Environment Protection Engineering

Vol. 45 2019 No. 1

DOI: 10.5277/epe190107

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GROWTH AND HEAVY METALS UPTAKE BY *VICIA FABA* IN MINING SOIL AND TOLERANCE OF ITS SYMBIOTIC RHIZOBACTERIA

Faba bean plants in the 1/8 mixture with soil had the ability to accumulate Pb, Zn and Cu. 95% of the absorbed Pb were in the roots, and Cu and Zn were found in the shoots by 35% and 45%, respectively. There was a decrease in the root hairs and the number of cell layers of the root cortex alongside epidermis lesions. From the 50 tested rhizobacterial strains, 20 were able to grow at 150 mg/dm^3 of Pb, 6 were resistant to 150 mg/dm^3 of Zn and 8 resisted to 20 mg/dm^3 of Cu. Best four strains had adsorption potentials and the biosorption was higher for Cu. These strains were capable of producing auxin and exopolysaccharides. The most tolerant strains (FD1 and FD2) isolated near the mining site produced siderophores and high amounts of exopolysaccharides. The use of such strains and *V. faba* could be of important biotechnological value in decreasing heavy metal pollution of mining soils.

1. INTRODUCTION

Mining activities generate pollution in the surrounding soils. They are a major source of ecosystem disturbance and greatly harm both vegetation and soil organisms. These activities generate a large amount of very unstable toxic waste in the soils, thus a loss of cultivable soils due to air and water pollution [1] and unlike many other pollutants, heavy metals are difficult to remove from the environment [2]. Mining is an essential activity of the Moroccan economy, and for that, like in other countries over the

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world, soil pollution has significantly been increasing [3]. Several metallogenic activities have taken place in the Marrakech region. Being abounded mines or in activity, these sites generate pollution in the surrounding ecosystem soils. Among the toxic substances involved, we quote Cu, Zn, Pb, Co, Mg, Cd, Fe and SO_4^{2-} [4]. The accumulation and transfer of heavy metals in the food chains were proved [2], and their toxicity can have a lot of ecological, health and socio-economic impacts. Various mechanical and chemical processes can be adopted to remove heavy metals from contaminated soils. They include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, membrane technologies and others [5]. Unfortunately, these conventional methods can be very expensive and cannot be considered as easy solutions for the removal of metals from soils.

In poor or degraded soils, legumes can adapt to very different environments, and are often the first colonizers. Previous essays of revegetation and restoration of arid and degraded ecosystems have been successfully established [6]. Rhizobacteria and legumes may influence metal solubility, bioavailability and mobility, and the plants act as bioremediation agents. Furthermore, both of them, contribute mutually to improve the nitrogen content of degraded soils [7]. Moreover, legumes are of great interest for the phytoremediation of soils contaminated with heavy metals and they are considered promising candidates for the successful revegetation and stabilization of ecosystems degraded by mining activities [8]. To develop a revegetation strategy, it is very important to have a good knowledge of the adaptability of the plants in extreme environmental conditions.

The present work aims to evaluate the impact of the Moroccan mining soil pollution on the development and heavy metals uptake by *V. faba* and the tolerance of its symbiotic rhizobacteria in a heavy metal contaminated soil surrounding Draa Sfar operating mine. This study was carried out with the legume plant *V. faba* L. (faba bean) which is one of the most common legumes grown in Morocco and occupies about 40% of the total area reserved for legumes. We have also examined the microscopic changes of root structure caused by this polluted soil.

2. MATERIAL AND METHODS

The site and sampling. Draa Sfar mining site is currently active and is localized at 13 km Northwest of Marrakesh city (31°42′54.1″N 8°08′04.7″W). This mine contains large polymetallic deposits highly loaded with zinc, zinc oxide, lead and copper. It is the deepest mine in North Africa, 1 km deep. The soil sample (10 kg) was taken at the mine downstream in an area with very low vegetation, after removing the first layer of surface soil (2 cm).

Mining soil analyses. pH of the mining soil sample was measured in triplicate using an AFNOR X31-103 pH-meter and the electrical conductivity (*EC*) was measured by

the method proposed by Aubert and Pinta [9]. These measurements were done in a suspension of soil in distilled water of 1:10 (soil:water) after 30 min shaking.

