

# Evaluation of enzyme activities in long-term polluted soils with mine tailing deposits of San Luis Potosí, México

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## Abstract

**Purpose** San Luis Potosí is one of the largest metal producers; mining activity has been responsible for metal emissions for over 100 years, from several sources (deposits, tailings, effluents, and dusts) generating effects in human and ecosystem health. The objective of this study was to evaluate the effect of the concentrations of heavy metals in the soil health of four municipalities of San Luis Potosí contaminated with mine tailings, using enzyme activity as a biochemical endpoint.

**Materials and methods** Four municipalities contaminated with residues of historical mining activity were analyzed (25 topsoil samples per type of site contaminated and reference). The parameters that were analyzed included pH; organic matter (OM); electrical conductivity (EC); percentage of clay, As, Cd, Cr, Cu, Hg, Pb, and Zn; and arylsulfatase (ARS),  $\beta$ -glucosidase (BG), urease (UR), and fluorescein diacetate hydrolysis (FDA) activities in soil. Differences among the parameters per municipality and type of site were evaluated using a factorial analysis of variance. The relationships were analyzed by Pearson's correlation and a stepwise distance-based linear model permutation test (DistLM). Results were visualized using a distance-based redundancy analysis

(dbRDA). A hazard quotient (HQ) for metals was calculated in order to estimate the effects on soil microbial processes. **Results and discussion** A concentration gradient (mg/kg) of Zn (4744.5–65,585.7), Pb (1321.0–31,932.2), As (ND–8736.7), and Cu (130.9–8475.4) was found in the contaminated sites. The HQ showed a very high hazard level for the elements detected in all contaminated sites (1.4–655.8). The pattern of enzymatic inhibition found was ARS (95.8 %), UR (90.6 %), FDA (86.9 %), and BG (76.0 %). Strong negative relationships were observed among enzymatic activities and heavy metals in the following inhibitory effect Cu > As > Zn > Pb. Metals and covariables explained from 84 to 86 % of variability in enzyme activity. EC, Cu, and As showed a strong inhibitory effect; and parameters such as OM, pH, and clay were found to have a slightly inducing effect. **Conclusions** In this study, the heavy metal concentrations were higher than the ones obtained in other reports for this region. The HQ reveals the presence of possible risks for the health of life in the region. The decrease of enzyme activities in soil could trigger adverse changes in the flow of matter and energy in ecosystems. This study provides a field baseline that could be part of a long-term monitoring program for these locations.

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**Keywords** Ecological risk · Enzymatic activities · Heavy metals · Mine tailings · Soil pollution

## 1 Introduction

The mining industry in México is one of the oldest and most important economic activities, and globally, the state of San Luis Potosí occupies the first place in arsenic (As), second in tin (Sn), third in copper (Cu) and zinc (Zn), fifth in lead (Pb), sixth in silver (Ag), and seventh in gold (Au) production

(Servicio Geológico Mexicano SGM 2007). Intensive mining activities have generated metal emissions from several as both effluents and dusts, leaving approximately 10,000 to 50,000 abandoned sites in the whole country. Metal pollution is a permanent threat to ecosystems and human health, inhibiting plant growth and reducing soil functionality through the selection of less metabolically efficient metal-resistant species, as well as the direct inhibition of enzyme activity (Mergeay 2000; D'Ascoli et al. 2006; Gomes et al. 2010).

Soil quality has been defined based on its capacity to support the agricultural production; however, soil also have important effects on the environment as it influence the quality of atmosphere and water and ultimately the biodiversity and human health (Karlen et al. 2004). A healthy soil has the ability to function as a living system, within the limits of the ecosystem and land use, and it is able to sustain biological productivity; promote quality of air and water environments; and maintain plant, animal, and human health (Dick 1997). Several indicators have been established in order to evaluate the quality and health of soil; the ones that are commonly used are the physicochemical, biochemical, and biological properties. Indicators can vary among localities depending on the type, use, function (interaction between soil biota and biological, chemical, and physical processes that can contribute to soil fertility) and formation factors (soil-forming processes that take place among the climate, relief, mountain rocks, organisms, and time) of the soil (Doran and Parkin 1994). Balanced, safe, and productive soil function exists when its biological, biochemical, physical, and chemical properties all correlate each other and contribute to sustainable evolution.

Enzyme activities play a fundamental role because of their intracellular or extracellular chemical reaction pathway in the nutrient cycling (Dick 1997). Several enzymes have been taken as an indicator of the rate of a whole metabolic process, mainly of microbial origin, and are regarded as sensitive to diverse groups of pollutants including heavy metals (Karaca et al. 2010). Urease and exopeptidases are important enzymes involved in N mineralization (Allison and Vitousek 2005), and urease is strongly inhibited by heavy metals (Hemida et al. 1997; Effron et al. 2004; Wyszowska et al. 2006; Yang et al. 2006; Mikanova 2006). Soil  $\beta$ -glucosidase activity plays a key role in the degradation of cellobiose-releasing glucose used as energy source by soil microorganisms (Eivazi and Tabatabai 1990); arylsulfatase releases sulfate from arylesters (Klose et al. 2011), its activity is normally related to the amount of organic carbon in soil, and it has shown a reduction in soil polluted with Cu and Cd (Geiger et al. 1998; Renella et al. 2003); and phosphatase activities (phosphomonoesterases and phosphodiesterase) are key enzymes in P cycling (Acosta-Martínez and Tabatabai 2011). Hydrolysis of fluorescein diacetate (FDA) has been used to determine the proportion of active fungi and

