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Rhizosphere bacterial populations of metallophyte plants in heavy metal-contaminated soils from mining areas in semiarid climate

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Summary

Rhizosphere bacterial populations associated with four metallophyte plants in one of major polymetallic (Pb–Zn–Cu) semiarid Moroccan Hercynian province (Draâ Sfar, Marrakech, Morocco) presenting long-term contamination mainly with Zn and Pb were analysed and compared to selected control soils. In the highly Zn-, Cu-, Pb- and Cd-contaminated soils, the total number of culturable heterotrophic bacteria were found in low proportions ($<2.6 \times 10^2$ – 1.6×10^4 g^{-1}soil). This bacterial content was slightly similar to that found in moderately polluted and controls soils ($6.7 \times 10^4 - 5.8 \times 10^6$). However, the bacterial diversity and the rhizosphere/soil ratio, which compares the bacterial content (or bacterial charge) around the metallophyte plants with that in non-rhizosphere soil, were the bacteriological parameters mostly affected by heavy metal contamination. The chronic Zinc-stress results in an increase of tolerance to this metal of both the rhizosphere and non-rhizosphere bacterial communities. However, in general, the rhizosphere bacterial populations exhibited less tolerance to Zn toxicity than the bacterial population of non-rhizosphere soils. This result suggests that toxic effects of Zn decrease in the rhizosphere soils of the metallophyte plants.

Introduction

Phytoextraction of soil heavy metals consists of growing hyperaccumulating plants on contaminated soils and harvesting the above ground plant material (leaves and stalks) which would then be dried and ashed (Baker *et al.* 1994). However, the successful plant-based decontamination of soils would requires crops able to concentrate metals in excess of 1–2% and biomass production. These characteristics severely limit the use of metallophyte plants for environmental decontamination. An alternative soil phytoremediation technology has been proposed using non-accumulator metallophyte plants for phytostabilization. This proposed technology consist of cultivating plants to establish a sustainable and durable vegetation in order to help the stabilization of the soil surface and eventuality to reduce heavy metal mobility (Cunningham 1995; Salt *et al.* 1995). To be effective, plants need to be able to take up large quantities of the metals or may harbour microbial communities with an efficient capacity to detoxify polluting substances by rendering then insoluble. In fact, soil micro-organisms are known to play a key role in the mobilization and immobilization of heavy metals (Hughes & Poole 1989), thereby changing their availability to plants. However, in spite of the increasing knowledge of metal-micro-organism interactions, few studies have attempted to characterize the rhizosphere soil bacterial communities of metallophyte plants *in situ* under metal stress. This is due, in part, to the complexity of soil systems. The complexity of soil and its micro-organisms is exacerbated by metal mobility, variations

induced by environmental factors and root exudates released by metallophyte plants. Consequently, more information is required to understand the metallophyte plants and their rhizosphere microbial population interactions and to be able to develop methods enhancing root growth in heavy metal-contaminated environments.

From the economic and environmental points of view, soils contaminated with heavy metals resulting from industrial and urban activities, are abundant and frequently support a distinct assemblage of metallophyte plants particularly resistant to heavy metals. The existence of these plants is attractive for their use in