The concentrations of heavy metals in soil were determined in triplicate using the standard AFNOR n X 31-151. 0.5 g of the sample was oven dried, then ashed at 550 °C in a crucible for 2 h. After cooling, the residue of calcination was treated in a Teflon® container, with 10 cm^3 of hydrofluoric acid 50%, and the residue was dried in a sand bath. Samples were then digested with *aqua regia* (HCl and HNO_3, in a ratio of 3:1 successively). This solution was adjusted to 10 cm^3 with distilled water. The samples were analyzed for metal concentrations (Cu^{2+}, Pb^{2+} and Zn^{2+}) using UNICAM 929 flame atomic absorption spectrophotometer (FAAS).

Effects of mining soil on V. faba. Seeds of *V. faba* (Moroccan Aguadulce variety) were planted in sterilized sand as control and in several samples of polluted mining soil mixed with sterilized sand, in medium sized pots (15 cm deep and 20 cm wide) with 3 seeds per pot. The mixtures were by 1/8, 1/4 and 1/2 of the mining soil. Irrigation was performed weekly with tap water. At flowering stage, the plants were harvested and several analyses were performed (root structure, fresh and dry weight, shoots height, roots length, number of nodes, number of flowers and heavy metal uptake).

Heavy metal uptake. The heavy metal uptake by *V. faba* plants was performed as described by Saber et al. [10]. 2 g of dry matter were kept at 450 °C for 4 h. Then few drops of diluted HNO_3 (5%) were added to the residue of calcination and again kept at 450 °C for one more hour. After that, 5 cm^3 of concentrated HNO_3 (63%) were added to the residue then dried it in a sand bath. The resulting residue was suspended in 5 cm^3 of H_2O_2, and then the volume was adjusted to 10 cm^3 by HCl 5%. Metal concentrations were measured using UNICAM 929 flame atomic absorption spectrophotometer.

For each metal two parameters were determined: translocation factor (*TF*) [11] and accumulation rate (*AR*) [12] based on the equations:

$$TF = \frac{\mathrm{SHMC}}{\mathrm{RHMC}}$$

$$AR = \frac{(\mathrm{SHMC}\times\mathrm{SDW}) + (\mathrm{RHMC}\times\mathrm{RDW})}{65(\mathrm{SDW} + \mathrm{RDW})}$$

where SHMC is the shoot heavy metal content (mg/kg), RHMC root heavy metal content (mg/kg), SDW – shoot dry weight, RDW – root dry weight. $TF > 1$ means a translocation of the metal from the roots to the shoots. *AR* is expressed in $mg \cdot kg^{-1} \cdot day^{-1}$.

Root structure. The roots are the main site of interaction between plant and rhizobacteria. In order to determine the changes of their structure caused by the mining soil,

transverse sections were performed by placing the root between two pieces of longitudinally cut pith, then, using a fine razor blade, fine sections were cute and stained using the technique of the double staining Mirande (staining with iodine green carmine), following these steps: 1) immersing from 10 to 20 min in 6 chlorometric degree sodium hypochlorite, 2) washing with abundant water (1 min), 3) washing in dilute acetic acid (1/2 diluted 10 min), 4) staining in iodine carmine-green 3–5 min, 5) washing with physiological water, and 6) observation under microscope (magnification 400×).

Tolerance of rhizobacterial strains to heavy metals. Rhizobacterial strains (FD1 and FD2) were isolated from root nodules of *V. faba* crops collected near the mining site and others (FR53, FR69 and RhOF strains) were isolated from nodules of faba bean crops in non-contaminated region (agricultural soils). These strains were purified and stored in glycerol 25% at –20 °C. Based on their 16S rDNA, the rhizobacterial strains FR53 and FR69 belong to *Achromobacter* sp., FD1 and FD2 are closer to *Ensifer* sp. and *Pseudomonas* sp. respectively, whereas the RhOF strains belong to *Rhizobium leguminosarum* except the strains RhOF3, RhOF4 and RhOF96 that were identified as *Ensifer meliloti* (formerly called *Sinorhizobium meliloti*) [13] and RhOF57A identified as *Rhizobium* sp.

