World Journal of Agricultural Sciences 3 (1): 111-122, 2007
ISSN 1817-3047

Responses of *Lablab Purpureus-rhizobium* Symbiosis to Heavy Metals in Pot and Field Experiments

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Abstract: *Lablab purpureus* (L.) Sweet (Kashrangeeg) is a native grain forage legume in Aswan area (Nubia). The effect of different levels of Cd, Zn, Co and Cu added at different levels (control, 50, 100, 150 and 200 mg kg^{-1} soil) in pot experiment and the effect of heavy metal contaminated soil in a field experiment on nodulation, nitrogen fixation and growth of *Lablab purpureus*. The accumulation of heavy metals in different plant parts and nodules were investigated. Nodule number and their mass were enhanced in soil treated with 100 mg kg^{-1} soil of Co (23.2/plant and 2.95 g^{-1} $plant^{-1}$) and Cu (21.8 plant and 2.8 g^{-1} $plant^{-1}$) respectively, while inhibited at the other levels of treatment. Cd (6.5 plant and 1.58 g^{-1} $plant^{-1}$) and Zn (10.4/plant and 1.6/g $plant^{-1}$) showed more inhibitory effect than Co and Cu. Nitrogenase activity was severely inhibited with the increased heavy metal concentration. Na, K and Ca content of shoot was decreased with the increased levels of Cd (35, 2.7 and 90 μg g^{-1} dry weight shoot) and Zn (51, 3.1 and 75 μg g^{-1} dry weight shoot) respectively. In the field experiment, nodules have accumulated heavy metals more than both root and shoot. The root accumulated 125 μg g^{-1} dry weight of Cd which is more than both nodules (100 μg g^{-1} dry weight) and shoot (93 μg g^{-1} dry weight). The accumulation factor of the heavy metals in nodules was higher than those of root and shoot with the exception of cadmium, where the accumulation factor in root (0.62) reached higher level than both nodules (0.48) and shoot (0.38). The accumulation of heavy metals in nodules of the plant is considered as a mechanism by which *Rhizobium/Lablab purpureus* increases the resistance to heavy metal toxicity. The results obtained suggest the possibility of utilizing *Lablab purpureus* in revegetation of soils contaminated by heavy metals. This species has the potential to be used as biomonitor of soil pollution by heavy metals.

Key words: Nitrogen fixation · nodulation · protein content · growth · phytoremediation

INTRODUCTION

Legumes constitute a major portion of human food. In addition, they are source of oils, fibers, timber and raw materials for many products. The incorporation of nitrogen rich leguminous crops is of great importance to soil fertility particularly in the newly reclaimed lands where soil is usually nutrient poor [1]. About 50 cultivars of *Lablab purpureus* (L.) Sweet (Kashrangeeg) are growing within a wide range of neotropical regions of the world as a native grain forage legume. In Egypt the species is growing in Aswan area (Nubia). Extensive production of *L. purpureus* as a high protein grain food and forage legume in Egypt has been reported by Younis [2]. Seed inoculation of *L. purpureus* with effective strains of specific Rhizobia is particularly of great importance for the newly cultivated desert lands to improve nodulation, as well as the efficiency of the root nodules and supports vigorous plant growth [3, 4].

Heavy metals released into the environment over long periods of time reflect the problems and impose environmental changes accurately [5, 6]. Heavy metals operate as stress factors in the environment causing physiological constraints that reduce growth of living organisms. A number of heavy metals, including zinc and copper are required as micronutrients in biological systems to act as cofactors and/or as part of prosthetic groups of enzymes in different metabolic and developmental pathways [7].

Heavy metals in water and soil are taken up by the growing plants, where they reduce the plant growth and impaired its metabolism [8, 9]. Heavy metals are frequently reached to the soil via human activities including sewage, industrial emissions, biosolid application and fertilizers.

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Numerous reports over the last 20 year have documented the adverse effects of metal contaminants on the soil ecosystem [10].

Adverse effects of heavy metals on nodulation and N_2 fixation have been reported for faba bean, soybean and lupine [11, 12]. Thus, [13] found that all Rhizobial strains isolated from nodules of white clover formed in metal-contaminated soils were ineffective in N_2 fixation. *Rhizobia* failed to survive at high Zn and Cd concentrations and there was no N_2-fixation [14, 15]. As pointed out by Dowdy and Ham [16], the growth of soybean plants and the number of nodules per root decreased in soil amended with sewage sludge. Whereas, Abd-Alla [11] reported that sewage sludge at low application rates may significantly improve legume growth on desert soils.

The effect of heavy metals on nodulation and nodule physiology of *Lablab purpureus* is under investigated in Egypt. The objective of the present work is to investigate, in pot experiment and in contaminated field soils, the effect of Cd, Zn, Co and Cu on N_2-fixation, growth and metal accumulation in plant organs and nodules.

