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Remediation Effect of Some N_2-Fixing Bacteria on Pea Plants Irrigated with Heavy Metals Polluted Drainage Water*

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A FIELD experiment at El-Hamoul district, Kafr El-Sheikh Governorate, Egypt, was conducted to study the effect of inoculation with some N_2-fixing bacteria(rhizobia and/or *Azotobacter*), and their combinations with farmyard manure and gypsum in reducing absorption of heavy metals by pea plants. The field of study was located at area irrigated with polluted drainage water. The heavy metals; Cd, Ni, Pb, Zn, Mn and Cu were studied. The results indicated that applied amendments generally showed notable decrease in heavy metals concentration in plant tissues (straw and grains) except the treatment (rhizobia + *Azotobacter*) which attained a positive effect of Cu only. The treatments (rhizobia) and (*Azotobacter* + farmyard manure + gypsum) were the superior; Rhizobial inoculation lowered straw heavy metal concentrations by 25.0, 25.7, 28.8, 37.6, 9.6 and 34.8% for Cd, Ni, Pb, Zn, Mn and Cu, respectively. The treatment (*Azotobacter* + farmyard manure + gypsum) resulted 28.6, 29.5, 26.3, 34.9, 24.9 and 42.2% decrease than control in the same order. In addition, studied amendments significantly increased dry weight of shoots. Rhizobia, rhizobia+ *Azotobacter* and rhizobia + *Azotobacter* + farmyard manure treatments significantly increased number and dry weight of nodules. Meanwhile, treatments contain rhizobia and/or *Azotobacter* saved 75% and 25% of N-fertilizers, respectively. On the other hand, available heavy metals (DTPA extraction) were increased except for Zn.

Keywords: N_2- Fixing bacteria, Soil conditioners, Heavy metals, Water pollution.

In the last years, it is noticed that soil pollution with heavy metals was increased

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as automobile and industrial activities increased, and also as the use of drainage and sewage water in crop irrigation. Zein *et al.* (1998) found that soils irrigated with polluted drainage water, such as wastewater effluent of oil and soap and sugar beet factories, were higher in Zn, Ni, Co, Cd and Pb content relative to that irrigated by Nile Water.

Also, irrigation with heavy metals polluted drainage water increased DTPA-extractable heavy metals from soils and their content in seeds of four soybean varieties than that irrigated by Nile or mixed water (Zein *et al.*, 1996-b). Plant absorption of these metals represents a big danger for human health and crop productivity. Thus, recent investigation are focused on decreasing absorption of these metals (Jensen *et al.*, 1994 and Baker *et al.*, 1994), by physicochemical methods, but these methods are not economical to be applied in a large scale except in the areas of high economical value. Other researchers focused on decreasing the absorption of these metals by immobilized it in soil to be in forms unavailable for plant utilization (Banks *et al.*, 1994 and Petruzzelli *et al.*, 1994). The later technique is more applied and less expensive (Bakey *et al.*, 1994). Recently, there has been an increasing awareness that much of the soluble metals in our environment has been chelated by organic members to form naturally sedimentable metal-rich particles (Beveridge and Koval, 1981). Bacteria are ubiquitous through nature and consist of a variety of high charged homo and heteropolymers, some of which (*e.g.* those making up the walls) are remarkably resistant to degradation. Cell walls have a high anionic charge density and consequently interact strongly with the electropositive metal rich ions dissolved in the environment to accumulate large quantities of bound metals. As a consequence, bacteria act as biological exchange resins and are surprisingly rich sources of particulate metals, especially in older cultures (Beveridge Koval, 1981 and Flemming and Trevors, 1989).

Organic matter may also be used as decontaminator for heavy metals as it increases the fixation of the heavy metals on the soil particles. Scialdone *et al.* (1980) reviewed studies which demonstrated that a negative effect of organic matter on heavy metals solubility was noticed.

Likewise, the presence of calcium ions in the soil decreased heavy metals uptake by plants, as it makes as antagonism to heavy metals and accordingly decrease its harmful effect (Zimdahl Koeppe, 1979; Diab *et al.*, 1991 and Nour El-Din, 1997).