Agar plates were incubated at 28 °C for 2–3 days. Individual colonies were re-streaked onto YEM agar plates and stored at 4 °C. The rhizobacterial strains were screened in triplicate for their tolerance to Cu, Pb and Zn using modified Duxbury medium as described by El Baz et al. [14]. After preliminary tests, the concentrations of heavy metals were hosen as follows: 5, 10, 15, 20 mg/dm^3 for copper, 50, 75, 100, 150 mg/dm^3 for zinc and 100, 150, 200, 250 mg/dm^3 for lead.

The heavy metals were added to the Duxbury agar medium using stock solutions of each heavy metal. Stock solutions of copper, lead and zinc were prepared from the diluted solution of $Cu(NO_3)_2{\cdot}H_2O$, $Pb(NO_3)_2$ and $Zn(NO_3)_2{\cdot}6H_2O$, respectively. Stock solutions of metal salts in distilled water were sterilized by filtration (0.20 μm). Plates were incubated at 28 °C for 3 days.

Biosorption potential of some rhizobacterial strains. Four strains (FD1, FD2, FR53 and FR69) were cultivated on YEM liquid medium without heavy metals. After incubation (30 °C for 5 days), biomass cultures (about 0.5 g) were centrifuged at 8000 rpm for 20 min; the pellets were washed four times with physiological water and twice with saline phosphate buffer. Then, the pellets were resuspended in sterile solution containing 50 mg/dm^3 of tested metal. The tubes were incubated at room temperature in a roller mixer. After 3 h of incubation, the cells were harvested by centrifugation at 8000 rpm for 20 min. The amount of residual metal present in the supernatant was measured by atomic absorption spectrophotometer (FAAS). Bioaccumulation of the metal in the bacterial biomass was expressed as the amount removed from the solution containing the tested metal based on the following equation [14]:

$$\text{Metal removal} = \frac{(C_0 - C_t)V}{M}$$

C_0 is the metal initial concentration in the solution (mg/dm^3), C_t is the metal concentration after 3 h in the solution (mg/dm^3), V is the total volume of the culture (dm^3), and M is the dry mass of the bacterial biomass (g).

Exopolysaccarides, siderophores and indole-3-acetic acid production. In order to determine other characteristics related to heavy metal resistance, the four strains (FD1, FD2, FR53 and FR69) were investigated for their capability to produce exopolysaccharides (EPS), siderophores and indole-3-acetic acid (IAA). The strains were incubated in 10 cm^3 of YEM liquid medium (for FR53 and FR69) or TSB medium (for FD1 and FD2) for 48 h at 28 °C. Cell density was determined by measuring optical density (*OD*) at 600 nm, then each bacterial sample was transferred to an Eppendorf tube to which we added Congo Red (CR) solution to obtain a final concentration of 40 μg/cm^3 of Congo Red. After incubation at 28 °C for 2 h with shaking, the mixtures were centrifuged at 14 000 rpm for 20 min. The amount of Congo Red remaining in the supernatant was determined by measuring the OD490 of the solution with the uninoculated medium as blank. The amount of Congo Red remaining in the supernatant is determined by reference to a standard range of Congo Red [15]. The amount of produced EPS was expressed in μg of Congo Red/DO600 by measuring bound-Congo Red (μg) by bacterial biomass measured at OD600.

The study of the secretion of siderophores was made on the Chrome Azurol S (CAS) medium [14]. First, a minimum medium agar (MM) without iron was prepared (glucose 10 g/dm^3, KNO_3 1 g/dm^3, $MgSO_4$ 0.5 g/dm^3, KCl 0.5 g/dm^3, K_2HPO_4 0.5 g/dm^3, agar 15 g/dm^3). Pure strains were grown on MM for 3 days at 28 °C and then streaked on CAS agar plates and incubated for 3 days at 28 °C. A positive reaction is indicated by a color change of CAS reagent from blue to orange-yellow, leading to a clearly visible halo around the colonies.

Bacterial cultures were washed according to the method described before, then 200 μl of the bacterial suspension (with an *OD* of 0.8) was cultured in Erlenmeyer flasks containing 100 cm^3 of Luria–Bertani (LB) medium supplemented with 1.02 g/dm^3 of L-tryptophan being the precursor of IAA. After incubation with continuous stirring at 28 °C for 4 days, each culture was centrifuged at 8000 rpm for 5 min.

