SO_4 0.03, $MgSO_4.7H_2O$ 0.2, $FeCl_3$ 0.01, $CaCl_2$ 0.376, K_2SO_4 0.845. The nutrient solution was almost free of nitrogen with small amounts of NH^+_4 to initial growth and nodulation of faba bean plants. While, the microelements were of the following composition (mg/L): H_3BO_3, 1.855; $MnSO_4.4H_2O$, 2.231; $ZnSO_4.7H_2O$, 0.288; $CuSO_4.5H_2O$, 0.25 and $NaMO_4$, 0.412. The pH of the nutrient solution was adjusted to 6.9 using diluted KOH solution.

c. Heavy metals used:

- Lead acetate [Pb $(CH_3COO)_2.3H_2O)$].
- Cadmium chloride ($CdCl_2$).

d. Seeds used:

Faba bean seeds (*Vicia faba* cv. Nobaria 1). were used in the pot experiment. Seeds were kindly supplied by Field Crops Research Institute, Department of Leguminous Crops, Sakha Agricultural Research Station.

e. Microorganisms studied:

Rhizobium leguminosarum biovar *Viceae* as N_2-fixing bacteria comprised 8 isolates. They isolated from three locations differs in pollution levels (El-Gabal El-Asfar, El-Hamoul and El-Reyad).

2. Study area:

Data in Table (1) showed the locations of collected samples.

Table (1):Location of samples collection.

No.	Governorate	Area	Pollution case	Sample type
1	Kafr El-Sheikh	El-Reyad	Control	Water Soil Plant
2		El-Hamoul	Moderately Polluted*	Water Soil Plant
3	Cairo	El-Gabal El-Asfar	Heavily Polluted**	Water Soil Plant

*** Talha, N.I. (2003).** **** Eman, M.A. (2005).**

3. Isolation and purification of rhizobial isolates:

To isolate the different rhizobium isolates, faba bean plants were uprooted from the soil and the root were gently rinsed in water to remove the adhered particles of soil. Healthy nodules were separated from the root, immersed for 10 seconds in 95% ethanol, then soaked in 0.1% acidified mercuric chloride for three minutes and rinsed several times in sterilized water, with a sterile glass rod individual nodules were crushed in sterile test tubes containing one milliliter of sterile distilled water. One loopful of each nodule suspension was streaked on yeast extract mannnitol agar (YEMA) medium (Vincent, 1970) containing either congored or bormothymol blue (BTB). Singel colony streaked on test tubes plant containing (YEMA) medium containing 1 g calcium carbonate L^{-1} (Somasegaran and Hoben, 1994).

4. Evaluation and survival of rhizobial isolates:

a. In laboratory (*in vitro*):

The growth and survival of rhizobial isolates were evaluated for their response to different concentrations of the tested heavy metals (Pb and Cd) in liquid media namely (YEM). The composition of different concentrations for heavy metals used are as follows:

- Media treated with Pb at the rates of zero, 100, 150, 200, 250, 300, 350 and 400 ppm Pb.
- Media treated with Cd at the rates of zero, 20, 40, 60, 80, 100 and 120 ppm Cd.

A starter cell suspension was prepared in (YEM) liquid medium for each isolate. Sterilized conical flasks (250 ml capacity) containing 100 ml of liquid media was prepared and enriched with different concentrations of heavy metals under investigation. Flasks were inoculated with 0.5 ml (10^8 cell/1 ml) of the starter rhizobial cultures previously prepared. The flasks were incubated on a rotary shaker (160 ppm min^{-1}) at 28ºC for 1-3 days according to the growth rate of rhizobial isolates. The growth was determined by counting the viable number by plate count method (Vincent, 1970) using yeast extract mannitol agar medium.

b. In green house (*in vivo*):

The experiment was a 4 x 8 x 5 complete factorial combination of each metal used. It was comprised 4 concentrations for each metal and 8 inoculation treatments (rhizobial isolates) with 5 replicates for each treatment.

Soil preparation:

The sandy soil was treated several times with 0.1 N HCl followed by washing with distilled water several times to remove nitrogen and other minerals (El-Nady, 2005).

Soil treated with Pb at the rates of zero, 2000, 4000 and 6000 g Pb/kg soil and Cd levels were at the rates of zero, 40, 80 and 120 g Cd/kg soil by dissolving them in distilled water according to the desired concentration and added to the soil and left for two weeks (Howaida, 2004).

