Effects of heavy metals on rhizobial growth

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Abstract

Effects of different concentrations of several heavy metals (Pb, Hg, Cd, Zn, Cu, Ni and Cr) on the growth of 16 strains of Bradyrhizobium japonicum, 15 strains of Sinorhizobum meliloti, 24 strains of Rhizobium leguminosarum (8 bv. phaseoli, 8 bv. viciae and 8 bv. trifolii), 4 strains of Rhizobium loti and 3 strains of Rhizobium galegae, all from a collection of the Soil Science Institute, were tested on YMA nutrient media containing Congo red.

The results clearly show different levels of intrinsic tolerance of rhizobial strains to the applied concentrations of heavy metals in terms of growth. All strains tested displayed the lowest intrinsic tolerance when growing on Ni and Cu, while most strains were found to have the highest intrinsic tolerance to Pb, Zn and Hg. Cd and Cr concentrations of 50-75 μgml^{-1} had inhibitory effect on the growth of most rhizobial strains tested. Differences in growth on different concentrations of heavy metals were found not only among different rhizobial species, but among rhizobia of identical species as well, which shows their different genetic structures resulting from indigenous biodiversity.

Keywords: Heavy metals, Rhizobia, Growth, Metal tolerance.

Introduction

Heavy metals are widely distributed in the biosphere, and their concentrations in soils and surface waters vary. Soils and waters can also be contaminated with heavy metals from different anthropogenic sources, such as smelters, mines, power stations, metal-rich pesticides, fertilizers, waste waters, etc. [14, 24, 5]. Over the past ten years, there has been a growing concern over increased heavy metal pollution of soils and waters, and the resulting toxicity to plants, animals and microorganisms, as well as their irreversible binding to various soil components [23, 25]. They have been found harmful to various associations of plants and microorganisms. Regarding mycorrhizae, the size of mycorrhizal roots has been found to decrease in soils containing high concentrations of heavy metals such as Cu, Pb and Zn [18]. Waste waters containing high concentrations of heavy metals have also been shown to inhibit mycorrhizal infection of soybean, especially in soils with low pH [11]. It has been proved that $CuSO_4$ affects nitrogen mineralization depending on soil pH, with acid soils (pH 5.1 and 5.9) being more tolerant to high concentrations of this agent (1000 $\mu g\ ml^{-1}$) and neutral soils (pH 7.3) showing a tendency of decreasing nitrogen mineralization under 100 $\mu g\ ml^{-1}$ concentration of $CuSO_4$. Accumulation of mineralized nitrogen under such pH conditions was complete when it was in nitrate form [16]. Plant uptake of heavy metals (Cu, Pb, Zn and Cd) from soil is highest in sandy soils, especially that of Zn and Cd, which are considerably more mobile than Pb and Cu. Total plant uptake of heavy metals was lowest in organic soils [7].

Pb and Cu are less mobile than Zn and Cd. Ni, Cd and Zn are potentially more serious contaminants of soil solutions than Cu and Pb [6]. The amount of heavy metals accumulated in a soil would depend on the degree of their emission from a source, their transport to an accumulation site and their retention in soil. Some metals, such as Zn, Cu, Ni and Cr, are essential and beneficial micronutrients to plants, animals and microorganisms and they uptake them by various metabolic processes, in contrast to non-essential ones (Cd, Hg and Pb), whose physiological function has not been fully clarified and uptake occurs by non-metabolic mechanisms [8, 20, 4]. Several studies so far have shown harmful effect of heavy metals on the growth, morphology and activity of microorganisms, including rhizobia, as well as on N_2 fixation [22, 19, 13, 1, 15].

Symbiotic nitrogen fixation has an important role in providing host plants with nitrogen and increasing their yield, as well as in soil enrichment with nitrogen for succeeding crops. Rhizobia are Gram-negative soil bacteria that are capable of performing N_2 fixation with leguminous crops, which is crucial for preserving soil fertility [17, 2]. As much as 25-400 kgN ha^{-1} in soil is retrieved annually through this association of legumes and rhizobia to provide for subsequent crops, and the process depends on the plant as a macrosymbiont and the rhizobial strain as a microsymbiont, as well as on a series of abiotic and biotic factors [8, 3, 12]. Substitution of mineral N fertilizers, especially in the production of staple legumes, with microbiological fertilizers containing active rhizobial agents enables substantial energy savings (> 25 10^9 J t^{-1}) in the production process and reduces threats of environmental pollution caused by continuing or excessive use of mineral N fertilizers.

