Effect of heavy metals on soil microbial quality of an abandoned mining area Sidi Kamber, North-East of Algeria

Nabil Charchar *, Laid Bouchaala, Hani Bouyahmed, Abd El-Fatteh Gherib, Amel Lehout

Center for Biotechnology Research (CRBt), Constantine, Algeria

Abstract

The ecological importance of soil bacteria is not limited to their number or biomass, although these parameters contribute greatly. Indeed, their main asset lies in their great genetic and functional diversity.This study aims to determine heavy metal contamination levels of the soils of an abandoned mining area of Sidi Kamber (Skikda), impact of heavy metals on bacterial communities and the possible risks that can affect the ecological balance of this area. Soil samples from three zones (Zone A, B and C) were collected from the top layer (0–20 cm) of mining area. Chemical analysis (pH, organic matter, total organic C, total N, available P, and cation exchange capacity, metal content of (Pb, Cu, Cd, Zn and Ni) and bacterial analysis were carried in center for biotechnology research CRBt. Our results show that the mining area is characterized by an acid pH. Significant variations were observed for edaphic parameters (organic matter, total organic C, total N, available P and cation exchange capacity) between three sampling zones. The overall area was severely polluted with Cu, Cd, Pb, Ni and Zn with a total concentration far exceeding international standards. The bacterial load and diversity were relatively high with a significant variation between the three zones. The PCA analysis of the soil's characteristics indicates that the organic matter and the cation exchange capacity affect the distribution of the metallic trace elements in the soil and allowed us thus to a clear separation of the studied zones.

Keywords: Heavy metals, mining area, Sidi Kamber, bacterial diversity.

Introduction

Mine tailings or mill tailings, which can account for more than 80-99% of the raw ore by weight (Edraki et al., 2014) are the remained materials after the economically extraction of the minerals from the ore (Diaby et al., 2007). The mine spoils and tailings generated by this industry generally present a hostile environment to the organisms' growth. Due to low nutriment availability, low organic matter content, high acidity and a very often elevated trace of metal content (Batty, 2005; Barrutia et al., 2011). Since these wastes aren't treated or even disposed of at the end of the activity, the former mine sites remain a local source of metal contamination (Navarro et al., 2008). Heavy metals are stable and environmentally persistent contaminants since they can neither be degraded nor destroyed (Sevgi et al., 2009). They produce an opposite effect on the human being's health as well as other living beings on both terrestrial and aquatic scale, as it can affect the food chain (Mishra and Nayak, 2009). Thus, it is absolutely necessary to evaluate the quality of the soil resources. Conceptually, the quality of the soil is defined as the capacity of the soil to function within ecosystem boundaries to sustain the biological productivity, to maintain the environmental quality and to

*	Nabil Charchar	✉ :	n.charchar@crbt.dz	iD :	0000-0001-5189-6562	(Corresponding author)
	Laid Bouchaala	✉ :	l.bouchaala@crbt.dz	iD :	0000-0001-8278-810x	
	Hani Bouyahmed	✉ :	h.bouyahmed@crbt.dz	iD :	0000-0003-0626-2581	
	Abd El-Fatteh Gherib	✉ :	a.gherib@crbt.dz	iD :	0000-0002-7354-8873	
	Amel Lehout	✉ :	a.lehout@crbt.dz	iD :	--	

promote both fauna and flora health (Alexander, 1977). The soil biology is a significant component of soil quality. Microorganisms play a vital role in the soil's fertility and the primary production through the organic matter decomposition and nutriment cycling (Doran and Parkin, 1994). Despite these unfavorable conditions, bacteria have evolved biological mechanisms to resist metal toxic contaminations and colonize this type of substrate (Whiting et al., 2004; Valverde et al., 2011). Bacteria are considered as efficient bio-indicators of the soil's quality because they respond faster and they are more sensitive to environmental changes than higher organisms. Either functional or structural bacterial diversity can be used as bio-indicators of the soil pollution (Nielsen et al., 2002). The ecological importance of the soils bacteria is not limited to their number of biomass even though these parameters contribute greatly. Indeed, their main asset lies in their great genetic and functional diversity (Torsvik and Øvreås, 2002). Our selected area was an abandoned mining area of Sidi Kamber in the North east of Algeria. Unexploited since 1984, with major mining wastes left. It represents a serious source of heavy metal contamination for this area and for the surrounding region.