As a consequence, the aim of the present study is to investigate the effect of some N_2-fixing microorganisms (rhizobia and *Azotobacter*) in addition to some naturally soil amendments (farmyard manure and/or gypsum) on decreasing heavy metals absorption by pea plants irrigated with heavy metals (Cd. Ni, Pb, Zn and Mn) polluted drainage water.

Material and Methods

Inoculant strains

a. *Rhizobium leguminosarum* biovar *viceae*: is kindly provided by Sakha Agricultural Research Station Lab. of Soil Microbiology .

b. Azotobacter spp.: is isolated by Sakha Agricultural Research Station Lab. of Soil Microbiology .

Soil amendments used

a. Farmyard manure : at level of 10 ton/fed.
b. Gypsum : at level of 3 ton/fed.

Plant species used : pea plant (Little Marvel var.)

Media used: Mannitol yeast extract medium:

Medium (1): (C.F. Somasegaran and Hoben, 1985) for cultivation of rhizobia: Mannitol, 10.0 g; K_2HPO_4, 0.5 g; $MgSO_4$- $7H_2O$, 0.2; g; NaCl, 0.1 g; yeast extract, 0.5 g and distilled water, 1.0 liter. pH was adjusted to 7 and medium was autoclaved at 121°C for 15 min.

Medium (2): (Vancura and Mancura medium, 1960) for isolation and cultivation of *Azotobacter* : Sucrose, 30.0 g; K_2HPO_4, 0.16 g; NaCl, 0.2 g; $MgSO_4$- $7H_2O$, 0.2 g; $CaCO_3$. 2.0 g; $Fe_2(SO_4)_3$ 0.005; g; Na_2MO_4, 0.005; $NaBO_3$, 0.005 g and distilled water, 1.0 liter. The medium was adjusted to pH 7 and autoclaved at 121°C for 15 min.

In order to study the remediation effect of some biological and soil amendments on pea plants grown in a field irrigated with heavy metals polluted drainage water, six treatments were included as follow:

(1) T : Traditional (fertilized with recommended doses of N (70 kg N/fed) and P (30 kg P_2O_5/fed).

(2) R : Inoculated with rhizobia and fertilized with 15 kg N/fed only.

(3) A : Inoculated with *Azotobacter* and fertilized with 55 kg N/fed.

(4) R+A : Inoculated with rhizobia + *Azotobacter* and fertilized with 15 kg N/fed only.

(5) R+A+F : Inoculated with rhizobia + *Azotobacter* and dressed with 10 ton /fed farmyard manure, and fertilized with 15 kg N/fed only.

(6) A+F+G : Inoculated with *Azotobacter* in addition to dressing with 10 ton /fed farmyard manure and 3 ton/fed gypsum and fertilized with 15 kg N/fed only.

The present experiment was conducted during winter season of 1998 at a farmer area at Al-Hamoul district, where the area at this location irrigated with drainage water (from El-Gharbia main drain). The aforementioned six treatments were incorporated in completely randomized design with four replications. The soil and irrigation water analysis were shown in Table 1. Experimental field was prepared and divided into plots, each of 10 x 20 m^2.

TABLE 1. Some chemical analysis of irrigation water and soil of studied location (El-Hamoul).

Soil and water sample	EC dSm^{-1}	Soluble ions ($meq\ L^{-1}$)								SAR
		$CO_3^{=}$	HCO_3^-	Cl^-	$SO_4^{=}$	Ca^{++}	Mg^{++}	Na^+	K^+	
Irrigation water	1.77	-	5.40	5.24	7.13	3.6	3.9	10.0	0.27	5.15
Soil paste extract	6.46	0.00	3.60	42.0	62.90	26.95	20.90	60.0	0.65	2.51
	Heavy metals content (ppm)									
	Cd		Ni		Pb		Mn		Zn	
Irrigation water	10		33		45		132		151	
Soils										
a) Available	0.2		1.33		3.92		21.4		1.9	
b) Total	2.3		78.4		37.5		436.0		92.0	

Plots of all treatments were fertilized with 30 kg P_2O_5 as super phosphate 15% and 24 kg K_2O/fed as potassium sulphate 48% before sowing. While nitrogen fertilizer is added with the aforementioned levels for each treatment.

Inoculation of seeds with rhizobia and/or *Azotobacter* were taken before sowing by mixing inoculant slurry and arabic gum with seeds and remain till dry far from sun light. Inoculated and uninoculated seeds were directly sown and irrigated.