It is noteworty that absence of nitrogen fixation in clover crops grown on soils contaminated with heavy metals over a long period of time has been found to result from a survival only of ineffective rhizobial strains [14]. Further more, rhizobia can be used as good indicator organism to estimate the level stress imposed on rhizobia to severel toksic chemicals including heavy metals [10, 21]. Consequently, our research focused on investigating the effect of heavy metals on rhizobial growth so as to be able to identify tolerant and highly effective strains suitable to be used as microbiological nitrogen fertilizers for major legumes production.

Materials and methods

The effects of different heavy metal concentrations were investigated on Yeast Mannitol Agar (YMA) medium supplemented with Congo red [9]. We tested 16 strains of *B. japonicum*, 15 strains of *S. meliloti*, 24 strains of *R. leguminosarum* (8 bv. *phaseoli*, 8 bv. *viciae* and 8 bv. *trifolii*), 4 strains of *R. loti* and 3 strains of *R. galegae*, all from a collection of the Institute of Soil Science.

Pb was applied as compound $Pb(CH_3COO)_2$ at concentrations of: 10, 25, 50, 75, 100, 150, 175 and 200 $\mu g \cdot ml^{-1}$; Hg as $HgCl_2$ (2, 5, 10, 20, 50, 75 and 100 $\mu g\ ml^{-1}$); Cd as $3CdSO_4\ 8H_2O$ (2, 5, 10, 20, 50 and 75 $\mu g\ ml^{-1}$); Zn as $ZnSO_4\ 7H_2O$ (5, 10, 25, 50, 75 and 100 $\mu g\ ml^{-1}$); Cu as $CuSO_4\ 5H_2O$ (2, 5, 10 ,20 and 25 $\mu g ml^{-1}$); Ni as $NiSO_4\ 7H_2O$ (2, 5, 10, 20 and 25 $\mu g\ ml^{-1}$); and Cr as $K_2Cr_2O_7$ (5, 10, 20, 25, 50 and 75 $\mu g\ ml^{-1}$).

The rhizobial strains were examined by adding their respective cultures, established on YMB medium with approximately 10^{-9} cells per ml, onto YMA media supplemented with appropriate heavy metal concentrations using multiple inoculator with 48 injectors, according to Handbook for Rhizobia [17] and with four replicates. After incubation in air bath at 26ºC for 4-7 days, visual assessment of growth was carried out using three marks: + (good growth), ± (weak growth) and – (no growth), as compared to control.

Results and discussion

Growth of unicellular microorganisms, including rhizobia, can be measured using two different parameters: cell mass and cells numbers, and the values acquired may not coincide. Mass of individual cells varies in different stages of growth and increases continuously over time, while cell numbers increase in a process that has interruptions caused by cell division at regular time intervals. Generation time for fast-growing rhizobia is 2-4 h, and for slow-growing 6-8 h. In this investigation, the growth of rhizobial strains applied to different heavy metals concentrations was determined visually based on cell mass increase.

Of the 16 *B. japonicum* strains tested, good growth at Pb concentration of 175 μg ml^{-1} was found in 10 strains (507, 518, 519, 520, 524, 526, 531, 533, 538 and 539), weak growth in 5 strains, and no growth in strain 542. Pb concentration of 200 μg ml^{-1} fully inhibited the growth of another 7 strains and significantly decreased growth of 5 more strains of *B. japonicum*. Only strains 507, 526 and 539 were found to grow well under that concentration (Table1).

Table 1. Highest concentration values of heavy metals affecting growth of *B. japonicum* strains.