The main purposes of this study were; a) To assess the soil heavy metals (Pb, Cu, Cd, Zn and Ni) contamination in the mining area called Sidi Kamber; b) To assess edaphic parameters such as pH, organic matter (OM), cation exchange capacity (CEC), total organic C, total N, available P; c) To spot the microbiological characteristics of the soil such as microbial communities and diversity; d) To determine the relation between total heavy metal content and bacteriological characteristics of the soils.

Material and Methods

The study area

This study was accomplished in the abandoned mine of Pb and Zn, precisely located in Sidi Kamber in the region of Oum Toub, Skikda,North eastern Algeria. (Boukhalfa, 2007) (Figure 1). The exploitation of this mine began in 1890 with a very low production and frequent stops and it's only in 1913 that a regular production began (Beddai, 1976). All the metallurgical activities on the site have been definitively ceased in 1984 (Oumdjbeur, 1986) (Figure 2). The studied area was characterized by rugged mountains which the altitudes are ranging between 200 to 500 m. The slope varies between 2 and 25%. It is very strong in the North Eastern land and low in the Southeastern valleys. According to the data collected from Skikda's weather station (SWS, 2014) for a period of 16 years (from 1997 to 2013). The studied area is characterized by a Mediterranean sub humid climate with a hot dry summer and mild winter. The annual temperature average is 19°C; the cumulative annual rainfall is estimated to 742.35mm. Monthly average value of relative humidity is 68.8 % (SWS, 2014). The vegetation inventory of the studied area allowed us to spot 104 plant species most of them are annual or perennial herbaceous plants that belong to the families of *Asteracea*e and *Poaceae* .The studied vegetation was characterized by therophytes to ruderals affinities. They are considered as the ultimate stage of ecosystem degradation*.* Spoil was colonized mainly by a short lawn composed of herbaceous such as *Rumex bucephalophorus*, *Lamarckia aurea*, *Virbascum sinuatum*, *Trifolium compestre* and *Medicago minima*. The ligneous stratum is small, it is mainly represented by *Cistus monspeliensis*, *Calicotome spinosa*, *Pistacia lentiscus* and some feet of *Erica arborea* (Lehout et al., 2017).
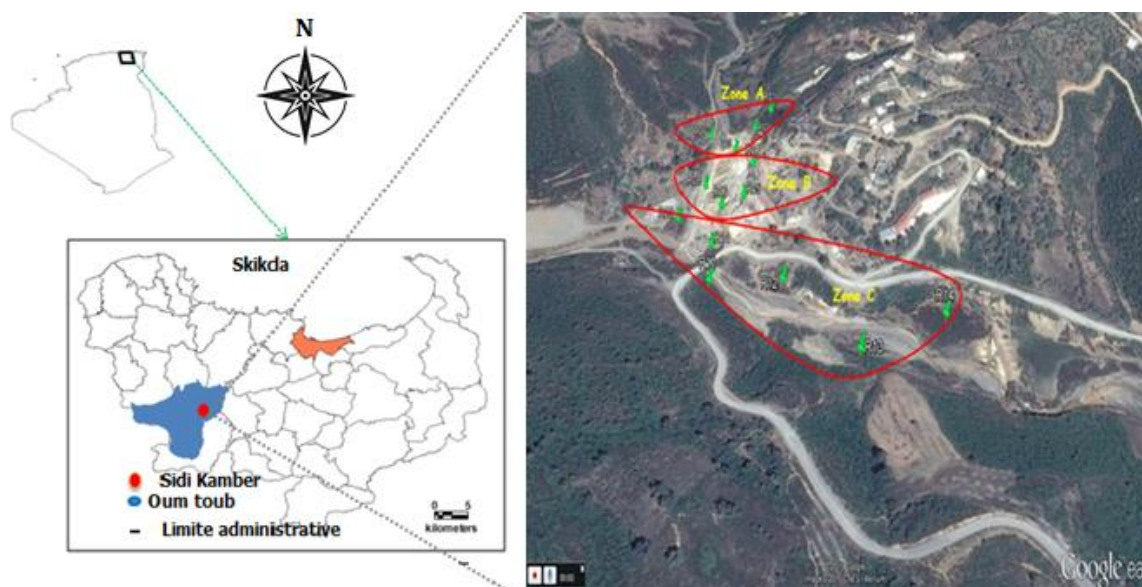

Figure 1. Geographic location of study area and sampling points.