Faba bean seeds were surface sterilized using alcohol 75% for 3 minutes and then 1 g/L $HgCl_2$ solution for 2 minutes and finally washed repeatedly with sterile water.

Seeds were planted in the pots (15 cm in diameter and 20 cm in depth) loaded with 3 kg air dried washed and sterilized sandy soil. Two seeds were sown in each pot and irrigated with distilled water and nutrient solution for twice weekly (Skradleta *et al.,* 1984). After 50 days from sowing, plants were uprooted and evaluated for plant dry weight, nodule dry weight and N%.

Chemical analysis:

a. Soil:

Total content of heavy metals (Pb and Cd) extracted by Aqua Regia solution (Cottenie *et al.,* 1982), while available heavy metals extracted by di-ethylene triamine penta acetic acid (DTPA), (Lindsay and Norvell, 1978). Both total and available extracted elements were determined spectrophometerically by atomic absorption technique PERKIN ELIMER 3300.

b. Plant:

Total nitrogen content of plant was determined by microkejeldahl method as described before by Page (1982).

Total content of heavy metals (Pb and Cd) were determined according to Bary (1988).

Statistical analysis:

Data obtained from experiment treatments were subjected to the analysis of variance and treatments means were compared using the L.S.D. method according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Records of Table 2 indicated the concentrations of Pb and Cd elements in soil, plant and water samples collected from agricultural locations with different degree of heavy metals pollution (El-Reyad, El-Hamoul and El-Gabal El-Asfar).

The data showed gradient concentrations of Pb and Cd (ppm). El-Gabal El-Asfar samples (soil, plant and water) contained the highest concentrations followed by El-Hamoul samples then El-Reyad samples. Plant organs contained different concentrations, whereas nodules had the highest Pb and Cd concentrations followed by roots then shoots. The concentrations of Pb and Cd mostly exceeded permissible levels especially those brought about from El-Gabal El-Asfar and El-Hamoul locations. Fields of these two locations regularly irrigated with polluted waste water since many years, the matter which cause severe pollution to these soils. (Talha, 2003 and Eman, 2005). Also, Mahmoud (2000) and found high Pb and Cd concentrations in faba bean plant grown in El-Gabal El-Asfar site and Talha (2003) measured high levels of Pb and Cd in soybean plant collected from El-Hamoul region.

***In vitro* evaluation of the effect of heavy metals on rhizobial isolates:**

Growing the investigated isolates of *R. leguminosarum* bv. *Viceae* on YEMA medium supplied with Pb at the levels of zero to 400 ppm, sowed a marked variation among these isolates (Table 3 and Figure 1).

There is a decrease in the growth on YEMA medium with increasing Pb concentrations. It was noticed that isolates of RH_6 and RH_8 were the most tolerant to higher applied lead concentrations as compared to the other isolates under study, where these isolates had the good ability to grow on YEMA medium supplemented with 350 ppm Pb which attained 2.01 and 2.04 log number respectively. On the other hand, some sensitive isolates had no ability to grow in 300 and 350 ppm.

Table (2): Lead and cadmium mean concentrations (ppm) of soil, plant and water samples from different heavy metals polluted locations.

Location	Soil (ppm)		Plant (ppm)			Water
	Total	Available	Nodules	Roots	Shoots	(ppm)
	Pb					
El-Reyad	102.72	2.46	238.80	167.80	143.90	0.172
El-Hamoul	170.90	3.06	230.70	181.11	161.56	0.471
El-Gabal El-Asfar	347.70	4.63	241.02	262.21	228.15	0.892
	Cd					
El-Reyad	1.53	0.01	11.6	9.6	8.6	0.014
El-Hamoul	3.07	0.04	15.1	11.1	9.3	0.090
El-Gabal El-Asfar	7.54	0.32	16.0	11.8	10.1	0.155
Permissible level of Pb	2-300*	-	0.2-20*	0.2-20*	0.2-20*	5.00**
Permissible level of Cd	0.01-2*	-	0.1-2.4*	0.1-2.4*	0.1-2.4*	0.01**

*** Kabata-Pendias, A. and Pendias, H. (1985)**
**** FAO (1985)**

It was noticed that, the highest level of applied Pb (400 ppm) completely inhibited the growth of all tested isolates.

These results are in agreement with those reported by Mahmoud (2000), who showed significant decrease in growth for *R. leguminosarum* bv. *Viceae* with increasing of lead concentrations in YEMA medium.