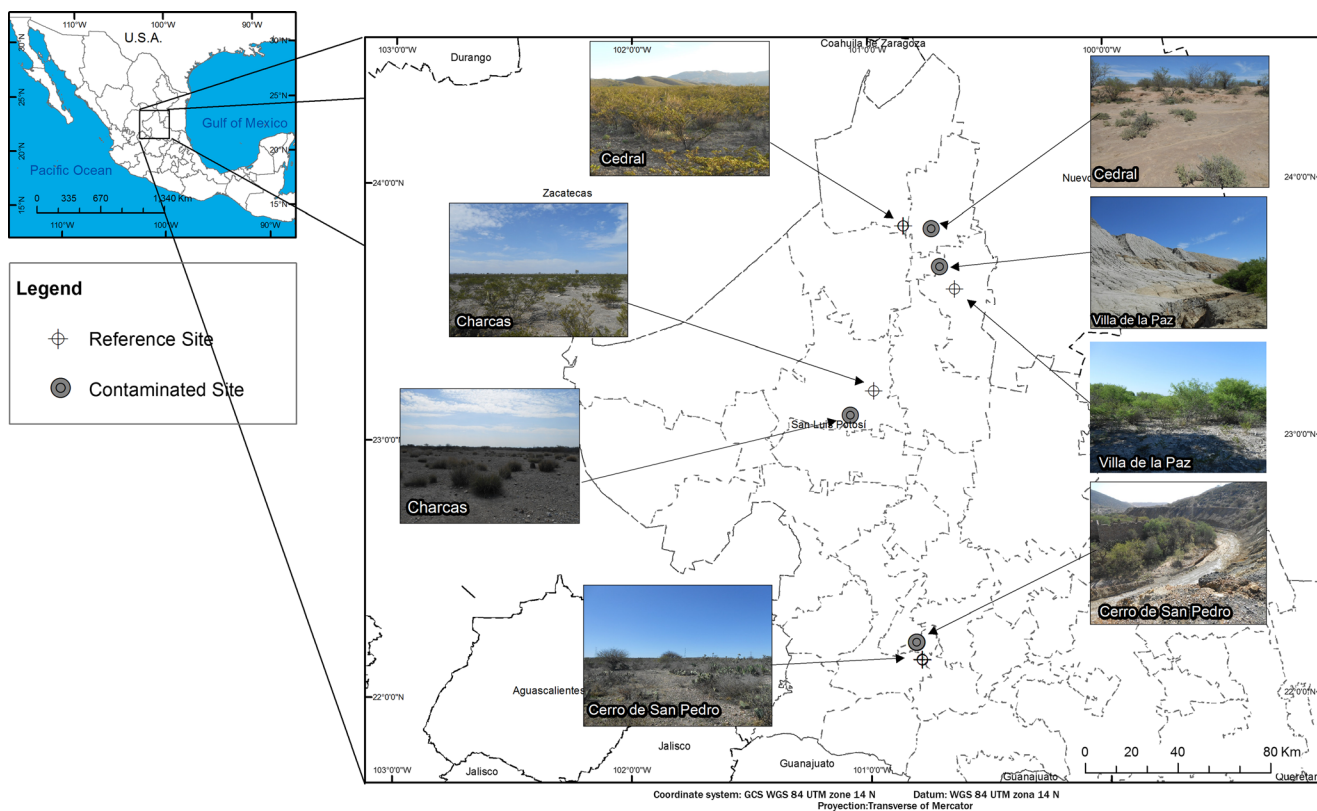
bacteria (Schnürer and Rosswall 1982; Dick 1997; Gaspar et al. 2001) and as a global indicator of microbial hydrolytic activity. Soil enzyme activities are inhibited by heavy metals and metalloids (Schnürer and Rosswall 1982; Haigh and Rennie 1994; Renella et al. 2003; Lorenz et al. 2006; Wyszowska et al. 2006; Li et al. 2009; D'Ascoli et al. 2006; Biró et al. 2012).

Soil enzyme activity also responds to other main soil properties such as organic matter content (OM), pH value, microbial biomass and activity, and soil use (Nannipieri et al. 2012). Therefore, more reliable sources of information about the impact of metal pollution on the biochemical activity of soil, such as the analysis of the reference site which is not influenced by the metal input, as well as the comparison of the measured activities with the chemical properties of soil are needed (D'Ascoli et al. 2006). The aim of this study was to evaluate the effects of As and other heavy metals in the soils of four municipalities of San Luis Potosí contaminated by historic mine tailings, in soil enzyme activity as a biochemical stress endpoint.

## 2 Methods

### 2.1 Study site

The arid region of the state of San Luis Potosí, México (Central Plateau), is characterized for having a bedrock of sedimentary origin made of limestone, lutite, and arsenite rock. Calcic Xerosols and Lithosols are the main types of soils distributed in the area. The predominant type of vegetation is the scrubland; the climate is dry, semi-desert steppe. The average altitude of the region is 2000 m above mean sea level, annual mean temperature is 17 °C, and there is an annual precipitation of 400 mm. Sampling was carried out during March–April on 2013 in four municipalities of the arid regions with environmental liabilities from mining activities in the state of San Luis Potosí, México (Fig. 1). The studied municipalities were (1) *Cedral* (CED), located in the plateau region of the northern part of the state (23° 49' N latitude and 100° 43' W longitude). The main mining area is located in the central part of the municipality, and it contains Hg and Sb deposits, with additional extraction of Zn, Cu, Pb, Ag, and Au taking place (skarn ore system). Even when mining activities in the municipality are currently active, there are numerous places with residues from as long as 243 years ago; (2) *Charcas* (CHA), located in the plateau region of the northern part of the state (23° 07' N latitude and 101° 06' W longitude). Numerous artisanal exploitations of Ag were found in this place, involving the use of Hg in the production processes (Gambusino method); this location has residues from as long as 442 years ago; (3) *Villa de la Paz* (VDP), located in the northern part of the state (23° 41' N latitude and 100° 43' W



**Fig. 1** Location of environmental sampling points in San Luis Potosí, México

longitude). The region shows large accumulations of current and postcolonial tailings; Pb-Zn-Ag (Cu-Au) skarn ore system has been mined for since 1870. High concentrations of pollutants as well as effects on health and environment have been reported in the site (Yañez et al. 2003; Jasso-Pineda et al. 2007; Espinosa-Reyes et al. 2014); and (4) *Cerro de San Pedro* (CSP), located in the central part of the state ( $22^{\circ} 12' N$  latitude and  $100^{\circ} 47' W$  longitude). The location has been exploited since the year 1545 and is still being exploited today, leaving many large current and colonial mine tailings exposed to open air.