Sampling points

Due to the big surface of the mining area (Sidi Kamber 700 h), the multiplicity and diversity of the metal extraction and the waste discharge sites. We have selected 14 sampling points distributed on three zones (Figure 2).

✓ Zone A: upstream of the mining zone with 4 sampling points.
✓ Zone B: in the center of the mine, close to both the waste discharges and the metal extraction sites with 4 sampling points.
✓ Zone C: the exit of the mining zone downstream, with 6 sampling points.

Figure 2. General view of the studied area

Some chemical analysis of soil samples

Soil samples (non-vegetated soil) were collected from the top layer (0-20cm) of the mining area. These samples were air-dried in a room temperature and sieved through 2mm mesh (to remove as far as possible plants materials and stones) before doing any further analysis of the physico-chemical characterization according to the standards methods. pH of soil (soil:distilled water; 1:2.5, w/v) was measured using the glass-electrode method, organic matter (OM%) and carbon content (C%) were determined using wet oxidation method of Walkey and Black (Walkley and Black, 1934), total nitrogen (TN) was measured using the Kjeldahl method described by Pansu and Gautheyrou (2006), available phosphorus (P) was determined by the method of Olsen (Olsen et al., 1954). Finally, cation exchange capacity (CEC) was measured by titration method with H_2SO_4 using ammonium oxalate in the presence calcium carbonate according to the method adopted by Pansu and Gautheyrou (2006).

Heavy metals analysis in soils

For heavy metal contents (Pb, Cu, Zn, Cd and Ni) analysis, 1g of soil was digested with 15 ml of Aqua-regia method (HNO_3: HCI in 3:1 ratio) in Teflon PFA vessels using speed wave accelerated reaction system (MWS-Berghof speed wave) at 80°C until obtaining a transparent solution according to the method of Allen (Allen et al , 1986). The digested samples were filtered and diluted with de-ionized water up to 50 ml. Heavy metals contents (Pb, Cu, Cd, Zn and Ni) have been carried out by ICP-MS Agilent 7700X at the Environmental Analysis Laboratory of CRBt.

Bacterial analysis

Soil samples were collected using sterile techniques, preserved at 4°C and analyzed within 48h. The total bacteria in the soil were determined by the plate count method. For each soil sample 10g of fresh soil shaken in 90ml of distilled water for 30 minutes, serially diluted in sterile saline buffer (0.85% NaCI) and 100 µl of each soil sample was placed on nutrient agar plates. The plates were incubated at 30°C for 48h and the formed bacterial colonies were counted according to the method described by Grunda (1985). These bacteria were streaked on fresh nutrient agar plates to procure for identification. The bacterial isolates were characterized based on cultural, morphological and biochemical characteristics as described in the Cowan and Steel's Manual for the identification of Medical Bacteria. Oxidase, catalase, methyl red, indole production, citrate and carbohydrate utilization (glucose, sucrose, maltose, xylose and lactose) were biochemically analyzed according to the method followed by Barrow and Feltham (1993).

Statistical analysis

Statistical analyses are performed using the XLSTAT (XlStat-Pro. V.7.5.2) software, including: ANOVA one-way, followed by a multiple comparison analysis test (Fischer LSD test and Duncan test) to compare differences between zones. Principal Component Analysis (PCA) and Pearson matrix correlation test to check the dependencies of studied parameters.