B. japonicum strain	Heavy metal concentration (μg ml^{-1})														
		Pb		Hg		Cd		Zn		Cu		Ni		Cr	
	∅	175	200	50	75	20	50	75	100	10	20	10	20	50	75
505	+	±	-	±	-	-	-	+	±	±	-	-	-	-	-
507	+	+	+	+	-	±	-	+	+	±	-	-	-	±	-
518	+	+	±	+	±	±	-	+	±	±	-	-	-	+	-
519	+	+	-	+	+	+	-	+	±	-	-	+	-	-	-
520	+	+	±	±	-	±	-	+	±	-	-	+	-	±	-
522	+	±	-	+	±	±	-	+	±	+	+	+	-	±	-
523	+	±	-	+	-	±	-	+	±	+	+	+	-	+	+
524	+	+	±	+	-	±	-	+	+	-	-	+	-	+	+
526	+	+	+	+	±	+	±	+	+	+	+	-	-	+	-
527	+	±	-	±	-	±	-	+	±	±	-	+	-	-	-
531	+	+	±	±	-	±	-	+	±	-	-	+	-	+	+
532	+	±	-	+	-	±	-	+	+	+	+	+	-	±	-
533	+	+	-	+	±	-	-	+	±	±	-	-	-	±	-
538	+	+	±	±	-	-	-	+	+	+	+	-	-	-	-
539	+	+	+	±	-	-	-	+	-	-	-	-	-	+	+
542	+	-	-	±	-	-	-	±	-	±	-	+	-	-	-

* Ø – control; (+) good growth; (±) weak growth; (-) no growth;

Nine strains of *B. japonicum* had good growth at Hg concentration of 50 μg ml^{-1} and 7 strains had weak growth, while the concentration of 75 μg ml^{-1} caused no growth of 11 strains and weak growth of 4 strains. Strain 519 alone was tolerant to that Hg concentration. Cd concentration of 20 μg ml^{-1} inhibited the growth of 5 strains and decreased growth of 9 strains of *B. japonicum*. Good growth under this Cd concentration was only found in strains 519 and 526, while 50 μg ml^{-1} concentration inhibited growth of all strains tested except 526, which had weak growth. Zn concentration of 75 μg ml^{-1} did not inhibit growth of all *B. japonicum* strains tested, and its concentration of 100 μg ml^{-1} was found to produce good growth of strains 507, 524, 526, 532 and 538, weak growth of 9 strains, and no growth of strains 539 and 542. All *B. japonicum* strains tested showed the lowest intrinsic tolerance to Cu and Ni. Cu concentration of 10 μg ml^{-1} inhibited the growth of 5 *B. japonicum* strains, while weak growth was recorded for 6 strains. Only strains 522, 523, 526, 532 and 538 displayed good growth at Cu concentration of 20 μg ml^{-1}, while the other strains had no growth at all. Ni concentration of 10 μg ml^{-1} had an inhibitory effect on the growth of 7 strains of *B. japonicum*, in contrast to the other strains that recorded good growth on that concentration, while 20 μg ml^{-1} concentration left all strains tested without any growth. Cr concentration of 50 μg ml^{-1}, and especially of 75 μg ml^{-1}, inhibited or reduced the growth of most tested strains of *B. japonicum*, except strains 523, 524, 531 and 539, which showed good growth under 75 μg ml^{-1} concentration.

Of the 15 *S. meliloti* strains tested, good growth was recorded for strains 215, 218, 219, 236, 241 and 247 at Pb concentration of 175-200 μg ml^{-1}, while growth of all other strains was weak or missing (Table 2).

Table 2. Highest concentration values of heavy metals affecting growth of *S. meliloti* strains.

S. meliloti strain	Heavy metal concentration (μg ml^{-1})														
		Pb		Hg		Cd		Zn		Cu		Ni		Cr	
	∅	175	200	50	75	20	50	25	50	5	10	10	20	10	20
204	+	±	-	±	-	+	-	+	-	-	-	±	-	±	-
215	+	+	+	+	-	+	-	-	-	+	-	+	-	+	-
218	+	+	+	+	-	+	-	-	-	±	-	+	±	+	-
219	+	+	+	+	-	±	-	+	-	±	-	-	-	+	±
222	+	±	-	±	-	±	-	-	-	-	-	±	-	±	-
224	+	±	-	+	+	+	-	+	+	+	+	+	±	+	-
236	+	+	+	+	+	+	+	+	+	+	-	+	±	-	-
239	+	±	-	+	-	+	-	±	-	±	-	+	-	±	-
241	+	+	+	+	+	+	±	±	-	+	+	-	-	+	-
242	+	-	-	+	-	±	-	-	-	±	-	+	-	+	-
244	+	±	-	+	-	-	-	-	-	±	-	±	-	+	-
245	+	-	-	+	+	-	-	-	-	±	-	±	-	-	-
246	+	-	-	-	-	-	-	-	-	-	-	±	-	±	-
247	+	+	+	+	-	+	-	-	-	±	-	+	-	+	-
249	+	±	-	+	+	±	-	-	-	+	-	±	-	-	-

