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EFFECT OF METAL TOLERANT PLANT GROWTH PROMOTING RHIZOBACTERIA ON BEAN GROWTH, CADMIUM AND ZINC UPTAKE AND STRESS RESPONSES

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Abstract

Plant growth promoting rhizobacteria (PGPR) serve as an alternative tool in sustainable agriculture. PGPR influence the heavy metal accumulation of crops in contaminated soils, either by decreasing or increasing the accumulation. This study focuses on the effect of plant growth-promoting rhizobacteria on the heavy metal uptake, plant growth and stress response of bean plants. The simultaneous treatment of Cd^{2+} and *Mitsuaria chitosanitabida* $T_3 10^{-2}/4$ (PGPR strain) as well as Zn^{2+} and bacteria resulted in the inhibition of root elongation in bean plants, but no differences were recorded in root biomass. A higher accumulation of the phytotoxic Cd^{2+} in the root compared to the shoot was observed in bean plants due to the limited translocation (varying between 7.95-23%). In the case of Zn^{2+} treatment the translocation from root to shoot was not limited. In the case of Cd^{2+} treatment the *Mitsuaria chitosanitabida* $T_3 10^{-2}/4$ decreased the accumulation of Cd^{2+} in bean plants. Differences in polyphenol oxidase (POD) and peroxidase activity (GPOX) were observed among metal stressed and control plants. *Mitsuaria chitosanitabida* $T_3 10^{-2}/4$ strain diminished the oxidative stress in the case of toxic metal (Cd^{2+}) treated bean plants most probably due to the inhibited metal uptake.

Key words: antioxidant enzymes, bean, heavy metal, plant growth promoting rhizobacteria, *Mitsuaria chitosanitabida*

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1. Introduction

The heavy metal pollution of the soil is a major environmental problem due to the rapid development of the industry. Heavy metals are non-biodegradable and persist prolonged in environment, affecting the soil microbial composition (Khan et al., 2009). Plants are sensitive to essential heavy metal deficiency (Cu, Zn, Fe, Mn, Mo, Ni, Co), and to excess of non-essential heavy metals (Cd, As, Hg, Pb) (Nagajyoti et al., 2010). Due to the plant metal uptake and transport, heavy metals are accumulated and affect the yield of the crops (Khan et al., 2009), caused by the alteration of metabolic processes, inhibited growth and injuries (chlorosis, browning) (Nagajyoti et al., 2010). Each plant has a different molecular mechanism to deal with the metal stress. The metal toxicity has three mechanisms: a). metals interacting directly with proteins due to their affinity for thioyl-, histidyl- and carboxyl-groups; b). metals modifying the antioxidant defense and generate oxidative stress, stimulating the production of reactive oxygen species (ROS); and c). metals displacing essential cations from specific binding sites inhibiting protein function (Sharma and Dietz, 2006). Plant responses to heavy metals are regulated biochemically by the homeostatic processes,

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which include the regulation of the metal-induced reactive oxygen species (ROS) signaling pathway. ROS generation and signaling plays an important role in heavy metal detoxification and tolerance (Lin and Aarts, 2012).

Plant growth-promoting rhizobacteria (PGPR) are found in association with a large range of host plants. Plant growth-promoting (PGP) mechanisms can be categorized as *1).* phytostimulating rhizobacteria, that enhance plant growth directly by providing nutrients and/or phytohormones; *2).* mycorrhiza and root nodule symbiosis helper rhizobacteria; and *3).* biocontrol rhizobacteria that protect plants from pathogens through the production of antimicrobial compounds, nutrient competence with the pathogen, or by stimulating plant resistance (Drogue et al., 2012). The PGPR in a heavy metal polluted environment need to deal with their toxic effect, evolving different mechanisms as: *a).* pumping the metal ions out of the cell; *b.)* accumulation and sequestration inside the cell; *c).* biosorption and metal transformation from a toxic to a less toxic metal (Khan et al., 2009). Due to the microbial effect, the plant bioavailability of trace elements can be either increased in the rhizosphere (Sessitsch et al., 2013) or decreased locally (Karthik et al., 2016). The bioavailability of heavy metals depends on their chemical properties, climate, soil conditions and its biological attributions (Miransari, 2011). Additionally, the plant associated bacteria, due to their effect on plant growth - increasing the root surface, length but also the number of root hairs - can influence the absorption, root-shoot translocation and complexation of trace elements (Sessitsch et al., 2013). The PGPR can also increase the metal accumulation due to the production of biosurfactants, siderophores and organic acids (Ullah et al., 2015). Some of the PGPR have mechanisms to diminish the heavy metal stress decreasing the metal concentration either in the soil or in the plant (Miransari, 2011). The PGPR bacteria can act as metal sink reducing the local concentration in soil by immobilization, chelation, exclusion, active removal, biosorption and bioaccumulation of heavy metal (Karthik et al., 2016). Some important genera of PGPR include *Serratia*, *Bacillus*, *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Erwinia* and *Klebsiella* (Ullah et al., 2015). For example *Pseudomonas sp.* produced siderophores that form high solubility complexes and improved the metal uptake of plants (Ullah et al., 2015). *Pseudomonas putida* increased the Cd (Kamran et al., 2015), Ni (Kamran et al., 2016) and Cr uptake and enhanced the growth of *Eruca sativa* plants (Kamran et al., 2017). Other strains from *Bacillus* and *Serratia* genera were reported as biosorbents of Cd, Cu and Zn (Khan et al., 2009). *Ensifer adherens* bacterial strain isolated by Oves et al. (2017) from chickpea root surface showed accumulation and biosorption potential of Cd, Cr, Cu, Zn and Ni, whereas *Rhizobium sp.* strain obtained from root nodules of *Phaseolus vulgaris* was able of Cr removal (Karthik et al., 2017b). Other PGPR have the ability to decrease the level of ethylene (stress hormone) in plants, due to the ACC deaminase (1-aminocyclopropane-1-carboxylate) production, that catalyzes the ACC (precursor of ethylene) transformation into ammonium and α-ketobutyric acid (Miransari, 2011; Ullah et al., 2015). Increased growth and plant protecting effect against heavy metal toxicity was reported in Pb and Zn resistant *Bacillus* strains on *Brassica juncea*, Cd resistant *Xanthomonas*, *Azomonas*, *Pseudomonas* and *Bacillus* strains on canola, and Cd resistant *Variovorax*, *Rhodococcus* and *Flavobacter* strains on Indian mustard (Khan et al., 2009).