1 cm^3 of the bacterial supernatant was mixed with Salkowski's reagent (a mixture of 15 cm^3 of 0.5 M $FeCl_3$, 500 cm^3 distilled water, and 300 cm^3 of concentrated H_2SO_4) and 2 drops of orthophosphoric acid (H_3PO_4), then the mixture was incubated in darkness at room temperature for 30 min. Using a spectrophotometer, the absorbance of auxin produced was read at 530 nm with LB medium supplemented with 2 cm^3 of Salkowski's reagent and 2 drops of orthophosphoric acid as blank [16]. The amount of

auxin produced by the bacterial cultures was calculated based on a standard range of synthetic auxin.

Statistical analysis. The experimental design was a randomized complete block. Analysis of variance (ANOVA) was performed for comparison of means ($P \leq 0.05$) using SPSS version 23. Results given are means±standard deviations. Growth values and nodulation parameters were means of four replicates per treatment. All results were subjected to analysis of variance, with a Student–Newman–Keuls for the comparison of means.

3. RESULTS

The studied site shows very low vegetation and presents multiple layers with different colors. The studied soil had a neutral reaction (pH 7.44) and an electrical conductivity of 3.45 mS/cm meaning that the soil has a high salinity (Table 1). The heavy metal concentrations were 602.55 mg/kg of Zn, 216.36 mg/kg of Cu and 343.92 mg/kg of Pb.

Table 1

pH, electrical conductivity and heavy metal content in the studied mine soil

pH	7.44±0.04
Conductivity, mS/cm	3.45±0.04
Cu, mg/kg	216.36±28.74
Pb, mg/kg)	343.92±67.53
Zn, mg/kg	602.55±24.62

Seeds of *V. faba* were planted in samples of polluted soil mixed with sterilized sand. These mixtures were by 1/8, 1/4 and 1/2 of the mining soil. In the 1/4th mixture, the growth of faba bean plants has been blocked since the first stages of growth and the death of the plants was noted after 8 days. Only the plants in the 1/8 mixture (mining soil/sand) were able to grow. These plants have taken 65 days to reach the beginning of the flowering stage, instead of 40 days for the control plants. The fresh and dry weights of *V. faba* plants in the mixture 1/8 (3.9 and 0.86 g/plant, respectively) were significantly weaker ($p < 0.05$) than those of the control plants (24.98 and 2.22 g/plant, respectively) (Fig. 1).

During our study, we noted at the presence of Draa Sfar mining soil, some changes of anatomical structure of roots resulting in a decrease in the number of cell layers of the root cortex and epidermis lesions in comparison to the control. Moreover, the size and the density of root hairs were also reduced (Fig. 2a, b). The roots became dark brown due to necrosis.

