

Salinization Depresses Soil Enzyme Activity in Metal-polluted Soils Through Increases in Metal Toxicity

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Abstract

Salinity may increase metal mobilization and toxicity with a potentially significant consequence for soil enzymatic activity and nutrient cycling. The goal of this study was to investigate changes in soil enzyme activity in response to salinization of a clay loam soil artificially polluted with cadmium (Cd) and lead (Pb) during an incubation experiment. Soil samples were polluted with Cd, Pb, and a combination of Cd and Pb, pre-incubated for aging, and then salinized with three levels of NaCl solution, and were finally incubated for 120 days. NaCl salinity consistently increased the mobilization of Cd and Pb with greater increases at high than low salinity levels. While the increased Cd mobilization was greater in co-polluted than Cd-polluted soils, the increase of Pb mobilization was lower in co-polluted than Pb-polluted soils at high salinity level. The salinity-induced increases in metal mobilization and toxicity significantly depressed soil microbial respiration, microbial biomass content and enzymatic activities. The increased soil electrical conductivity, Cd mobilization and pH after salinization were the most important factors governing microbial activity and biomass in metal-polluted soils. Changes in microbial biomass and mobile metal pool with increasing salinity had the major effects on enzyme activities, particularly under the combined metals. Secondary salinization of metal polluted soils would impose an additional toxicity stress on enzymatic activities as biochemical indicators of soil quality, and therefore should be avoided for the maintenance of soil microbial and biochemical functions, especially in arid regions. In metal-polluted soils, the observed responses of enzymes to salinity can be used to advance our knowledge of microbial processes when modelling the carbon and nutrient cycling.

1 Introduction

Enzymes are the key soil components for their multifaceted functions in organic matter decay, nutrient cycling and biodegradation of potentially toxic compounds in the soil, and for plant growth promotion (Nannipieri et al. 2002; Rao et al. 2017). Their activities are often used as biochemical indicators of soil fertility, quality and health (Dick 1997; Karaca et al. 2010a; Rao et al. 2017), and provide integrative information on soil microbial processes and nutrient dynamics in soil ecosystems (Dick 1997). Soil enzymes are significant because of their quick response to any changes in the environmental conditions (Burns et al. 2013; Nannipieri et al. 2018) and due to their important role in sustainable crop production (Rao et al. 2017; Datt and Singh 2019; Dotaniya et al. 2019). The measure of soil enzyme activities can also be used as a suitable surrogate for quantifying and monitoring changes in the microbial community structure and activity, carbon sequestration as well as nutrient transformations in response to anthropogenic inputs (Karaca et al. 2010b; Rao et al. 2017; Datt and Singh 2019). Enzymes, whether extracellular or intracellular, could be used as reliable biosensors for monitoring the risks of soil pollution by heavy metals (Rao et al. 2014) and soil degradation by salinization (Rath and Rousk 2015; Boyrahmadi and Raiesi 2018), especially in arid environments.

The input of heavy metals into the soil from different anthropogenic sources is a global concern for soil degradation because they potentially have important consequences for soil microbial community and enzymatic activity (Karaca et al. 2010b; Khan et al. 2010; Zheng et al. 2017; Xu et al. 2019; Raiesi and Dayani 2020). Secondary salinization is also another serious soil degradation problem worldwide, particularly in the landscape of arid and semiarid regions, which can inhibit the activity and biomass of microbial community, and the key biochemical functions in salt-impacted soils (Wichern et al. 2006; Rath and Rousk 2015; Singh 2016; Boyrahmadi and Raiesi 2018; Rath et al. 2019). Soil extracellular and intracellular enzymes have been widely employed as early and sensitive indicators of the abiotic stresses induced by heavy metals (Karaca et al. 2010b; Khan et al. 2010; Pan and Yu 2011; Zheng et al. 2017) and salinity (Rietz and Haynes 2003; Boyrahmadi and Raiesi 2018; Yang et al. 2020) in agroecosystems. More importantly, soil pollution with heavy metals and salinity are the major soil degradation processes and important ecological stresses, which can also co-occur in arid and semiarid regions of the world (Ghallab and Usman 2007;

Abbas et al. 2018; Raiesi et al. 2018). Under these unfavorable conditions, salinity may increase metal solubility and mobility through the formation of less charged or uncharged metal-chloride complexes or the strong competition between cations and metals for adsorption onto soil exchange sites (Ghallab and Usman 2007; Abbaspour et al. 2008; Acosta et al. 2011; Filipović et al. 2020). Therefore, the salinization of metal polluted soils would impose an additional stress, which can aggravate metal ecotoxicity to growing plants (Ghallab and Usman 2007; Ondrasek et al. 2012; Usman 2015; Abbas et al. 2018; Wang et al. 2019b) as well as to living soil organisms and enzymatic reactions (Zheng et al. 2017; Raiesi et al. 2018; Raiesi and Sadeghi 2019; Filipović et al. 2020). Salinity-induced stress was also found to change the composition and structure of the bacterial (Wang et al. 2019a) and archaeal (Wang et al. 2019c) communities in metal polluted soils. Such effects may have large consequences for energy flow and nutrient cycling as well as crop productivity in terrestrial ecosystems (Ghallab and Usman 2007; Zheng et al. 2017; Wang et al. 2019b).

Although, the effects of salinity on soil enzyme activity have been studied more frequently in soils polluted with a single metal (Zheng et al. 2017; Raiesi et al. 2018; Raiesi and Sadeghi 2019; Filipović et al. 2020), how enzymatic reactions are influenced by salinization in co-polluted soils remains elusive. Indeed, the effect of metal co-pollution on soil enzyme activity could differ from that of individual metal pollution (Chaperon and Sauvé 2007; Khan et al. 2010; Huang et al. 2017). For example, the co-pollution of soil with cadmium (Cd) and lead (Pb) inhibited enzyme activities more than the soils polluted with single Cd or Pb (Khan et al. 2010; Pan and Yu 2011). In another work, responses of urease and dehydrogenase activities to metal combinations were synergistic in an agricultural soil exposed to different metals as a result of their toxicity interaction (Chaperon and Sauvé 2007). In this context, our aim was to investigate the impacts of NaCl salinity on the enzymatic activity in soils polluted simultaneously with Cd and Pb during an incubation experiment. We hypothesized that salinity would lower enzyme activity in metal-polluted soils by mobilizing metals and this salinity effect would be greater under the combined metals than under the single metals because of the toxicity interactions between metals. The outcome of this study may have an important implication for soil potential ecological risk assessments, particularly in saline co-polluted environments.

2 Materials And Methods

2.1 The study soil and experimental design

A clay loam soil (clay = 31.5%, silt = 40%, and sand = 28.5%) was collected from a farmland field plot (0–20 cm) in the Shahrekord Plain (mean annual rainfall = 325 mm and mean annual temperature = 10.5 °C), air-dried and passed through a 2-mm mesh sieve before analysis. Subsamples were analyzed for chemical and physical properties. The study soil had the following physical and chemical properties: pH (1:2 ratio) = 7.9, electrical conductivity of the saturated-paste extract (ECe) = 2.0 dS m⁻¹, organic carbon content = 4.70 g kg⁻¹, total nitrogen content = 0.39 g kg⁻¹, moisture content at field capacity (FC) = 21.2% (w/w), total Cd content = 0.20 mg kg⁻¹ and total Pb content = 20 mg kg⁻¹ soil.