The aim of our research was to select PGPR bacterial strains based on metal tolerance, and to determine the effect of a PGPR strain on bean heavy metal accumulation (Cd^{2+}, Zn^{2+}) and stress response. In the present research the plant growth (length, weight), heavy metal accumulation and the stress protein amount (GPOX, POD) were determined from plants treated with different heavy metal concentrations in the presence or lack of the PGPR strain *Mitsuaria chitosanitabida* $T_3 10^{-2}/4$ respectively.

2. Material and methods

2.1. PGPR strains

Three bacterial strains: *Serratia proteomaculans* ($T_1 10^{-2}/2$), *Serratia sp.* ($T_5 10^{-1}/2$) and *Mitsuaria chitosanitabida* ($T_3 10^{-2}/4$) were isolated from wild leguminous plant rhizosphere from Ciuc Mountains. *Serratia proteomaculans* ($T_1 10^{-2}/2$) showed the following PGP characteristics: nitrogen fixation, siderophore production, organic phosphate mobilization and indole acetic acid production. The *Serratia sp.* ($T_5 10^{-1}/2$) bacterial strain has PGP traits both as organic and inorganic phosphate mobilization, indole acetic acid production and nitrogen fixation. *Mitsuaria chitosanitabida* ($T_3 10^{-2}/4$) showed positive values for all five plant growth promoting characteristics listed above.

2.2. Heavy metal tolerance of PGPR

The ability of the strains to grow under increasing concentrations of Cd^{2+} and Zn^{2+} was tested using plate assay. Nutrient agar media (0.5% peptone, 0.3% yeast extract, 1.5% agar and 0.5% NaCl, pH adjusted to 7.4) was supplemented independently either with $ZnSO_4*7H_2O$ salt from 0.5 mM to 25 mM or with $CdSO_4*8H_2O$ salt from 0.5 mM to 6 mM. 10µl bacterial suspension (OD_{600}=0.3) was inoculated on agar plates, and incubated at 28°C for 5 days. The experiments were carried out in triplicate. The inhibitory effect of the heavy metals was determined using colony diameter measurement. Agar plates without metals were used as controls (Sun et al., 2010).

2.3. Plant experiments

Based on the bacterial metal tolerance the *M. chitosanitabida* ($T_3 10^{-2}/4$) strain was selected for plant experiments.

Table 1. Controls and samples used in the experiments

Controls	***Mitsuaria chitosanitabida $T_3 10^{-2}/4$***	***Heavy metals***	***Heavy metal concentration (mM)***
K1	-	-	-
K2	+	-	-
K3	-	Cd^{2+}	0.1
K4	-	Cd^{2+}	0.5
K5	-	Zn^{2+}	0.5
K6	-	Zn^{2+}	3
Cd0.1	+	Cd^{2+}	0.1
Cd0.5	+	Cd^{2+}	0.5
Zn0.5	+	Zn^{2+}	0.5
Zn3	+	Zn^{2+}	3

Legend: (-) absence / (+) presence

The bean seeds (*Phaseolus vulgaris*) were surface disinfected, following the above described protocol. Seeds were soaked in 70% ethanol for 10 seconds followed by a 2% sodium hypochlorite treatment for 10 minutes, then rinsed in sterile distilled water five times. The seeds were germinated in dark, on wet filter paper in Petri dishes at 28°C for 7 days in Memmert Incubator.

The soil samples were treated with different concentrations of heavy metals and PGPR suspension (Table 1). Each seedling was inoculated with 1 mL of bacterial suspension (*M. chitosanitabida*, OD_{600}=1.5). In order to detect the effect of bacteria and heavy metals, 6 control samples were used (K1-K6, Table 1).