Plant and soil samples were collected at 45 and 75 days of sowing. Plant dry weight, number and dry weight of nodules were determined. Nitrogen content of seeds and heavy metals concentrations of plant samples were determined according to Chapman and Pratt (1961). Available heavy metals of soil were extracted by DTPA as mentioned by Lindsay and Norvell (1978) and metals were measured using the atomic absorption spectrophotometer of Perkin Elmer 3300. Also, bioconcentration ratio were calculated as shown by Alloway (1995) as the following:

$$\text{Bioconcentration ratio} = \frac{\text{Concentration of plant heavy metals (ppm)}}{\text{Concentration of available heavy metals in soil (ppm)}}$$

All data described in this study are the means of 4 replicates. Data of dry weight of plant, number and dry weight of nodules and nitrogen content were statistically analyzed on the bases of one way analysis of variance according to Snedecor and Cochran (1968).

Results and Disussion

Number and dry weight of nodules, dry weight of shoot, and nitrogen content

Data represented in Table 2 indicated that inoclation with rhizobia (R) solitary or in combination with *Azotobacter* (A) and/or gypsum (G) significantly enhanced the nodulation process on the roots of pea plants (number and dry weight of nodules). Whereas the treatments which, received rhizobia gave a number of nodules represented by 20, 14 and 12 nodules/plant at 45 days old and 22, 13 and 10 at 75 days old for the treatments of R, R+A+F and R+A, respectively, compared to 6 and 8 for traditional treatment (T) at 45 and 75 days old respectively. While, the un-inoculated treatment (T) or those inoculated with A or A + F + G gave the least number of nodules (6, 2 and 3 at 45 days and 8, 3 and 4 at 75 days, respectively). Dry weight of nodules, similarly, attained the same trend.

Data recorded with Armanios *et al.* (1996) are coincided with this results, whereas they indicated that inoculation of faba bean with *Rhizobium* leguminosarum biovar *vicieae* increased number and dry weight of nodules. In the same time, Chaudri *et al.* (1992) found that polluted soils with heavy metals had a low number of native rhizobia.

TABLE 2. Effect of bacterial inocualation and soil amendments on number and dry weight of nodules, dry weight of shoot and nitrogen content of pea plants.

Treatment	Number of nodules/plant		Dry weight of nodules (mg/plant)		Dry weight of shoot (g/plant)		Nitrogen content (mg/plant)
	at 45 days	at 75 days	at 45 days	at 75 days	at 45 days	at 75 days	at 75 days
T	6.0 b	8.0 b	4.0 a	6.0 a	3.7 a	4.8 a	71.2 a
R	20.0 e	22.0 d	31.0 d	41.0 d	5.2 e	7.8 e	105.7 e
A	2.0 a	3.0 a	3.0 a	5.0 a	4.2 b	5.7 b	78.2 b
R + A	12.0 c	10.0 bc	23.0 c	31.0 c	4.6 c	6.9 d	89.8 c
R + A + F	14.0 d	13.0 c	19.0 b	26.0 b	4.8 d	6.2 c	87.7 c
A + F + G	3.0 a	4.0 a	6.0 a	7.0 a	4.5 c	6.6 d	94.2 d

T. traditional (control), R; inoculated with *R. leguminasarum* biovar *viceae*, A; inoculated with *Azotobacter* spp.,
R + A; inoculated with rhizobia and *Azotobacter* spp., R + A + F; rhizobia + *Azotobacter* spp. + farmyard manure,
A + F + G; *Azotobacter* + farmyard manure + gypsum.
Means followed by the same letters are not significantly different according to Duncan's multiple test at 5% level.

In addition, from abovementioned nodulation parameters (specially control treatment) it is suspected that soil of the experimental field did not have a suitable number of indigenous rhizobia to attain a better nodulation. This phenomenon may be due to continuos irrigation with polluted drainage water, which contain high levels of heavy metals like Cd, Ni, Pb, Cu. Mn and Zn as well as its moderate salinity level. Therefore, it is a vital importance of continuous inoculation of the soil with effective, persistence, competitive, and heavy metals tolerant strains of rhizobia for these types of soils.