Experimental factors were: (i) metal treatment, including soils polluted with Cd (10 mg Cd kg⁻¹) and Pb (150 mg Pb kg⁻¹), both representing single-metal pollution, and soil polluted with Cd + Pb (the same levels as in individual pollutions), representing co-pollution; and (ii) NaCl salinity levels, including 0 mM NaCl (control), 20 mM NaCl (low salinity) and 40 mM NaCl (high salinity). The experiment was arranged in a completely randomized 3×3 factorial design with nine combined treatments, each replicated three times (n = 3). The soil was artificially polluted with Cd and Pb chloride solutions to increase metal levels. Metal-polluted soils were mixed and then distilled water was used to adjust gravimetrically the soil moisture content at 60–70% of the FC. The soils were incubated at room temperature

(about 20 ± 5 °C) for 30 days to achieve an equilibrium condition and for metal aging process. After this period, the polluted soils were treated with NaCl salt. The soils were pre-incubated at 60–70% FC and in the dark at room temperature for 20 days to minimize the effects of soil preparation and disturbance, and to restore autochthonous microbial populations. Ultimately, the soil samples were gravimetrically maintained at $65 \pm 5\%$ FC and placed in an incubator at 25 ± 1 °C for 120 days.

2.2 Soil microbial analysis

To study the potential effects of salinity on the metal mobility and toxicity, several soil microbial metrics, including microbial respiration and biomass as well as enzyme activities, were assayed. Fresh subsamples were obtained from the incubated soils and analyzed for chemical, microbial and enzyme activities at the end of the incubation period. Microbial respiration (MR), as an indicator of the overall microbial catabolic activity, was measured by analyzing the CO₂ accumulated in sealed plastic containers incubated at constant soil moisture (60% FC) and temperature (25 °C) over 30 days (Alef and Nannipieri 1995). Soil microbial biomass C (MBC), a potential indicator reflecting the size of the soil microflora, was determined using the fumigation–extraction method (Joergensen 1995). Microbial metabolic quotient ($q\text{CO}_2$) was calculated from MR and MBC values to provide an indicator of stress within the soil microbial community (Anderson and Domsch 1993; Fließbach et al. 1994), and was expressed as the CO₂-C respired per unit MBC and day. Enzyme activities were determined based on the release of the product upon the enzyme action by incubating soil subsamples with an adequate substrate under standard conditions, following the methods described by Alef and Nannipieri (1995). The enzyme activities assayed are those important in nutrient (N, P and S) cycling, including urease (URE), alkaline phosphomonoesterase (ALP), arylsulphatase (ARY), and those involved in microbial oxidoreductase metabolism, including dehydrogenase (DEH) and catalase (CAT). The fluorescein diacetate (FDA) hydrolysis activity was also determined. The FDA hydrolysis, reflecting protease, esterase and lipase activities, has been widely used for measuring total microbial activity in saline soils (Rietz and Haynes 2003; Raiesi and Sadeghi 2019). The geometric mean of enzyme activities (GME) was calculated using ALP, URE, DEH, ARY, CAT and FDA activity values according to Paz-Ferreiro et al. (2012) and Hinojosa et al. (2004b). Available Cd and Pb concentrations in subsamples were extracted with DTPA-TEA (Lindsay and Norvell 1978) using an atomic absorption spectrophotometer (AAS Model GBC 913 plus). For each salinity level, the percentage of metal mobilization was calculated using the following equation:

$$\text{Metal – Mobilization (\%)} = \frac{M_{(\text{SAL})} - M_{(\text{CON})}}{M_{(\text{CON})}} \times 100$$

where, $M_{(\text{CON})}$ is the DTPA-extractable metal concentration in the control soil and $M_{(\text{SAL})}$ is the DTPA-extractable metal concentration in the saline soil.

2.3 Statistical analysis

Two-way analysis of variance (ANOVA) was utilized to establish the significant effects of treatments on each variable with Tukey test to compare the significant differences ($P < 0.05$) among the treatments. Soil properties were also subjected to correlation analysis (CA), factor analysis (FA) and redundancy analysis (RDA). The FA was performed with quartimax rotation to group soil variables and discriminate metal and salinity treatments. The RDA was used to identify the most important soil predictors (explanatory variables) of microbial properties and enzyme activities. Likewise, soil explanatory variables were determined for enzyme activities separately, considering microbial biomass and respiration. The degree of multicollinearity among the independent (explanatory) variables was assessed by calculating variance inflation factor (VIF). Explanatory variables with VIF value > 3 were excluded from the RDA model.

to avoid the collinearity amongst variables. The ANOVA, CA and FA were carried using Minitab 19 and the RDA using Canoco 4.5 for Windows.

3 Results

High salinity level slightly increased soil pH by 2% in Pb-polluted soils and by 4% in co-polluted soils (Fig. 1). This is due to the addition of higher Na^+ ions at high salinity level. A significant positive correlation ($r = 0.73$, $P < 0.001$, $n = 27$) was observed between soil pH and ECe (Table 1). Salinity consistently increased the availability of Cd (12–22%) and Pb (5–16%) with a greater increase at high (9–22%) than low (5–12%) salinity levels. While the increased Cd availability was greater in co-polluted (22%) than Cd-polluted (17%) soils at high salinity level, the increase of Pb availability was lower in co-polluted (9%) than Pb-polluted (16%) soils.

Responses of microbial properties of polluted soils to salinity are shown in Fig. 2. The effects of salinity on soil microbial properties varied widely, depending on metal pollution and salinity level. Microbial respiration (MR) responded negatively to NaCl salinity and decreased up to 43% compared to the control soils (Fig. 2). The reductions in microbial respiration were greater in Cd-polluted (30–43%) than Pb-polluted (23–36%) soils at both salinity levels, and in single metal-polluted (23–43%) than co-polluted (8–103%) soils. However, the decreased microbial respiration in co-polluted soils did not differ between low and high salinity levels (Fig. 2). Similarly, the microbial biomass carbon (MBC) content decreased by 35–63% with addition of NaCl and the increases were much greater under high than low salinity levels only in single metal-polluted soils. Salinity decreased the MBC in Cd-polluted soils (47–63%) more than in Pb-polluted soils (40–57%). As with microbial respiration, the reductions of microbial biomass were greater in single metal-polluted soils (40–63%) than co-polluted soils (35–37%). In contrast, the microbial metabolic quotient ($q\text{CO}_2$) was increased by salinity with greater increases under high than low salinity conditions only in single metal-polluted soils. There were significant negative correlations between MR and MBC, and soil pH or ECe, and a negative correlation between $q\text{CO}_2$ and soil pH ($P < 0.001$) or ECe ($P < 0.001$) across treatments (Table 1).