Eight equally developed bean seedlings were sown in plastic pots (top diameter 17.5 cm, bottom diameter 11.5 cm, height 13.5 cm) filled with approximately 1L sterilized soil (autoclaved for 30 min at 105°C), that contained macronutrients (mg L^{-1}) N, 50-400; available P_2O_5, 50-300; K_2O, 80-400; pH 5.5-6.5; salt content, 1-2 g KCl L^{-1}. The plants were grown under controlled conditions in a plant growth chamber (Sanyo MLR-351, Versatile Environmental Test Chamber), maintained at 22±1°C, 70% relative humidity with a 12/12h dark/light cycle 2500 lx illumination for 30 days. The pots were irrigated with 100 mL distilled water every second day. After 10, 20 and 30 days one whole plant from each treatment was removed for enzyme activity determination. At the end of the experiment plants were harvested, the soil was carefully removed from the roots and further analyzed for plant growth parameters, accumulation and enzyme activity.

2.4. Plant growth parameter measurements

Shoot and root length, fresh and dry weight were measured (n=5). The length of shoot and root of the treated plants were measured using a digital caliper whereas for the biomass measurement a technical scale (KERN EMB 200-2) was used. The obtained results were compared to controls. In order to determine the moisture content, the plants were dried 48 h at 105°C in a G-Therm 115 (F-lli Galli) thermostatic oven to constant weight. The tolerance index (TI) was calculated for both shoot and root elongation and biomass using Eq. (1):

$$TI = V_m/V_c*100 \quad (1)$$

where V_m is the measured value (shoot and root length or weight) in the presence of metal, and V_c is the measured value for the control (Wilkins, 1978).

2.5. Heavy metal accumulation

The concentrations of Cd^{2+} and Zn^{2+} were determined by atomic absorption spectrophotometry (AAS). Five replicates of each treatment were oven dried at 105°C to constant weight. Plant sample preparation was carried out using cremation in Gefran 1001 incinerator. The samples were placed in porcelain jars, followed by incineration. The temperature was gradually increased from 250°C with 100°C/h to 450°C and then was kept at 450°C for 4 h. The ash obtained was dissolved in 5 mL of 25% HNO_3 premixed solution and then filtered on filter paper. The volume was completed to 10 mL with distilled water.

The prepared samples were atomized in the atomic absorption spectrophotometer (Varian Spektra AA 110) and the absorbance values were read. In order to determine the concentration of the unknown solution, calibration curves were determined. Metal concentration in tissues will be expressed as µg g^{-1} dry matter.

2.6. Antioxidant enzyme activity and protein content determination

An amount of 0.2 g of plant tissue (shoot), three repetition per treatment was measured in Eppendorf tubes, porcelain beads were added with 1 mL QB buffer (100 mM KPO_4 (pH 7.8), 1 mM EDTA, 1% Triton X-100, 10% glycerol, and 1 mM DTT added before use). The cells were disrupted in FastPrep-24 mill two times (30 seconds) with 5 m/s speed.

The samples were centrifuged at 4°C and 10000g for 30 min, and the supernatants were removed in new Eppendorf tubes and stored at -20°C until use. The amount of total protein was determined

based on the Bradford method using a BSA calibration curve (Elavarthi and Martin, 2010).

The activity of polyphenol oxidase (POD) was determined by adding 50 µL crude protein extract to 950 µL of a solution containing 0.2 mM phosphate buffer (pH 7.5) and 50 mM 3-methyl-catechol (substrate). The absorbance was measured every 30 seconds for 10 minutes at 400 nm until the end of reaction (Cheema and Sommerhalter, 2015).

The activity of guaiacol peroxidase (GPOX) was determined by adding 25 µL crude protein extract to 975 µL of a solution containing 0.2 mM phosphate buffer (pH 7.5), 20 mM H_2O_2 and 20 mM guaiacol. The absorbance was measured every minute for 5 minutes at 480 nm until the end of reaction. One enzymatic unit was defined as the change of 1.0 absorbance unit per mL enzymatic extract, and expressed as units of enzyme activity per g fresh matter (U/g) (Cavalcanti et al., 2004).

2.7. Statistical analysis

Microsoft Office Excel Worksheet 2007 and Past.exe 2.17c statistical program were used for statistical analysis. Analysis of variance (ANOVA) was used to compare datasets.

3. Results and discussion

3.1. Heavy metal tolerance of PGPR

The growth of the selected PGPR strains in the presence of heavy metals is presented in Table 2. The average diameter of bacterial colonies and the standard deviation values are listed only for the strains and concentrations where growth was observed. *Mitsuaria chitosanitabida* $T_3 10^{-2}/4$ and *S. proteomaculans* $T_1 10^{-2}/2$ strains were able to tolerate up to 1 mM Zn^{2+} and 0.5 mM Cd^{2+} concentrations, showing growth on agar medium. The growth of *Serratia sp.* $T_5 10^{-1}/2$ bacterial strain was inhibited by the higher concentrations of both metals.

Based on our results the *M. chitosanitabida* $T_3 10^{-2}/4$ bacterial strain showing higher heavy metal tolerance was selected for further experiments.

3.2. Plant growth in the presence of heavy metals

The effect of different Cd^{2+} and Zn^{2+} concentration on plant growth was examined under controlled conditions. The length and weight of the root and shoot of bean plants were measured. Table 3 presents the obtained values and the standard deviations.