Likewise, data of Table 2 indicated that all studied treatments significantly increased dry weight of shoots at each of the two stages 45 and 75 days old. The treatments that inoculated with rhizobia were superior. Where, they attained 5.2, 4.6 and 4.8 g/shoot at 45 days and 7.4, 6.9 and 6.2 g/shoot at 75 days old for the treatment R, R + A and R + A + F, respectively compared with 3.7 and 4.8 g/shoot for traditional treatment at 45 and 75 days old, respectively. These results are coincide with that recorded by Somasegaran and Hoben (1985), where they found that rhizobial inoculation increased dry weight of chickpea. Also, Zein and El-Kady (1997) found that *Rhizobium* inoculation significantly increased the concentrations of chlorophyll a, chlorophyll b, carotenoieds, total photopigements content of soybean leaves as well as seed yield, straw yield, biological yield and harvest index. Ewada and Valassak (1988) indicated that inoculation of maize with *Azotobacter chrococcum* increased dry weight of plant, in addition many investigators reported that application of farmyard manure caused marked increase in dry weight of plant as Saleh *et al.* (1992). On the other hand, Zein *et al.* (1996 a and c) and El-Yamani *et al.* (1997) found that application of gypsum improved chemical and physical characteristics of soil which positively reflected on plant growth.

Nitrogen contents (g/shoot) sharply increased due to applied treatments than that of traditional one. These results may be interpreted as inoculation with symbiotic or non-symbiotic N_2-fixing microorganisms and/or application of farmyard manure and gypsum act as the following manner:

1. Increased bioavailability of nitrogen for plant utilization by N_2-fixing microorganisms and addition of organic matter (Nour El-Din 1997 and El-Yamani *et al.*, 1997).

2. Improved chemical and physical characteristics of soil (Saleh *et al.*, 1992 and Zein *et al.*, 1996a, c).

3. Secretion of phytohormones by N_2-fixing bacteria in the rhizospheric area of plant roots (Franssen *et al.*, 1992 and El- Silk 1995).

Available heavy metals of soil

Results of Table 3 indicated that available concentrations of Cd, Ni, Pb and Mn of soil increased, in general, than control with the addition of amendments. Contrarily, Zn concentration exhibited a general trend of decrease than traditional. The increase in available heavy metals caused by applied treatments (bacterial inoculation and/or soil amendments) may be resulted from one or more of the following factors:

1. Increasing organic acids secretions by either indigenous or introduced bacteria, as mentioned by Alexander (1977).

2. Decaying of farmyard manure, due to increasing microbial loads and release of different organic and inorganic acids (Flemming and Trevors, 1989).

3. Forming of HCO_3 resulted from solubilization of CO_2, evolves through microbial respiration, in rhizospheric water (Alexander, 1977).

4. The improving action of applied farmyard manure and gypsum upon physical and chemical properties of soil making up a good conditions for both plant and microbial growth, which in turn produce more acids (Taha *et al.*, 1966 and Zein *et al.*, 1996a).

But in case of Zn, its high absorbence by plants, as a nutrient element, may be the reason for decrease of its available form in soil, especially the applied amendments lead to increase in plant growth.

TABLE 3. Effect of bacterial inoculation and soil amendments on available heavy metals concentration of soil.

Treatment	Available heavy metals (ppm)				
	Cd	Ni	Pb	Zn	Mn
T	0.10	0.09	0.39	0.61	4.0
R	0.06	0.10	0.79	0.44	9.1
A	0.14	0.09	0.80	0.36	7.08
R + A	0.18	0.35	0.77	0.49	5.34
R + A + F	0.10	0.85	0.80	0.74	14.12
A + F + G	0.06	0.20	0.60	0.42	4.75

T; traditional (control), R; inoculated with *R. leguminosarum* biovar *viceae*, inoculated with *Azotobacter* spp.,
R+A; inoculated with rhizobia and *Azotobacter* spp., R+A+F; rhizobia + *Azotobacter* spp. + farmyard manure,
A + F + G; *Azotobacter* + farmyard manure + gypsum.

Heavy metals concentration of pea plants

Results shown in Table 4 and Fig. 1 generally indicated that the applied amendments considerably reducd plant heavy metals concentration in both straw and seeds of pea plants. The effect of rhizobia alone, in most cases, was efficient than that in combination with *Azotobacter*, farmyard manure and/or gypsum.