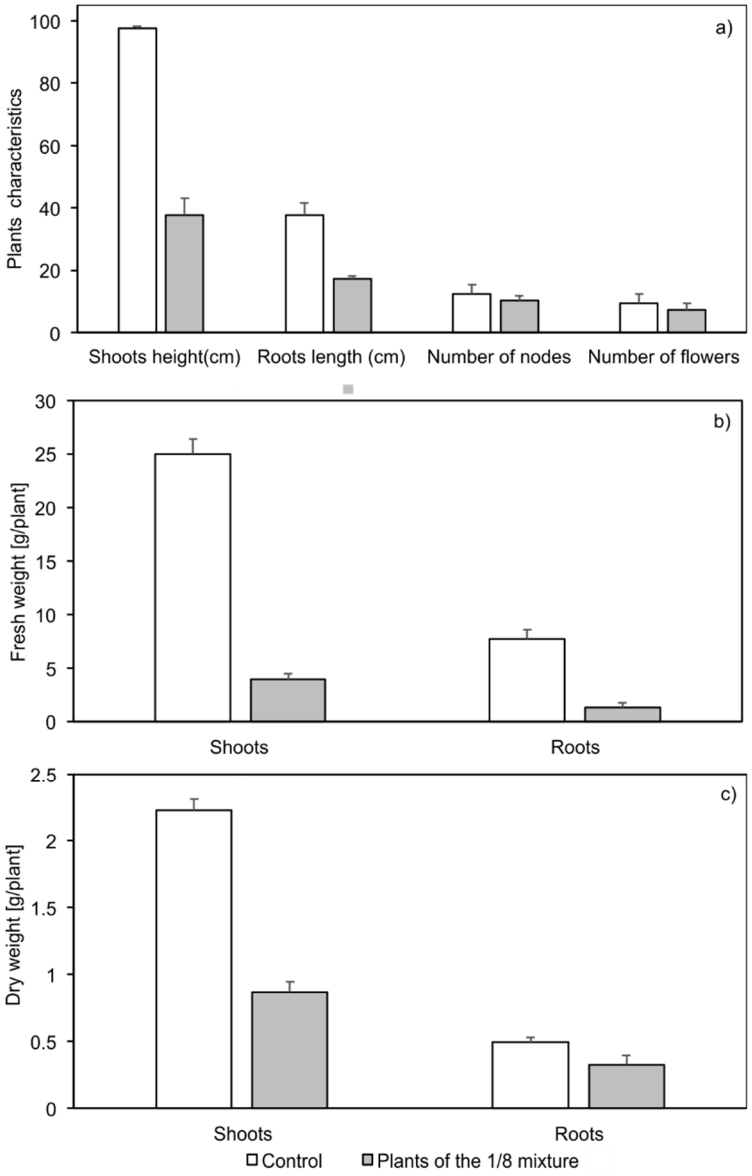

Fig. 1. Effect of mining soil on the growth of faba bean plants:
a) plants characteristics, b) fresh weight, c) dry weight

Harvested plants were analyzed in order to locate where the metals were more accumulated. Heavy metals quantities accumulated in the shoots and the roots of the harvested plants are presented in Table 2. *V. faba* plants accumulated Zn (101.96±2.10 mg/kg), followed by Cu (26.55±0.71 mg/kg) and Pb (3.12±0.22 mg/kg). These metals

were more accumulated in the roots than in the shoots especially for Pb. Indeed, 95% of the absorbed Pb were located in the roots of the plants, whereas 5% were in the shoots. As for Cu and Zn, the shoots contained more these two metals with 35% and 45%, respectively.

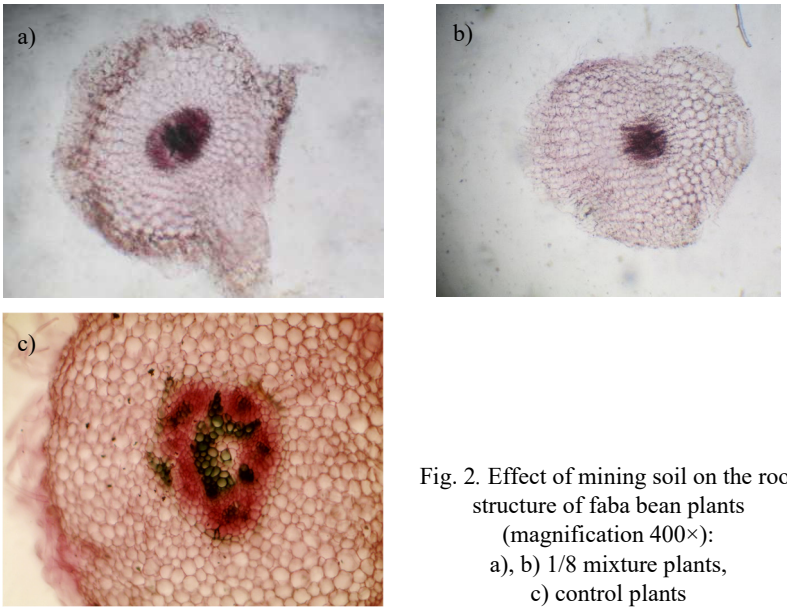

Fig. 2. Effect of mining soil on the root structure of faba bean plants (magnification 400×): a), b) 1/8 mixture plants, c) control plants

Table 2

Uptake of heavy metals by *V. faba* plants

Metal	Shoots	Roots	Total uptake
Cu, mg/kg	9.20±0.32	17.35±0.72	26.55±0.71
Pb, mg/kg	0.14±0.04	2.98±0.22	3.12±0.22
Zn, mg/kg	45.64±3.20	56.32±4.49	101.96±2.10

Table 3

Translocation factors and accumulation rates of *V. faba* plants

Metal	Translocation factor	Accumulation rate [$mg \cdot kg^{-1} \cdot day^{-1}$]
Cu	0.53±0.005	0.18±0.007
Pb	0.047±0.01	0.01±0.002
Zn	0.81±0.05	0.75±0.009