Table 2. Growth of PGPR colonies Nutrient agar media, supplemented with $ZnSO_4*7H_2O$ and $CdSO_4*8H_2O$

Heavy metal	***Concentration***	***Bacterial strain***	***The average diameter of the colonies (mm)***
Zn^{2+}	0.5 mM	*Mitsuaria chitosanitabida* $T_3 10^{-2}/4$	12.94 ± 2.49
		Serratia sp. $T_5 10^{-1}/2$	9.86 ± 2.10
		Serratia proteomaculans $T_1 10^{-2}/2$	10.97 ± 2.22
	1 mM	*Mitsuaria chitosanitabida* $T_3 10^{-2}/4$	10.04 ± 0.59*
		Serratia sp. $T_5 10^{-1}/2$	8.70 ± 0.64*
		Serratia proteomaculans $T_1 10^{-2}/2$	9.67 ± 0.55*
	5 mM	*Mitsuaria chitosanitabida* $T_3 10^{-2}/4$	6.45 ± 1.12*
		Serratia sp. $T_5 10^{-1}/2$	0.00 ± 0.00
		Serratia proteomaculans $T_1 10^{-2}/2$	6.51 ± 1.36*
Cd^{2+}	0.5 mM	*Mitsuaria chitosanitabida* $T_3 10^{-2}/4$	11.21 ± 1.75*
		Serratia sp. $T_5 10^{-1}/2$	9.09 ± 0.55*
		Serratia proteomaculans $T_1 10^{-2}/2$	9.84 ± 1.03*
	1 mM	*Mitsuaria chitosanitabida* $T_3 10^{-2}/4$	7.22 ± 1.63*
		Serratia sp. $T_5 10^{-1}/2$	0.00 ± 0.00
		Serratia proteomaculans $T_1 10^{-2}/2$	6.93 ± 2.04*
Control		*Mitsuaria chitosanitabida* $T_3 10^{-2}/4$	15.48 ± 0.82
		Serratia sp. $T_5 10^{-1}/2$	12.88 ± 0.30
		Serratia proteomaculans $T_1 10^{-2}/2$	11.73 ± 0.62

(- significantly different from control at p<0.05 level)*

Table 3. Length and biomass of the root and shoot (n=5) recorded in bean plants

			Cd^{2+}		***Zn^{2+}***		***Control***
			0.1 mM	***0.5 mM***	***0.5 mM***	***3 mM***	
Without bacteria	Shoot	Length (cm)	31.3±1.85	32.6±1.67	32.2±0.00	30.0±1.94	30.7±3.78
		Biomass (g)	4.9±0.72	5.6±0.48	5.2±0.00	4.4±0.51	4.3±1.68
	Root	Length (cm)	13.3±4.66	11.7±5.23	8.5±0.00*	7.1±2.52*	17.2±4.43
		Biomass (g)	0.4±0.09	0.6±0.24	0.5±0.00	0.7±0.30	0.7±0.45
Mitsuaria chitosanitabida $T_3 10^{-2}/4$	Shoot	Length (cm)	33.7±6.49	30.2±7.93	33.6±1.53	31.6±4.26	33.3±3.67
		Biomass (g)	4.5±1.17	4.1±1.31	4.6±0.80	4.0±0.42	4.4±0.40
	Root	Length (cm)	10.1±3.56*	10.3±3.49	9.2±5.74*	9.0±1.51*	18.±3.21
		Biomass (g)	0.46±0.12*	0.5±0.16	0.42±0.16	0.51±0.16	0.69±0.21

(- significantly different from control at p<0.05 level)*

The presence of Cd^{2+} (0.1 and 0.5 mM), with or without bacterization with *M. chitosanitabida* $T_3 10^{-2}/4$, had no significant effect on the shoot elongation. The root elongation in bean plants was inhibited by the simultaneous presence of Cd^{2+} and bacteria, statistically significant differences were observed (ANOVA, p=0.038 in the case of Cd0.1 and p=0.045 in the case of Cd0.5). No statistically significant differences were recorded in the case of the shoot and root biomass in the presence of Cd^{2+} with or without bacterial inoculation (Fig. 1). In a similar study, the effect of cadmium in the presence or absence of *Pseudomonas putida* was studied on growth and biomass of *Eruca sativa* by Kamran et al. (2015). Cadmium treatment caused decreased growth and biomass, whereas the inoculation with *P. putida* increased both parameters (Kamran et al., 2015). Our results are different from those presented by Kamran et al. (2015), which can be explained by the contrasting effect of bacterization on Cd uptake, being increased in *E. sativa* and decreased in *P. vulgaris* plants. Karthik et al. (2016) observed enhanced growth (shoot and root length and biomass) and limited Cr accumulation in PGPR inoculated *Phaseolus vulgaris*. An increased root length was reported by Karthik et al. (2017a) in *Zea mays*, *Vigna mungo*, *V. radiata*, *P. vulgaris* and *Sesbiana aculeata* plants when inoculated with *Cellulosimicrobium funkei*-like bacteria.