In order to reach the objective of this study, two sampling stations were selected for every municipality and they were grouped according to the type of site: (1) contaminated, sites with residues of historical mining activity, and (2) reference, sites without mining activity located within a 6–10-km distance range from the contaminated soils and with similar biophysical conditions.

## 2.2 Soil sampling

Twenty-five pooled samples were taken per site; each pooled sample consisted of five subsamples of  $10 \text{ cm}^3$  of soil collected in a  $1\text{-m}^2$  surface area (center and vertex) every 10 m following a random transect up to 250 m.

The samples were homogenized, divided, sieved (2 mm), and put in polyethylene bags, so that they could be transported in order to be analyzed in the laboratory. Fifty-gram aliquots of the samples were put inside sterile conical tubes for enzyme analysis and kept at  $4^{\circ} \text{C}$  during transport to the laboratory, where they were then stored for 7 days at  $-20^{\circ} \text{C}$ .

## 2.3 Metal analysis

Element quantification was performed using X-ray fluorescence (XRF) with a portable analyzer (Thermo Scientific® Niton XL3t). This technique has the benefit of being non-destructive and gives a response in real time of the different elements present in a soil sample, which is why its use is widely recommended for environmental studies (Vogel-Mikuš et al. 2010). The measurement was done *ex situ* following the operating protocol of the instrument. Of the homogenized and previously oven-dried soil ( $40^{\circ} \text{C}$ ), 100 mg was used for the quantification. Each sample was analyzed in triplicate using 60 s of analysis per beam/filter as default. Element concentrations were expressed in milligrams per kilogram of dry soil ( $\text{mg/kg d.w.}$ ). Standard reference material analysis (Montana soil NIST-2711a) was conducted for quality control. Accuracy and precision of the XRF data were  $<20\%$  of difference (%diff) and  $<7\%$  of relative standard deviation (%RSD), respectively. The reported detection limits

(mg/kg) for the analyzed elements were As (7.0), Cd (12.0), Cr (22.0), Cu (6.5), Hg (9.0), Pb (8.0), and Zn (10.0).

## 2.4 Physicochemical parameters

pH and electrical conductivity (EC) of soil were determined through the measurement method in water in a 1:2 (*w/v*) and 1:5 (*w/v*) soil:water relationship, respectively (Jones 2001). The measurements were achieved using a potentiometer (Hanna HI 2216). OM in the soil was obtained using the method of humid digestion (Combs and Nathan 2010; Metson et al. 1979); 1 g of each of the soil samples was mixed with 10 mL of Na<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, 2H<sub>2</sub>O (0.5 M), and 20 mL of concentrated H<sub>2</sub>SO<sub>4</sub>. The samples were kept in constant agitation for 30 min. Of the deionized water, 70 mL was then added and they were then left unaltered for 24 h. Absorbance was measured using a spectrophotometer (Biomate 3 s Thermo Fisher Scientific) at 660 nm in 2 mL of the supernatant. OM in soil samples was expressed in percentage (%). The hydrometer method (Diario Oficial de la Federación DOF 2002) was used to analyze the clay content of soil samples.

## 2.5 Enzyme activity assays

Urease (UR) activity was determined according to the Kandeler and Gerber (1988) method, modified by Kandeler et al. (2011).  $\beta$ -Glucosidase (BG) was determined using the method developed by Eivazi and Tabatabai (1988), modified by Deng and Popova (2011). Arylsulfatase (ARS) activity was determined according to the method reported by Tabatabai and Bremner (1970), reported by Klose et al. (2011). Fluorescein diacetate hydrolysis (FDA) activity was determined according the method reported by Adam and Duncan (2001). Enzyme activities were determined 7 days after soil sampling. Concentrations of enzymes were adjusted to the moisture content of soil. All chemicals used were of analytical grade, and all measurements were carried out in triplicate and the mean was reported.

## 2.6 Hazard quotient

The estimation of the hazard quotient (HQ) of each of the analyzed metals was carried out by comparing the concentrations with the international guidelines, employing the following equation:  $HQ = C_e/C_r$ , where  $C_e$  is the environmental concentration and  $C_r$  is the reference concentration of Ecological Soil Screening Level (Eco-SSL) for protection against microbes and microbial processes in soil (Sutter 2007; Buchman 2008). The HQ is the ratio of potential exposure to the substance and the level at which no adverse effects are expected. If the HQ is greater than 1, then it is possible for adverse environmental health effects to appear.

## 2.7 Statistical analysis

Data are reported as the mean, standard error, and range. Samples with non-detectable values of arsenic and heavy metals were assigned half of the detection limit, when the number of data was at least half of the number for the variable. The data were transformed logarithmically in order to obtain a normal distribution. Differences among the concentrations of arsenic, heavy metals, enzymes, and covariables per municipality (CSP, VDP, CED, and CHA) and per type of site (contaminated or reference) were evaluated using a factorial analysis of variance followed by a multiple mean comparison test (least significant difference (LSD)). Relationships among arsenic, heavy metals, cofactors (physicochemical parameters), and enzymes were analyzed using Pearson's correlation and a stepwise distance-based linear model permutation test (DistLM, selection criterion, AICc; 9999 permutation). Results were visualized using a distance-based redundancy analysis (dbRDA; Anderson et al. 2008). Statistical significance was determined using 5 and 1 %. Analysis was performed using Statistica version 12.0 (StatSoft®, Tulsa, Oklahoma, USA) and Primer 7+ Permanova add-on software package (v7.0.10 and v1.0.6; PRIMER-E Ltd., Ivybridge, UK) for univariate and multivariate analyses, respectively.