Results showed that salinity significantly affected soil enzymatic activities ($P < 0.001$), depending on the salinity level and the metal treatment (Table 2). Salinity decreased the activity of ALP only in Pb-polluted (16–24%) and co-polluted soils (10–14%), with a larger decrease at high (16%) than low (24%) salinity levels only in Pb-polluted soils. URE activity was inhibited only at high salinity level (18–23%) in single metal-polluted soils and at both salinity levels (17–42%) in co-polluted soils. Increasing NaCl level resulted in a significant reduction in ARY activity in Cd-polluted (25–42%) and co-polluted soils (42–87%), and the decline in the activity of this enzyme was similar at both salinity levels in Pb-polluted soils. CAT activity was affected by salinity, independence of the metal pollution treatment (no interaction between salinity and metal treatment; Table 2). The added NaCl salts reduced CAT activity by 50%, and this effect did not differ between the two NaCl levels. Increasing NaCl salinity level resulted in significant reductions in the activity of DEH in all metal-polluted soils, with greater reductions in co-polluted soils (10–29%) than Cd-polluted (6–17%) or Pb-polluted (9–19%) soils (Table 2). As with ARY, the FDA values decreased with the increasing salinity level by 10–63%, with greater decreases in co-polluted (63%) than single metal-polluted (33%) soils at high salinity level. Similar to individual soil enzymes, the geometric mean of enzyme activity (GME) was significantly ($P < 0.001$) affected by both salinity and metal pollution treatment (Table 2). Salinity consistently decreased the GME, and the declined GME values were much greater in co-polluted (17–50%) than single metal-polluted (11–27%) soils, especially at high salinity level (50% vs. 22%). Soil enzyme activities showed significant correlations with soil pH and ECe (Table 1). The ALP and DEH activities and GME values were negatively correlated only with available Cd concentration. Soil enzyme activities were positively correlated with microbial metrics, particularly MBC, and negatively correlated with $q\text{CO}_2$ values (Table 1).

The changes in soil microbial properties and enzyme activities with salinity were also analyzed by factor analysis (FA). The results of FA (Fig. 3, Table 3) showed that microbial properties and the activities of soil enzymes were significantly different between salinity and metal treatments (Fig. 3), and this difference was related to the changes in soil ECe, pH and available metal (Cd and Pb) concentrations. Three factors were significant (eigenvalue > 1) and together were responsible for about 89% of the total variance in soil properties (Table 3). The first factor accounted for 65%, the second factor explained 13% and the third factor was responsible for 11% of the total variability in the data. Ordination of metal treatments was primarily related to the second factor, which loaded heavily on available Pb (-0.91) and MR (-0.74) parameters (Table 3). This factor successfully separated the metal treatments (Fig. 3A). Discrimination of salinity treatments was linked to the first factor, which loaded heavily on pH, ECe and the rest of microbial properties (Table 3). The first factor effectively separated the salinity levels (Fig. 3B). The third factor included available Cd content and ALP activity with a positive loading for available Cd (0.90) and a negative loading for ALP (-0.65). This points out that the third factor was also associated with the effect of metal pollution treatment.

In order to find the dependent relationship between soil chemical properties (ECe, pH, and available Cd and Pb concentrations) and microbial properties, a redundancy analysis (RDA) was applied to all the soil data, including microbial metrics and enzyme activities, as the response (dependent) variables (Fig. 4A). Two canonical axes effectively contributed to the explanation of the response variables. The first two RDA axes explained 90% and 1% of the total variation in the microbial data, respectively. In the RDA ordination diagrams, ECe (75%, $F = 75.3$, $P = 0.001$, $VIF = 2.27$), available Cd (9%, $F = 13.2$, $P = 0.002$, $VIF = 1.40$), pH (4%, $F = 8.0$, $P = 0.008$, $VIF = 2.54$) and available Pb (3%, $F = 6.54$, $P = 0.013$, $VIF = 1.20$) were accounted for 91% of the variability in soil microbial properties. The results of RDA also revealed clear separation of soil properties among salinity treatments (Fig. 4A). Redundancy analysis was also used separately to identify the main soil variables that explained the variance in soil enzyme activities (Fig. 4B). Axes 1 and 2 explained 71.2% and 10.5% of the total variation, respectively (Fig. 4B). MBC (65%, $F = 46.5$, $P = 0.001$, $VIF = 1.63$), available Cd (10%, $F = 9.25$, $P = 0.001$, $VIF = 1.36$), available Pb (6%, $F = 7.31$, $P = 0.003$, $VIF = 1.24$) and pH (2%, $F = 3.5$, $P = 0.038$, $VIF = 2.54$) explained 83% of the variability in soil enzyme activities (Fig. 4B). Soil ECe and MR had high VIF values (> 3) and were excluded from the RDA model to avoid potential multicollinearity problem.

4 Discussion

4.1 Salinity effects on metal mobilization

The results indicated that soil salinization mobilized Cd and Pb in both single metal- and co-polluted soils. This observation suggests that salinity was the key factor controlling the solubility and mobility of the metals in polluted soils. The finding supports our hypothesis that the mobility and availability of metals would increase with increasing salinity, with a potentially significant consequence for soil microbial processes and functions. Similarly, NaCl salinity increased the mobility of soil Cd (Ghallab and Usman 2007; Abbaspour et al. 2008; Ondrasek et al. 2012; Raiesi and Sadeghi 2019; Wang et al. 2019b; Filipović et al. 2020) and Pb (Usman et al. 2005; Acosta et al. 2011; Wang and Song 2019; Raiesi et al. 2020) in short-term pot and incubation experiments. In another study, NaCl salinity increased DTPA-extractable Cd pool in soils polluted with raw city effluent, with the greatest available Cd concentration at 50 mM salts (Abbas et al. 2018). The possible underlying mechanisms by which salinity would increase metal mobility and availability in salt-affected environments are (i) the formation of soluble complexes between metal ions and Cl^- ions through cation exchange, (ii) the strong competition between cations such as Na^+ and free metal ions for adsorption on the negatively charged exchange sites of soil particles, and (iii) the high ionic strength induced by addition of NaCl salt (Ghallab and Usman 2007; Abbaspour et al. 2008; Acosta et al. 2011). These mechanisms can result in a shift of metals from soil solid phase to soil solution phase, which increases their mobility in the soil (Acosta et al. 2011; Li et

al. 2019). As Cl^- ion is the most important inorganic ligands with regard to metal binding in salt-affected soils (Ghallab and Usman 2007), the formation of chloro-metal complexes is a leading mechanism for the mobilization of Pb and Cd in multi-metal polluted soils (Acosta et al. 2011). Li et al. (2019) reported a positive correlation between the concentration of Cl^- added by NaCl and the concentrations of the soluble Cd in the pore water and the water-extractable Cd in the soil.

Metal mobilization by salinity depends on a number of factors, including the type of metal and salinity, as well as soil properties (Abbaspour et al. 2008; Acosta et al. 2011). Results showed that NaCl salinity mobilized more Cd than Pb in both single metal- and co-metal polluted soils. This suggests that Cd was more readily complexed with Cl^- ions or more easily replaced by / exchanged with Na^+ ions from exchange sites than Pb while Pb had a greater tendency to be retained on soil exchange sites compared with Cd, especially in co-polluted soils. Similarly, Acosta et al. (2011) reported that addition of NaCl with high ionic strength ($>0.2\text{ M}$) resulted in a higher release of Cd than Pb in multi-metal polluted soils around industrial areas in Spain. This is likely due to a high affinity of Cl^- ions for Cd to form Cl-Cd complexation (Rieuwerts et al. 1998; Acosta et al. 2011; Filipović et al. 2020). Cadmium can easily form stable complexes with Cl^- ions in salinized soils (Usman et al. 2005; Filipović et al. 2020). Under competitive conditions (i.e., the presence of more than one metal in the soil), Pb can maintain its strong affinity with the soil surfaces while Cd can be displaced from the soil surfaces (Lu and Xu 2009; Mouni et al. 2017). Another possible explanation could be related to the initial total concentrations of the metals, which was 10 mg kg^{-1} for Cd and 150 mg kg^{-1} for Pb. In saline soils, a higher total metal concentration resulted in a smaller release of heavy metals (Acosta et al. 2011). When the proportion of a metal bound to soil particles is high, it cannot easily be released by salinization (Acosta et al. 2011). Another explanation for less increases in Pb availability in co-polluted soils might be the greater affinity of Pb for reaction with functional groups in soil organic matter (Appel and Ma 2002). Generally, metal attributes such as the atomic mass, electronegativity, dehydrated ionic radii, hydrated ion diameter and hydration energy might explain metal bonding and selectivity to the soil surfaces (McBride 1994; Lu and Xu 2009). Cadmium is lighter than Pb (Cd = 112.4 u , Pb = 207.2 u), has a lower electronegativity (Cd = 1.69 , Pb = 2.10), a larger hydrated radius (Cd = 4.26 \AA , Pb = 4.01 \AA), a greater hydration energy (Cd = -1807 kJ mol^{-1} , Pb = -1481 kJ mol^{-1}) and a higher hydrolysis constant (Cd = 10.1 , Pb = 7.71); and probably is less strongly bound to soil constituents (McBride 1994; Appel and Ma 2002). The greater release and mobilization of Cd by salinity might also be due to its smaller polarizing power and the weak interaction forces between carbon-Cd compared with Pb, as the desorption increases with the decreasing polarization power of the exchangeable cation (McBride 1994). All the above factors make Cd to less readily undergo inner-sphere surface sorption reactions and complexation with soil surface functional groups than Pb (Appel and Ma 2002; Mouni et al. 2017).