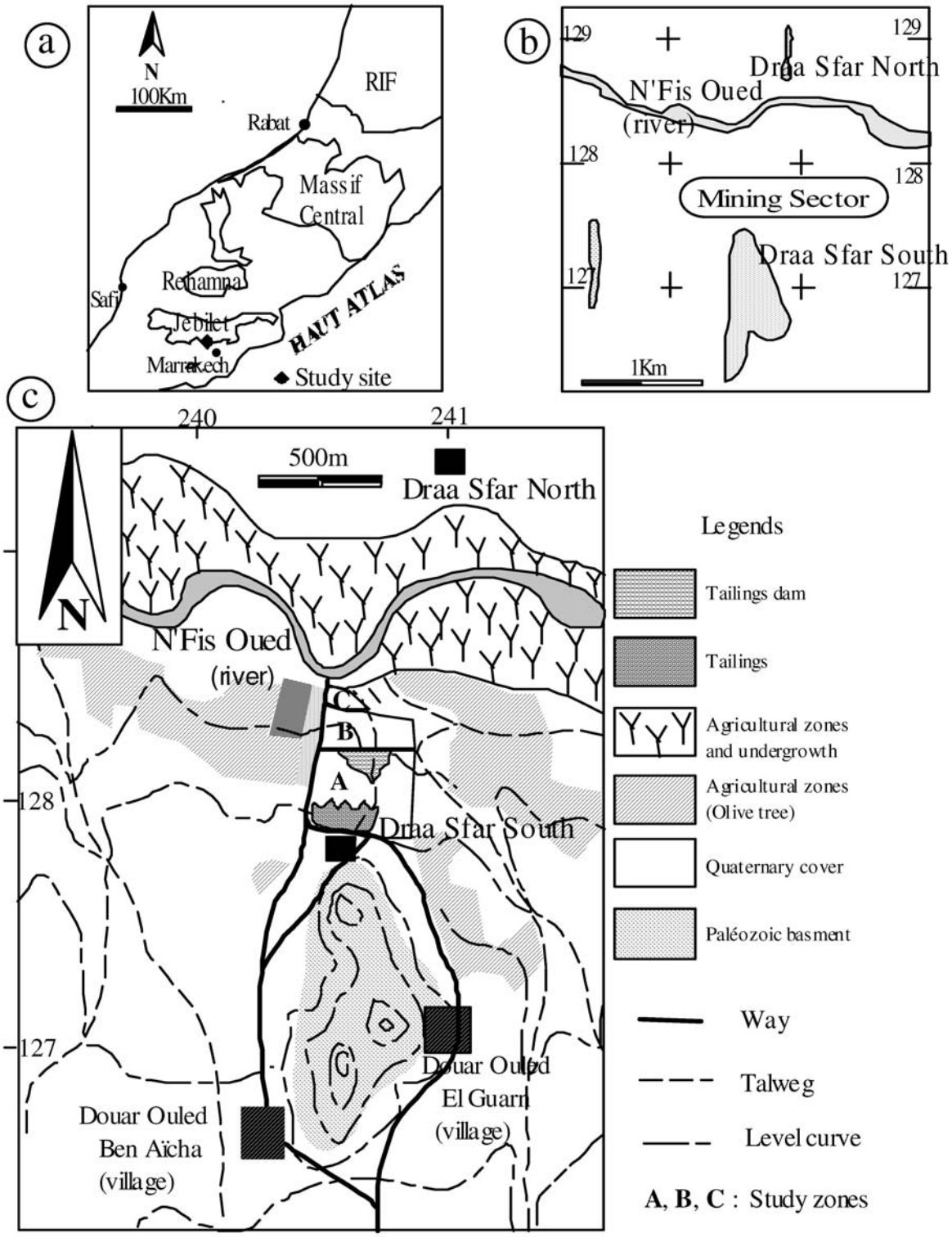

Figure 1. Simplified map of the massive sulphide deposit Draâ Sfar (Marrakech, Morocco): (a) localization of the site from Marrakech city within the central part of the Jebilet hills; (b) localization of the site from the river (Oued N'Fis); (c) study area (A–C). For geological details, see legends within the (c).

phytostabilization proceses. In this study, we selected contaminated soils located in the vicinity of outcrops of metalliferous minerals (Draâ Sfar, Marrakech, Morocco) presenting long-term contamination, mainly with Zn and Pb resulting from mining operations. We investigated the density, diversity and Zn tolerance of the rhizosphere bacterial populations in four dominant non-accumulator metallophyte plant species collected on contaminated soils.