Table (3): Log number of *Rhizobium legminosarum* bv. *Viceae* isolates grown on YEMA medium under stress of increased Pb concentrations (ppm).

Isolates	Concentration (ppm)								
	0	100	150	200	250	300	350	400	Mean
RH_1	9.19 **b**	7.11 **d**	5.10 **d**	3.07 **d**	1.85 **e**	0.00 **d**	0.00 **f**	0.00 **a**	3.76
RH_2	9.22 **ab**	7.18 **c**	5.12 **d**	4.08 **c**	3.04 **d**	1.88 **c**	0.00 **f**	0.00 **a**	4.36
RH_3	9.24 **a**	7.21 **ab**	6.198 **ab**	5.16 **ab**	4.12 **c**	3.09 **ab**	1.86 **d**	0.00 **a**	5.27
RH_4	9.24 **a**	7.22 **ab**	6.19 **ab**	5.17 **a**	4.13 **bc**	3.06 **b**	1.80 **e**	0.00 **a**	5.26
RH_5	9.24 **a**	7.20 **bc**	6.15 **c**	5.13 **b**	4.12 **c**	3.09 **ab**	1.89 **c**	0.00 **a**	5.26
RH_6	9.25 **a**	7.24 **a**	6.22 **a**	5.19 **b**	4.17 **a**	3.11 **a**	2.01 **a**	0.00 **a**	5.31
RH_7	9.24 **a**	7.22 **ab**	6.17 **bc**	5.13 **b**	4.10 **c**	3.09 **ab**	1.92 **b**	0.00 **a**	5.26
RH_8	9.25 **a**	7.23 **ab**	6.20 **a**	5.18 **a**	4.16 **ab**	3.10 **a**	2.04 **a**	0.00 **a**	5.31
Mean	9.23	7.20	5.92	4.76	3.71	2.55	1.44	0.00	4.97