4.2 Salinity effects on soil microbial activity and biomass

The results showed that an increase in metal mobilization and toxicity induced by NaCl salinity generally lowered the microbial activity and biomass. This indicates that salinity increased the mobilization of metals with further increases in their biotoxicity to soil microbial community. Our observations are similar to the few studies that reported salinization consistently decreased microbial respiration rate and biomass content in soils polluted with Cd (Usman 2015; Raiesi et al. 2018; Raiesi and Sadeghi 2019) and Pb (Zheng et al. 2017; Raiesi et al. 2020) in laboratory incubation experiments. The results of RDA indicated that ECe, pH and metal availability (more strongly the available Cd pool) were the key factors regulating microbial activity and biomass in saline polluted soils. Based on our RDA results, the inhibition of microbial activity and biomass by soil salinization was partly direct, induced by the elevated ECe and partly indirect, caused by the increases in metal toxicity and pH. Similarly, soil ECe and pH increased with increasing salinity, which hampered the activity of soil microorganisms (Yang et al. 2020). Nevertheless, the variation

in microbial metrics explained by soil pH (4%) or Pb availability (3%) was low, suggesting ECe (75%) and Cd toxicity (9%), which together explained much of the variability (84%), are the most important factors regulating microbial activity and biomass in saline polluted soils. Our finding indicates that the increased mobility of heavy metals induced by the soil salinization could impose an additional stress on the microbial population, growth and activity in metal-polluted soils. This is supported by the increased $q\text{CO}_2$ values in metal-polluted soils exposed to NaCl salinity, particularly at a high level of NaCl addition (Fig. 2). The $q\text{CO}_2$ (i.e., the amount of C respired per unit microbial biomass) can be used as an eco-physiological indicator of microbial stress (Anderson and Domsch 1993; Fließbach et al. 1994) and maintenance energy demand (Anderson and Domsch 2010) or as a useful measure of microbial C assimilation efficiency (Wardle and Ghani 1995). Its increasing value exhibits the presence of stressful conditions or a low microbial C use efficiency. Therefore, the finding of this study indicates an increase in salinity induced by NaCl promoted salt and metal stresses within the soil microbial community with high maintenance energy requirements, in consistent with previous studies (Raiesi and Sadeghi 2019). This suggests that the soil microbial communities under additional stress induced by salinity would direct more energy from growth and production to maintenance, and in turn respire more CO_2 to support cellular function and maintain viability (Fließbach et al. 1994; Anderson and Domsch 2010).

Although not determined in the current study, changes in the microbial $q\text{CO}_2$ could also be linked to a shift in microbial community composition, which is also known to alter by metal toxicity (Khan et al. 2010; Xu et al. 2019) and salinity (Wichern et al. 2006; Wang et al. 2019b). Compared with soil fungi, bacteria are assumed to respire more C to maintain biomass (Six et al. 2006) and thus are characterized by a higher $q\text{CO}_2$. Hence, there is a possibility that the salinization of metal-polluted soils would favor the bacterial community. Soil bacteria with lower C assimilation and higher $q\text{CO}_2$ have been reported to be less sensitive to soil salinization (Sardinha et al. 2003; Yuan et al. 2019) and heavy metal (Cd and Pb) pollution (Lin et al. 2019) than soil fungi. In Cd-polluted soils, bacterial communities were more active in response to the NaCl salinity-induced stress than fungal communities (Wang et al. 2019b).

In the present study, the increase in the $q\text{CO}_2$ was due to the larger decreases in microbial biomass size (35–63%) than microbial respiration rate (8–43%). The greater absolute correlation coefficient between $q\text{CO}_2$ and MBC (-0.88) than between $q\text{CO}_2$ and MR (-0.62) may further confirm that the observed increases in the microbial $q\text{CO}_2$ of salt-stressed polluted soils is mainly associated with the reduction in the size of microbial community. This result also implies that microbial biomass was more sensitive than microbial activity to the effect of metal mobilization induced by soil salinization. In support of this, there was a stronger negative correlation between ECe and MBC ($r=-0.87$) than between ECe and MR ($r=-0.70$) across treatments (Table 1). This further verifies that part of the salinity effect on microbial metrics was direct, probably through lowering the osmotic potential and thus water availability in soil, and through ion toxicity (here Na^+ and Cl^- ions) of excessive salts (Rath and Rousk 2015; Singh 2016; Boyrahmadi and Raiesi 2018).

Our study indicated that NaCl salinity had a greater negative impact on microbial respiration and biomass in Cd-polluted soils than Pb-polluted soils, and in single-metal polluted soils than co-polluted soils. This could be related to the more solubility and ecotoxicity of Cd under saline conditions compared with Pb, as outlined above (Sect. 4.1). The RDA model further confirms the most important role of Cd mobilization in controlling microbial activity and biomass. In contrast, Khan et al. (2010) and Xu et al. (2019) reported a larger reduction in microbial biomass and respiration in soils co-polluted with Cd and Pb than soils polluted with individual metals in the short-term laboratory incubation experiments.

4.3 Salinity effects on soil enzyme activities

Soil microorganisms are the major source of biotic and abiotic enzymes in soil (Nannipieri et al. 2002, 2018) and these biocatalysts permit heterotrophic microbes to acquire energy and nutrients from decomposing complex organic compounds in soil (Nannipieri et al. 2002; Rao et al. 2017). It is, therefore, anticipated that any change in the growth and biomass of soil microbial community in response to salinity may also affect the synthesis and production of enzymes. In line with the microbial respiration and biomass, NaCl salinity inhibited the activities of extracellular enzymes involved in P, N and S cycling, and intracellular enzymes that are involved in microbial metabolism (up to 87%). Similarly, the potential activities of ALP, ARY, CAT, URE, DEH and FDA in Cd-polluted soils (Raiesi et al. 2018; Raiesi and Sadeghi 2019; Filipović et al. 2020) and those of CAT and URE in Pb-polluted soils (Zheng et al. 2017) were inhibited by salinity induced by NaCl addition. In a Cd-polluted soil, DEH activity was depressed by NaCl salinity, and Cl-Cd complexes with low sorption affinity had a greater inhibitory effect on DHA activity than other Cd species (Filipović et al. 2020).