Materials and methods

Field site description and study area

The massive sulphide deposit Draâ Sfar, located 16-km Northwest of Marrakech city is one of the major polymetallic (Pb–Zn–Cu) occurrences in the Moroccan Hercynian province (Figure 1). It was discovered in 1962 in the central part of the Jebilet hills and emerged from exploratory research in this area after the discovery of the Kettara deposit. Site descriptive details have been reported in Hibti *et al.* (1999). The study area extended from the mine to a very large river called Oued N'Fis and located 550-m downstream from the mine. In order to study rhizosphere bacterial populations in metallophyte plants, the polluted area was divided into three sampling zones (A–C). Zone A is located between the mine installations and the first tailing dam 200-m downstream. Climatic variations and rainfall leaching of rocks and trailing are responsible for environmental damage caused by acid production in this zone causing a rarity of flora in this area. Plants were tentatively identified as *Rhus pentaphylla*, *Mesembryanthemum nodiflorum*, *Salsola vermiculata* and *Peganum harmala*. Zone B is located immediately downstream of zone A. It is bounded by the tailing dam of A. Zone B is formed mainly of soils broken down in some places leading to drainage water. It extends for 300 m to the north and is bounded downstream by a second tailing dam. It is characterized by the same plant species as zone A. Zone C is located immediately downstream of B and is bounded in the north by the Oued N'Fis river. Unless otherwise indicated, the plant species are similar to those observed in zones A and B, with exception of *Mesembryanthemum nodiflorum*, which was not found in zone C.

Soil and plant sampling

Three samples from each soil and plant were collected at three separate zones within the mining area and uncontaminated soils. Uncontaminated soils (control soils) were collected form a site located approximately 150 km north of Draâ Sfar. This control zone presents approximately the same soil type and plant species (*R. pentaphylla* and *P. harmala*) as the heavy metal-contaminated soils. Samples of soils and plants were taken according to a simple randomized schematic strategy (Van Elsas & Smalla 1997). Samples of rhizosphere soils were taken using the method described by Pochon & Tardieux (1965). Plant roots were, therefore, enclosed in a plastic bag to maintain a modest degree of moisture during shipment. In the laboratory, the soil was obtained by shaking the plant. Samples of non-rhizosphere soil samples were collected 2 m from each plant at a depth of 10 cm. Soils were sieved through a 2 mm-mesh size screen. An aliquot of each soil was air-dried before physico-chemical analyses. The remaining soils were stored in sterile glass vials at cold 4 °C for microbiological analyses.

Physico-chemical analyses

Heavy metals in soils and plant tissues were analysed on a Varian 475-Atomic Adsorption spectrophotometer. Total metal concentrations in soils and in plants dried at 105 °C were determined according to the analytical procedures recommended by the Association Française de Normalisation (AFNOR 1997); detection limit of the atomic absorption spectrometry was 0.02 mg kg^{-1} Available metals in the soils were measured by extraction with 0.1 M $CaCl_2$ (McGrath & Cegarra 1992). Soil pH was measured in soil: water mixtures (1:5 w/v). Total soil organic matter was measured by using an incineration method and soil granulometry was performed following the international method of Robinson (Aubert 1978).

Microbiological analyses

Bacteria were extracted from soil by shaking 2 g of soil at 150 rev/min^{-1} in 50-ml of sterile NaCl solution (8.5 g l^{-1}) for 30 min. Viable heterotrophic bacteria were counted by plating appropriate dilutions on Luria-broth (LB) consisting of tryptone (10 g l^{-1}), yeast extract (5 g l^{-1}), NaCl (5 g l^{-1}) and agar (15 g l^{-1}) at pH 7.5 supplemented with cycloheximide (50 μg ml^{-1}) to prevent fungal growth. Colonies were counted after 5 days of incubation at 30 °C. Zn-tolerant heterotrophs were counted by supplementing the LB agar, with appropriate amounts of filtration-sterilized Zinc (as $ZnSO_4 \cdot 7\ H_2O$) stock solution to give final concentrations of 0.1, 0.5, 1, 5, and 10 mM Zn. Bacteria were isolated from the LB agar plates used for the bacterial counts. Morphological dissimilarity was used as a criterion for isolate selection (Duxbury & Bicknel 1983). Isolated bacteria were identified using the directives given in *Bergey's Manual of Determinative Bacteriology* (1994). Preliminary biochemical tests including motility, oxidase reaction, respiration type, nitrate respiration, nitrate reduction and the Hugh-Leifson O/F test were conducted to complement the microscopic observations (fresh state and Gram staining). Gram-negative, non-fermenting bacteria were differentiated using indole production and gelatine hydrolysis tests. Gram-negative, facultative anaerobic, negative oxidase and fermentative bacteria were identified as *Enterobacteriaceae*. Generics of this family were identified using the following physiological tests: β-galactosidase and urease reactions, growth on citrate as sole carbon