* Ø – control; (+) good growth; (±) weak growth; (-) no growth;

Hg concentration of 50 μg ml^{-1} inhibited growth of strain 246, while 75 μg ml^{-1} concentration caused good growth of strains 224, 236, 241, 245 and 249 and no growth of the other strains tested. Cd concentration of 20 μg ml^{-1} inhibited growth of strains 244, 245 and 246, while its concentration of 50 μg ml^{-1} was found to cause good growth of strain 236 and weak growth of strain 241. Zn concentration of 25 μg ml^{-1} inhibited growth of 9 strains of *S. meliloti*, while 50 μg ml^{-1} concentration was found to cause good growth of strains 224 and 236, and no growth of all other strains tested. All *S. meliloti* strains tested displayed the lowest intrinsic tolerance to Cu and Ni. Cu concentration of 5-10 μg ml^{-1} inhibited or reduced growth of most strains tested. Good growth was only found in strains 215, 224, 236, 241 and 249. At 10 μg ml^{-1} concentration of Ni, weak or no growth were found in most tested strains of *S. meliloti*, except strains 215, 218, 224, 236, 239, 242 and 247, which grew well. Ni concentration of 20 μg ml^{-1} produced no growth of most strains tested. Cr concentration of 10 μg ml^{-1} inhibited growth in strains 236, 245 and 249, while none of the strains showed any growth at 20 μg ml^{-1} concentration.

Concerning the 8 strains each of the tested *R. leguminosarum* bv. *phaseoli* and *viciae*, most of them were found to achieve good growth at Pb concentration of 175 μg ml^{-1}. Pb concentration of 200 μg ml^{-1} inhibited growth of two *R. leguminosarum* bv. *phaseoli* strains (133 and 134), in contrast to most *R. leguminosarum* bv. *viciae* strains that had good growth (Table 3).

Table 3. Highest concentration values of heavy metals affecting growth of *R. leguminosarum* bv. *phaseoli* and *viciae* strains.

Rhizobia			Heavy metal concentration (μg ml^{-1})													
			Pb		Hg		Cd		Zn		Cu		Ni		Cr	
species	strain	∅	175	200	50	75	50	75	50	75	5	10	5	10	50	75
R. leguminosarum bv. *phaseoli*	110	+	+	+	±	-	±	-	±	-	-	-	±	-	-	-
	120	+	+	+	±	-	±	±	-	-	±	-	±	-	±	-
	123	+	+	±	±	-	-	-	±	-	±	-	±	-	±	-
	126	+	+	±	+	-	±	-	±	-	±	-	±	-	±	-
	130	+	+	±	+	±	±	-	+	±	-	-	+	±	±	-
	132	+	+	+	+	+	+	±	+	±	±	-	+	±	+	±
	133	+	±	-	+	±	+	±	±	-	-	-	-	-	-	-
	134	+	±	-	+	±	-	-	±	-	+	±	-	-	±	-
R. leguminosarum bv. *viciae*	301	+	+	+	-	-	±	-	-	-	-	-	-	-	-	-
	311	+	+	+	+	±	±	-	±	-	+	±	+	±	+	±
	312	+	+	+	±	-	+	±	+	±	+	±	+	±	-	-
	316	+	+	±	-	-	±	-	±	-	±	-	-	-	-	-
	318	+	+	+	±	-	+	±	-	-	±	-	±	-	-	-
	320	+	+	±	+	±	±	-	-	-	±	-	-	-	-	-
	321	+	+	+	+	±	-	-	-	-	+	±	+	±	±	-
	322	+	+	+	-	-	±	-	-	-	+	±	-	-	±	-

* Ø – control; (+) good growth; (±) weak growth; (-) no growth;