The *TF*s of our plants (Table 3) were less than 1, which indicates less translocation to aerial parts. These factors were decreasing in the order Zn > Cu > Pb. As for the accumulation rates, the plant shoots accumulated over the 65 days of the experiment

0.18, 0.01 and 0.75 $mg \cdot kg^{-1} \cdot day^{-1}$ of Cu, Pb and Zn, respectively, showing that Zn was the most accumulated metal as only a few amounts of Pb were taken by the plant shoots. The metals were more accumulated in the roots of the plants.

The rhizobacterial strains isolated from nodules of faba bean plants were tested for their ability to grow on Duxbury medium amended with different concentrations of heavy metals (Cu, Pb and Zn). These metals were chosen being the major metallic elements in the studied site (Table 1). From the 50 rhizobacterial strains tested, 20 strains were able to grow at high levels of Pb (150 mg/dm^3), 6 strains resisted until 150 mg/dm^3 of Zn and 8 strains resisted to 20 mg/dm^3 of Cu (Table 4).

Table 4

Resistance of rhizobacterial strains to heavy metals [mg/dm^3]

Strain	Pb	Zn	Cu	Strain	Pb	Zn	Cu
RhOF1	150	150	20	RhOF60	150	100	20
RhOF2	150	150	15	RhOF61	0	0	15
RhOF3	100	0	15	RhOF63	100	50	15
RhOF4	150	0	15	RhOF65	100	0	15
RhOF5	150	0	15	RhOF66	150	75	15
RhOF6	150	50	15	RhOF68	150	0	15
RhOF7	150	0	15	RhOF70	100	0	15
RhOF8	100	75	15	RhOF71	100	50	15
RhOF9	150	0	15	RhOF72	0	75	10
RhOF10	150	0	10	RhOF90	150	0	20
RhOF11	150	0	15	RhOF91	100	75	15
RhOF12	100	0	15	RhOF92	150	0	15
RhOF13	100	0	15	RhOF93	100	0	15
RhOF14	100	0	15	RhOF94	100	50	15
RhOF16	100	0	15	RhOF96	100	75	15
RhOF17	100	0	15	RhOF105	100	50	20
RhOF19	100	0	15	RhOF107	100	0	15
RhOF20	100	0	15	RhOF113	100	0	15
RhOF21	100	0	15	RhOF117	100	0	15
RhOF22	100	0	10	RhOF118	100	0	15
RhOF34	150	0	15	RhOF122	100	0	15
RhOF50	100	100	15	FD1	250	150	20
RhOF54	100	75	10	FD2	250	150	20
RhOF56	150	75	20	FR53	150	150	15
RhOF57A	100	0	15	FR69	150	150	20

We noticed that the tolerance of rhizobacterial strains to Zn is weaker due to the big amount of strains that could not grow even at 50 mg/dm^3 (the lowest concentration tested). The strains isolated near the mining site were more tolerant to heavy metals than the strains isolated from non-contaminated soils. For example, the strains FD1 and FD2

were tolerant towards 250 mg/dm^3 of Pb, 150 mg/dm^3 of Zn and 20 mg/dm^3 of Cu, whereas RhOF1, RhOF2, FR53 and FR69 were the most tolerant strains isolated from agricultural soils.

Four strains (FD1, FD2, FR53 and FR69) were evaluated for their biosorption potentials being resistant to the tested heavy metals. The obtained results showed that the rhizobial strains could accumulate Pb, Cu and Zn (Fig. 3). Moreover, the strains FD1 and FD2 (isolated near the mining site) accumulated more Zn than the other strains.