Results

Soil physico-chemical properties

The results of the edaphic parameters of soils samples are given in Table 1. The overall area was characterized by an acid pH (5.58-6.05), organic carbon (C%) was relatively high (2.31 to 4.75%), total nitrogen (TN) was also uniformly high (0.35-0.63) resulting a low C/N ratios (5.41-9.44). The CEC ranged from (7.29-13.44 cmol.kg1). The available phosphorus (P) content varies between (3.26-7.11 mg kg1) compared to soil fertility standards, the soil of the studied area is moderately poor in phosphorus. Organic matter (OM%) is the store house of plant and bacteria nutrients and mineral recycling (Rattan et al., 2005) ranged between (3.97-8.18%). The comparison of these edaphic parameters of the three sampling areas shows that, for the pH values there were not a significant variation only the zone B which presented a lower pH 5.58. However, for the other parameters we have recorded that zone A is characterized by the lowest values of C%, TN, OM%, CEC, C/N and a high concentration of available phosphorus 7.11 mg.kg^{-1} comparing to zone B and C.

Heavy metal content in the soil

The soil samples heavy metal contents from all the sites are shown in Table 1. Generally, the concentrations of metallic trace elements are higher compared to the international soil standards. Total Cd, Pb, Zn, Cu and Ni ranged from 5.54 to 200.01 mg Cd kg^{-1}, 1844.08 to 18048.83 mg Pb kg^{-1}, 987.78 to 42928.99 mg Zn kg^{-1} 259.56 to 995.10 mg Cu kg $^{-1}$ and 56.02 to 220.32 mg Ni kg^{-1}. Heavy metal concentrations show a significant variation between the zones; mainly, the lowest concentrations were observed in zone A compared to the other zones, whereas, the highest concentrations of heavy metals are recorded in zone B.

Bacteriological analysis

Plate counting analysis revealed that, bacterial load (bacterial biomass) of zone A is thirty times higher than the one from zone B (Table 1). According, to the bacteria load, we can classify the sites into two groups; Group I includes the zones B and C with the highest concentration of heavy metals and a less number of bacteria, Group II includes zone A, with a low concentration of heavy metals and high amount of bacteria. The differences in bacteria quantity of each zone are shown in Table 1. From the prospected mining area, we have isolated a sum of 31 strains that belong to 31 genus. *Pseudomonas* genus was the most represented with 5 species, followed by *Aeromonas, Enterobacter* and *Seratia* with 3 species, for *Pasteurella* and *Chrysebacterium* we have isolated 2 species for each genus and one species for the rest of the genus Table 3. Zone A shows the highest value of bacterial diversity with an index of Shannon Weaver calculated H=1.81 and 19 species, followed by zone B with H=1.56 and 15 species and finally comes zone C with H=0.95 and only 11 species (Figure 3).
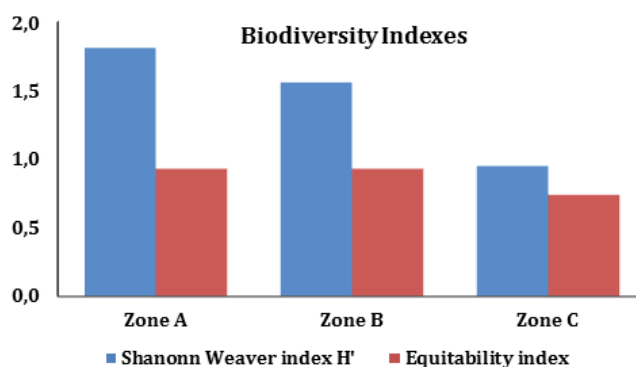

Figure 3. Biodiversity indexes of bacterial communities

Statistical analyses

Processing data by the LSD test (least significant difference) using one–way Anova analysis revealed a significant difference (p<0.05) of Pb, Cd, Cu, Zn and Ni content between zone B and the zones A and C. No-significant differences were recorded between zone B and C with respect to pH and TN. A significant difference was also observed between zone B and the zones A and C for OM%, C%, P, CEC and bacterial load (bacterial biomass). The correlation coefficients computed between the some chemical and bacterial parameters are presented in the Table 2. A strong correlation was observed between CEC and Cd, Zn and Ni (r=0.59*, r=0.58 * and r=0.36 * respectively). A low correlation between pH and organic matter (r=-0.44*). A correlation between bacterial biomass and organic matter, Ni and available phosphorus (r=-0.34*, r=-0.44* and r=0.60* respectively).