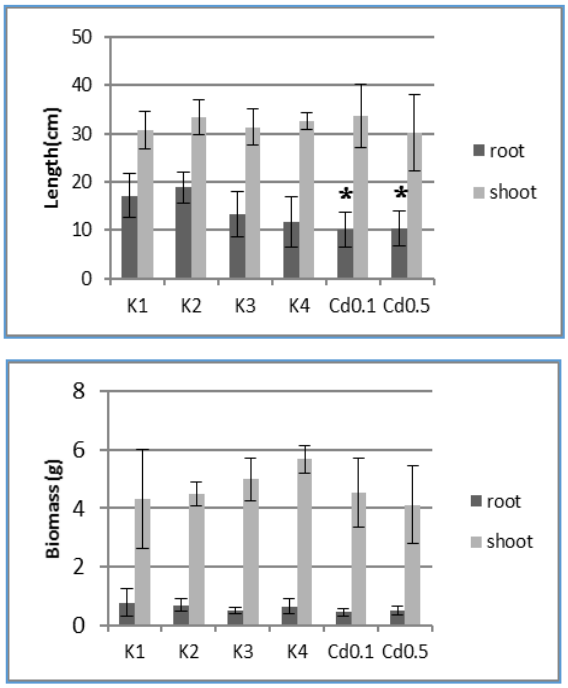

Fig. 1. Bean plant growth parameters in the presence of Cd^{2+} (∗ - significantly different from control at p<0.05 level), treatments: K1 (absolute control), K2 (bacterized control), K3 (0.1 mM Cd), K4 (0.5 mM Cd), Cd0.1 (0.1 mM Cd and bacteria), Cd0.5 (0.5 mM Cd and bacteria)

The presence of Zn^{2+} in the used concentrations (0.5 and 3 mM), with or without bacterization, had no significant effect on shoot elongation (Fig. 2). The root elongation was inhibited by the presence of Zn^{2+} with or without bacterial inoculation. The differences between control (K1) and samples were statistically significant (ANOVA, p=0.01 in case of K5, p=0.004 in case of K6, p=0.009 in case of Zn0.5 and p=0.02 in case of Zn3), but also between control with inoculation and samples (ANOVA, p=0.003 in case of K5, p=0.0008 in case of K6, p=0.002 in case of Zn0.5 and p=0.006 in case of Zn3). No statistically significant differences were recorded in the case of the shoot and root biomass in the presence of Zn^{2+} with or without bacterial inoculation (Fig. 2).

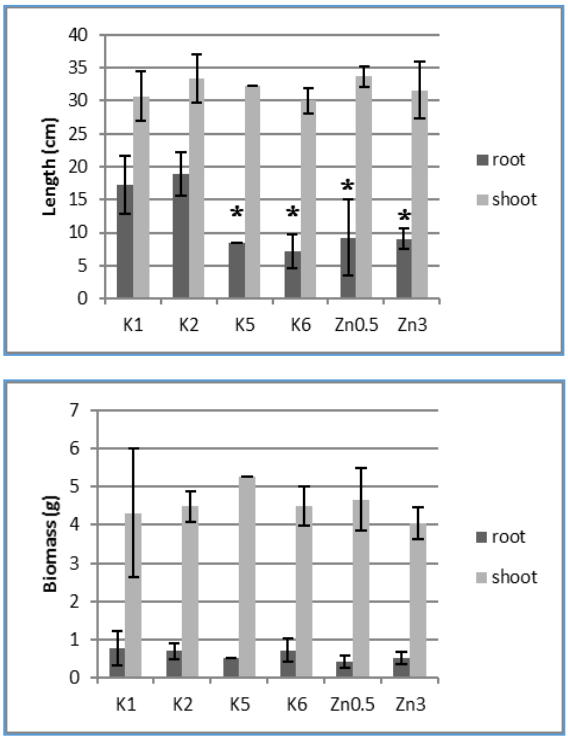

Fig. 2. Bean plant growth parameters in the presence of Zn^{2+} (∗ - significantly different from control at p<0.05 level), treatments: K1 (absolute control), K2 (bacterized control), K5 (0.5 mM Zn), K5 (3 mM Zn), Zn0.5 (0.5 mM Zn and bacteria), Zn3 (3 mM Zn and bacteria)

The lowest TI was observed in the bean root biomass and elongation (Fig. 3). The observed tolerance index for shoot length and biomass in the case of the bean plants was higher. The shoot growth was less inhibited by Cd^{2+}, with or without PGPR, than the root growth. The observed TI for the length and biomass of the root showed lower values whereas in the case of the shoot the tolerance index showed higher values (except the sample treated with 3 mM Zn^{2+} concentration and PGPR).

The decrease of the tolerance index of shoot length and biomass was observed in the case of the combined treatment with Zn^{2+} and PGPR (Fig. 3). The limited growth in root length and biomass can be attributed to its increased exposition to heavy metal treated soil as well as higher accumulation and metal stress.