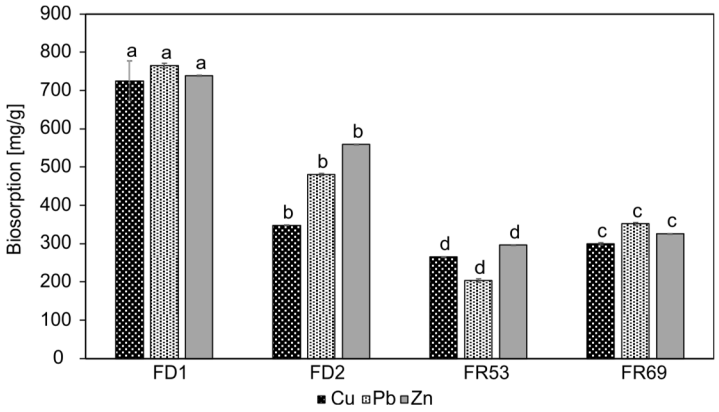

Fig. 3. Biosorption of Cu, Pb and Zn by rhizobacterial strains

Table 5

EPS, Siderophores and IAA production by rhizobacterial strains

Strain	FR53	FR69	FD1	FD2
EPS, µg CR/OD600	369.03±31.86 c	601.07±15.53 b	1452.19±6.18 a	1462.05±28.07 a
IAA, µg/cm^3	24.21±0.63 c	22.28±1.41 c	47.24±2.69 a	30.71±1.32 b
Siderophores	–	–	+	+

Means followed with different letters in each line are significantly different at $p \leq 0.05$.

Metal accumulation and sorption are alternative mechanisms for metal detoxification used by bacteria. The four tested strains showed a good potential of EPS production as well as the strains FD1 and FD2 were capable of producing siderophores. The IAA production varied; FD1 and FD2 isolates were better than rhizobial strains (Table 5).

4. DISCUSSION

Table 1 illustrates Draa Sfar soil characteristics. The high quantities of heavy metals and salinity are related to the activity of Draa Sfar mine generating pollution and causing toxicity, therefore low state of vegetation is observed in the site.

Faba bean plants in the 1/8 mixture of mining soil/sterilized sand are affected by the mine soil. The plants dry weight (0.86 g/plant) was significantly lower than that of the control plants (2.22 g/plant). The same case was for shoots heights and roots lengths. Wang et al. [17] showed that Pb can cause phytotoxicity to *V. faba*, which was illustrated by the significant decrease in shoot heights. Karimi et al. [11] have also reported that the growth of *V. faba* was significantly affected by heavy metals as there was a consistent decrease in plant growth alongside increasing the level of heavy metals. Similarly, Probst et al. [18] described the negative effect of metals on *V. faba* plants. They noticed the reduction of *V. faba* growth at high concentrations of metals, especially Zn and Pb, in the mine tailings in which they cultivated *V. faba* plants. We also noted that the size and the density of root hairs were reduced, also the roots became dark brown due to necrosis. Previous studies [1] have described other symptoms of toxicity such as leaf chlorosis, necrotic lesions, progressive yellowing, leaf folding and fading. Similarly, Karimi et al. [11] have reported that *V. faba* roots are strongly affected by Pb toxicity, while Singh et al. [19] noted that heavy metals decrease the length of the roots and the number and size of root hairs in *Vigna radiata*.

The main reason for the physiological alterations is that heavy metals trigger oxidative stress in plants leading to the oxidation of proteins and membrane lipids, even after a short-term exposure [20]. *V. faba* plants had the ability to accumulate Pb, Zn and Cu (Table 2). These metals were more accumulated in the roots than in the shoots especially for Pb. In general, the metal content in plants increases with the increase of metal concentrations in the soil, and the metal accumulation in root is usually significantly higher than in shoots. Karimi et al. [11] reported that Pb was preferentially accumulated by the roots of *V. faba*. However, the heavy metals uptake varied with plant species and the kind of metals. The ability of *V. faba* to uptake heavy metals from the mining soil is beneficent as this legume plant can ameliorate soil fertility as well. Dary et al. [11] have noted that legumes are of great interest for the phytoremediation and restoration of degraded soils.

Table 3 shows the TFs of faba bean plants. All of the factors were lower than 1 which is the case of non-accumulator plants. Studies like Pajuelo et al. [21] stated that phytostabilization in the roots where metals are not bioavailable, is more efficient than translocation to shoots where metals become bioavailable and can be dispersed.