MATERIALS AND METHODS

Pot experiment: Plant culture and experimental conditions: Surface-sterilized Lablab seeds were inoculated with 5 ml *Rhizobium* sp. strain I_4 (approximately 10^8 cells ml^{-1}) for 1 h at 30°C. Each five seeds were planted into plastic pots (14 cm diameter) containing 5 kg sterilized clay-sandy soil. The soil was treated with different levels (0 (control), 50, 100, 150 and 200 mg kg-1) of the tested heavy metals (cadmium, zinc, cobalt and copper) by adding 500 ml of each heavy metal solution (added as chloride) separately per 5 kg soil. The soil was thoroughly mixed and allowed to equilibrate for a week and packed into the plastic pots. The seeded pots were irrigated with tap water every other day and with nitrogen-free nutrient solution after 10 days. The nutrient solution contained (μmol l^{-1}) K_2SO_4 175, KCl 25, $MgSO_4$ 125, KH_2PO_4 25, $CaCl_2$ 500, Fe-EDTA 5, $MnSO_4$ 0.0125, $CuSO_4$ 0.05, $ZnSO_4$ 0.025, H_3BO_3 2.5, $(NH4)_2MoO_4$ 0.05, $CoCl_2$ 0.125 and $NiSO_4$ 0.0025. Seedlings were thinned out to three per pot after five days. The plants were maintained in open greenhouse. Pots were arranged in a Randomized Block Design with three replicates for each treatment. Plants were harvested at 50 days age. Nodule number and nodule fresh weight were recorded. Plant shoot and root were separated and dried at 80°C for 48 h and their dry weight determined.

Field experiment: Two field plot experiments were conducted in a site having soil contaminated with sewage (Bamban region-Aswan-Egypt). The main source of irrigation water in this site depends mainly upon underground water, with irregular mixed supply of sewage water. Surface-sterilized *Lablab* seeds were inoculated with 75 ml *Rhizobium sp.* strain I_4 (approximately 10^8 cells ml^{-1}) and incubated for 1 h at 30°C. Total of 75 seeds were planted into each plot (4 m^2) in the sewage contaminated field site. Plants were harvested after 50 days. Nodules number and fresh weight were recorded. Plant shoot and root were separated and dried at 80°C for 48 h before determination of the dry weight.

Soil analysis: Soil samples taken for pot experiment and field plot experiment were screened in a 2 mm sieve and the gravel content was discarded. The remainder was kept for mechanical and chemical analysis. For mechanical analysis, the different soil size particles were determined by using the pipette method [17]. For the chemical analysis, chlorides, bicarbonates, calcium, magnesium, sodium and potassium were determined using the saturated soil paste extract method [18]. The electrical conductivity (E.C.) was determined by conductivity meter and pH by pH meter. Carl-Zeiss flame photometer was used for sodium and potassium determination.

Rhizobium inoculum: *Rhizobium* sp. (native strain isolated, identified and donated as *Rhizobium* sp. strain I_4 by Prof. Dr. M. El-Sebaaey, Desert Research institute, El Materya, Cairo Egypt) was used. This bacterial strain was grown in 250 ml Erlemeyer flask containing 40 ml yeast mannitol broth [19] on a rotating incubator shaker for 3 days at 28°C. The composition of the medium was as follows: Mannitol l0, K_2HPO_4 0.5, MgSO4.7H_2O 0.2, NaCl 0.1 (g L^{-1}). Yeast extract 0.5. Volume was made up to one litter with distilled water and adjusted to pH 6.8.

Nitrogenase activity: Nitrogenase activity was assayed by the acetylene reduction method [20]. The test material was incubated in an atmosphere containing 10% acetylene in a closed 250 ml vessel. The amount of ethylene produced after a period of incubation is then measured by gas chromatography (Hewlett Packard 6890) using a glass column (130 x 0.25 cm) filled with activated alumina (80 to 100 mesh), with N_2 as a carrier gas (20 ml min^{-1} at 50° C) and the injector and detector temperature at 150°C. Acetylene reduction was expressed directly as μmol C_2H_4 produced per plant (absolute activity) or per nodule per hour (specific activity).

Determination of protein and leghaemoglobin content: Protein content of nodules (cytosol and bacteroids) was determined according to Lowery [21] after nodule fractionation and bacteroid isolation by employing the methods of Becana [22]. Leghaemoglobin content of nodule cytosol was measured colorimetrically as described by Johnson and Hume [23].

Chemical analysis of plant material: Exact 0.2 g powdered tissue was digested in 2 ml concentrated HNO_3 in a heating block at 180°C until the remaining solution appeared clear. One ml of 30% (w/v) H_2O_2 was added and heating continued to 120°C until effervescence stoped from the decolorized digests. The mixture was made up to 25 ml with distilled water. The insoluble centrifugation fractions were similarly digested in 5 ml concentrated HNO_3. The levels of Cd, Zn, Co, Cu, Na, K and Ca were measured by atomic absorption spectroscopy (Model Varian AA55).

Statistical analysis: The data were analyzed by one-way analysis of variance (PC-state computer program) and the least significant difference (LSD) was used to test the differences between treatments.

RESULTS

Soil analysis:

Pot experiment: Results of the mechanical and chemical analysis of the experimental soil are presented in Table 1. Percentages of sand, silt and clay also were calculated. It is clear that the used Nile valley soil for pot experiment embodied relatively higher proportion of 61.9 and 27.4% as course sand and clay respectively. According to this texture the soil was classified as sandy clay loam. In addition, the soil paste revealed an alkaline pH of 8.12 and E.C. of 1.97 (Mmhos/cm). Chemical analyses showed a higher percentage of Na^+ (12.18 meq l^{-1}) as cations compared with other cations and higher content of Cl^- (16.63 meq l^{-1}) as anions.

Field site: The soil of the two field experiment sites is classified as sandy clay loam texture. The soil is slightly alkaline with pH values of 8.32 and 8.56 and E.C. of 2.1 and 1.78 (Mmhos/cm) in each of site 1 and site 2, respectively. Chemical analyses showed high percentage of Na^+ (10.5 and 13.2 meq l^{-1}) cations and Cl^- (13.85 and 17.51 meq l^{-1}) anions in each of the two sites, respectively (Table 1). The total soil concentrations of Cd, Zn, Co and Cu are shown in Table 2. In general site 2 is more polluted with heavy metals than site 1.