source, production of acid from mannitol, gelatine hydrolysis, and the glucose fermentation metabolic pathway. Gram-positive bacteria were characterized by the following tests: catalase reaction, sporulation tests, and acid-alcohol tolerance. All the assays were performed according to methods described by Smibert & Krieg (1981) and/or using, in the case of Gram-negative bacteria, API20NE and API20E strips for non-fermentative and fermentative bacteria respectively.

Calculation of diversity indices

Diversity analysis was carried out by using the Shannon species diversity indices, $H' = -\Sigma\ pi \log pi$, where *pi* corresponds to the number of individuals in each cluster divided by the total number of isolates in the sample (Barkey et al. 1985; Dean-Ross 1990).

Statistical analysis

One way analyses of variance (ANOVA) were performed on the results using the SPSS statistical program. Least significant differences were obtained using Duncan's test. Results were considered to be significantly different at $P < 0.05$.

Results and discussion

Characteristics and metal concentrations of the soils sampled

The characteristics of the tested soil samples are presented in Table 1. Soils in zones A–C and the control zone are sandy (data not shown). Organic matter content was found to be variable in all soils, ranging from 3.5% to 16%. The pH of all soils was generally neutral to alkaline with the exception of the non-rhizosphere and *R. pentaphylla* rhizosphere soils in zone A, which were very acid (3.6 and 3.3 respectively). When considering the non-rhizosphere soils, the most severely contaminated was zone A with Zn, and Cu metal concentrations 10 times higher (1059 and 424 vs. 74 and 29 mg kg^{-1}) and Pb 1000 times higher (1040 vs 1 mg kg^{-1}) respectively than the control soil. Zones B and C show lower Zn and Pb concentrations than A, and total Cu and Cd concentrations that are within the background levels (Table 1). It is important to note that in the highly polluted zone (A), a significant decrease in heavy metals was observed in the rhizosphere soils as compared to non-rhizosphere. This observation could be attributed to the metal detoxification by adsorption on the plant cell walls and intracellular sequestration (Kabata-Pendias & Pendias 1992). Table 2 shows high concentrations of Zn, Cu, Pb and Cd in both shoots and roots in the highest polluted soils. It is also interesting to note that in all polluted soils (A–C), the largest Cu, Pb and Cd concentrations were retained essentially in the roots (Table 2). The plants thereby appeared capable of restricting the translocation of these metals towards their aerial parts within the adequate range below the phytotoxic level as seen in soils B and C. In contrast Zn is retained in the roots only in the highest polluted soils (A), and concentrated essentially in the shoots in the moderate polluted soils (B and C) (Table 2).

On the other hand, since determination of total content of metals has only limited value, as they occur

Table 1. Total and metal concentrations and soils physicochemical characteristics.