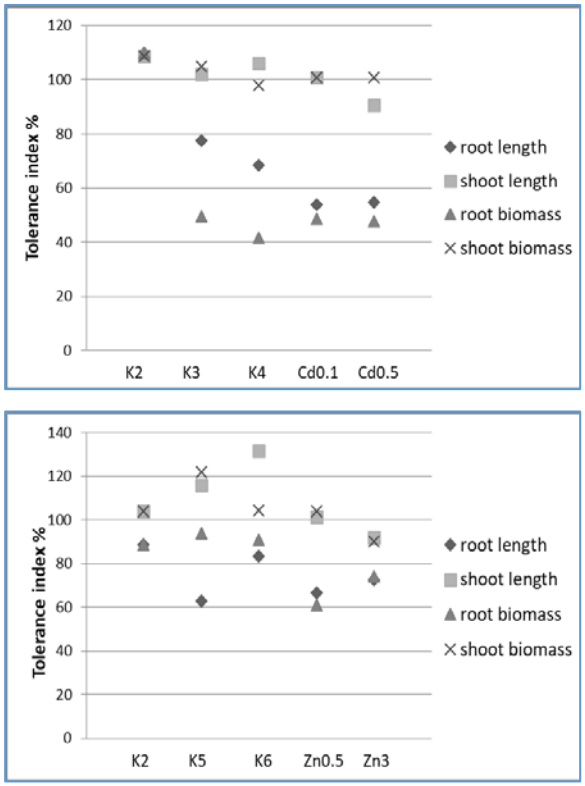

Fig. 3. Tolerance index of bean plants in the presence of heavy metal, treatments: K2 (bacterized control), K3 (0.1 mM Cd), K4 (0.5 mM Cd), Cd0.1 (0.1 mM Cd and bacteria), Cd0.5 (0.5 mM Cd and bacteria), K5 (0.5 mM Zn), K6 (3 mM Zn), Zn0.5 (0.5 mM Zn and bacteria), Zn3 (3 mM Zn and bacteria)

3.3. Heavy metal accumulation

The uptake of Cd^{2+} and Zn^{2+} by the bean root and shoot, under inoculated and uninoculated conditions, were determined by AAS. We observed that higher amounts of Cd^{2+} were accumulated in the root than in the shoot of the bean plants (Fig. 4). The heavy metal accumulation was decreased, in both shoot and root, by the bacterial inoculation. The use of *M. chitosanitabida* $T_3 10^{-2}/4$ decreased the Cd uptake of bean root with 68% and 41%, and shoot with 23% and 46% in Cd0.1 and Cd0.5 samples. The difference was statistically significant (ANOVA, p=0.008) only in case of root accumulation for the higher Cd^{2+} concentration (K4 to K1 absolute control). In case of Cd^{2+} resistant PGP rhizobacterial strains (*Pseudomonas sp.* and *Mycobacterium sp.*) the protection of *Brassica napus* plants against the toxic effect of the heavy metal was already reported (Dell'Amico et al., 2008). In contrast, the improved accumulation of Cd^{2+}, Pb^{2+}, Zn^{2+} and growth enhancement by *Enterobacter sp.* and *Klebsiella sp.* bacterial strains on the same plant (*Brassica napus*) were reported by Jing et al. (2014). Enhanced Cd^{2+} plant uptake by PGPR was reported by several authors in the case of *Solanum nigrum* by *Pseudomonas sp.* (Chen et al., 2014), *Lycopersicon esculentum* and *Zea mays* by *Burkholderia sp.* (Jiang et al., 2008), *Eruca sativa* by *Pseudomonas putida* (Kamran et al., 2015). Variations in cadmium and zinc uptake among plant species exist due to their individual response (Kamran et al., 2014), which can be modified by PGPR and plant association.

The Zn^{2+} accumulation in the shoot and root was determined with or without PGPR inoculation (Fig. 4). The heavy metal accumulation was slightly decreased by bacterial inoculation both in the case of the shoot and root. The differences were statistically significant in the case of root accumulation for the higher Zn^{2+} concentration in both treatments: without bacterization (K6, ANOVA, p=0.0006 and p=0.0004 compared to K1 and K2 control) and with bacterization (Zn3, ANOVA, p=0.002, p=0.001 and p=0.004 compared to K1, K2 and K6 control). Statistically significant differences of shoot accumulation were observed among control, inoculated control and Zn^{2+} amended plants, but no differences were observed between PGPR inoculated and uninoculated plants. The effect of inoculation was reported in the case of *Brassica napus*, where the inoculation with *Rahnella sp*. caused an increase in Zn^{2+} accumulation (He et al., 2013). Tiwari et al. (2012) also reported an enhanced Zn^{2+} accumulation in *Brassica juncea* inoculated with *Paenibacillus sp.* and *Bacillus sp.* strains.

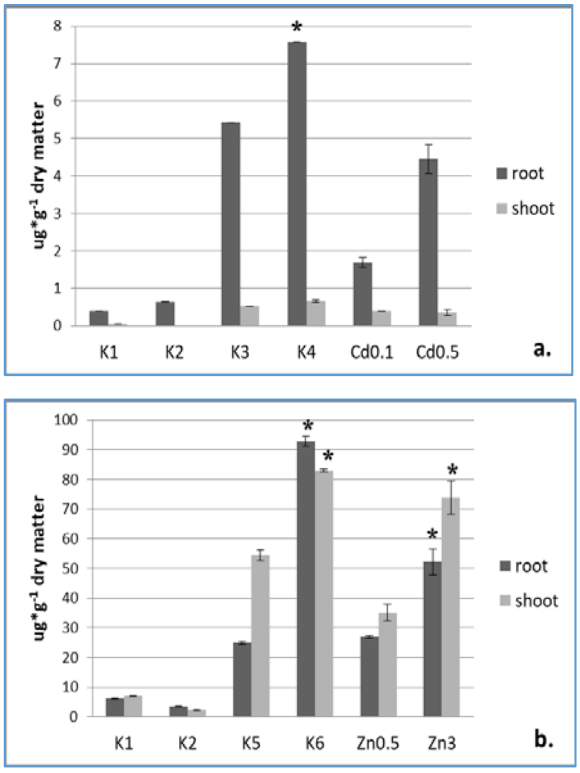

Fig. 4. Accumulation of Cd^{2+} (a) and Zn^{2+} (b) in shoot and root (* - significantly different from control at p<0.05 level), treatments: K1 (absolute control), K2 (bacterized control), K3 (0.1 mM Cd), K4 (0.5 mM Cd), Cd0.1 (0.1 mM Cd and bacteria), Cd0.5 (0.5 mM Cd and bacteria), K6 (0.5 mM Zn), K5 (3 mM Zn), Zn0.5 (0.5 mM Zn and bacteria), Zn3 (3 mM Zn and bacteria)