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

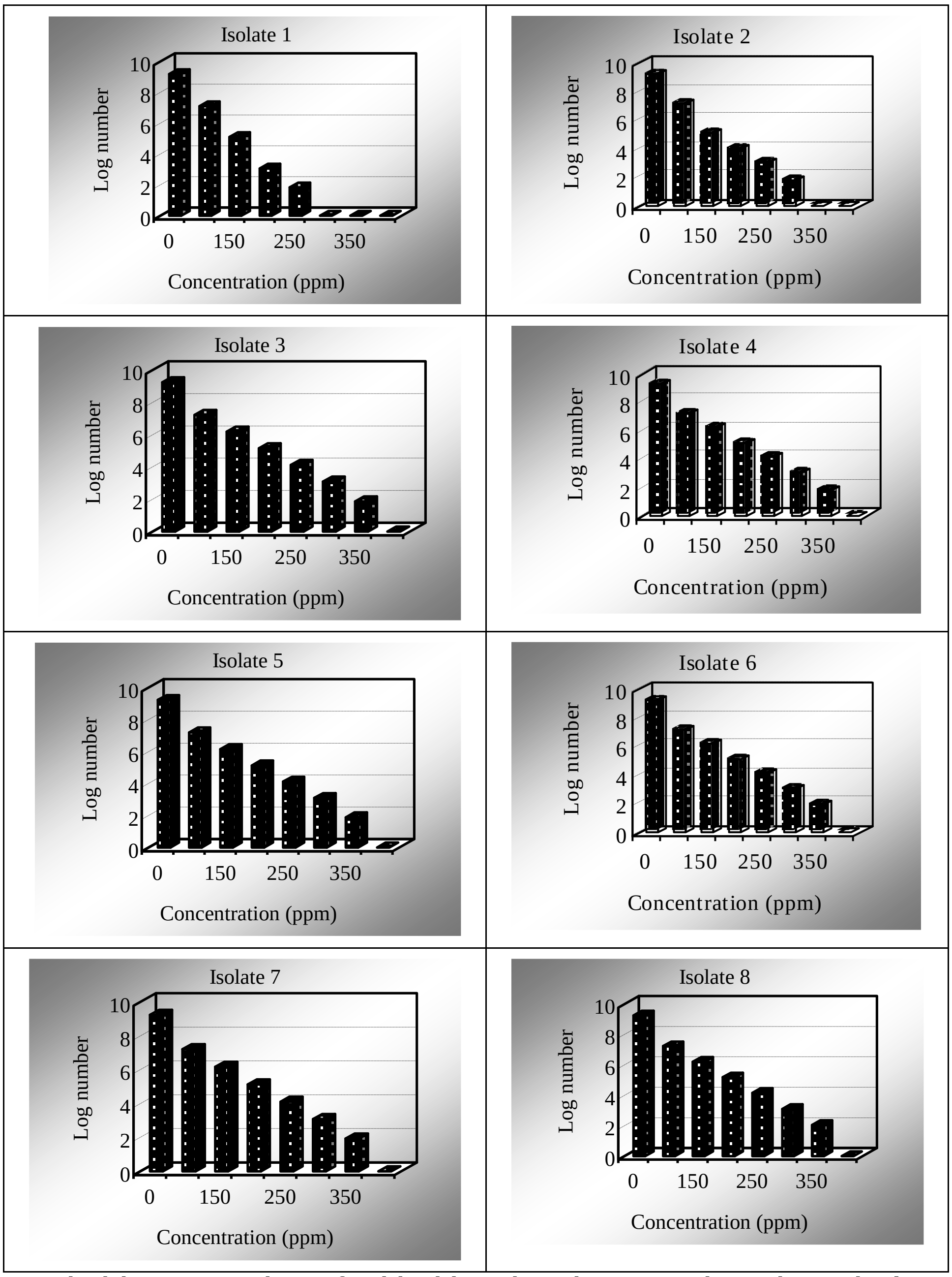

Fig (1): Log number of *Rhizobium legminosarum* bv. *Viceae* isolates grown on YEM-medium under stress of increased Pb concentrations (ppm).

b. Effect of cadmium (Cd):

Data presented in Table 4 and Figure 2 revealed that the increase of Cd concentrations negatively affected the growth of all studied rhizobial isolates. It was found also that the values of cell log number of the tested isolates grown on YEMA medium significantly decreased by increasing Cd concentrations. It was noticed that isolates of RH_1 and RH_2 were the most sensitive to Cd concentrations when grown on solidified YEM medium especially in 80, 100 and 120 ppm. On the other hand, isolates of RH_6 and RH_8 were the more tolerant to higher applied Cd concentrations especially in 100 ppm where gave log values of 1.53 and 1.60, respectively. In addition, the highest level of applied Cd (120 ppm) completely inhibited the growth of all tested isolates.

El-Aziz *et al.* (1991) reported that the ability of the isolates to tolerate metal salts in YEM medium is depended on the isolate of rhizobia and the level of metal-salts used. El-Gamal (1988) showed that the sensitivity of *R. sesbania* increased with increase of Cd concentrations in YEM medium.

Table (4): Log number of *Rhizobium legminosarum* bv. *Viceae* isolates grown on YEMA medium under stress of increased Cd concentrations (ppm).

Isolates	Concentration (ppm)							
	0	**20**	**40**	**60**	**80**	**100**	**120**	**Mean**
RH_1	9.20 **c**	6.11 **c**	3.06 **e**	1.89 **d**	0.00 **f**	0.00 **e**	0.00 **a**	2.89
RH_2	9.21 **bc**	6.10 **c**	3.04 **c**	1.85 **e**	0.00 **f**	0.00 **e**	0.00 **a**	2.88
RH_3	9.24 **ab**	7.33 **b**	5.15 **d**	3.17 **c**	1.50 **e**	0.0 **e**	0.00 **a**	3.77
RH_4	9.27 **a**	7.35 **ab**	6.12 **c**	3.32 **a**	1.57 **d**	1.37 **d**	0.00 **a**	4.14
RH_5	9.26 **a**	7.35 **ab**	6.12 **c**	3.27 **b**	1.64 **c**	1.40 **d**	0.00 **a**	4.15
RH_6	9.28 **a**	7.36 **ab**	6.16 **ab**	3.31 **a**	1.81 **ab**	1.53 **b**	0.00 **a**	4.21
RH_7	9.26 **a**	7.34 **ab**	6.13 **bc**	3.27 **b**	1.78 **b**	1.47 **c**	0.00 **a**	4.18
RH_8	9.28 **a**	7.37 **a**	6.17 **a**	3.32 **a**	1.84 **a**	1.60 **a**	0.00 **a**	4.22
Mean	9.25	7.04	5.24	2.92	1.27	0.92	0.00	3.80

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Effect of heavy metals on different rhizobial isolates *In vivo* evaluation:

a. Effect of Pb on plant dry weight, nodules dry weight and N%:

The effect of inoculation with rhizobial isolates on plant dry weight (g/plant), nodules dry weight (g/plant) and N% of faba bean plant under different concentrations of Pb was presented in Table 5.