Table 1. Physico-chemical and bacteriological characteristics of soil samples

	Zone A (n=4)			Zone B (n=4)			Zone C (n=6)		
	Max	Min	Mean± SD	Max	Min	Mean± SD	Max	Min	Mean± SD
pH	7.18	4.64	6.05 ± 0.99	7.05	3.81	5.58 ± 1.02	6.58	3.74	5,66 ± 1.00
C, %	5.48	0.87	2.31 ± 1.76	9.17	3.17	4.75 ± 1.81	4.52	0.51	2,89 ± 1.08
P, mg kg^{-1}	8.18	4.68	7.11 ± 1.19	7.84	1.83	4.19 ± 2.3.	7.30	1.87	3,26 ± 1.26
TN, %	0.59	0.22	0.35 ± 0.18	1.45	0.22	0.61 ± 0.35	1.46	0.27	0,63 ± 0.28
OM, %	9.43	1.50	4.97 ± 3.04	15.77	5.45	8.18 ± 3.11	8.82	3.23	5,48 ± 1.55
CEC, cmol kg^{-1}	9.75	2.75	7.29 ± 1.90	24.00	3.00	13.44 ± 6.89	12.75	3.00	7,55 ± 2.49
C/N	18.20	2.00	6.80 ± 4.88	17.55	4.52	9.44 ± 3.76	13.08	1.00	5,41 ± 3.00
Pb, mg kg^{-1}	7066.99	270.53	1844.08 ± 2697.60	61004.44	1800.12	18048.83 ± 23209.94	8468.52	1269.49	3615,60 ± 1967.16
Cd, mg kg^{-1}	16.87	1.86	5.54 ± 6.21	742.27	13.89	200.01 ± 293.22	46.30	14.18	23,60 ± 7.66
Zn, mg kg^{-1}	4178.03	16.87	987.78 ± 12841.14	156140.42	3457.27	42928.99 ± 32165.50	11376.63	3369.72	6811,11 ± 2504.62
Cu, mg kg^{-1}	3787.12	39.06	543.48 ± 706.52	2459.22	285.64	995.10 ± 834.92	608.48	100.75	259,56 ± 149.97
Ni, mg kg^{-1}	59.12	46.45	56.02 ± 4.81	357.33	73.00	169.89 ± 106.93	301.73	45.78	220,32 ± 81.56
Bacterial number in soils, CFU g^{-1}	9.2x10^7	1.2x10^6	4.2x10^6 ± 1.8x10^6	3.9x10^4	2.02x10^3	1.3x10^4 ± 1.05x10^4	4.6x10^6	3.05x10^4	4.3x10^5±, 2.4x10^4

Table 2. Matrix of correlations

Variables	Pb	Cd	Zn	Cu	Ni	pH	C	P	N	OM	CEC	Biomass
Pb	1											
Cd	0,0357	1										
Zn	0,0576	0,9976***	1									
Cu	0,6785*	0,1607	0,1602	1								
Ni	-0,1306	0,5194*	0,5415*	-0,1127	1							
pH	-0,2761	0,2100	0,2203	-0,2538	0,3381	1						
C	0,7089*	0,1145	0,1215	0,6699**	-0,0997	-0,4465*	1					
P	0,0046	0,3100	0,3049	0,0281	-0,1767	0,4111	-0,2035	1				
N	0,4602	0,2149	0,2385	0,2338	0,4273	-0,0885	0,4166	0,0011	1			
OM	0,7159**	0,0990	0,1076	0,6689*	-0,0562	-0,4665*	0,9137	-0,2535	0,4312	1		
CEC	0,3213	0,5891*	0,5794*	0,2958	0,3585	0,1576	0,2249	0,1936	0,1389	0,1505	1	
Biomass	-0,2309	-0,2312	-0,2424	0,0195	-0,4382	0,2657	-0,2614	0,5916*	-0,3382	-0,3368	-0,1864	1