The inhibitory impact of salinity could be linked to the increases in available metal pools, particularly Cd, induced by salinity and lower microbial biomass content with salinization. This hypothesis is supported by the results of correlation analysis (Table 1) and RDA (Fig. 4) for the most part. Specifically, the RDA confirmed that the soil enzymatic activity had been altered in response to significant changes in microbial biomass and metal mobilization, especially Cd (Fig. 4). This finding suggests an indirect effect of salinity on soil enzyme activity through the decreased MBC and the increased metal mobilization, since soil E_{Ce} with a VIF value greater than 3 was removed from the RDA model. The RDA results also showed the amount of variability explained by soil pH was quite low (2%) compared with the variability explained by MBC (65%) and available metal concentrations (in total 16% for both metals), suggesting microbial biomass, which is assumed to be the main source of soil enzymes, and metal mobilization played more important roles than the E_{Ce} and pH, and therefore were the most influential factors driving changes in the soil enzymatic activity. In saline soils, a lower quantity of microbial biomass is a possible mechanism that can explain the lower enzymatic activity in the soil, due to the less production and release of enzymes (Rietz and Haynes 2003; Rath and Rousk 2015; Boyrahmadi and Raiesi 2018). The strong positive correlations between the MBC values and the activity of all the assayed enzymes (Table 1) further suggest that the synthesis and release of soil enzymes by living microbes might be low under saline conditions. Enzyme activities are frequently correlated with microbial biomass in salt-affected soils (Rietz and Haynes 2003; Boyrahmadi and Raiesi 2018). Our findings indicate that the salinity-induced metal ecotoxicity that reduces microbial biomass also reduces enzyme activity in saline metal-polluted soils. Several studies have found that salinity has the potential to depress enzyme activities in metals-polluted soils and consequently nutrient cycling by declining the size of microbial biomass (Raiesi et al. 2018; Raiesi and Sadeghi 2019). However, results showed that while the activities of soil enzymes were closely linked to microbial biomass, they were more sensitive to soil salinization than microbial respiration rate or biomass content in metal polluted soils. This is probably due to the direct effect of increased metal toxicity under salinity conditions. Heavy metals may directly interact chemically with the enzyme structure or substrate, and they usually inhibit enzyme activity through their multiple effects, such as complexation with the enzyme substrate, their reaction with the active functional groups and thus active sites of the enzyme, denaturing the enzyme protein or interacting with the enzyme-substrate complex in soil (Hinojosa et al. 2004a; Karaca et al. 2010b). Therefore, the salinization of metal polluted soils may decrease enzyme activity indirectly through its direct negative effects on microbial biomass and its direct positive effects on metal mobilization. The latter would in turn directly inhibits enzyme activity further.

Lower enzyme activities might also be related to changes in the microbial functional groups, since changes in enzyme activity can be accompanied by a shift in microbial community composition (Waldrop et al. 2000; Allison et al. 2007). The indirect evidence for this hypothesis is the strong negative correlations between enzyme activity and $q\text{CO}_2$ value across treatments (Table 1). Hence, it is possible that the salinization of metals-polluted soils would favor the

bacterial community with low C use efficiency (i.e., high $q\text{CO}_2$), which would be less efficient at enzyme production or have less active biomass to release enzymes. This hypothesis is yet to be tested further in the future.

The current study demonstrated that the magnitude of reductions in soil enzyme activity with increasing salinity level depended on the type of soil enzyme assayed. ARY and FDA were more impacted by salinity, especially in co-polluted soils under high salinity level, than the other enzymes assayed in this study. In addition, salinity impact was greater on the activity of CAT than DEH, both intracellular enzymes involved in the life processes of soil microorganisms. Differential responses of soil enzyme activity to salinity have also been demonstrated by other studies in unpolluted soils (Rietz and Haynes 2003; Boyrahmadi and Raiesi 2018). They reported that the inhibitory effect of salinity is enzyme-specific. Considering our experimental setting and data, the varying reductions of enzyme activities in metal-polluted soils in response to salinization cannot be elucidated evidently and thus remain inconclusive. We observed that soil salinization has a greater negative influence on enzymatic activity in co-polluted than single metal-polluted soils, particularly at high salinity level. This is probably due to the synergistic interaction under the conditions of multiple-metal pollution (Chaperon and Sauvé 2007). Our results are in agreement with previous observations (Khan et al. 2010; Pan and Yu 2011; Huang et al. 2017) that reported Cd and Pb co-pollution had more suppressive impact on the activities of soil URE, DEH and acid phosphatase.

The GME was used as a single numerical index to integrate or summarize soil enzyme activities that are very similar in nature and strongly interconnected but have different units and numeric ranges (Hinojosa et al. 2004b; García-Ruiz et al. 2008; Paz-Ferreiro et al. 2012). The GEM values indicated a consistent and more clear response to the increasing salinity level in all polluted soils. The greatest reduction (50%) in GME was observed in co-polluted soil exposed to high salinity and the lowest reduction (11%) in Cd-polluted soils subjected to low salinity level. The negative effect of increasing salinity on the GME values was stronger in co-polluted than single metal-polluted soils and at high salinity level than low salinity level. This index was highly correlated negatively with soil pH, E_{Ce}, available Cd concentration and $q\text{CO}_2$, but positively with microbial biomass and respiration rate (Table 1). This would mean that salinity may inhibit soil enzymatic activity indirectly by lowering microbial activity and biomass in soils polluted by heavy metals. Based on all these observations, we proposed that specifically the GME is a good indexing tool to integrate enzyme activities in a single value, which is sensitive to the stresses indirectly (through decreased microbial biomass and increased metal mobility) induced by salinity in metal-polluted soils. Hence, it is essential to develop suitable soil management practices for alleviating the adverse effects of salinity on enzyme activity and thus maintaining nutrient cycling in metal-polluted soils through the existing remediation and amelioration techniques. These strategies should improve soil microbial stability (resistance and resilience) and enzyme activity under environmental stresses induced by both metal pollution and salinity.

5 Conclusions

The results demonstrated that salinity increases metal mobility and toxicity and hence exaggerated the harmful effect of metal biotoxicity on the activity and biomass of microbial community as well as enzyme activity in soils polluted with Cd and Pb, in accordance with our hypothesis. The aggravating effect of salinity was more pronounced at high salinity level and in Cd-polluted soils for microbial respiration and biomass, and in co-polluted soils for enzyme activities. Multivariate analysis such as the FA and RDA of the soil data were useful tools for the strong separation among the salinity and metal treatments. The RDA further indicated that reduced soil microbial activity and biomass were mainly associated with increased E_{Ce}, Cd toxicity and pH, while depressed enzyme activities were strongly related to reduced microbial biomass and increased metal mobilization. The salinization of heavy metals-polluted soils in arid zones would lead to a high ecological risk for the potential activities of enzymes involved in the cycling of

important nutrients and microbial metabolism. Therefore, adoption of a suitable management strategy is needed to mitigate the potential risk of metal biotoxicity associated with secondary salinization in metal-polluted soils, and to improve soil microbial resistance and resilience to multiple stresses.

Declarations

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The authors declare that they have no competing interests.

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Tables

Table 1 Matrix of paired correlation coefficients (*r*) between soil properties (n=27).