From the results, it is evident that increase of Pb in the soil significantly decreased the three plant parameters. It is worthily to mention that not inoculated control plants had no nodules, this because of sterilization of used soil. Many studies obtained similar responses as that of Ali (1982) on the effect of Pb or dry weight of faba bean, Angle and Chaney (1991) on dry weight of alfalfa, Kumar *et al.* (1993) on dry weight of nodules of soybean plants and Poonam *et al.* (2004) on N% of Egyptian clover. On the other hand, inoculation treatments mostly increased the above mentioned plant characters with significant levels inn some cases Nour El-Din *et al.* (2003) found that inoculation of soybean plants with *Bradyrhizobium japonicum* under normal or polluted conditions with Mn increased growth parameters as well as N-content over not inoculated plants.

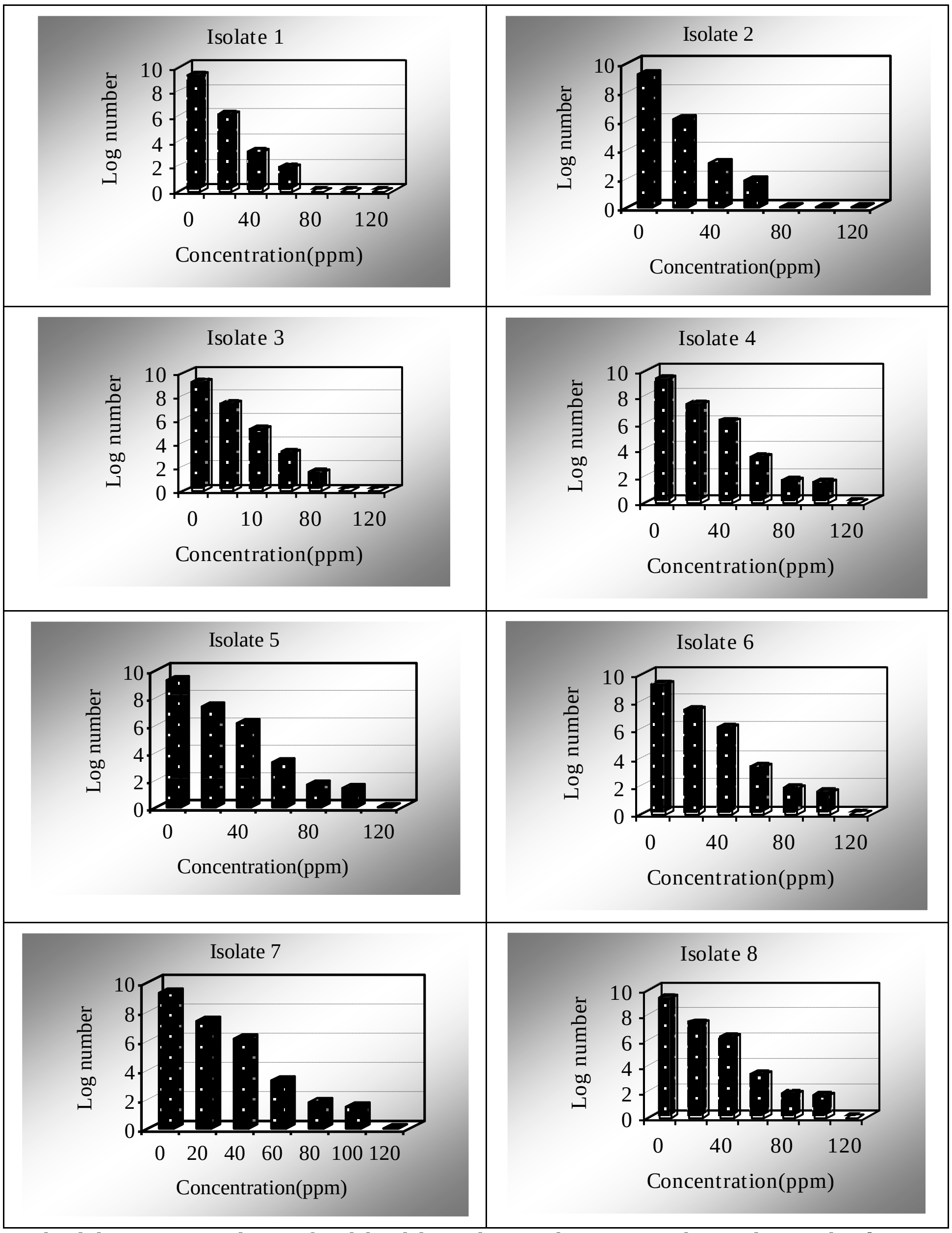

Fig (2): Log number of *Rhizobium leguminosarum* bv. *Viceae* isolates grown on YEMA under stress of increased Cd concentrations (ppm).

Table (5): plant dry weight, nodules dry weight and N% of faba bean plants under inoculation with *Rhizobium* isolates as affected by different concentrations of Pb.

Isolates	Concentration (ppm)				
	0	2000	4000	6000	Mean
	Plant dry weight (g/plant)				
RH_1	3.58 **bc**	1.50 **d**	2.23 **ab**	1.97 **b**	2.32
RH_2	3.45 **bc**	2.31 **bc**	2.00 **ab**	2.50 **ab**	2.57
RH_3	4.34 **a**	1.73 **cd**	2.44 **a**	2.41 **ab**	2.73
RH_4	3.73 **b**	1.85 **cd**	2.09 **ab**	2.29 **ab**	2.49
RH_5	3.71 **b**	1.69 **d**	2.24 **ab**	2.07 **ab**	2.42