*P<0,05, **P<0,01, ***P<0,001

The PCA analysis of the soil characteristics (Figure 4) led to a reduction in the initial size of the two-component data set, accounting for 58.92% of the variation in the data (F1 33, 47% and F2 25, 44% of the variance). F1 shows that organic matter and CEC affect positively the distribution of metallic trace elements in the soil where we observed a group of metals that preferred to bind to the organic matter (Pb and Cu) and another group that preferred a inorganic ligand (Cd, Zn and Ni). With the PCA analysis, it was possible to obtain a clear separation of the studied zones. Thus, the zone A contains high values of pH and available phosphorus contents , a very important bacterial charge as well as low concentrations of Cd and Ni, Cu, Pb and Zn. Zone B was mainly characterized by its high concentrations of Pb, Cu, Zn, Cd and Ni also its high contents of organic matter and CEC. Whereas, zone C shows intermediate values between the two first zones.

Table 3. Results of identified bacteria populations

| Identified species		
Pseudomonas puteda	*Enterobacter intermedus*	*Proteus fenneri*
Pseudomonas alcaligens	*Enterobacter sakazaki*	*Photobacterium damsella*
Pseudomonas luteola	*Enterobacter cloacae*	*Burkhoderia cepacia*
Pseudomonas aerogenosa	*Chrysebacterium meningosepticum*	*Mannheimia haemolytica*
Pseudomonas fluoresens	*Chrysebacterium indologens*	*Hafnia alvei*
Aeoromonas hydrophyla	*Pasteurella sp.*	*Pantoea sp.*
Aeoromonas hydrophyla	*Pasteurella pneumotropica*	*Brecella sp.*
Aeromonas salmonicida	*Providencia alcaligens*	*Vibrio fluviatis*
Seratia plumuthyca	*Klebseilla pneumonia*	*Echerichia coli*
Saratia odorifera	*Acenitobacter lwofii*	*Shewanella putrefaciens*
Seratia laquefaciens		
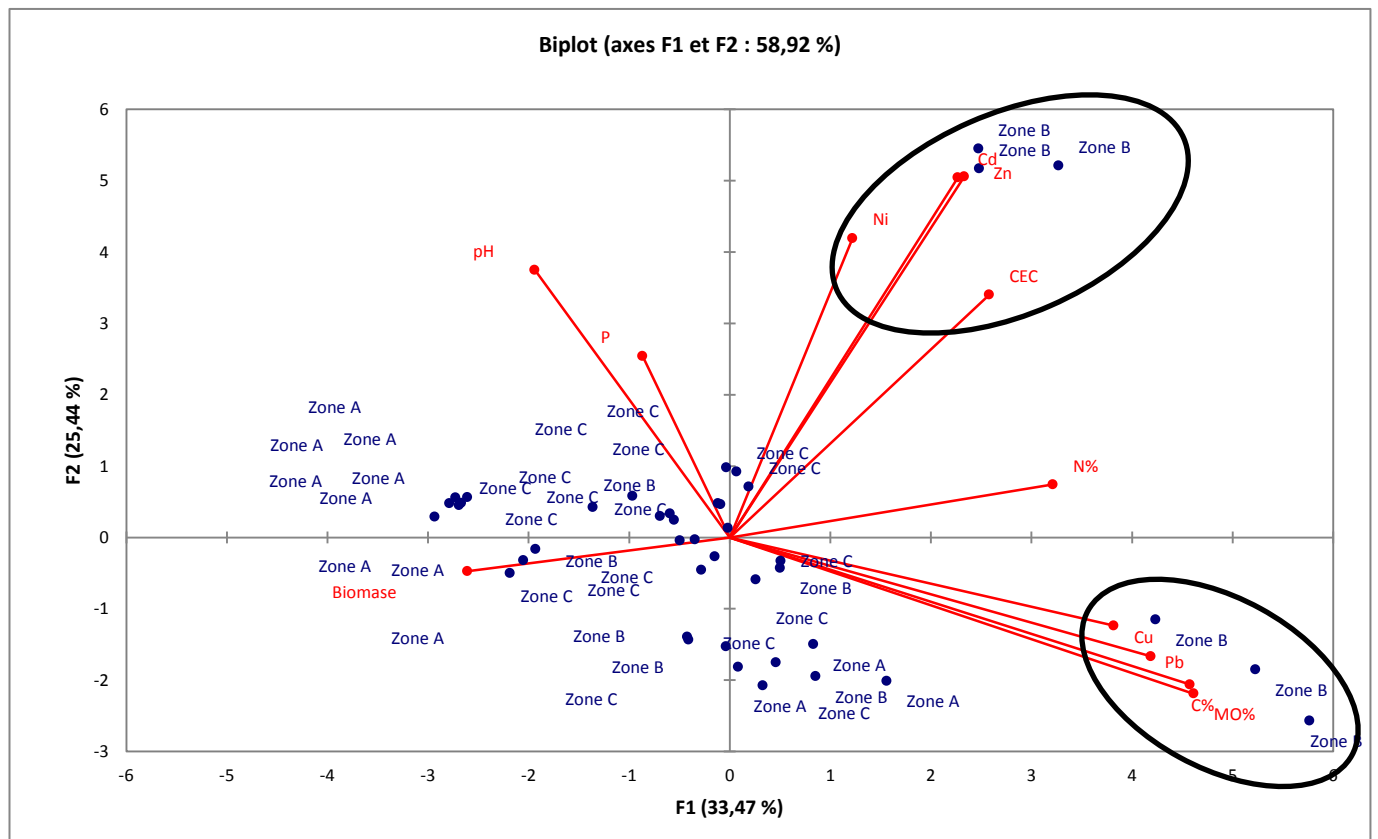
Figure 4. Factorial map applied to the bacterial and physic-chemical variable data conducted by Principal components analysis (PCA)