## 3 Results

### 3.1 Concentrations of As and heavy metals in soil samples

Heavy metal concentrations are shown in Table 1. No detectable levels of Cd, Cr, and Hg were found. In general, the levels of heavy metals and As were higher in the contaminated sites compared to the reference sites (from 40 to 1193 times). The observed concentration gradient in the sampled sites was Zn > Pb > Cu > As. Results of the ANOVA factorial test indicate the effect of the interaction of factors (municipality  $\times$  site type) for all quantified elements, cofactors, and enzyme activities (Table 2). The post hoc analysis of the contaminated sites showed that CHA had the highest levels of Cu, Pb, and Zn. VDP had the highest concentrations of As; As was not detected in CHA or in CED. A positive correlation was found among the levels of Cu with Pb ( $r = 0.843$ ,  $p < 0.01$ ) and Zn ( $r = 0.871$ ,  $p < 0.01$ ) as well as among levels of As and Zn ( $r = 0.171$ ,  $p < 0.05$ ).

### 3.2 Hazard coefficients (HQ)

According to the limits set by Eco-SSL, HQ analysis performed for the microorganisms in the contaminated sites was as follows: in CSP, the results were Pb (51.0) > As (15.9) > Cu (13.0) > Zn (8.1). In VDP, they were As (87.37) > Zn (51.2) > Cu (13.2) > Pb (1.4). In CED, they were Zn



**Table 1** Concentration of As, metals, and covariables in soils from San Luis Potosí, México

Parameter	Cerro de San Pedro (CSP)		Villa de la Paz (VDP)		Cedral (CED)		Charcas (CHA)	
	Reference ( <i>n</i> = 25)	Contaminated ( <i>n</i> = 25)	Reference ( <i>n</i> = 25)	Contaminated ( <i>n</i> = 25)	Reference ( <i>n</i> = 25)	Contaminated ( <i>n</i> = 25)	Reference ( <i>n</i> = 25)	Contaminated ( <i>n</i> = 25)
As (mg/kg)	3.7 ± 0.2** (3.5–10.5)	1589.3 ± 148.7 <sup>a</sup> (128.7–3028.0)	12.7 ± 1.7** (3.5–31.9)	8736.7 ± 1176.8 <sup>b</sup> (1461.9–28,274.0)	27.9 ± 2.3 (3.5–49.7)	<LOD	3.9 ± 0.2 (3.5–8.9)	<LOD
Cu (mg/kg)	<LOD	130.9 ± 15.0 <sup>a</sup> (6.5–286.8)	7.2 ± 0.7** (6.5–25.7)	1322.7 ± 131.7 <sup>b</sup> (208.1–2345.2)	8.3 ± 1.2** (6.5–31.9)	1786.0 ± 89.7 <sup>b</sup> (1107.5–2619.7)	7.1 ± 0.6** (6.5–22.7)	8475.4 ± 613.7 <sup>c</sup> (6.5–13,927.5)
Pb (mg/kg)	64.2 ± 3.0** (34.5–100.6)	7281.0 ± 774.3 <sup>b</sup> (1124.5–18,537.8)	22.1 ± 2.7** (4.0–47.8)	1321.0 ± 161.5 <sup>a</sup> (466.1–3486.4)	26.2 ± 2.5** (8.0–55.7)	7863.0 ± 252.6 <sup>b</sup> (5814.6–10,108.0)	32.6 ± 1.0** (19.4–42.8)	31,932.2 ± 2213.9 <sup>c</sup> (153.5–43,201.7)
Zn (mg/kg)	93.6 ± 1.9** (77.6–113.7)	5101.2 ± 902.0 <sup>a</sup> (360.7–17,080.1)	90.0 ± 2.1** (69.4–109.7)	5120.4 ± 693.6 <sup>a</sup> (1165.9–13,684.7)	117.0 ± 2.1** (101.0–138.6)	4744.4 ± 294.2 <sup>a</sup> (3109.3–7277.4)	104.3 ± 1.6** (82.8–119.8)	65,585.7 ± 4615.1 <sup>b</sup> (400.9–95,892.7)
pH	8.3 ± 0.04** (7.4–8.7)	4.1 ± 0.3 <sup>c</sup> (1.5–6.7)	8.4 ± 0.01** (8.3–8.6)	7.7 ± 0.04 <sup>b</sup> (7.1–8.2)	8.6 ± 0.02** (8.5–9.0)	7.9 ± 0.01 <sup>ab</sup> (7.9–8.1)	8.4 ± 0.03** (7.7–8.6)	7.6 ± 0.04 <sup>b</sup> (7.3–8.1)
Electric conductivity (dS/m)	0.1 ± 0.01** (0.05–0.3)	1.6 ± 0.1 <sup>a</sup> (1.0–4.2)	0.05 ± 0.001** (0.04–0.08)	0.7 ± 0.03 <sup>b</sup> (0.2–1.2)	0.06 ± 0.002** (0.05–0.1)	0.7 ± 0.02 <sup>b</sup> (0.4–1.0)	0.06 ± 0.004** (0.05–0.1)	0.8 ± 0.07 <sup>b</sup> (0.1–2.4)
Organic Matter (%)	3.7 ± 0.1** (2.03–5.1)	2.0 ± 0.1 <sup>a</sup> (1.0–3.5)	4.1 ± 0.1** (2.9–7.0)	2.4 ± 0.3 <sup>ab</sup> (1.2–11.6)	3.7 ± 0.07** (3.1–4.8)	0.7 ± 0.1 <sup>c</sup> (0.3–2.9)	4.6 ± 0.04** (4.2–5.1)	2.9 ± 0.1 <sup>b</sup> (2.0–4.4)
Clay (%)	16.7 ± 0.58** (12.0–22.0)	10.4 ± 0.62 <sup>a</sup> (6.0–16.3)	25.6 ± 0.49** (20.3–28.3)	9.2 ± 1.2 <sup>ab</sup> (3.6–25.6)	17.2 ± 0.95** (2.0–22.0)	6.5 ± 0.64 <sup>b</sup> (1.6–16.3)	24.2 ± 0.43** (22.0–30.0)	13.3 ± 1.95 <sup>c</sup> (4.0–46.0)