Variable	pH	ECe	Cd	Pb	MR	MBC	<i>q</i> CO ₂	ALP	URE	ARY	CAT	DEH	FDA
ECe	0.73												
Cd	0.32	0.07											
Pb	0.03	0.11	-0.38										
MR	-0.41	-0.70	-0.28	0.15									
MBC	-0.58	-0.87	-0.35	0.18	0.90								
<i>q</i> CO ₂	0.64	0.87	0.35	0.13	-0.62	-0.88							
ALP	-0.55	-0.44	-0.82	0.34	0.58	0.67	-0.58						
URE	-0.81	-0.81	-0.32	-0.32	0.33	0.62	-0.81	0.47					
ARY	-0.84	-0.86	-0.41	-0.05	0.56	0.80	-0.84	0.68	0.87				
CAT	-0.45	-0.80	-0.26	-0.12	0.64	0.85	-0.87	0.56	0.62	0.74			
DEH	-0.74	-0.69	-0.61	-0.25	0.34	0.64	-0.80	0.72	0.89	0.85	0.67		
FDA	-0.86	-0.83	-0.35	-0.26	0.41	0.66	-0.79	0.52	0.95	0.90	0.65	0.91	
GME	-0.86	-0.81	-0.50	-0.15	0.46	0.72	-0.83	0.69	0.94	0.96	0.70	0.95	0.96

Bold correlation coefficients ($r \geq |0.49|$ and $\geq |0.60|$ are significant at $P < 0.01$ and $P < 0.001$, respectively.

(abbreviations: ECe, electrical conductivity of the saturated-paste extract; Cd, available cadmium; Pb, available Pb; MR, microbial respiration; MBC, microbial biomass carbon; *q*CO₂, metabolic quotient, ALP, alkaline phosphomonoesterase activity; URE, urease activity; ARY, arylsulphatase activity; CAT, catalase activity, DEH, dehydrogenase activity; FDA, fluorescein diacetate hydrolysis activity, GEM, geometric mean of enzyme activity).

Table 2 Effect of salinity on the activities of soil enzymes in metal-contaminated soils. Values are mean \pm standard error of the mean (n=3).

Salinity treatment	Pollution treatment			Source
	Cd	Pb	Cd+Pb	
Alkaline phosphomonoesterase activity (μmol PNP g ⁻¹ h ⁻¹)				
Control	9.78±0.18DE	14.16±0.08A	10.50±0.11CD	Pollution (P)***
Low salinity	9.60±0.18E	11.87±0.23B	9.49±0.14E	Salinity (S)***
High salinity	9.50±0.02E	10.80±0.26C	9.06±0.02E	P×S***
Urease activity (μmol NH ₄ -N g ⁻¹ h ⁻¹)				
Control	2.47±0.01A	2.37±0.05AB	2.36±0.02AB	Pollution (P)***
Low salinity	2.26±0.01AB	2.24±0.03B	1.96±0.02C	Salinity (S)**
High salinity	1.90±0.02C	1.95±0.05C	1.37±0.08D	P×S***
Arylsulphatase activity (μmol PNP g ⁻¹ h ⁻¹)				
Control	2.36±0.08C	3.18±0.01A	2.74±0.04B	Pollution (P)***
Low salinity	1.78±0.02D	1.83±0.02D	1.60±0.03DE	Salinity (S)***
High salinity	1.37±0.05E	1.62±0.04DE	0.36±0.04F	P×S***
Catalase activity (μmol O ₂ g ⁻¹ h ⁻¹)				
Control	2.31±0.01A	2.35±0.02A	2.24±0.02A	Pollution (P) ^{ns}
Low salinity	2.15±0.06B	2.18±0.04B	2.08±0.01B	Salinity (S)***
High salinity	2.09±0.04B	2.08±0.04B	2.11±0.01B	P×S ^{ns}
Dehydrogenase activity (μmol TPF g ⁻¹ h ⁻¹)				
Control	0.167±0.001B	0.181±0.001A	0.148±0.001D	Pollution (P)***
Low salinity	0.156±0.001C	0.164±0.001B	0.133±0.002F	Salinity (S)***
High salinity	0.138±0.001E	0.146±0.001D	0.104±0.001G	P×S***
Fluorescein diacetate hydrolysis activity (μmol Fluorescein g ⁻¹ h ⁻¹)				
Control	0.163±0.001A	0.161±0.001A	0.142±0.001B	Pollution (P)***
Low salinity	0.132±0.002C	0.142±0.001B	0.128±0.002C	Salinity (S)***
High salinity	0.109±0.001D	0.108±0.001D	0.052±0.001E	P×S***

Geometric mean of enzyme activity				
Control	2.10±0.02B	2.37±0.01A	2.06±0.01B	Pollution (P)***
Low salinity	1.86±0.01D	1.97±0.01C	1.71±0.01E	Salinity (S)***
High salinity	1.63±0.01F	1.73±0.01E	1.03±0.01G	P×S***

Values followed by the same letter are not significantly different at the $P < 0.05$ probability level (Tukey test at $P \leq 0.05$). *** and ^{ns} indicate significant at $P < 0.001$ and not significant ($P > 0.05$), respectively.

Table 3 Variable loading coefficients (eigenvectors) of the three quartimax (with Kaiser normalization) rotated factors extracted using soil properties, their eigenvalues (total variance), and individual percentage of total variance explained by each factor.

Variable	Factor1	Factor2	Factor3	Communality
pH	-0.84	0.07	0.01	0.94
ECe	-0.93	0.15	-0.32	0.99
Cd	-0.36	0.16	0.90	0.97
Pb	-0.16	-0.91	-0.30	0.96
MR	0.59	-0.74	0.06	0.97
MBC	0.84	-0.41	0.01	0.98
qCO_2	-0.91	0.03	0.01	0.92
ALP	0.63	-0.31	-0.65	0.92
URE	0.93	0.24	0.00	0.95
ARY	0.96	-0.06	-0.08	0.94
CAT	0.80	-0.07	0.01	0.93
DEH	0.89	0.24	-0.36	0.99
FDA	0.95	0.16	-0.02	0.96
GME	0.97	0.09	-0.18	0.99
Variance (eigenvalue)	9.04	1.86	1.60	12.50
Variance explained (%)	65	13	11	89

Boldface factor loadings (> 0.60) are considered highly weighted.

(abbreviations: ECe, electrical conductivity of the saturated-paste extract; Cd, available cadmium; Pb, available Pb; MR, microbial respiration; MBC, microbial biomass carbon; qCO_2 , metabolic quotient, ALP, alkaline phosphomonoesterase activity; URE, urease activity; ARY, arylsulphatase activity; CAT, catalase activity, DEH, dehydrogenase activity; FDA, fluorescein diacetate hydrolysis activity, GEM, geometric mean of enzyme activity).