Zones	Soils	OM %	pH	Total metal concentrations(mg kg^{-1})				Extractable metal concentrations(mg kg^{-1})			
				Zn	Cu	Pb	Cd	Zn	Cu	Pb	Cd
A	Non-rhizosphere soil	3.5	3.6	1059 a	424 a	1040 a	3.5 a	43.00 b	47.00 b	73.20 b	1.90 a
	R. pentaphylla rhizosphere soil	11.0	3.3	984 b	312 b	676 b	2.9 b	66.00 a	76.00 a	128.00 a	0.30 b
	M. nodiflorum rhizosphere soil	5.5	7.8	429 f	105 c	163 cd	2.9 b	2.50 c	0.25 d	0.32 c	0.05 d
	S. vermiculata rhizosphere soil	6.5	7.7	856 c	77 e	53 e	2.3 c	0.50 f	0.50 d	0.02 d	0.04 d
	P. harmala rhizosphere soil	7.5	7.6	861 c	65 f	42 ef	0.7 gh	1.50 d	0.70 d	0.02 d	0.10 c
B	Non-rhizosphere soil	6.5	7.9	667 e	35 gh	73 d	0.4 gh	1.14 e	0.60 d	0.04 d	< 0.02 d
	R. pentaphylla rhizosphere soil	5.0	8.3	761 d	48 g	29 g	1.5 e	0.58 f	0.32 d	0.03 d	0.03 d
	M. nodiflorum rhizosphere soil	6.0	8.3	192 g	33 gh	34 f	0.5 gh	0.11 g	0.07 e	< 0.02 d	< 0.02 d
	S. vermiculata rhizosphere soil	3.6	7.7	847 c	85 d	115 cd	0.2 gh	1.07 e	0.38 d	0.03 d	0.03 d
	P. harmala rhizosphere soil	3.5	7.2	506 f	63 f	236 c	1.0 g	0.41 f	0.13 e	0.02 d	< 0.02 d
C	Non-rhizosphere soil	7.0	7.8	674 e	36 gh	74 d	1.9 d	0.93 e	0.42 d	0.03 d	0.02 d
	R. pentaphylla rhizosphere soil	7.0	8.5	744 de	28 h	34 f	3.8 a	0.95 e	0.31 d	0.03 d	0.02 d
	S. vermiculata rhizosphere soil	12.5	7.6	745 de	26 h	30 g	3.2 a	1.02 e	2.33 c	0.04 d	0.02 d
	P. harmala rhizosphere soil	8.0	7.9	49 h	8 i	6 h	0.1 h	0.31 f	0.65 d	0.08 d	0.02 d
Control	Non-rhizosphere soil	5.0	7.7	74 h	29 h	1 h	1.3 f	0.70 f	0.60 d	0.03 d	0.02 d
	R. pentaphylla rhizosphere soil	6.0	8.1	73 h	35 gh	5 h	0.8 gh	0.56 f	0.70 d	0.02 d	0.04 d
	P. harmala rhizosphere soil	16.0	7.5	67 h	25 h	12 g	0.9 gh	0.20 g	0.40 d	0.03 d	0.02 d

OM, organic matter. *M. nodiflorum* was not found in C and control zones, *S. vermiculata* was not found in control zone. Means in each column followed by the same letter did not differ significantly ($P < 0.05$) by one-way analysis of variance (ANOVA) and Duncan's test.

Table 2. Shoot and root metal concentrations (mg kg^{-1} dry matter).