Mean ± standard error (minimum – maximum). The different letters indicate the significant differences at 5 % between contaminated sites

LOD limit of detection

\*\*Significant at 1 % compared by contaminated

**Table 2** ANOVA for the results of elements, enzyme activity, and covariables

Effect	df	Mean squares											
		Elements				Enzymes				Covariables			
		As	Cu	Pb	Zn	ARS	GLU	FDA	URE	pH	OM	EC	CLA
Municipality (A)	3	35.55**	7.24**	6.05**	3.93**	1.02**	0.94**	1.09**	7.78**	0.34**	1.40**	0.81**	1.22**
Site type (B)	1	65.11**	234.88**	266.35*	174.98**	49.69**	12.97**	22.90**	41.61**	0.66**	7.80**	60.95**	0.13
AXB	3	43.19**	6.75**	2.82**	3.55**	1.13**	0.32**	1.30**	0.55**	0.30**	0.95**	0.06**	0.29**
Residue	192	0.04	0.10	0.08	0.07	0.06	0.03	0.07	0.08	0.01	0.02	0.01	0.06

ARS arylsulfatase, GLU β- glucosidase, FDA fluorescein diacetate, URE urease, OM organic matter, EC electric conductivity, CLA clay  
 \*Significant at 5 %, \*\*Significant at 1 %

(47.4) > Cu (17.8) > Pb (8.7). In CHA, they were Zn (655.8) > Cu (84.7) > Pb (35.4). Indexes show a very high hazard level for the elements detected in all contaminated sites. HQ levels for the other organisms (e.g., Eco-SSL for plants and mammals) including humans were 10 times higher than those obtained from microorganisms.

**3.3 Physicochemical characteristics of soil**

The results of the physicochemical characteristics of the studied sites are presented in Table 1. pH values varied within a range of 8.3 to 8.6 in the reference sites and from 4.1 to 7.9 in the contaminated sites. The pH of contaminated sites was lower compared to the reference sites by 18.6 % (LSD, MS = 0.005, p < 0.01). According to the classification established by the Mexican regulations (Diario Oficial de la Federación DOF 2002), pH values for the reference sites go from neutral to strongly alkaline and for the contaminated sites from neutral to moderately alkaline, except for CSP where soils were found to be strongly acidic (4.1). pH values showed a negative correlation with As, Pb, and Zn (Table 3).

EC (dS/m) levels found in reference sites ranged from 0.05 to 0.1 and from 0.7 to 1.6 in contaminated sites. EC of contaminated soils increased by 92.5 % compared to reference soils (LSD, MS = 0.018, p < 0.01). CSP was found to have the highest levels of electrical conductivity. According to the criteria established by Canadian Council of Ministers of the Environment (Canadian Council of Ministers of the

**Table 3** Pearson’s correlation coefficients between physicochemical parameters and metal concentrations (mg/kg) in soils

Parameter	As	Cu	Pb	Zn
pH	-0.459*		-0.430*	-0.252*
EC (dS/m)	0.475*	0.781*	0.904*	0.842*
OM (%)		-0.585*	-0.595*	-0.508*
Clay (%)	-0.303*	-0.622*	-0.625*	-0.598*

\*Significant at 5 %

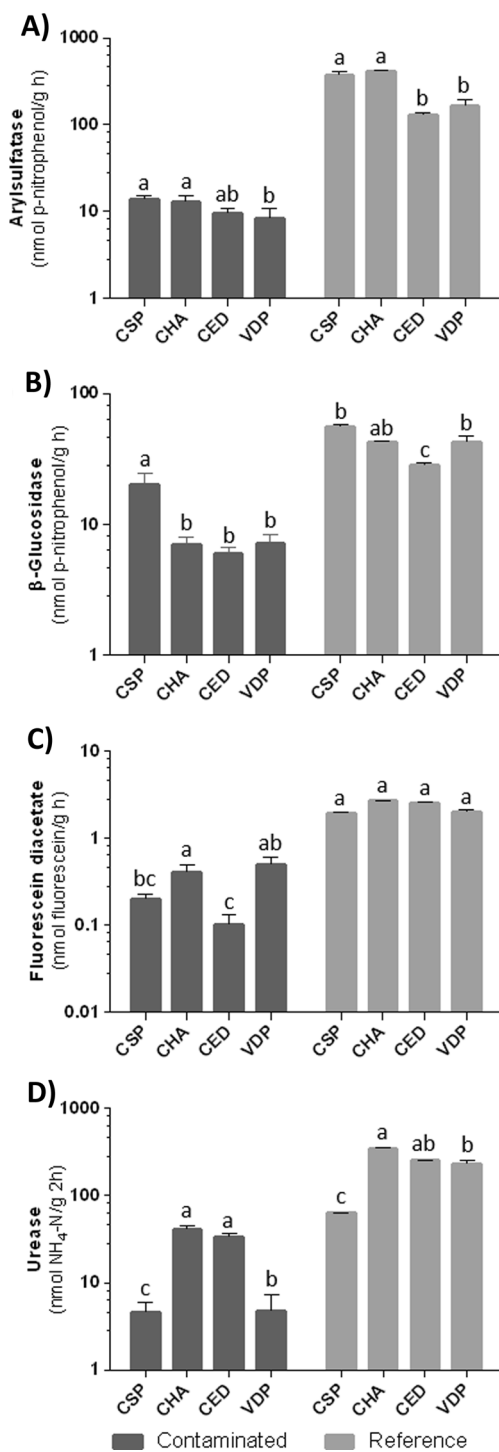
Environment CCME 2014), the electrical conductivity in the reference sites of CSP and CHA indicated low and medium salinities, while in CED and VDP, there was low salinity. Contaminated sites of VDP and CED had soils ranging from medium to high salinity. In CSP, salinity ranged from very high to high levels, and in CHA, salinity went from low to medium. EC showed a positive association among Pb, Zn, Cu, and As (Table 3).