Figures

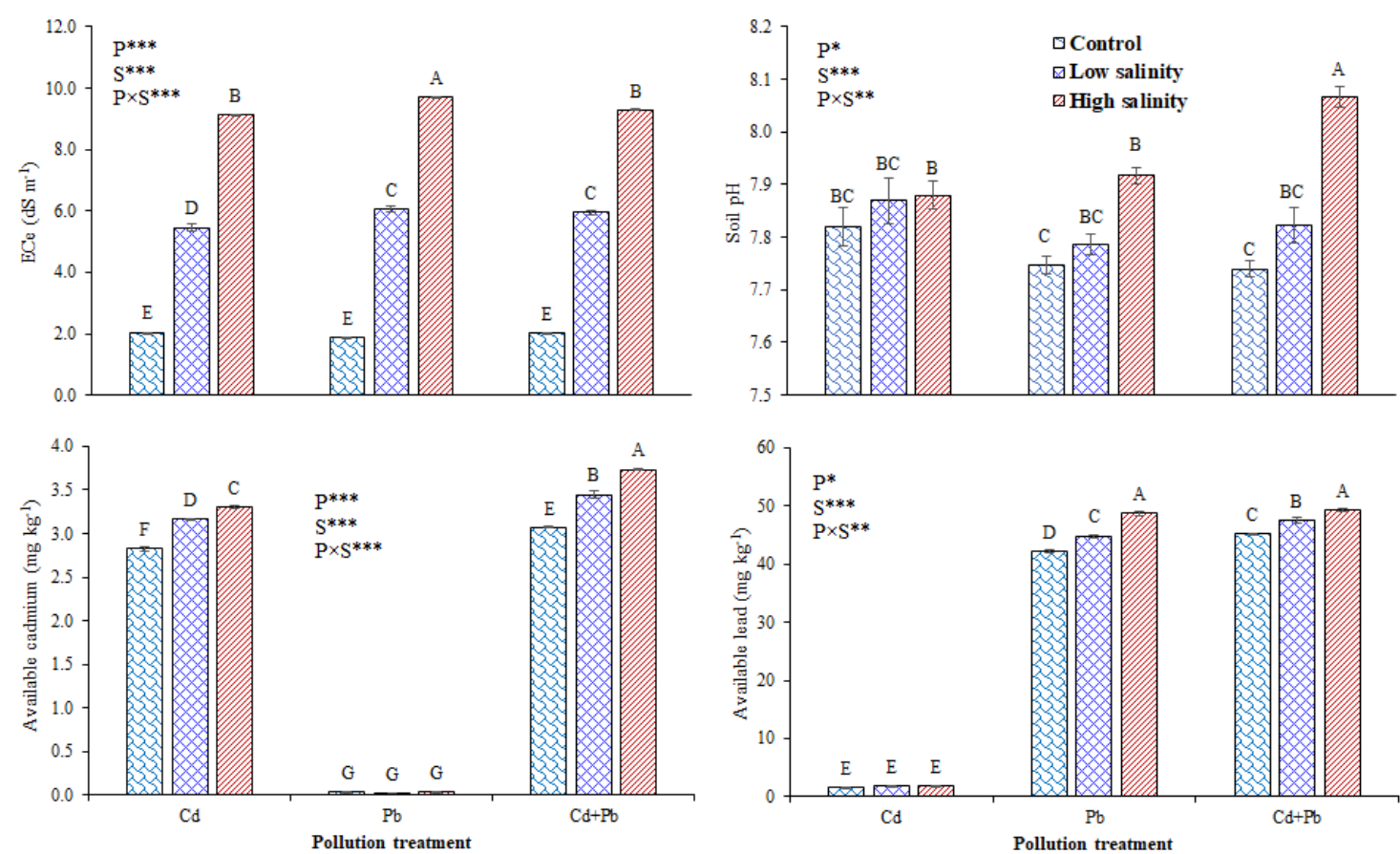


Figure 1

Effect of salinity (S) on the electrical conductivity of the saturated-paste extract (ECE), pH and the concentrations of DTPA-extractable cadmium (Cd) and lead (Pb) in metal-polluted (P) soils. Values are mean and the vertical bars represent standard error of the mean (n=3). Different letters represent significant differences by Tukey test at $P \leq 0.05$. *, ** and *** indicate significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

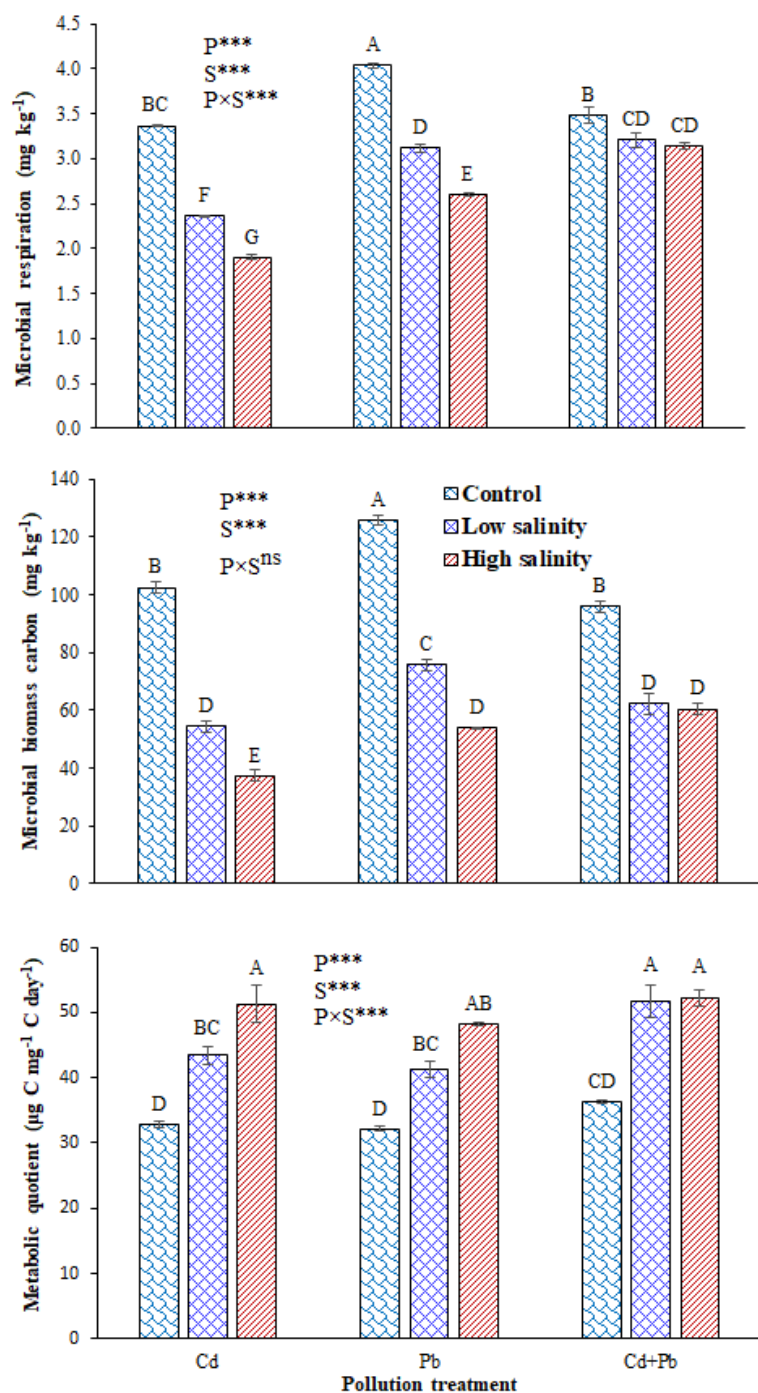


Figure 2

Effect of salinity (S) on microbial respiration and biomass, and metabolic quotient in metal-polluted (P) soils. Values are mean and the vertical bars represent standard error of the mean (n=3). Different letters represent significant differences by Tukey test at $P \leq 0.05$. *** and ns indicate significant at $P < 0.001$ and not significant ($P > 0.05$), respectively.