Due to the differences recorded in the case of the accumulation values for shoot and root, the

shoot/root accumulation ratio was calculated for Cd^{2+} and Zn^{2+} treated plants. The shoot/root ratio was lower in the case of Cd^{2+} and higher in the case of Zn^{2+} treated plants (Fig. 5). Our results show that there is a limited translocation between bean root and shoot in the case of the Cd^{2+} being a toxic metal, both in inoculated (23% and 7.95% in Cd0.1 and Cd0.5 treatments) and uninoculated (9.5% and 8.7% in K3 and K4 treatments) plants. Due to the fact that Zn^{2+} is an essential micronutrient, an allowed translocation was observed, the accumulation in the shoot being more accentuated. Low translocation was observed for Pb, Cr, Cd, Co, Cu, Zn and Ni in *Populus nigra* (Barbeș and Bărbulescu, 2017).

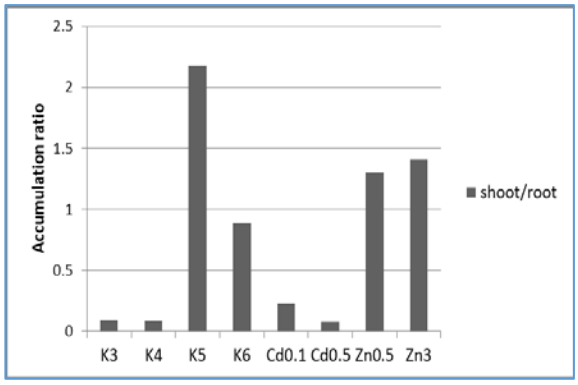

Fig. 5. Accumulation ratio in the case of Cd^{2+} and Zn^{2+} treatment in inoculated and uninoculated bean samples, treatments: K3(0.1 mM Cd), K4 (0.5 mM Cd), Cd0.1 (0.1 mM Cd and bacteria), K5 (0.5 mM Zn), K6 (3 mM Zn), Cd0.5 (0.5 mM Cd and bacteria), Zn0.5 (0.5 mM Zn and bacteria), Zn3 (3 mM Zn and bacteria)

3.4. Enzyme activity

The enzyme activity for POD was higher in the case of plants treated only with Cd^{2+} than in the plants treated with Cd^{2+} and PGPR. *Mitsuaria chitosanitabida* $T_3 10^{-2}/4$ decreased the oxidative stress caused by toxic metals (Fig. 6). Similar data are presented in Karthik et al. (2016) study, when chromium treated *P. vulgaris* plants were inoculated with rhizosphere bacteria that reduced the toxic effect and lowered the polyphenol oxidase activity. In the case of Zn^{2+} treatment, the POD enzyme activity increased together with the used concentration, and the PGPR strain accentuated both the accumulation and the oxidative stress.

Increase in antioxidant enzyme activity was reported previously by Zhang et al. (2012) in inoculated *Elymus dahuricus* under cadmium stress. Very strong (r=0.82, for cadmium treatment) and moderate (r=0.54, for zinc treatment) positive correlations were observed between the mean POD enzyme activity and metal accumulation. This strengthens the presumption that the PGPR inoculation diminished the oxidative stress due to the limited accumulation. The values of POD enzyme activity vary among sampling.

In case of GPOX no significant differences were detected (Fig. 7). The values of GPOX decreased while the accumulated Cd^{2+} and Zn^{2+} in plants increased. Moderate (r=-0.5, for cadmium treatment) and weak (r=-0.3, for zinc treatment) negative correlations were observed between the mean GPOX enzyme activity and metal accumulation. The values of GPOX enzyme activity varied among sampling.

4. Conclusions

In this work we investigated the effect of inoculation with the Cd^{2+} and Zn^{2+} tolerant PGP bacterial strain *M. chitosanitabida* $T_3 10^{-2}/4$ on the growth, accumulation and stress of bean plants supplemented with Cd^{2+} and Zn^{2+}. We observed that the root elongation of bean plants was inhibited by the simultaneous treatment of Cd^{2+} and PGPR, as well as Zn^{2+} and PGPR.