Pot experiment: The effect of different levels of Cd, Zn, Co and Cu on nodulation is presented in Table 3. The nodule numbers and mass were enhanced in soil treated up to 100 μg of Co (23.2 plant^{-1} and 2.95 g^{-1} plant^{-1}) and Cu (21.8 plant^{-1} and 2.8 g^{-1} plant^{-1}), then there was a significant decrease in nodulation (nodules number and fresh weight) by increasing the metal concentration of Cu and Co to 150 and 200 μg. Cadmium and zinc concentration showed no significant effect on nodulation and nodule fresh weight up to 100 μg when compared with the control. Increasing the application rate of cadmium (6.5 plant^{-1} and 1.58 g^{-1} plant^{-1}) and zinc (10.4 plant^{-1} and 1.6 g^{-1} plant^{-1}) at 200 μg caused significant decrease in nodules number and their fresh weight.

Protein content of both nodule cytosol and bacteroid fractions and the leghaemoglobin concentration of nodule cytosol were increased as the tested heavy metal supply

Table 1: Mechanical and chemical analysis of the soil used in the pot and field experiments

	Mechanical analysis (%)				
	Course sand	Fine sand	Silt	Clay	Textural class
Pot soil	61.9	8.4	2.3	27.4	Sandy clay loam
Field soil					
Site 1	57.3	6.9	2.1	33.5	Sandy clay loam
Site 2	66.5	9.6	3.2	29.7	Sandy clay loam

	Chemical analysis of cations and anions (meq l^{-1})							
	EC (Mmhos/cm)	Na^+	Ca^{++}	Mg^{++}	K^+	HCO_3	Cl^-	pH
Pot soil	1.97	12.18	6.85	4.69	1.76	3.19	16.63	8.12
Field soil								
Site 1	2.1	10.5	5.28	3.47	1.22	4.13	13.85	8.32
Site 2	1.78	13.2	4.98	5.21	1.92	5.46	17.51	8.56

Table 2: Total heavy metal concentration in soil used in the field experiment (μg kg^{-1} dry soil)

Field experiment	Cd	Zn	Co	Cu	Heavy metal content
Site 1	138	96	210	71	Less polluted
Site 2	206	148	405	129	More polluted

Table 3: Effect of heavy metals in pot and field experiments on nodulation of *Lablab purpureus* (L.) inoculated with *Rhizobium* sp. Strain I_4. The control is the same for both pot and field experiments

	Nodules/plants							
Treatment heavy metal	Numbers $plant^{-1}$				Fresh weight g^{-1} $plant^{-1}$			
(μg)	Cd	Zn	Co	Cu	Cd	Zn	Co	Cu
0 (control)	17.0	18.0	17.6	17.2	2.40	2.45	2.51	2.49
Pot experiment								
50	16.4	18.5	20.5	20	2.34	2.42	2.81	2.73
100	16.2	17.8	23.2	21.8	2.35	0.39	2.95	2.80
150	11.0	12.5	19.5	15.4	2.15	0.20	2.81	2.62
200	6.5	10.4	15.5	11.2	1.58	1.60	2.10	0.05
Field experiment								
Site 1	16.4	17.5	19.5	21.5	2.60	1.42	2.95	2.80
Site 2	15.8	12.8	13.5	20.6	2.15	0.29	2.22	2.50
LSD at 5%	2.23	2.91	3.15	2.72	0.19	0.22	0.27	0.24
LSD at 1%	2.31	3.14	3.21	2.81	0.31	0.39	0.57	0.51

increased up to 100 μg. When the tested heavy metals concentrations were increased more than100 μg, the protein content of nodules and leghaemoglobin content of nodule cytosol showed significant decrease (Table 4).

Insignificant differences in both nodule protein and nodule legheamoglobin (expressed as mg g^{-1} nodule fresh weight) between treated and untreated soil with heavy metal concentration up to 100 μg are pronounced. Both protein and legheamoglobin content of the tested nodules were decreased by increasing the rates of the applied heavy metals. The absolute nitrogenase activity and specific nitrogenase activity (per gram fresh weight nodules) of the plant were enhanced by application of Co and Cu at rate of 100 μg, while a remarkable decrease recorded at an application rate of 200 μg for Co and Cu (Fig. 1a and b). Heavy metals showed no effect at concentrations up to 100 μg of Cd and Zn, while attained significant decrease at concentrations 150 and 200 μg of Cd and Zn.

A stimulatory effect of Co (2.5 and 4.6 g dry weight $plant^{-1}$) and Cu (2.05 and 3.63 g dry weight $plant^{-1}$) on the dry weight of root and shoot were apparent up to 100 μg (Table 5). High levels of Co and Cu (200 μg) showed an inhibitory effect on dry matter accumulation of the plant (1.3 and 1.18 g dry weight $plant^{-1}$ for root and 1.9 and 1.48 g dry weight $plant^{-1}$for shoot. On dry weight basis, calculation of shoot/root ratio showed 1.84 of Co and 1.77 of Cu at treatment level of 100 μg. High application rates of Co and Cu up to 200 μg produced values of 1.46 and 1.25 dry weight $plant^{-1}$ respectively. Shoot growth inhibited more than that of the root resulting in a remarked decrease in shoot/root ratios.