Translocation factors were decreasing in this order Zn > Cu > Pb, as Zn was relatively the most translocated to shoots ($TF = 0.81$). That is why some studies recommend the use of legumes to enhance cultures of Zn from contaminated soils. Pastor et al. [22] have also proposed *Lupinus* as a Zn accumulator, capable of accumulating up to 3600 mg/kg of Zn in the aerial parts but only in acidic soil. The plants accumulated over the 65 days of the experiment 0.18, 0.01 and 0.75 mg/kg of Cu, Pb and Zn respectively, showing that Zn was the most accumulated metal as only few amounts of Pb were taken by the plant. From the 50 rhizobacterial strains isolated from nodules of *V. faba* plants, 20 strains were able to grow at 150 mg/dm^3 of Pb, 6 strains were resistant to 150 mg/dm^3 of Zn

and 8 strains resisted to 20 mg/dm^3 of Cu (Table 4). Cu, among other metals, has been reported to inhibit the growth, and the activities of various groups of microorganisms including rhizobia. In fact, it has been reported that elevated levels of copper are harmful to both free-living rhizobia and their corresponding symbiotic associations with legumes [23]. The obtained results are in consistence with those of Broos et al. [24] who have reported that Zn is the most toxic metal to free living rhizobia in soils as Zn induces mutations affecting the synthesis and/or export of exopolysaccharides making rhizobia more vulnerable to Zn.

The biosorption of four resistant rhizobacterial strains was tested. They had adsorption potentials and the biosorption was higher for Cu than Zn and Pb (Fig. 3). These strains were capable of producing auxin and high amounts of exopolysaccharides and siderophores (Table 5). These results confirm that the biosorption of heavy metals may be associated with the ability of produced polysaccharides to bind minerals [25]. Some reports described a number of microorganisms that have been shown to produce exopolysaccharides and other biopolymers with metal-binding properties [25]. Among others, rhizobia, well known for their exopolysaccharides secretion and their role in plant host specificity, have been investigated for their metal sorption capacities [25].

The metal can be mobilized in the plant tissues to be accumulated. Indeed, Vigliotta et al. [25] found that certain mechanisms take place in the bacteria, *Lysinibacillus* spp., *Stenotrophomonas* spp., *Pseudomonas* spp. and *Bacillus* spp. in order to protect themselves from the toxicity of heavy metals. The same mechanisms could at the same time promote the development of plants with which these bacteria are associated. Among these mechanisms, there are production of siderophores, indole-3-acetic acid (AIA) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase [25].

Besides, the metal resistance of rhizobial strains might be also attributed to changes in the metal efflux of microbial cell membranes; intracellular chelation due to the production of metallothionein proteins and the transformation of heavy metals to a less toxic oxidative form through microbial metabolism [26].

5. CONCLUSION

Mining activities generate pollution in the nearby ecosystem soils. The tested Moroccan mining soil is saline and contains Zn (602.55 mg/kg), Cu (216.36 mg/kg) and Pb (343.92 mg/kg). Our study demonstrated that *V. faba* plants had the ability to accumulate Pb, Zn and Cu in the roots and can also translocate Zn and Cu to the harvestable parts even if the roots are affected, as there was a change of anatomical structure of roots illustrated by a decrease in the number of cells layers of the root cortex, epidermis lesions and intense necrosis. Also, the size and the density of root hairs decreased in comparison to the control.

Of the 50 rhizobial strains isolated from faba bean plants, and tested for their tolerance to heavy metals, 20 strains were able to grow at 150 mg/dm^3, 6 strains resisted to 150 mg/dm^3 of Zn and 8 strains resisted to 20 mg/dm^3 of Cu. The strains isolated nearby the mining site were more tolerant to heavy metals than the strains isolated from non-contaminated soils.

Four rhizobial strains, evaluated for their biosorption potentials, can adsorb the tested heavy metals and the biosorption was higher for Cu than Zn and Pb. The most tolerant strains (FD1 and FD2) isolated near the mining site, demonstrated their ability to produce indole acetic acid, siderophores and high amounts of exopolysaccharides.

The rhizobacterial strains tolerant to heavy metals and the legume plant V. faba may be of great value and friendly biotechnological pathway in order to reduce the pollution of mining soils.

Further research will be conducted using heavy metal tolerant bacteria in combination with V. faba in order to decrease contamination of mining soil by phytostabilisation.

ACKNOWLEDGMENTS

This work is financially supported by the project Toubkal n° 16/21–Campus France n° 34717WA, the project PPR2/2016/42, CNRST Morocco and the Mediterranean project ENVIMED-MISTRALS.

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