Although the applied treatments had a general reducing effect on heavy metals concentration in plant tissues (Table 4), there was a specialized influence for each treatment towards different heavy metals. Where, under the experimental conditions, the A+F+G treatment was the superior in decreasing plant Cd and Ni elements by 44.4% and 28.6% for Cd and 25.4% and 25.5% for Ni in grain and straw, respectively. *Rhizobium* treatment was found to be most efficient one for Pb and Zn (35.8 % and 20% for grain; 28.8% and 37.6% for straw, respectively). While R + A + F treatment was the superior for Mn (23.3% for grain and 12.8% for straw). However, regarding to heavy metals together, it was found generally that A + F + G and R treatments had the best influence for decreasing concentrations in plant tissues.

TABLE 4. Effect of bacterial inoculation and soil amendments on heavy metals concentration of pea plants.

Treatment	Heavy metals (µg/g dry weight)											
	Cd		Ni		Pb		Zn		Mn		Cu	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T	0.091	0.84	0.51	5.33	5.03	5.48	125.0	136.3	78.3	97.5	187.5	230.0
R	0.072	0.63	0.48	3.96	3.23	3.90	100.0	85.0	77.0	88.2	150.0	150.0
A	0.083	0.75	0.44	4.98	4.60	4.31	130	102.5	78.3	78.0	150.0	140.0
R + A	0.080	0.83	0.52	5.31	4.36	5.06	128.3	115.0	82.5	86.3	125.0	130.3
R + A + F	0.085	0.62	0.42	5.12	4.98	4.91	120.0	117.5	60.0	85.0	187.5	150.0
A + F+ G	0.057	0.60	0.36	3.76	4.96	4.03	120.0	88.8	70.0	73.3	183.3	133.0

T; traditional (control), R; inoculated with *R. leguminosarum* biovar *viceae*, A; inoculated with *Azotobacter spp.*,
R+A; inoculated with rhizobia and *Azotobacter* spp., R + A F; rhizobia + *Azotobacter* spp. + farmyard manure,
A + F + G; *Azotobacter* + farmyard manure + gypsum.

These results are coincided with many investigators, where Kurek and Kobus (1990) found that plants inoculated with *R. trifolii* accumulated less Cd than plants fertilized with mineral nitrogen fertilizer, Nour El-Din (1997) found the same effect when soybean plants were inoculated with *B. japonicum*. The reason of this phenomenon still totally unclear. But many investigators mentioned some consequences in this respect summarized in the following points:

1. Bacteria have a high anionic charge and consequently act as a biological exchange resins and are rich sources of particulate metals, especially in older cultures (Flemming and Trevors, 1989).

2. The nodules formed in the plant roots due to efficient inoculation with specialized rhizobia was found by Rabie *et al.* (1989) to accumulate high amounts of heavy metals than roots and shoots, and thus may be partially prevent translocation of them via root and shoot.