Zones	Plants	Zn		Cu		Pb		Cd	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
A									
	R. pentaphylla rhizosphere soil	178 d	295 c	45.0 a	56.0 a	17.0 d	44.0 b	0.8 cd	2.9 a
	M. nodiflorum rhizosphere soil	433 a	381 b	21.0 b	46.0 a	13.0 d	25.0 c	1.5 b	1.0 c
	S. vermiculata rhizosphere soil	49 fg	72 f	14.0 d	13.0 d	6.0 e	51.0 a	0.7 d	0.5 d
	P. harmala rhizosphere soil	32 g	60 fg	4.4 de	29.0 b	0.7 f	6.3 e	0.3 e	0.3 e
B									
	R. pentaphylla rhizosphere soil	74 f	43 fg	8.6 de	18.0 c	3.4 ef	21.0 c	0.4 de	0.3 e
	M. nodiflorum rhizosphere soil	37 fg	19 h	4.2 de	12.0 d	1.8 ef	11.0 d	0.3 e	0.6 d
	S. vermiculata rhizosphere soil	75 f	38 g	3.4 e	14.0 d	1.7 ef	52.0 a	0.4 de	0.8 cd
	P. harmala rhizosphere soil	66 fg	58 fg	7.2 de	16.0 c	1.4 ef	7.1 e	0.1 e	0.4 de
C									
	R. pentaphylla rhizosphere soil	31 g	22 gh	6.3 de	13.0 d	1.1 ef	2.5 ef	0.1 e	0.1 e
	S. vermiculata rhizosphere soil	105 e	48 fg	3.1 e	7.4 de	1.4 ef	4.3 ef	0.2 e	0.6 d
	P. harmala rhizosphere soil	45 fg	42 fg	3.2 e	9.1 de	0.6 f	10.0 d	0.1 e	0.3 e
Control									
	R. pentaphylla rhizosphere soil	22 gh	14 h	6.2 de	8.9 de	1.3 ef	2.1 ef	0.1 e	0.2 e
	P. harmala rhizosphere soil	35 g	27 g	3.1 e	4.9 de	1.0 ef	1.3 ef	0.1 e	0.7 d

nd, not determined. *M. nodiflorum* was not found in C and control zones, *S. vermiculata* was not found in control zone. Means in each metal followed by the same letter did not differ significantly ($P < 0.05$) by one-way analysis of variance (ANOVA) and Duncan's test.

in various forms with different bioavailability and biotoxicity, the available metal fraction in the soils was also evaluated by a chemical extraction with $CaCl_2$ solution to estimate concentrations of metals in the soil solution and really-exchangeable fraction. The amounts of extractable heavy metals were very low in the neutral soils (Table 1). Therefore, its accurate measurement was restricted only to the acidic soils including *R. pentaphylla* rhizosphere and the non-rhizosphere soils in the zone A. From this result, the pH greatly influenced the solubility of heavy metals. Consequently, bacterial populations in the acidic soils could be exposed to higher and more toxic *in situ* concentration of heavy metals than from neutral soils.

Counting and diversity of culturable heterotrophic bacteria

Culturable heterotrophic bacteria in the non-rhizosphere soils were 3–10 times less abundant in the contaminated soils A–C than in the control soils (1.6×10^4, 6.7×10^4, 1.3×10^5 vs. 4.0×10^5 c.f.u. g^{-1}, respectively) (Table 3). Despite the apparently much lower density of bacteria in the presence of metal-stressed soils compared to those without metal-stressed soils, Table 3 shows that there were no significant differences in the mean culturable heterotrophic bacteria count between polluted and control non-rhizosphere soils, with the exception of the acidic soils which present the higher bioavailable heavy metal concentrations (Table 1). Thus, determination of bioavailable metal concentrations is imperative to better understand bacterial exposure to metal ions (Roane & Kellog 1996).

One numerical measure of the rhizosphere effects is the rhizosphere/soil ratio, which compares the number of bacteria in the rhizosphere with the number in the non-rhizosphere soil, i.e. the soil unaffected by root exudates. The rhizosphere/soil ratio was higher in the contaminated soils than in the control soils. This was expected, since it is well known that root exudates serve as a source of carbon and energy to micro-organisms. These therefore occur in far higher numbers in rhizosphere soils than in non-rhizosphere soils, where the lack of organic matter input may reduce the bacterial populations (Kuperman & Carreiro 1997).

Data describing the generic groups identified among the rhizosphere and the non-rhizosphere soils, and the diversity index are presented in Table 3. Based on the

Table 3. Enumeration of the aerobic heterotrophic bacteria and diversity indices in the soils.