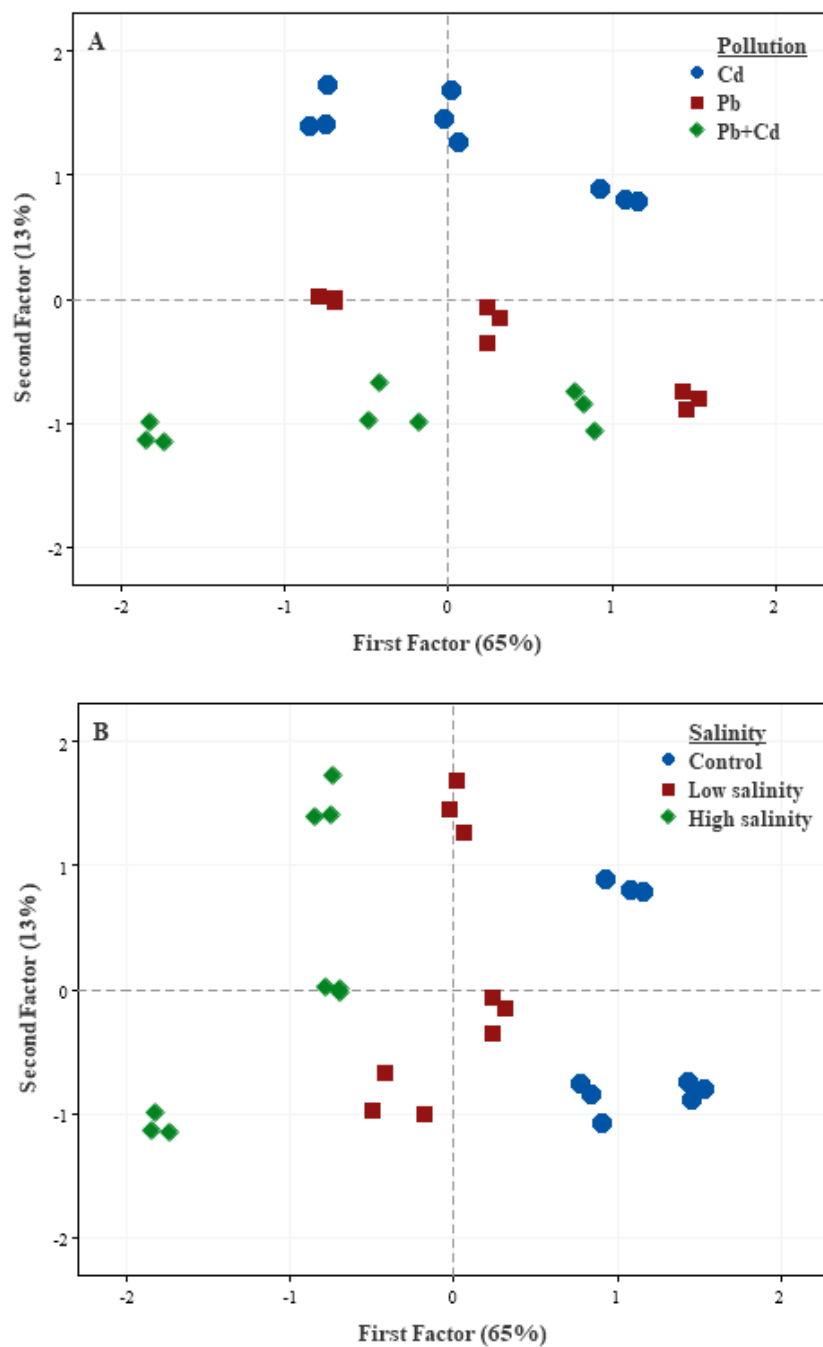


Figure 3

Factor loading plot based on soil microbial properties and enzyme activities, showing separation of metal pollution (A) and NaCl salinity (B) treatments on the first two extracted factors (Cd, cadmium; Pb, lead).

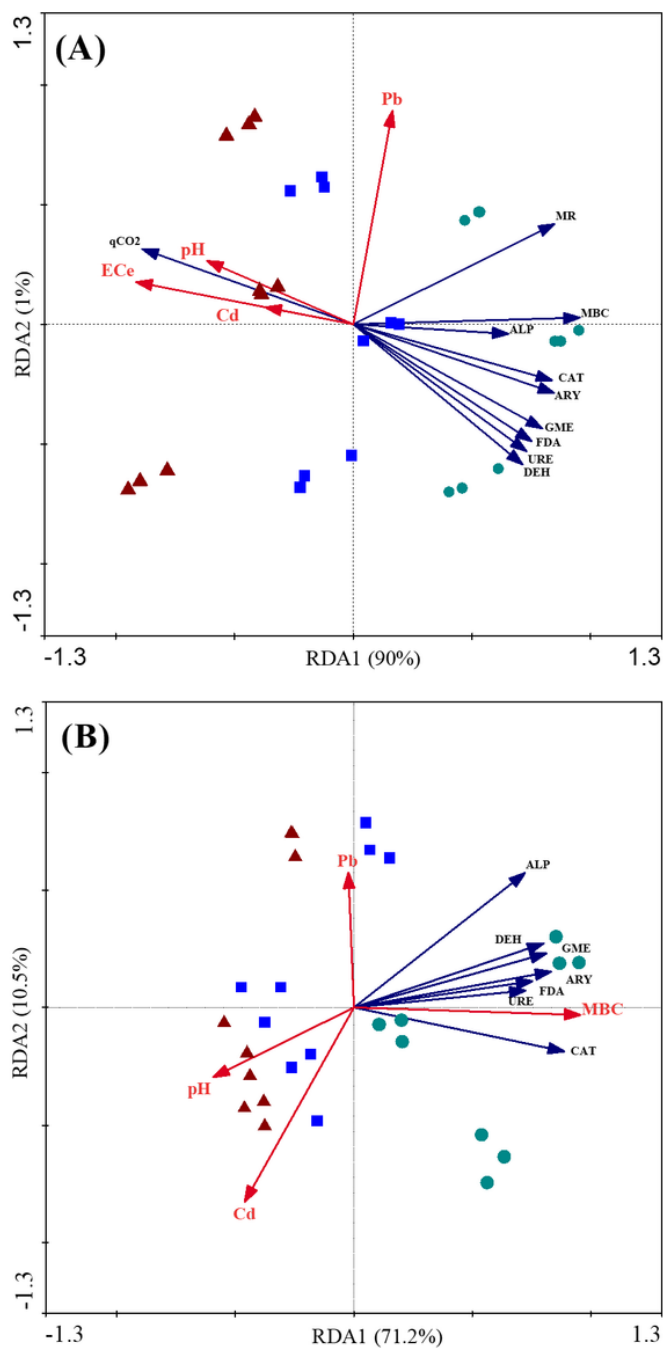


Figure 4

Redundancy analysis (RDA) ordination of the microbial properties and enzyme activities (A) constrained by chemical properties (pH, ECe, Cd and Pb) and enzyme activities (B) constrained by both chemical properties (pH, Cd and Pb) and microbial biomass (MBC) in metal-polluted soils salinized by NaCl. The red arrows display explanatory variables (chemical properties) and response variables (microbial properties and enzyme activities) are shown in blue vectors. Filled circles show salinity treatments (abbreviations: ECe, electrical conductivity of the saturated-paste extract; Cd, available cadmium; Pb, available lead; MR, microbial respiration; MBC, microbial biomass carbon; qCO₂, metabolic quotient, ALP, alkaline phosphomonoesterase activity; URE, urease activity; ARY, arylsulphatase activity; CAT, catalase activity; DEH, dehydrogenase activity; FDA, fluorescein diacetate hydrolysis activity, GEM, geometric mean of enzyme activity).