RH_6	3.74 **b**	3.05 **a**	2.45 **a**	2.40 **ab**	2.91
RH_7	3.74 **b**	2.82 **ab**	2.14 **ab**	2.72 **a**	2.85
RH_8	3.79 **ab**	3.01 **a**	2.55 **a**	2.48 **ab**	2.96
C	3.07 **c**	2.09 **cd**	1.78 **b**	1.94 **b**	2.22
Mean	3.68	2.23	2.21	2.31	2.61
	Nodules dry weight (g/plant)				
RH_1	0.160 **bc**	0.073 **d**	0.167 **b**	0.57 **bc**	0.114
RH_2	0.177 **abc**	0.267 **b**	0.220 **b**	0.197 **a**	0.215
RH_3	0.207 **abc**	0.180 **c**	0.087 **c**	0.073 **b**	0.137
RH_4	0.183 **abc**	0.130 **cd**	0.173 **b**	0.087 **b**	0.143
RH_5	0.137 **c**	0.187 **c**	0.190 **b**	0.107 **b**	0.155
RH_6	0.183 **abc**	0.363 **a**	0.220 **b**	0.210 **a**	0.244
RH_7	0.250 **a**	0.070 **d**	0.157 **b**	0.117 **b**	0.148
RH_8	0.217 **ab**	0.303 **ab**	0.290 **a**	0.217 **a**	0.257
C	0.00 **d**	0.00 **e**	0.00 **d**	0.00 **c**	0.00
Mean	0.168	0.175	0.167	0.118	0.157
	N (%)				
RH_1	2.167 **a**	1.500 **a**	1.303 **b**	1.323 **a**	1.573
RH_2	2.127 **a**	1.337 **a**	1.157 **b**	1.130 **a**	1.438
RH_3	1.957 **a**	1.360 **a**	1.380 **b**	1.357 **a**	1.013
RH_4	1.703 **a**	1.333 **a**	1.450 **b**	1.383 **a**	1.468
RH_5	1.663 **a**	1.523 **a**	1.520 **b**	1.440 **a**	1.537
RH_6	1.890 **a**	1.697 **a**	1.653 **b**	1.547 **a**	1.697
RH_7	1.767 **a**	1.537 **a**	1.597 **b**	1.503 **a**	1.601
RH_8	1.943 **a**	1.820 **a**	1.760 **b**	1.637 **a**	1.790
C	1.223 **a**	1.297 **a**	1.110 **b**	1.010 **a**	1.160
Mean	1.827	1.489	2.548	1.370	1.808

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Comparison	S.E.D.	L.S.D. (5%)	L.S.D. (1%)
I*C for plant dry weight	0.278	0.554	0.735
I*C for nodules dry weight	0.034	0.068	0.090
I*C for N %	2.353	4.690	6.225