On the contrary, an inhibitory effect of cadmium and zinc on the dry weight of root (0.95 and 0.88 g dry weight $plant^{-1}$) and shoot (1.05 and 0.75 g dry weight $plant^{-1}$) respectively, were apparent at concentration of 200 μg (Table 5). A slight effect on dry matter accumulation was revealed at 100 μg of cadmium (1.25 and 2.03 g dry weight $plant^{-1}$) and zinc (1.52 and 2.25 g dry weight $plant^{-1}$) respectively. The shoot/root ratio showed that the dry matter decreased when cadmium and zinc treatment was equally partitioned between shoot and root up to 100 μg Cd and Zn treatment, while at 200 μg an adverse effect on shoot rather than on root was observed on dry matter accumulation.

The mineral ion content (Table 6) revealed that Na^+ and K^+ were significantly decreased in the shoot with the increase of cadmium content (103 and 6.23 μg g^{-1} dry weight) and zinc (116 and 6.83 μg g^{-1} dry weight) at 100 μg treatment level. On the other hand, Na+and

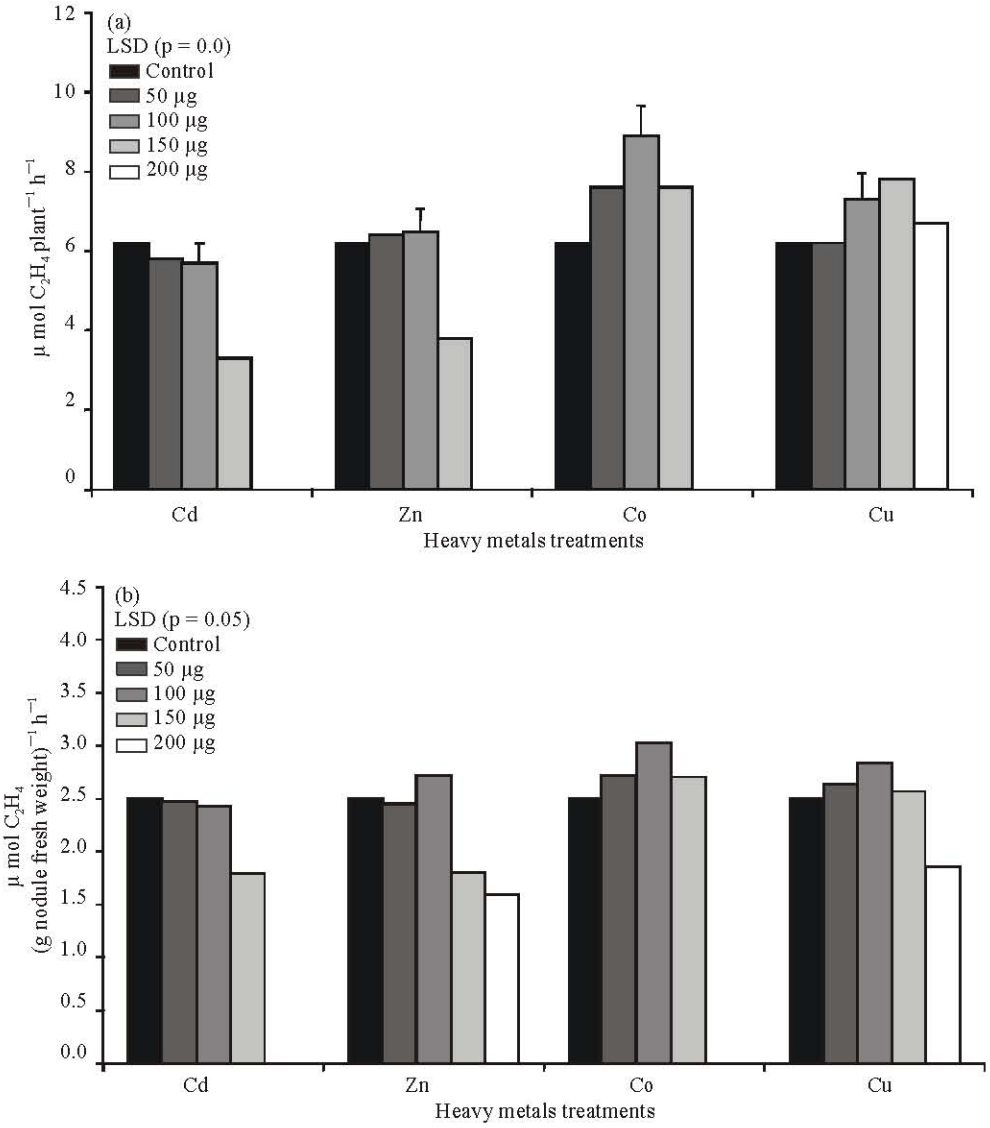

Fig. 1: Effect of different levels of heavy metals on total (a) and specific (b) nitrogenase activity of *Lablab purpureus*. Values represent the mean of three replicates and vertical bars are LSD at 0.05 level

K^+ content increased in the shoot with the increase of cobalt (188 and 9.5 µg g^{-1} dry weight) and copper (169 and 8.95 µg g^{-1} dry weight) at 100 µg treatment, respectively. A remarkable decrease in Na^+ and K^+ in the shoot at 200 µg of each cadmium (35 and 2.7 µg g^{-1}dry weight) and zinc (51 and 3.1 µg g^{-1} dry weight) were observed. Ca^{2+} uptake was considerably declined to 90 and 75 µg g^{-1} dry weight with the increase of cadmium and zinc at 200 µg treatment. Alternatively, Ca^{2+} revealed the highest amounts of 182 and 157 µg g^{1-} dry weight with cobalt and copper treatment at 100 µg, while it revealed a slight decrease to 120 µg g^{-1} and 108 µg g^{-1} dry weight with cobalt and copper at 200 µg treatment level.