OM values (%) ranging from 3.7 to 4.6 for the reference sites and from 0.7 to 2.9 for the contaminated sites were obtained. OM in contaminated soils showed a 50.5 % decrease when compared to reference soils (LSD, MS = 0.020, p < 0.01). CED had the lowest levels of OM followed by CSP and VLP. CSP, CED, and VLP had an OM concentration ranging from medium to high in accordance with the Mexican regulations (Diario Oficial de la Federación DOF 2002). OM showed a negative correlation with Pb, Cu, and Zn (Table 3).

Presence of clays (%) ranged from 16.7 to 25.6 in the reference sites and from 6.5 to 13.3 in the contaminated sites. There was a 52.9 % decrease in the percentage of clays in the contaminated sites regarding the reference sites (LSD, MS = 0.031, p < 0.01). CED was found to have the lowest clay levels followed by VLP and CSP. Clay was also negatively correlated with Pb, Cu, Zn, and As (Table 3).

**3.4 Soil enzyme activity**

Results regarding enzymatic activities in the sampling sites are shown in Fig. 2. Contaminated sites were found to have less enzymatic activities than the reference sites. ARS activity (nmol p-nitrophenol/g h) ranged from 130.5 to 411.5 for the reference sites and from 8.2 to 13.5 for the contaminated sites. The inhibiting pattern for each of the sites was as follows: CHA (96.8 %), CSP (96.3 %), VDP (95.0 %), and CED (92.7 %). BG activity (nmol p-nitrophenol/g h) ranged from 28.2 to 55.7 and from 6.0 to 20.2 for reference and contaminated sites, respectively. Inhibition of BG per reference site was as follows: CHA (83.6 %), VPD (83.1 %), CED (78.2 %), and CSP (63.7 %). FDA activity (nmol fluorescein/g h)



**Fig. 2** Enzyme activity in soil by municipally and site type (reference and contaminated). The vertical bars indicate the standard error. The different letters indicate the significant differences ( $p < 0.01$ ) by municipally

ranged from 1.9 to 2.7 for the reference site and from 0.18 to 0.54 for the polluted site. FDA inhibition regarding reference sites was as follows: CED (92.5 %), CSP (89.0 %), CHA (83.1 %), and VDP (73.5 %). URE showed values (nmol  $\text{NH}_4\text{-N/g}$  2 h) ranging from 62.9 to 346.9 for reference sites

and from 4.5 to 40.7 for contaminated sites. The inhibiting pattern for URE was as follows: VDP (97.9 %), CSP (92.8 %), CHA (88.2 %), and CED (86.6 %). All enzyme activities showed correlation with each other (UR between FDA  $r = 0.70$ ,  $p < 0.01$ ; BG between ARS  $r = 0.928$ ,  $p < 0.01$ ; ARS between FDA  $r = 0.857$ ,  $p < 0.01$ ). The DistLM results (Table 4) show the multivariate relations among the elements, covariables, and enzymatic activities. All enzymes show a strong relationship with metals and covariates (from 84 to 0.87 %). EC, Cu, and As showed a strong inhibitory effect, and parameters such as OM, pH, and clay were found to have a slightly inducing effect. Figure 3 shows the dbRDA analysis, and it explains 84.1 of the total variation, where axis 1 determines the grouping of samples mainly based on the type of site (77.7 % of the total variation). The variation between samples is much longer in contaminated sites compared to reference sites, suggesting that contamination disturbance determines diverging responses. Axis 1 shows a negative association ( $p < 0.05$ ) among Zn ( $r = -0.44$ ), Cu ( $r = -0.41$ ), EC ( $r = -0.39$ ), As ( $r = -0.38$ ), and Pb ( $r = -0.29$ ) as well as a positive one between clay ( $r = -0.35$ ), OM ( $r = -0.33$ ), and pH ( $r = -0.11$ ).

## 4 Discussion

### 4.1 Metals in soil samples and hazard coefficients (HQ)

Sites with mining activity were found to have significantly higher levels of metals than the reference sites. The main sources of contamination in these sites include tailings, dams, and slag deposits, from which pollutants mobilize, by being transported through wind and runoff (Monroy et al. 2002). The presence of this distribution pattern of pollution corresponds to what was observed in different studies done in similar environments (Razo et al. 2004a, b; Jasso-Pineda et al. 2007; Espinosa-Reyes et al. 2014). Even though previous studies have not been performed in all municipalities, the levels of metals found in this study were higher than the ones reported previously in studies conducted in VDP and CHA (Monroy et al. 2002; Razo et al. 2004a, b; Espinosa-Reyes et al. 2014). Pollutant concentrations exceed environmental protection criteria established by the international guidelines Squirt-NOOA (Eco-SSLs for soil microbiological effects). HQ results show a high-risk potential for bacterial life in the soil of the region. It is worth mentioning that hazard coefficients for protecting health of humans and biota (Avian, invertebrates, mammals, and plants) are even higher (ranging from 10 to 1000). In vitro bioaccessibility was determined in soil samples of VDP and CSP, obtaining values of  $>80$  % for As and Pb (Razo et al. 2004b; Jasso-Pineda et al. 2007; Vazquez-Vazquez 2012). The combination and integration of these indexes, together with high bioaccessibility, suggest that