RH: *Rhizobium* isolate

In the same time, there is a remarkable variations between the response of the plant to inoculation with different isolates. The rhizobial isolates RH_6 and RH_8 exhibited the most tolerant ones to Pb pollution, whereas they attained best records especially at high levels of Pb pollution. On contrast, not inoculated N-fertilized plants were the most sensitive ones. They exhibited the lowest records in plant dry weight, nodules dry weight and N%, especially under high concentrations of Pb. It is of interest to remember that rhizobia isolates RH_6 and RH_8 isolated from heavy metals polluted fields

(El-Gabal El-Asfar) which irrigated with sewage water since 50 years and its soil contained high amounts of heavy metals as shown from (Table 2). Also, Nour El-Din (1997) found that inoculation of soybean plants with *B. japonicum* rhizobia enabled efficiency of plant growth even at 2000 ppm Pb pollution.

b. Effect of Cd on plant dry weight, nodules dry weight and N%:

Observation of the effect of inoculation of faba bean plants with different rhizobial isolates on plant dry weight, nodules dry weight and N% in presence or absence of Cd pollution were presented in Table 6.

Table (6): Plant dry weight, nodules dry weight and N% of faba bean plants under inoculation with *Rhizobium* isolates as affected by different concentrations of Cd.

Isolates	Concentration (ppm)				
	0	40	80	120	Mean
	Plant dry weight (g/plant)				
RH_1	3.55 **bc**	2.01 **b**	1.46**ab**	1.17 **ab**	2.04
RH_2	3.45 **bc**	1.97 **b**	164 **ab**	1.45 **ab**	2.13
RH_3	4.34 **a**	2.31 **ab**	1.63 **ab**	1.38 **ab**	2.41
RH_4	3.73 **abc**	2.79 **a**	1.53 **ab**	1.03 **ab**	2.27
RH_5	3.71 **abc**	2.50 **ab**	2.01 **a**	1.45 **ab**	2.42
RH_6	3.74 **abc**	2.61 **ab**	1.65 **ab**	1.60 **ab**	2.40
RH_7	3.50 **ab**	2.39 **ab**	1.24 **b**	1.39 **ab**	2.13
RH_8	4.00 **c**	2.38 **ab**	1.81 **ab**	1.67 **a**	2.46
C	3.11	1.96 **b**	1.58 **ab**	0.97 **b**	1.90
Mean	3.68	2.32	1.62	1.34	2.24
	Nodules dry weight (g/plant)				
RH_1	0.160 **bc**	0.197 **a**	0.087 **c**	0.127 **ab**	0.143
RH_2	0.170 **bc**	0.200 **a**	0.127 **bc**	0.100 **ab**	0.149
RH_3	0.207 **abc**	0.173 **ab**	0.103 **c**	0.80 **b**	0.141
RH_4	0.183 **abc**	0.210 **a**	0.083 **c**	0.077 **b**	0.138
RH_5	0.137 **c**	0.167 **ab**	0.140 **abc**	0.100 **ab**	0.136
RH_6	0.183 **abc**	0.233 **a**	0.203 **a**	0.137 **ab**	0.189
RH_7	0.250 **a**	0.110 **b**	0.150 **abc**	0.157 **a**	0.167
RH_8	0.217 **ab**	0.217 **a**	0.183 **ab**	0.137 **ab**	0.188
C	0.00 **d**	0.00 **c**	0.00 **d**	0.00 **c**	0.00
Mean	0.167	0.167	0.120	0.101	0.139
	N (%)				
RH_1	2.16 **a**	1.53 **c**	1.34 **f**	1.34 **d**	1.59
RH_2	2.12 **a**	1.38 **d**	1.03 g	0.96 **e**	1.37
RH_3	1.95 **b**	1.39 **d**	1.42 **ef**	1.39 **cd**	1.54
RH_4	1.70 **c**	1.37 **d**	1.49 **de**	1.41 **cd**	1.49
RH_5	1.66 **c**	1.56 **c**	1.56 **cd**	1.48 **bc**	1.56
RH_6	1.89 **b**	1.73 **c**	1.68 **b**	1.57 **ab**	1.72
RH_7	1.76 **c**	1.57 **c**	1.63 **bc**	1.53 **b**	1.62
RH_8	1.94 **b**	1.86 **a**	1.78 **a**	1.67 **a**	1.81
C	1.22 **d**	1.32 **d**	0.96 g	0.91 **e**	1.10
Mean	1.827	1.527	1.435	1.367	1.53