Heavy metal content of root, shoot and nodules are shown in Fig. 3. The content of cadmium and zinc in the plant organs increased gradually as cadmium and zinc level increased in the soil up to 200 µg (Fig. 3a and b). On the other hand, the root accumulated cadmium and zinc at higher level than the shoot while nodules accumulated cadmium and zinc more than both root and shoot. The content of cobalt and copper in the plant organs increased at high levels of 200 µg of cobalt and copper treatment (Fig. 3c and d). The root accumulated cobalt and

Table 4: Effect of different heavy metals in pot and field experiments on protein content (nodule cytosol and Bacteroid fractions) and Leghaemoglobin content of nodule cytosol of *Lablab purpureus* (L.) nodulated with *Rhizobium* sp. strain I_4

	Protein content (mg g^{-1} nodule fresh weight)											
	Nodule cytosol				Bacteroid fractions				Leghaemoglobin (mg g^{-1} nodule fresh weight)			
Treatment heavy metal (μg)	Cd	Zn	Co	Cu	Cd	Zn	Co	Cu	Cd	Zn	Co	Cu
0 (control)	8.62	8.73	8.35	8.56	5.19	5.33	5.22	5.73	3.51	3.31	3.25	3.05
Pot experiment												
50	8.13	8.83	8.94	8.79	4.92	5.39	5.63	5.92	3.42	3.15	3.87	3.51
100	7.99	8.69	9.37	8.97	4.95	5.42	6.83	6.21	3.32	3.27	4.27	3.71
150	6.31	8.31	8.87	8.81	4.05	5.01	6.41	6.11	2.60	2.83	3.65	3.53
200	6.09	8.07	8.46	7.98	3.83	4.25	4.83	4.51	1.85	2.52	3.25	2.81
Field experiment												
Site 1	8.52	8.43	8.75	8.88	5.11	5.25	6.12	6.20	3.33	3.25	3.91	3.62
Site 2	7.20	8.41	8.54	8.65	4.83	4.88	5.24	6.05	2.82	2.95	3.31	3.22
LSD at 5%	1.12	1.25	1.67	1.45	0.59	0.79	1.02	0.83	0.25	0.34	0.65	3.95
LSD at 1%	1.19	1.32	1.73	0.52	0.65	0.87	1.09	0.81	0.34	0.40	0.73	0.52

Table 5: Effect of different heavy metals in pot and field experiments on growth of *Lablab purpureus* (L.) nodulated with *Rhizobium* sp. strain I_4

	Dry weight (g $plant^{-1}$)								Shoot/Root			
	Root				Shoot				Ratio			
Treatment Heavy metal (μg)	Cd	Zn	Co	Cu	Cd	Zn	Co	Cu	Cd	Zn	Co	Cu
0 (control)	1.92	2.1	2.2	1.95	3.25	3.50	3.7	3.22	1.69	1.67	1.68	1.65
Pot experiment												
50	1.55	1.83	2.3	2.12	2.60	2.80	4.1	3.65	1.68	1.53	1.78	1.72
100	1.25	1.52	2.5	2.05	2.03	2.25	4.6	3.63	1.62	1.48	1.84	1.77
150	1.10	1.05	2.1	1.89	1.35	1.33	3.5	2.84	1.22	1.27	1.66	1.50
200	0.95	0.88	1.3	1.18	1.05	0.75	1.9	1.48	1.10	0.85	1.46	1.25
Field experiment												
Site 1	1.61	1.65	1.56	2.07	2.55	2.20	2.75	3.44	1.60	1.33	1.45	1.89
Site 2	1.36	1.15	1.25	1.80	1.72	1.52	1.52	2.22	1.25	1.15	1.19	1.42
LSD at 5%	0.65	0.45	0.87	1.03	0.32	0.42	1.1	0.93	0.59	0.39	1.09	0.79

Table 6: Effect of different heavy metals in pot and field experiments on mineral ion contents of *Lablab purpureus* (L.) shoot nodulated with *Rhizobium* sp. strain I_4

	Mineral content (μg g^{-1} dry weight)											
	Na^+				K^+				Ca^{++}			
Treatment heavy metal (μg)	Cd	Zn	Co	Cu	Cd	Zn	Co	Cu	Cd	Zn	Co	Cu
0 (control)	153	172	163	155	8.13	8.62	8.13	8.22	135	130	125	127
Pot experiment												
50	110	135	175	163	7.75	8.21	9.1	8.74	130	127	153	139
100	103	116	188	169	6.23	6.83	9.5	8.95	125	120	182	157
150	75	93	167	142	3.80	4.50	8.62	7.21	120	120	143	121
200	35	51	121	96	2.70	3.10	5.75	3.70	90	75	120	108
Field experiment												
Site 1	95	103	175	166	6.91	7.45	8.85	8.62	117	87	148	135
Site 2	77	87	89	125	4.35	5.12	6.12	7.55	86	64	123	115
LSD at 5%	21	36	65	32	1.09	2.05	2.36	1.29	66	33	76	52
LSD at 1%	36	47	87	77	2.32	2.83	3.95	2.55	87	57	98	89