**Table 4** Results of the distance-base multivariate model (DistLM) for enzyme activity

Enzyme	AIC	R <sup>2</sup>	RSS	No	Covariables	Pseudo-F	Prop	Cumul	Effect
Arylsulfatase	-401.3	0.872	24.81	7	EC	435.46	0.687	0.687	-
					Cu	64.73	0.077	0.764	-
					As	78.19	0.067	0.831	-
					OM	33.27	0.024	0.855	+
					Pb	15.63	0.010	0.865	-
					Zn	6.79	0.004	0.869	-
					Clay	5.70	0.003	0.872	+
β-Glucosidase	-361.0	0.845	30.06	8	Cu	525.60	0.726	0.726	-
					Clay	39.49	0.045	0.771	+
					pH	24.63	0.024	0.795	-
					OM	21.96	0.018	0.813	-
					Zn	18.09	0.016	0.829	-
					As	9.75	0.010	0.739	+
					Pb	6.88	0.005	0.844	-
					EC	2.21	0.001	0.845	-
Fluorescein diacetate	-395.7	0.871	25.52	7	EC	612.03	0.755	0.755	-
					OM	50.69	0.050	0.805	+
					Zn	40.39	0.033	0.838	-
					pH	16.16	0.012	0.851	+
					Clay	16.88	0.011	0.863	+
					As	10.30	0.006	0.870	-
					Pb	2.54	0.001	0.871	-
Urease	-359.4	0.841	31.54	4	EC	332.30	0.626	0.626	-
					As	229.45	0.200	0.827	-
					Pb	9.11	0.007	0.835	-
					Clay	7.76	0.006	0.841	+
All enzymes	-100.0	0.860	110.84	8	EC	365.53	0.648	0.648	-
					Cu	60.47	0.082	0.731	-
					As	70.84	0.071	0.802	-
					OM	25.41	0.022	0.825	+
					pH	16.52	0.013	0.839	+
					Zn	12.47	0.009	0.848	-
					Clay	8.71	0.006	0.855	+
Pb	7.38	0.005	0.860	-					

AIC Akaike’s information criterion, RSS residual sum of squared errors, Prop proportional R<sup>2</sup>, Cumul cumulative proportional, - inhibitory effect, + inductive effect

immediate evaluation of the effects on human and ecological receptors ought to be performed, as well as the risk assessment associated with the exposure to polluted soils.

#### 4.2 Effect on physicochemical parameters

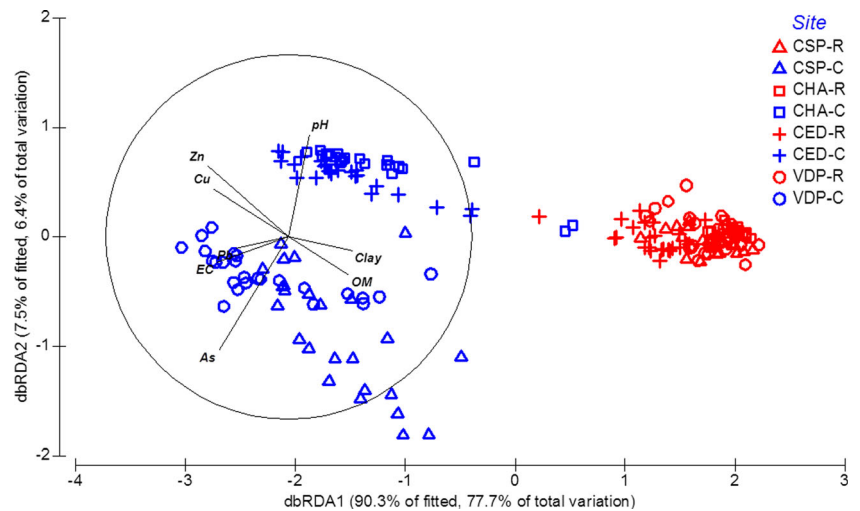
When metals enter soil in great amounts, pH can be altered, causing it to acidify (Effron et al. 2004); this could be the explanation of the high correlation among heavy metal concentrations and pH values. pH values, temperatures, and oxidation of sulfide minerals, which are present in these areas, can favor the generation of acid mine drainage (Sherene

2010); CSP was the only place that showed evidence of the generation of acid mine drainage. On the other hand, in arid regions, sodium can accumulate in the form of sodium carbonate and its hydrolysis can produce strong alkali (NaOH), which is why the pH value can vary within a range of 8.5 to 10 in these soils, generating a buffer effect of pH on the ground.

Arid and dry locations generally tend to have soils with higher salinity levels because there is more evaporation taking place rather than precipitation and because they tend to have a pH that approximates or is close to 7. According to the Canadian Council of Ministers of the Environment (Canadian Council of Ministers of the Environment CCME



**Fig. 3** Distance-based redundancy analysis (dbRDA) ordination plot for all enzyme activities with metals and covariables



2014), electrical conductivity in residential soil or parks should not exceed 2 dS/m; however, contaminated soil from CSP doubled the established value (4.2 dS/m), and the contaminated site from CHA was at the maximum limit (2 dS/m). Higher salinity can increase the mobility of heavy metals through the following two possible means: positively charged ions associated to salts (Na and K) which can replace heavy metals in the absorption sites and negatively charged ions of chloride which can form stable soluble compounds with heavy metals (Cd, Zn, and Hg), with a tendency of generating acidic soils (Sherene 2010).

OM plays an important role in metal binding, complexation by organic ligands, control of metal solubility, and mobility. High amounts of heavy metals in polluted soil could change the mineralization rate of organic matter in soil and affect its accumulation and distribution (Zhang and Wang 2007). According to the classification established by Mexican regulations (Diario Oficial de la Federación DOF 2002), the amount of organic matter in soils from reference sites was considered high, and for contaminated sites, it was reduced to a medium level (50.5 % quantitatively).