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Comparison	S.E.D.	L.S.D. (5%)	L.S.D. (1%)
I*C for plant dry weight	0.289	0.576	0.765
I*C for nodules dry weight	0.033	0.065	0.086
I*C for N %	0.051	0.102	0.135

RH: *Rhizobium* isolate

Inoculated treatments increased the above mentioned plant parameters as compared with the not inoculated treatment, with significant variations in most cases. Results of Mahmoud (2000) were in accordance with the present results, in which the increase in growth parameters of faba bean plants was due to inoculation with specific rhizobia. On the other hand, increasing Cd concentrations significantly decreased these plant parameters. Results of Ali (1982) are in agreement with these observations as they reported that increasing Cd concentrations lead to decrease of growth of faba bean plants.

In addition, there is an obvious variation among the effect of different rhizobial isolates in relation to Cd pollution. The isolates RH_6 and RH_8 showed good tolerance to Cd, as they caused the highest increase in all studied parameters, especially under high levels of Cd pollution. So that, the inoculation of plants grown under Cd pollution with heavy metals tolerant rhizobia is very ungent to help the plant to circumvent the unfavourable conditions resulted from the Cd pollution.

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سمية الرصاص والكادميوم على العلاقة التكافلية بين الفول البلدى والريزوبيا والأثر المعالج باستخدام الريزوبيا

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*** قسم الميكروبيولوجيا الزراعية ـ كلية الزراعة ـ جامعة المنصورة**

**** قسم الميكروبيولوجيا الزراعية ـ معهد بحوث الاراضى والمياه والبيئة ـ مركز البحوث الزراعية**

اجريت تجربتى معمل وصوبة لدراسة تأثير التلوث بالرصاص والكادميوم على ريزوبيا الفول البلدى ونبات الفول البلدى وكذلك دور التلقيح بالريزوبيا المتحملة للعناصر الثقيلة فى تخفيف تأثير سميه العناصر الثقيلة.

تم عزل العديد من عزلات ريزوبيا الفول البلدى من حقول الفول المروية بمياه صرف ملوثة (بالجيل الاصفر ومنطقة الحامول). والتى احتوت التربه بها على درجات مختلفة من التلوث بالعناصر الثقيلة بالاضافة الى موقع مرى بمياه نظيفة (منطقة الرياض)

تبين ان عينات التربة والماء والنبات المأخوذة من الجبل الاصفر احتوت على اعلى تركيزات للرصاص والكادميوم تبعها عينات منطقة الحامول ثم عينات منطقة الرياض.

عرضت عزلات الريزوبيا لتركيزات مختلفة من الرصاص والكادميوم وضعت بالبيئة السائلة للريزوبيا وتبين ان اعداد الريزوبيا كانت تقل وبدرجات مختلفة مع زيادة تركيزات العناصر الثقيلة. العزلات RH_8, RH_6 كانت اكثر العزلات تحملا للعناصر الثقيلة وهى التى عزلت من مواقع ملوثة بينما العزلات رقم RH_2, RH_1 والتى عزلت من تربة نظيفة اظهرت اعلى حساسية للعناصر الثقيلة.

قل الوزن الجاف للنبات (جرام/نبات) والوزن الجاف للعقد الجذرية (جرام/نبات) ونسبة النتروجين فى النبات (%) كلما زاد تركيز الرصاص والكادميوم وكانت الزيادة معنوية.

زاد التلقيح لنبات الفول البلدى بعزلات الريزوبيا المختلفة من خصائص النبات المدروسة عنه فى معاملة الكنترول المسمد وبدون تلقيح وذلك تحت الظروف الطبيعية والملوثة.

حققت النباتات الملقحة بالعزلات RH_8, RH_6 اعلى تحمل لسميه العناصر الثقيلة. ولقد اعطوا اعلى نتائج للوزن الجاف للنبات والعقد الجذرية ونسبة النتروجين حتى فى وجود التركيزات المرتفعة من الرصاص والكادميوم.

لذلك نوصى بتلقيح نباتات الفول البلدى المنزرعة فى مواقع ملوثة بالعناصر الثقيلة بسلالات الريزوبيا المتحملة للعناصر الثقيلة وذو الكفاءة العالية فى التثبيت الازوتى لتخفيف التأثير الضار لهذه العناصر السامة على النبات.