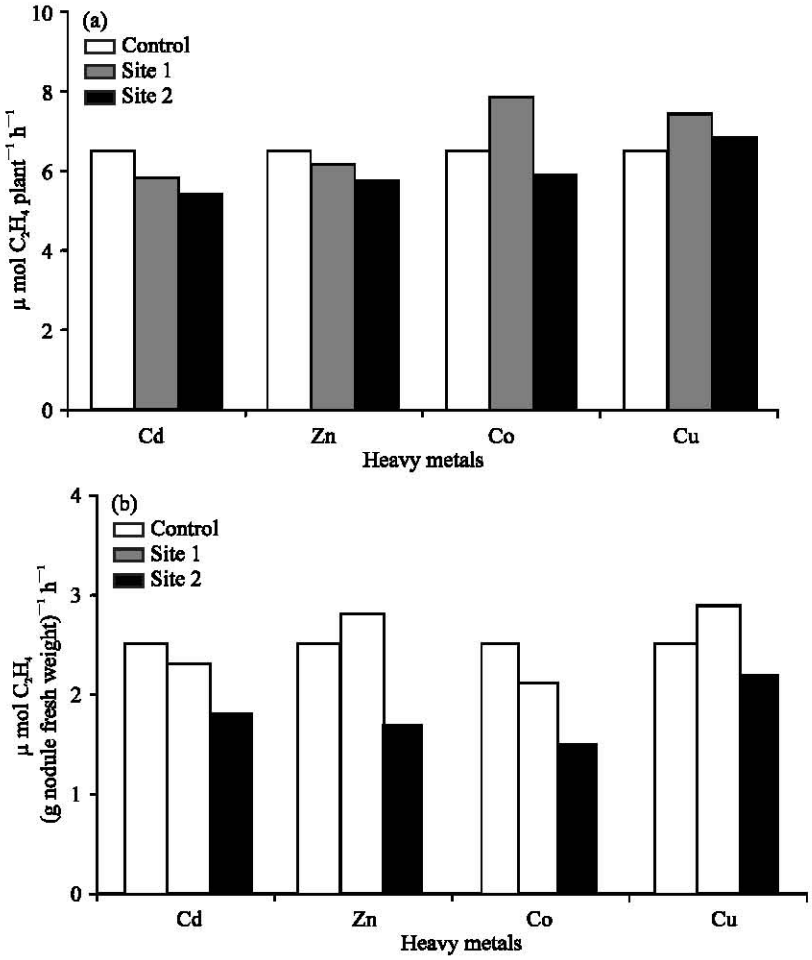

Fig. 2: Effect of heavy metal contaminated soil on total (a) and specific (b) nitrogenase activity of *Lablab purpureus*

copper more than shoot, while nodules accumulated cobalt and copper more than both root and shoot at treatment level of 200 μg.

Field experiment: Nodule numbers and mass are significantly enhanced in plants raised in soil contaminated with Co and Cu in the two field study sites. Slight decrease is observed in plants raised in soils contaminated with Cd and Zn in both field study sites (Table 3). The nodule number per gram root dry weight showed that heavy metals have an effect on growth rather than on nodule formation in both pot and field experiments.

The protein content of both nodule cytosol and bacteroid fractions and leghaemoglobin concentration revealed an increase in soils contaminated with Co and Cu and slight decrease in soil contaminated with Cd and Zn in the two study sites (Table 4).

The absolute nitrogenase activity of lablab plants is enhanced in soils contaminated with Co and Cu, while not affected in soils contaminated with Cd and Zn in the field study site 1. On the other hand, specific nitrogenase activity is enhanced in soils contaminated with Zn and Cu, while slightly decreased in soils contaminated with Cd and Co. Alternatively, the absolute and specific nitrogenase activity is significantly decreased in soils contaminated with Cd, Zn, Co and Cu in both study field sites (Fig. 2).

A stimulatory effect up to 3.44 g dry weight plant^{-1} increase in shoot and to 2.07 g dry weight plant^{-1} increase in root in soils contaminated with Cu is apparent in the field site 1 and an inhibitory effect to 2.22 and 1.8 g dry weight plant^{-1}in site 2 (Table 5). As for Cd, Zn and Co, the opposite is true.

Table 6 reveals that Na+and K+decreased to 95 and 6.91 μg g^{-1} dry weight in soils contaminated with Cd and decreased to 103 and 7.45 μg g^{-1} dry weight in soils contaminated with Zn. On the other hand, Na^+ and K^+ are increased to 175 and 8.85 μg g^{-1} dry weight in soils contaminated with Co and increased to 166 and