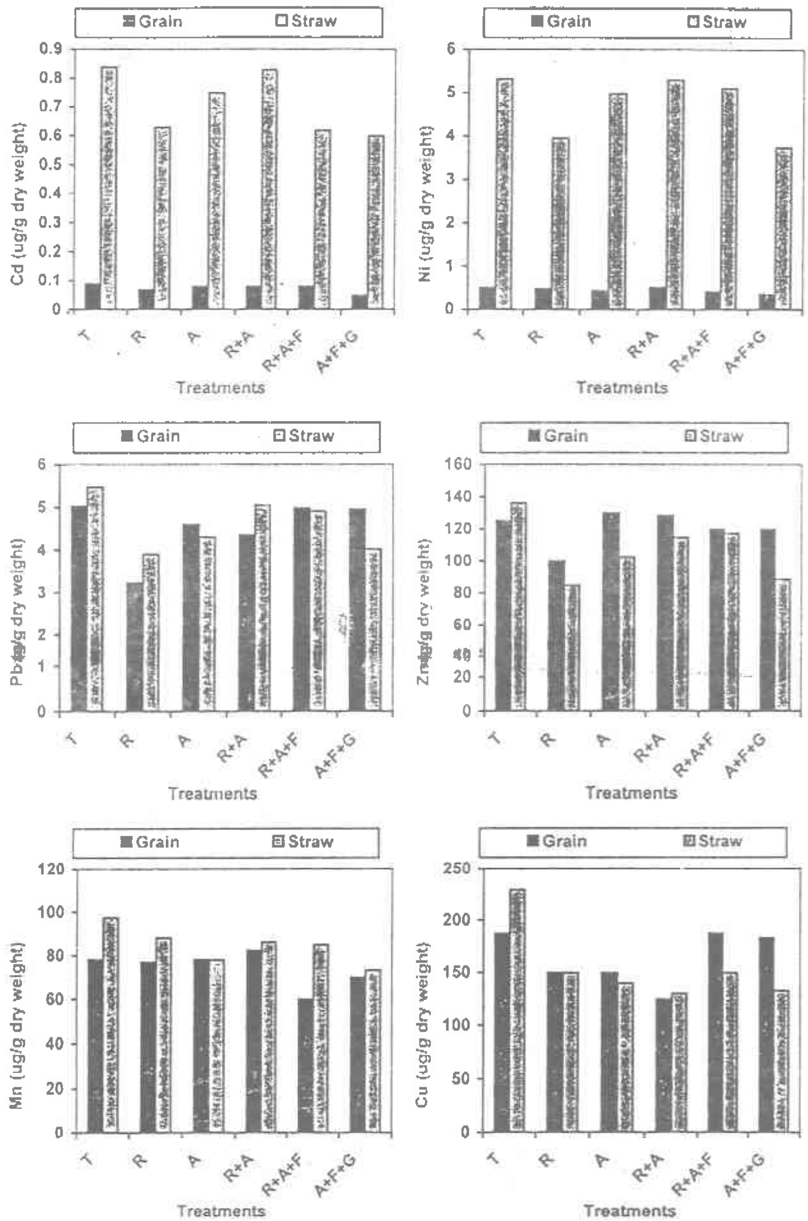

Fig. 1. Effect of bacterial inoculation and soil amendments on heavy metals concentration of pea plants.

3. The polysaccharides found in bacteria as rhizobia and *Azotobacter* cell envelopes may play an important role in scavenge and chelated heavy metals from its media (Banerjee and Sharma, 1994). Similar finding were found by Gueven *et al.* (1995), they indicated that lead was retained in highest amounts by algal polysaccharides.

4. Bacterial inoculation makes plants more vigorous and activate their metabolic activities to tolerate heavy metals influence by different ways as for example: (a) Exclusion mechanism: which means that the organisms have an ability of active discrimination and selective sorption of potentially available ions or compounds in the substrate (Tyler *et al.*, 1989). They added that of great importance is a selective transport to the shoots of ions sorbed from the substrate by the roots. (b) Immobilization mechanism: as species tolerance to heavy metals may possess the abilities of such immobilization exist, including binding to cell wall constituents to thioneins, organic acids, sulphides..., etc. This occurs either diffusely in or on the tissue and cells or in particular storage organs. (c) Excretion mechanisms: Tyler *et al.* (1989) suggested many mechanisms for excretion of heavy metals. Some metal salts are secreted as gutation from leaves, but the mechanisms of this foliar release still obscure.

Organic matter, also, were found by many authors to chelate heavy metals to be unavailable for plant absorption (Scialdyne *et al.*. 1980 and Nour El-Din, 1997). Zimdahl and Koeppe (1979) in this connection, considered the addition of organic matter as a feasible means of reducing the effects of lead pollution. Likewise, Diab *et al.* (1991) and Brown & Brinkmann (1992) concluded that the p+esence of calcium ions in soil decrease lead uptake by plant and consequently decrease its harmful effect. The mechanism of calcium in decreasing effect of lead may be ascribed to Ca-Pb competition for initial exchange sites (*e.g.* those of proteins in the endoplasmic reticulum of cell).