Discussion

The studied mining area of Sidi Kamber was severely polluted with Cu, Cd, Pb, Ni and Zn far exceeding international standards and maximum permitted levels for the protection of the ecosystem and the human health as well as the commercial and industrial land use which is higher than their respective standard levels set for agricultural soils (Baiz, 2000; CCME, 2007; VROM, 2009). Total metal contents around this site have already shown a high level of heterogeneity, a typical characteristic of many mine tailings (Kock and Schippers 2008; Streten-Joyce et al., 2013; Chodak et al., 2013). However, the high level of metal concentrations found in the soil of this mine could well be responsible of its high values of OM content (4.97,

8.18 and 5.48 respectively) and the low values of TN, P and C/N ratio because heavy metals might affect biological mineralization cycles (Chander and Brookes, 1991). The impairment of the biological activity of the soil due to metal loading leads basically to a reduction in the decomposition and turnover rates of organic matter (Babich and Stotzky, 1985). The relationship between organic matters and heavy metals lies in its important role to the heavy metal binding and complexity (Manskaia and Drozdova, 1968; Rashid and Leonard, 1973). Indeed, organic matter is considered as a preferential support for trace elements especially Pb and Cu (Singer, 1977). Organic matter influence the behavior of the heavy metals in the soil by: i. releasing heavy metals associated with the organic matter; ii. extracting or mobilizing heavy metals from the complexes and; iii. improving soil microbial populations which affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes. The other elements have preferred to bind to inorganic ions which is the case of Cd, Zn and Ni this explained the correlation between these elements and CEC. Pérez-Esteban et al (2014) have found that the organic matter and the organic carbon could increase the metal mobility through the formation of soluble metal organic complexes facilitating in this way the metal uptake by the plants. In the studied soils, OM amounts differ significantly between the zones. Accordingly, it can be assumed that this soil property would have it due to the variation in the bacterial load, which contributes in the mineralization of this organic matter, where we observed a high bacterial load in zone A compared to the other two zones with a pH close to neutrality favoring the decomposition of this organic matter. Since pH has great effects on the solubility of heavy metals, the difference of pH may have influence on other soil characteristics as well as bacterial community diversity (Giller et al., 1998). Taylor et al (2002) also found that the soil pH would affect microbial community structure and diversity. In the present study an acidic pH (Table I) is observed in the soil samples and there was no significant difference between the three sampling sites. Similar pH values have been reported in other studies (Lin et al., 1987; Tam et al., 1995). The acidic pH was partly resulted from the oxidation of FeS_2 and FeS to H_2SO_4 and partly from active microbial decomposition of litter and hydrolysis of tannin in plants, which released various types of acids. These microbial meditated reactions are important determinants of the acidity generation, metal mobilization and the release of key nutrients (such as phosphate and metal cations) required not only for their own metabolism but for the plants growth and eutrophication potential (Welch et al., 1999; Uroz et al., 2009). Tailing are oxidized in contact with air and water with the help of bacteria and produce acid mine drainage that can contaminate surface and groundwater (Liao, 1990). It has been repeatedly reported that the high values of toxic elements in the soils tend to decrease the soil's pH (Alloway, 2010; Hohl and Varna, 2010). Plate counts have been seen as the most appropriate method to determine the effect of heavy metals on soil bacteria compared to culture-independent approaches (Ellis et al., 2003). The diversity and activity of microbial communities are important