Zones	Soils	c.f.u. g^{-1}	RS/NRS	Diversity index	Gram-negative bacteria (%)	Gram-positive bacteria (%)
A	Non-rhizosphere soil	1.6×10^4 e		0.60	6	94
	R. pentaphylla rhizosphere soil	$< 2.6 \times 10^2$ f	< 0.01	nd	nd	nd
	M. nodiflorum rhizosphere soil	2.5×10^5 cd	15.6	1.17	64	36
	S. vermiculata rhizosphere soil	5.8×10^6 a	362.5	1.08	89	11
	P. harmala rhizosphere soil	6.1×10^5 c	38	0.37	96	4
B	Non-rhizosphere soil	6.7×10^4 d		0.81	0	100
	R. pentaphylla rhizosphere soil	3.4×10^5 cd	5	1.48	67	33
	M. nodiflorum rhizosphere soil	8.3×10^4 d	1.2	1.1	94	6
	S. vermiculata rhizosphere soil	1.1×10^6 c	16.4	1.65	55	45
	P. harmala rhizosphere soil	4.4×10^5 cd	6.5	1.07	38	62
C	Non-rhizosphere soil	1.3×10^5 d		0.92	40	60
	R. pentaphylla rhizosphere soil	3.6×10^6 b	27.3	1.64	75	25
	S. vermiculata rhizosphere soil	1.4×10^6 c	10.7	0.69	86	14
	P. harmala rhizosphere soil	2.4×10^5 d	1.8	1.29	16	84
Control	Non-rhizosphere soil	4.0×10^5 cd		1.44	49	51
	R. pentaphylla rhizosphere soil	6.3×10^5 cd	1.5	1.72	76	24
	P. harmala rhizosphere soil	3.4×10^6 b	8.7	2.1	75	25

nd, not determined; C.F.U. colonies forming units; RS/NRS, number of bacteria in the rhizosphere soil/ number of bacteria in the non-rhizosphere soil ratio. *M. nodiflorum* was not found in C and control zones, *S. vermiculata* was not found in control zone(see materials and methods). Means in c.f.u. column followed by the same letter did not differ significantly ($P < 0.05$) by one-way analysis of variance (ANOVA) and Duncan's test.

diversity indices, non-rhizosphere soils A had the lowest diversity index (0.60). In contrast the diversity indices of the non-rhizosphere soils in zones B and C, which had lower heavy metal concentrations, were 0.81 and 0.92 respectively. These values are very low compared to the control non-rhizosphere soil that exhibited a high diversity index (1.44). In each polluted zone, the rhizosphere soils, with the exception of *P. harmala* in zone A and *S. vermiculata* in zone C, exhibited much higher diversity indices (>1) than the corresponding non-rhizosphere communities (<1). It was noted that the rhizosphere soils had indices of 0.37 to 1.17 in zone A, 1.07 to 1.65 in zone B, and 0.69 to 1.64 in zone C. Rhizosphere control soils showed much higher diversity indices that ranged from 1.72 to 2.08. Our results are in agreement in reporting extremely low bacterial diversity as an immediate consequence of bacterial adaptation to heavy metal pollution (Barkay *et al*. 1985; Roane & Kellog 1996). Therefore, heavy metals induce stress that shifts bacterial community composition to lower diversity. However, we also noted differences in bacterial community structure between rhizosphere and non-rhizosphere soils. Hence, the rhizosphere may be considered as a rich medium (heterotrophic) and naked soil as a poor medium (oligotrophic). Therefore, it is difficult to compare our data with those reported in the literature because of the lack of information concerning rhizosphere metallophyte plant flora. The fact that the rhizosphere bacterial community was different from that in the non-rhizosphere soils is of considerable interest. Another significant interest is the fact that Gram-negative bacteria was mostly represented in bacterial population of rhizosphere soils (Table 3). The dominant Gram-negative generic groups isolated were *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Xanthomonas* and *Acinetobacter*, whereas Gram-positive bacteria were less abundant and were represented mainly *Bacillus* genera (Bennisse et al. 2003). The predominance of Gram-negative over Gram-positive bacteria in the rhizosphere has already been reported for rhizosphere soils (Gilbert et al.1994) and for forest soils where root biomass is high (Pennanem et al. 1996).