Bioconcentration ratio (BC)

Bioconcentration ratio is represented in Table 5. This parameter is an indicator for the bioavailability of metals absorbed by plants. Except for Cd, values of these ratios are generally lower in case of amended treatments than that of traditional one (control). Thus, it was found that most the used amendments were efficient in decreasing bioavailability of studied metals. Therefore, these amendments can be beneficial materials in decreasing plant uptake of heavy metals. In this connection, Babukutty and Jacob (1995) reported that bioconcentration ratio is useful in quantifying the metal bioavailability. Finally, it may be concluded that the studied amendments are with considerable importance not only in decreasing heavy metals concentration of plant but also in increasing crop productivity, saving large amounts of nitrogen fertilizer as well as sustainable improvement of chemical and physical characteristics of soil.

TABLE 5. Effect of bacterial inoculation and soil amendments on bioconcentratios of heavy metals in pea plants.

Treatment	Cd	Ni	Pb	Zn	Mn
T	4.8	59.2	14.05	223.4	24.9
R	10.5	39.6	4.94	193.2	9.7
A	5.4	55.3	5.39	284.7	11.0
R + A	4.6	15.2	6.57	234.6	16.2
R + A + F	6.2	6.0	6.14	158.8	6.0
A + F + G	10.0	18.8	6.72	211.4	15.4

T; traditional (control), R; inoculated with *R. leguminosarum* biovar *viceae*, A; inoculated with. *Azotobacter* spp.,
R+A; inoculated with rhizobia and *Azotobacter* spp., R+A+F; rhizobia +*Azotobacter* spp. + farmyard manure,
A + F + G; *Azotobacter* + farmyard manure + gypsum.

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تأثير المعالجة ببعض البكتريا المثبتة للازوت الجوى ومصلحات التربة على نباتات البسلة التى تروى بمياه صرف ملوثة بالمعادن الثقيلة

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أقيمت تجربة حقلية بمركز الحامول محافظة كفر الشيخ - مصر لدراسة تأثير التلقيح ببعض البكتريا المثبتة للأزوت الجوى (ريزوبيا و/ أو ازوتوباكتر) والاسمدة العضوية Farmyard manure (FYM) والجبس وتوليفات بينها وذلك على تقليل امتصاص نبات البسلة للعناصر الثقيلة. ويقع الحقل الذى أجريت فيه الدراسة فى منطقة تروى بمياه صرف ملوثة. وكانت العناصر الثقيلة التى تم دراستها هى الكادميوم، والنيكل ، والرصاص ، والزنك والمنجنيز والنحاس. وقد أوضحت النتائج بصفة عامة أن معظم المعاملات أدت إلى انخفاض ملحوظ فى تركيز العناصر الثقيلة فى الاجزاء النباتية (قش وحبوب) فيما عدا معاملة التلقيح بالـــ (ريزوبيا + ازوتوباكتر) التى أدت إلى زيادة فى النحاس فقط. وكانت أفضل المعاملات هى التلقيح بالريزوبيا و (ازوتوباكتر + مخلفات عضوية + جبس) حيث خفضت معاملة التلقيح بالريزوبيا بتركيز العناصر الثقيلة بالسيقان بنسب ٢٥ ، ٧ . ٢٥ ، ٨ . ٢٨ ، ٦ . ٣٧ ، ٦ . ٩ ، ٨ . ٣٤ ٪ لكلا من الكادميوم والنيكل والرصاص والزنك والمنجنيز والنحاس على الترتيب. كما أدت معاملة (ازوتوباكتر + مخلفات عضوية + جبس) إلى خفض هذه العناصر بنسب ٦ . ٢٨ ، ٥ . ٢٩ ، ٢ . ٢٦ ، ٩ . ٣٤ ، ٩ . ٢٤ ، ٢ . ٢٤ ٪ بنفس الترتيب عن معاملة المقارنة

بالاضافة إلى ذلك فإن المعاملات المدروسة زادت بدرجة معنوية الوزن الجاف للسيقان. وقد أدت كلا من المعاملات: ريزوبيا وريزوبيا + أزوتوباكتر ، ريزوبيا + ازوتوباكتر + مخلفات عضوية إلى زيادة معنوية فى عدد ووزن للعقد البكترية الجاف وفى نفس الوقت فإن المعاملات التى أحتوت على التلقيح بالريزوبيا والأزوتوباكتر وفرت ٧٥٪ ، ٢٥ ٪ من النتروجين السمادى على الترتيب. وعلى الجانب الأخر فإن العناصر الثقيلة الميسرة (المستخلصة بالـــ DTPA) قد زات ما عدا الزنك.