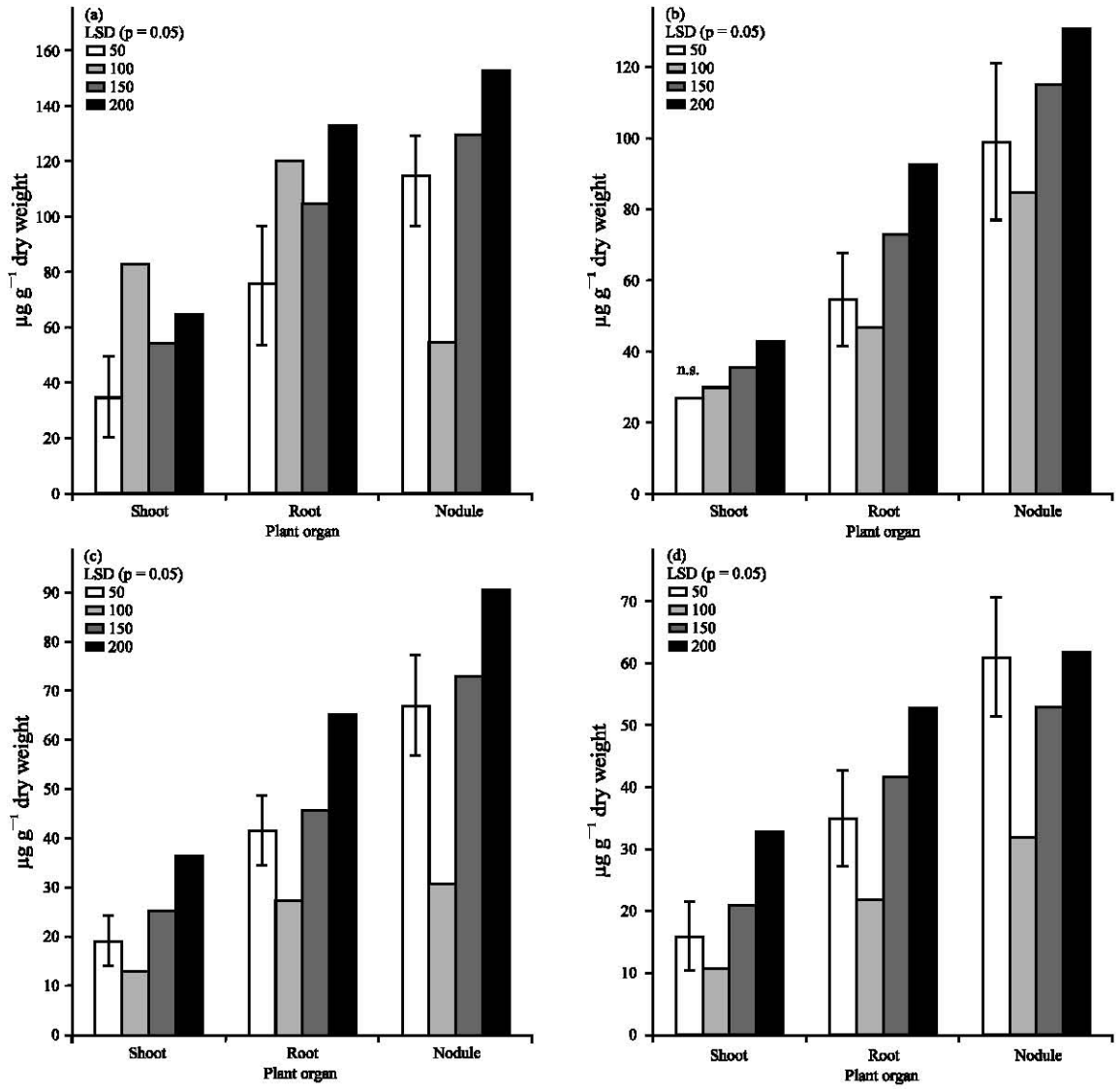

Fig. 3: Metal accumulation (µg g^{-1} dry weight) in root, stem and nodules of *Lablab purpureus* plants nodulated with *Rhizobium* sp. strain I_4 grown at different levels of heavy metals Cadmium (a), Zinc (b), Cobalt (c) and Copper (d). Values represent mean of three replicates for each treatment within a plant organ and vertical bars are LSD at 0.05 level. n.s. denote non-significant differences among treatment

8.62 µg g^{-1} dry weight in soils contaminated with Cu. A noticeable decrease is observed in the field site 2 where amounts up to 89 and 6.12 µg g^{-1} dry weight are found in soils contaminated with Co, while values decreased to 125 and 7.55 µg g^{-1} dry weight in soils contaminated with Cu.

The accumulation of the heavy metals in the plant organs in field site 2 is higher than in site 1, with the exception of Co, where the accumulation of Co in site 1 more that in site 2 (Fig. 4). On the other hand, nodules accumulated more heavy metals than both root and shoot, with the exception of cadmium, where root accumulated more heavy metals than both nodules and shoot.

The results recorded in Table 7 showed that plants grow in soils contaminated with Cd, Zn, Co and Cu have metal accumulation factors defined as the ratio of shoot to root or nodule heavy metal concentration to the total heavy metal concentration in soil. The accumulation factor of the heavy metals in nodules is higher than that of the root and shoot, with the exception of cadmium, where the accumulation factor in root is higher than that of nodules and shoot (Table 7). One may conclude that

Table 7: Concentration of heavy metals and Accumulation Factors (AF) in stem, root and nodules of *Lablab purpureus* nodulated with *Rhizobium* sp. strain I_4 grown on heavy metals contaminated soil (μg g^{-1} dry matter)

Item		Cd	AF	Zn	AF	Co	AF	Cu	AF
Shoot	Site 1	46	0.33	33	0.34	56	0.27	18	0.25
	Site 2	92	0.38	45	0.3	38	0.09	30	0.23
Root	Site 1	78	0.57	51	0.53	85	0.40	47	0.67
	Site 2	128	0.62	93	0.63	62	0.15	59	0.46
Nodule	Site 1	116	0.84	76	0.79	98	0.47	53	0.75
	Site 2	98	0.48	113	0.76	75	0.19	64	0.49

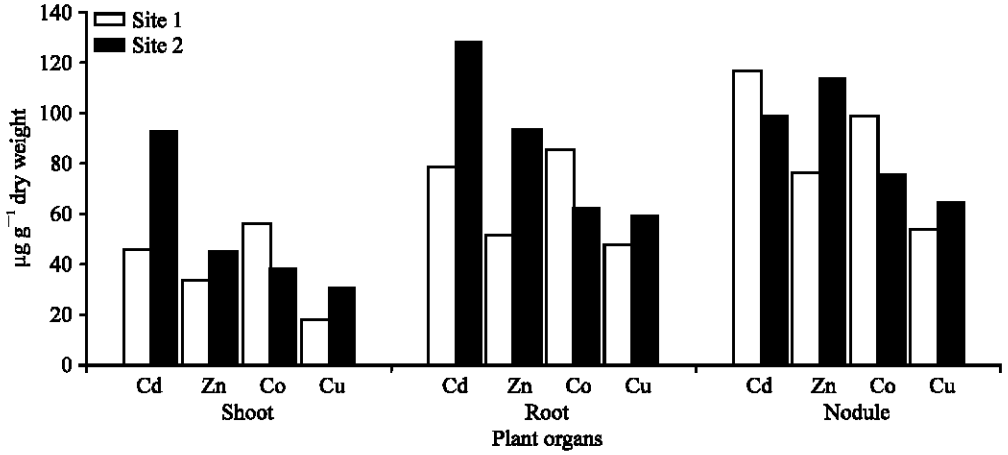

Fig. 4: Metal accumulation (μg g^{-1} dry weight) in stem, root and nodules of *Lablab Purpureus* plants nodulated with *Rhizobium* sp. strain I_4 grown on soil contaminated with heavy metals

Lablab purpureus can be used as phytoremediator and in revegetation of soils contaminated with heavy metals.