Zn tolerance of the bacterial community

The impact of heavy metals on the number, biomass and activity of micro-organisms, or on the species diversity of microbial populations, has been extensively studied (Huysman *et al*. 1984; Barkay *et al*. 1985; Frostegard *et al*. 1993; Roane & Kellogg 1996). It is well known that all the microbiological variables cited above are non-specific as they could be affected by different environmental factors (temperature, pH), and not only by heavy metals. Information obtained from metal impact studies is therefore difficult to evaluate because of the problem inherent to separating the effect of metal toxicity from that of other environmental factors. A more direct method of studying the effect of heavy metal pollution on micro-organisms may be to estimate the number of tolerant micro-organisms. In our study, the degree of Zn tolerance of the culturable bacterial community was assessed by comparing viable counts on unsupplemented medium to those obtained on media supplemented with several Zn concentrations. After log transformation of total colonies-forming units (CFU) counts, data had a nearly linear pattern. This assessment allowed us to use

Table 4. Relative toxicity of Zn to soil bacterial populations as expressed in regression coefficient *a* values from a resistance pattern[a].

Soils	Zones			
	A	B	C	Control
R. pentaphylla rhizosphere soil	nd	−0.51 d	−0.34 h	−0.54 c
M. nodiflorum rhizosphere soil	−0.27 i	−0.46 e	nd	nd
S. vermiculata rhizosphere soil	−0.43 f	−0.36 h	−0.54 c	nd
P. harmala rhizosphere soil	−0.20 j	−0.35 h	−0.55 b	−0.62 a
Non-rhizosphere soil	−0.14 k	−0.39 g	−0.47 d	−0.54 c

[a] see the text; nd, not determined. Number followed by the same letter did not differ significantly ($P < 0.05$) by one-way analysis of variance (ANOVA) and Ducan's test.

the linear regression $\ln(y) = ax + b$ (Tichy *et al.* 1993) where a and b are regression coefficients, x the concentration of Zn in testing plates and $\ln(y)$ is the natural logarithm of frequency of surviving c.f.u. Regression coefficient a well describes general decrement of a number of surviving c.f.u. with increasing concentration of Zn in testing medium. Changes in spectrum of Zn-tolerance soil c.f.u. via using of the coefficient a are expressed in Table 4. On the three non-rhizosphere polluted soils A–C, a values are lower than in the control soils (0.14, 0.40, 0.47 vs. 0.54 respectively). These results suggest that the bacterial population under Zn stress became more resistant to Zn. This agrees well with other studies using the same plate count method and showing that the fraction of metal-tolerant bacteria can be used as a sensitive measure of the metal impact (Doelman *et al.* 1994; Huysman *et al.* 1984). The most tolerant bacterial population in the present work were found in the most toxic soils (non-rhizosphere soil in the zone A) which exhibit the highest bioavailable Zn concentration.

Comparison between a values of rhizosphere and bulk soils in the same zone showed different degrees of tolerance to Zn, in spite of the similar Zn concentration observed in these soils by the chemical analyses (Table 1). In most situations the rhizospheric bacterial populations displayed less tolerance to Zn toxicity than the corresponding bulk soils. Since the degree of tolerance to heavy metal indicates the level of toxicity in soils as showing by previous findings (Huysman *et al.* 1984) we could conclude that toxic effects of Zn decrease in the rhizosphere soils. This contrasts with the chemical analyses, which show similar level of Zn bioavailable. However, in some cases, rhizospheric bacteria showed higher tolerance than the non-rhizosphere soils, this suggests that Zn tolerance in the rhizosphere soils is not a result of a stress by heavy metal contamination only, but is probably due to the structure of the bacterial communities. There, Gram-negative bacteria in the rhizosphere soil were dominant compared to the non-rhizosphere soils. Many reports have shown that Gram-negative bacteria are more tolerant of heavy metal stress than Gram-positive bacteria (Huysman *et al.* 1984; Frostegard *et al.* 1993; Roane & Kellogg 1996; Pennanen *et al.* 1996). This metal-tolerance may be attributed to the interactions between microbial cell wall components and metal ions resulting in their detoxification.

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