Hg inhibited growth of 3 *R. leguminosarum* bv. *viciae* strains (301, 316 and 322) at concentration of 50 μg ml^{-1}, while 75 μg ml^{-1} concentration caused either weak growth or no growth of most strains of *R. leguminosarum* bv. *phaseoli* and *viciae*. Cd concentration of 50 μg ml^{-1} acted inhibitory on the growth of strains 123 and 134 of *R. leguminosarum* bv. *phaseoli* and strain 321 of *R. leguminosarum* bv. *viciae*, while most strains of the two rhizobia species had weak growth or no growth at 75 μg ml^{-1} concentration. At 50 μg ml^{-1} concentration of Zn, good growth was recorded for strains 130 and 132 of *R. leguminosarum* bv. *phaseoli* and strain 312 of *R. leguminosarum* bv. *viciae*, while concentration of 75 μg ml^{-1} caused all strains of both rhizobial species to display weak growth or no growth at all. All strains tested in both rhizobial species showed the lowest intrinsic tolerance to Cu and Ni. At Cu concentration of 5 μg ml^{-1}, good growth was found only in strain 134 of *R. leguminosarum* bv. *phaseoli* and 4 strains of *R. leguminosarum* bv. *viciae* (311, 312, 321 and 322), while its concentration of 10 μg ml^{-1} caused all strains to show weak growth or no growth in both rhizobial species. Good growth was found in strains 130 and 132 of *R. leguminosarum* bv. *phaseoli* and 3 strains of *R. leguminosarum* bv. *viciae* (311, 312 and 321) at 5 μg ml^{-1} concentration of Ni, while weak growth or no growth were recorded for all strains of both species at 10 μg ml^{-1} concentration. Cr concentration of 50 μg ml^{-1} was found to cause good growth only of strain 132 of *R. leguminosarum* bv. *phaseoli* and strain 311 of *R. leguminosarum* bv. *viciae*, while growth of all other strains tested was weak or missing, and this was especially so at 75 μg ml^{-1} concentration.

Regarding the 8 tested strains of *R. leguminosarum* bv. *trifolii*, most had good growth at Pb concentration of 175 μg ml^{-1}, while the 200 μg ml^{-1} concentration resulted in weak growth or no growth of most strains, the exception being strains 407 and 409 (Table 4).

Table 4. Highest concentration values of heavy metals affecting growth of *R. leguminosarum* bv. *trifolii* strains.

R. leg. bv. *trifolii*	Heavy metal concentration (μg ml^{-1})														
		Pb		Hg		Cd		Zn		Cu		Ni		Cr	
	∅	175	200	20	50	50	75	50	75	5	10	5	10	50	75
407	+	+	+	±	-	±	-	-	-	+	±	±	-	+	-
409	+	+	+	+	±	±	-	±	-	-	-	-	-	+	±
451	+	±	-	±	-	±	-	±	-	-	-	+	±	±	-
459	+	+	±	-	-	-	-	-	-	±	-	±	-	-	-
461	+	+	±	±	-	±	-	±	-	±	-	+	±	-	-
464	+	+	±	±	-	±	-	±	-	±	-	+	±	-	-
467	+	+	±	±	-	+	±	±	-	+	±	-	-	-	-
469	+	±	-	±	-	-	-	+	±	±	-	+	+	±	-

* Ø – control; (+) good growth; (±) weak growth; (-) no growth;

At 20 μg ml^{-1} concentration of Hg, good growth of *R. leguminosarum bv. trifolii* strains was recorded only for strain 409, while all other strains had weak growth or no growth, and this especially applied to 50 μg ml^{-1} concentration. Cd concentration of 50 μg ml^{-1} resulted in no growth of strains 459 and 469, while the growth of all other strains was weak or there was no growth at all, and this was even more evident at 75 μg ml^{-1} concentration. Similar effect was recorded by Zn concentration of 50 μg ml^{-1}, which caused good growth of strain 469, while all other strains had weak growth or no growth, especially at 75 μg ml^{-1} concentration. All *R. leguminosarum* bv. *trifolii* strains tested showed the lowest intrinsic tolerance to Cu and Ni. Good growth was found only in strains 407 and 467 at 5 μg ml^{-1} concentration of Cu, while the growth of all other strains was weak or there was no growth, and this was especially so at 10 μg ml^{-1} concentration. Ni concentration of 5 μg ml^{-1} was found to cause good growth of 3 strains (461, 464 and 469), while all other strains had weak or no growth at all. Strain 469 was found to have good growth at its concentration of 10 μg ml^{-1}, while all other strains had weak growth or no growth. Good growth of *R. leguminosarum* bv. *trifolii* strains tested was recorded for strains 407 and 409 at 50 μg ml^{-1} concentration of Cr, while most other strains had weak or no growth, at 75 μg ml^{-1} concentration in particular.

Of the 4 *R. loti* and 3 *R. galegae* strains tested, good growth at Pb concentration of 175 μg ml^{-1} was found for 2 strains of *R. loti* (601 and 612) and 2 strains of *R. galegae* (801 and 802) (Table 5).