DISCUSSION

The results showed that *Lablab purpureus* plants nodulated with *Rhizobium* sp. strain I_4 has an ability to remove Cd, Zn, Co and Cu from polluted soils, an important asset for using the species in phytoremediation of soils polluted with heavy metals. When compared with other findings, these results are in agreement with El-Enany and Abd-Alla [9], and Heckman *et al.* [24] findings, where low levels of cadmium in polluted soils showed no effect on growth, nodulation and nitrogen fixation of soybean and faba bean. Other studies demonstrated that nodule number and fresh weight and nitrogenase activity were severely inhibited by increasing the levels of the heavy metals, especially cadmium and zinc concentrations [15]. The delay in early nodulation events is most likely related to root hair damage [25]. The effect of different Zn concentrations on mineral nutrition, growth, nodulation and nitrogenase activity of nodulated *Lupinus albus* demonstrated that Zn causes phytotoxicity problems hampering the cell division and cell elongation [26].

In this context [27] found that low levels of Cu and Co pollution increased nodulation and nitrogenase activity of faba bean, where they interact with radicals such as O_2 and make it toxic to many microorganisms [28, 29]. Copper toxicity is based on production of hyperoxide radicals which interact with cell membranes [30]. The decline in nitrogenase activity at higher rates of heavy metals could be attributed to the impaired leghaemoglobin synthesis and limited bacteroid proliferation.

The decrease in N_2 fixation and leghaemoglobin content in nodules of soybean plants treated with Cd, Ni, Cu and Zn is due to the reduction of Fe availability as a result of competition [31]. Studies on heavy metal inhibition [32] showed that growth, nodulation and nitrogen fixation in *Vigna radiate* was not affected at low levels of pollution, while Jonak *et al.* [33] reported the effect of heavy metal stress on activation of distinct nitrogen-activated protein kinase pathways by copper and cadmium. Moreover, El-Enany and Abd-Alla [9] found that cadmium concentration up to 100 μg showed no effect on nitrogenase activity,

leghaemoglobin and protein content of faba bean nodules.

The effect of electroplating factory effluent containing heavy metals in different concentrations on the germination and growth of *Lablab purpureus* seeds was studied by Ajmal and Khan [34]. The reduction of dry matter accumulation correlated with noxious effect of cadmium on several aspects of plant metabolism [35]. In this context [36, 37] found that growth and dry matter yield increases with low levels of Zn concentration, but excess amounts inhibited the plant growth. Heavy metals are much toxic to plants than to free-living rhizobia [38]. Cadmium is more toxic to rhizobia than Zn, where activity of Cd for inhibition of plant growth and N_2 fixation is several folds lower than that of Zn [15].

The results of the present work suggest that heavy metals may exert deleterious effect on absorption of some mineral elements from the soil. The decreased uptake of K and Ca due to accumulation of heavy metals suggests a role of Ca and K in the reduction of the N_2-fixing ability. The regulation of calcium level is a major mechanism of plant cell metabolism. Ca ions may play a role in the maintenance of membrane integrity [39], delay of senescence [40] and several other biological processes. The observed decrease of Ca content seem to be attributed to the loss of membrane stability [40, 41]. The decreased uptake of K and Ca due to accumulation of heavy metals suggests the role of Ca and K in the reduction of N_2 fixation.

The decrease of cobalt and copper in the plant organs at 100 μg of cobalt and copper levels indicated that the heavy metals are consumed through the plant metabolism and become stimulatory substrates [42, 43]. The findings of [9] showed that nodules of faba bean formed Cd-binding protein complexes which contained between 38-53% of the Cd-complexes. The Cd-binding protein is considered as a mechanism by which *Rhizobium*-legume symbiotic system elevates the plant resistance to Cd-toxicity. Studies of Domazlika and Opatrny [44] demonstrated that about 42% of cadmium were retained in roots of potato. It was suggested that roots may act as a barrier restricting the transport of cadmium to shoots [45], while Chaudhary *et al.* [46] found that all the N_2 fixing parameters in pea and Egyptian clover were adversely affected by the presence of heavy metals. Results obtained in the present study and the cited authors may present an explanation for the accumulation of heavy metals in nodules and the formation of heavy metal-binding protein that play an important role in the detoxification of excess Cd and other heavy metals and explain the resistance of *lablab-Rhizobium* sp. strain I_4 symbiosis to heavy metal toxicity Moreover, low concentrations of cobalt and copper may play a role in the plant growth and as a co-factors or co-enzymes for increasing the process of lablab-Rhizobium symbiosis up to 100 μg of cobalt or copper. This finding supports the findings of [47] who suggested that the essential role of Co in N_2-fixing legumes may be to allow synthesis of adequate vitamin B_{12}, which in turn is probably required for the synthesis of leghaemoglobin.

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