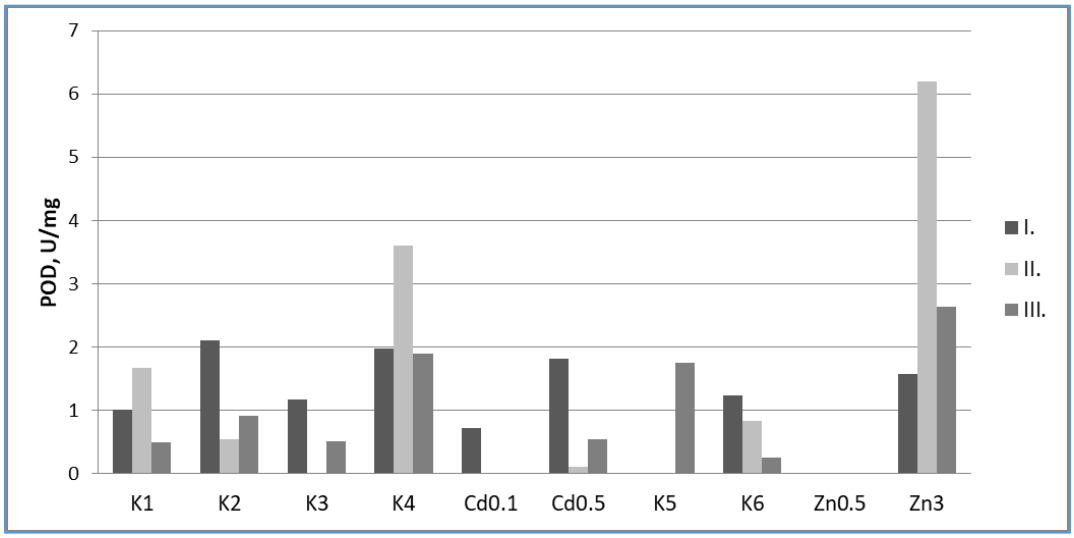

Fig. 6. POD activity (I-first sampling after 10 days, II- second sampling after 20 days, III.-sampling after 30 days), treatments: K1 (absolute control), K2 (bacterized control), K3(0.1 mM Cd), K4 (0.5 mM Cd), Cd0.1 (0.1 mM Cd and bacteria), K5 (0.5 mM Zn), K6 (3 mM Zn), Cd0.5 (0.5 mM Cd and bacteria), Zn0.5 (0.5 mM Zn and bacteria), Zn3 (3 mM Zn and bacteria)

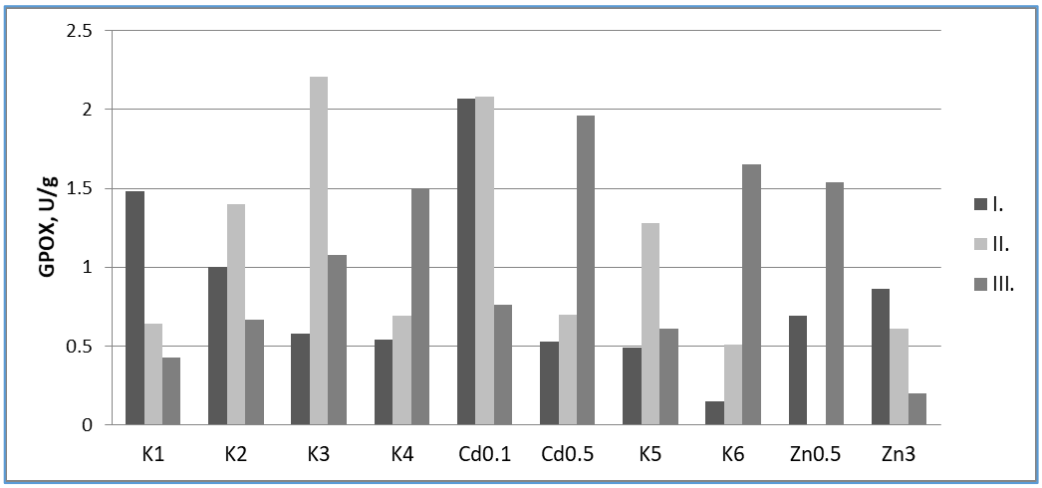

Fig. 7. GPOX activity (I-first sampling after 10 days, II- second sampling after 20 days, III.-sampling after 30 days), treatments: K1 (absolute control), K2 (bacterized control), K3 (0.1 mM Cd), K4 (0.5 mM Cd), Cd0.1 (0.1 mM Cd and bacteria), K5 (0.5 mM Zn), K6 (3 mM Zn), Cd0.5 (0.5 mM Cd and bacteria), Zn0.5 (0.5 mM Zn and bacteria), Zn3 (3 mM Zn and bacteria)

No significant effect of heavy metal treatment with or without PGPR was observed in the case of shoot development, root biomass and shoot biomass. A higher accumulation in the root, with respect to shoot, was observed in bean plants treated with the phytotoxic Cd^{2+}, due to a limited translocation. The inoculation with *M. chitosanitabida* $T_3 10^{-2}/4$ lowered the amount of accumulated Cd^{2+}. The higher accumulation in shoot, with respect to root, was observed in plants treated with Zn^{2+}, and the amount accumulated Zn^{2+}, was increased with *M. chitosanitabida* $T_3 10^{-2}/4$ inoculation. The POD enzyme activity increased with the used heavy metal concentration, and the inoculation with PGPR diminished the oxidative stress in case of Cd^{2+}. *Mitsuaria chitosanitabida* $T_3 10^{-2}/4$ reduced the accumulation of Cd^{2+} and the antioxidant enzyme (POD) activity in the bean plants compared with the control.

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