Strains 801 and 802 of *R. galegae* were found to have good growth at its concentration of 200 μg ml^{-1} as well, while *R. loti* strains produced weak or no growth at the same concentration. Good growth of *R. loti* strain 612 and *R. galegae* strain 804 were recorded at 50 μg ml^{-1} concentration of Hg, while growth of all other strains was weak or there was no growth at all, especially *R. loti* strains. Strain 804 grew well at Hg concentration of 75 μg ml^{-1}, while most strains tested had no growth. Good growth of strain 612 of *R. loti* was found at Cd concentration of 50 μg ml^{-1}, while all other strains of both rhizobial species had weak or no growth, especially at 75 μg ml^{-1} concentration. Zn concentration of 50 μg ml^{-1} was found to result in good growth of *R. loti* strains 612 and 615 and *R. galegae* strain 801, while all other strains had weak or no growth. Zn concentration of 75 μg ml^{-1} acted inhibitory on the growth of most strains in both rhizobial species. Strains of both rhizobial species showed the lowest intrinsic tolerance to Cu and Ni. At 5 μg ml^{-1} concentrations both of Cu and Ni, good growth was recorded for *R. loti* strain 612 and *R. galegae* strain 804, while 10 μg ml^{-1} concentrations resulted in weak growth or no growth of either rhizobial species. Good growth was found in *R. loti* strain 612 at 50 μg ml^{-1} concentration of Cr, while growth was weak or there was none recorded for any other strain in either rhizobial species, especially at 75 μg ml^{-1} concentration.

Table 5. Highest concentration values of heavy metals affecting growth of *R. loti* and *R. galegae* strains.

Rhizobia			Heavy metal concentration (μg ml^{-1})													
			Pb		Hg		Cd		Zn		Cu		Ni		Cr	
species	strain	∅	175	200	50	75	50	75	50	75	5	10	5	10	50	75
R. loti	601	+	+	±	-	-	-	-	-	-	-	-	±	-	±	-
	608	+	±	-	±	-	-	-	-	-	-	-	±	-	±	-
	612	+	+	±	+	±	+	±	+	±	+	±	+	±	+	±
	615	+	±	-	-	-	±	-	+	±	-	-	±	-	±	-
R.galegae	801	+	+	+	±	-	±	-	+	±	±	-	±	-	±	-
	802	+	+	+	±	-	±	-	±	-	±	-	±	-	±	-
	804	+	±	-	+	+	±	-	±	-	+	±	+	±	±	-

* Ø – control; (+) good growth; (±) weak growth; (-) no growth;

Tolerance of the strains investigated in this study was inconsistant with data reported by Tong and Sadovska (1994) from a similar research on *R. leguminosarum* bv. *trifolii*. The concentrations of Cd and Zn are consistant for all rhizobial species, but the minimum inhibitory concentration of Ni for *R. leguminosarum* bv. *trifolii* strains tested in our study was considerably lower (10 and 20μg ml^{-1}), compared to 80 μg ml^{-1} for strains Tal 1820 and Tal 1824 reported by the authors. Of a total of 62 strains, 29 were found to be susceptible to 10 μgml^{-1} Ni. The strains tested in this study were found to display higher tolerance to Hg, with 19 strains being tolerant to concentration of 75 μg ml^{-1}, whereas the other authors reported MIC<1 μg ml^{-1} Hg. Based on the significant difference in degrees of tolerance to some heavy metals, both among different rhizobial species and within them, various authors have made recommendations regarding selective media for differentiation between different groups of rhizobia. Tong and Sadowsky (1994) had recommended a selective medium for *B. japonicum* (BJSM). They also found that fast-growing rhizobia were more susceptible to Zn and Co (20-40 μg ml^{-1}) than slow-growing (80-480 μg ml^{-1}), and less susceptible to Mo.

Conclusion

The results of this investigation clearly indicate different levels of intrinsic tolerance of the investigated rhizobia strains in terms of their growth under various heavy metal concentrations applied.

All rhizobial strains tested were found to display the lowest intrinsic tolerance to Ni and Cu. Most strains showed the highest intrinsic tolerance to Pb, Zn and Hg. Cd and Cr concentrations of 50-75 μgml^{-1} acted inhibitory to growth of most rhizobial strains tested.

Differences in growth at various concentrations of heavy metals were recorded not only for different rhizobial species but strains of the same species as well, which shows differences in their genetic structure originating from their indigenous biodiversity.

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