indicators of soil's quality. The influence of soil physic-chemical properties (including metal concentrations) on the soil's microbial properties and vice versa is worth mentioning. An increasing piece of evidence suggests that heavy metals have a strong impact on both bacterial and fungal communities (Kozdrój and van Elsas, 2001). In this study, we have observed a strong heavy metals effect on the bacterial biomass. The average bacterial biomass in zone A was 39 times higher compared to zone B and C respectively. This indicates that metal concentrations modified in a strong way the present microbial community in the most contaminated samples (B and C) either ways direct or indirect as verified by the changes in the relative abundance of several plant species. (Epelde et al., 2010; Lehout et al., 2017). The presence of heavy metals decreased the microbial biomass directly, when the heavy metal contents exceeded the threshold of tolerance or by inhibiting some biochemical properties of the soil which are essential for their survival. Moreover, the bacterial biomass can be influenced by many other environmental factors such as pH, temperature, nutrient content and his root exudates nature (Chodak et al., 2013). It is noteworthy that the structure and diversity of bacteria communities in the soil are not only influenced by heavy metal concentrations but also by pH (Kock and Schippers, 2008), organic matter (Bouskill et al., 2010) and the interactions between these factors. The vegetation in this area provides high levels of organic matter. According to Lehout et al. (2017), zone A showed abundance and a vegetal richness compared to the other zones. The natural nutrient content of this soil might therefore play a key role in the evolution of the bacterial diversity. On the other hand, regarding the species richness of bacterial community diversity it appears that it was not strongly influenced by the contamination level in the studied area. Indeed, so many studies have shown a notable decrease in microbial diversity and other animals and plants organisms in the soil contaminated by the metals due to the toxicity of the element (Bamborough and Cummings, 2009; Singh et al., 2014). However, recent studies have shown that after a century of exposure to acid drainage, the bacterial community tends to stabilize and increase its diversity, resulted in an environment with a microbial diversity similar to that of the non-impacted areas (Reis et al., 2013; Bouskill et al., 2010; Pereira

et al., 2014). Nonetheless, soil micro-organisms have developed highly efficient systems of metal detoxification which, in bacteria, can be grouped into five categories: intracellular sequestration, export, reduced permeability, extracellular sequestration and extracellular detoxification (Epelde et al., 2010; Pereira et al., 2015). Heavy metal contamination provides a strong pressure selected for the recruitment of multiple resistance phenotypes that encode resistance to the predominant metals in the site (Ryan et al., 2005).

Conclusion

The present study shows that the mining area of Sidi Kamber is characterized by high levels of metals far exceeding international standards. These high levels of heavy metals may have both direct and indirect effect of nutrients content. Negative impact of heavy metals on bacterial communities in the soil is shown by several studies. These were significant differences in the structure of microbial communities depending on the level of heavy metal contamination. However, bacterial biomass can be influenced by other environmental factors such as pH and nutrient content. Moreover, total metal concentration in the soil does not give enough information on metal mobility, bioavailability and phytotoxicity. Instead, assessment of soil contamination can also be determined by estimating the bio-available fraction of toxic metals. Finally, an ecological understanding of how contaminants, ecosystem functions and biological communities interact in the long term is needed for a proper management of these fragile metalliferous ecosystems.

Acknowledgments

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