Sites with higher concentrations of metals had fewer amounts of clay (predominantly sandy-loam soils). The negative association pattern in this study has also been observed in soils with fine textures contaminated mainly with lead (Sherene 2010). It is likely that the washing of clays which is an additional process to contamination can derive from the mechanical effect to which these soils have been exposed to.

#### 4.3 Effect on enzyme activities

A strong negative relationship was also observed in the present study among enzymatic activities and heavy metals; the determination coefficients show the following inhibitory effect  $\text{Cu} > \text{As} > \text{Zn} > \text{Pb}$ . The effects of metals are modified when other variables are considered (Table 4).

Generally, enzyme activities are often enhanced by increasing pH in soil (except for acid phosphatase, which is predominant in acidic soils; Deng and Tabatabai 1997). According to the DistLM models, the inducing effect of pH was mainly found in BG and FDA (between 24 and 12 %, respectively). pH changes can affect the distribution of fungal and bacterial biomass in soil and can consequently modify patterns and soil enzyme activities, which can also heavily affect substrate-enzyme kinetics (Baath et al. 1992).

EC showed a great inhibitory effect in the enzymes evaluated in this study as well as a strong association with the toxic elements (Cu, As, Zn, and Pb). It has previously been reported that enzyme activities were reduced with increasing EC and that the degree of inhibition varied among the enzymes assayed and the nature and amounts of salts added. FDA, BG, and ARS were inhibited exponentially by increasing salinity and linearly by increasing sodicity (Zahran 1997). Carbonates can play an active role in metal immobilization such as Zn, Co, Ni, Hg, Ag, and Au (Adamo et al. 2006), but at alkaline pH values, the availability of the other elements (e.g., As, Cr, Mo, Se, and V) can increase.

ARS levels were inhibited in a 95.2 %, and the elements related to this inhibition were Cu, As, Pb, and Zn. Jadwiga et al. (2010) found a decrease of ARS activity of 46.8 % in the presence of 450 mg/kg of Cu. Speir et al. (1999) reported a 50 % inhibition of ARS derived from exposure to As. Borowik et al. (2014) found an inhibition of 85 % of ARS in soil polluted with Zn (2400 mg/kg). Hernández et al. (2006) found an inhibition of 64.3 % of ARS in soil polluted with Zn (18,900 mg/kg), Pb (4930 mg/kg), Cd (15.10 mg/kg), and Cu (11.9 mg/kg). This study also found that Cu was a strong inhibitor of BG. In this regard, Jadwiga et al. (2010) reported an inhibition of 37.5 % in the presence of 450 mg/kg of Cu, and D'Ascoli et al. (2006) found a negative association among concentrations of BG and Cu ( $229.34 \pm 17.0$  mg/kg on average). Kuperman and Carreiro (1997) reported inhibitions of

85 % of BG in soils contaminated with a mixture of heavy metals (As, Cd, Cu, Pb, and Zn in concentrations ranging from 121.0 to 3446.6 mg/kg of all evaluated metals).

FDA activity was significantly decreased by 84.5 %; metals involved in the inhibition were Zn, Pb, and As. Bhattacharyya et al. (2008) found that Zn (239 mg/kg) and Pb (333 mg/kg) caused a negative effect on FDA in contaminated soils experimentally. Kuperman and Carreiro (1997) reported an 81 % decrease in FDA activity in areas contaminated with metals derived from military operations. UR activity was inhibited by 77.2 % due to the presence of As and Pb. Yang et al. (2009) described an inhibition of the activity of UR by 75 % in agricultural soils contaminated with Cd (100 mg/kg) and Pb (500 mg/kg). Friedlová (2010) observed an inhibition of UR activity in alluvial soils containing high concentrations of Pb (5286 mg/kg), Zn (9288 mg/kg), and Cu (62.85 mg/kg). Wyszowska et al. (2006) showed that UR activity was inhibited by 76.7 % in concentrations of 450 mg/kg of Cu when combined with Zn and Pb (50 mg/kg each). It is important to mention that in previously reported studies, inferior concentrations of heavy metals to the ones reported in this study were found or were determined in controlled conditions (laboratory experiments).

FDA was strongly associated ( $p < 0.01$ ) with the rest of the enzymes in the following way: ARS ( $r = 0.85$ ), BG ( $r = 0.83$ ), and URE ( $r = 0.70$ ). This can suggest that the decrease of enzymatic activity is mainly caused by the direct suppression of bacterial growth derived from the adverse conditions present in contaminated soils and subsequently by the direct interactions among enzymes and heavy metals (Kuperman and Carreiro 1997). In this regard, other studies carried out by our group (unpublished data) have shown strong inhibition (24 to 42 %) of microbial respiration of soil (%CO<sub>2</sub>) in the contaminated sites.

About the effects of metal aging in soil, Ciarkowska et al. (2016) reported an increase of soil enzymatic activity in soils with metal aging from 30 to 400 years. Our results did not show a temporary recovery pattern of the enzymatic activity. An important factor that influences the metal effects on soil enzymatic activities is the metal bioavailability that does not depend linearly on the metal total concentration. Assessment of the metal bioavailability in the studied soils deserves more attention in the future to understanding the potentials of soils to restore their ecological functionality on the long term.

## 5 Conclusions

In this study, heavy metal concentrations were higher than the ones obtained in other reports for contaminated sites in this region. The concentration and type of heavy metals, according to the HQ, reveal the presence of possible risks for the health of life in the region (biota and humans), which is why the

location requires urgent intervention measures as well as constant monitoring and regulations of the levels of heavy metal in soil. Our results showed the relationship among different physicochemical parameters (pH, OM, EC, clay) and a mix of heavy metals on enzymatic activity in chronically contaminated soil under field conditions. The inhibition on enzymatic activity in this condition could be attributed to metal bioavailability. Furthermore, no decrease in toxic effects was observed through time in these sites; however, it could be inferred that the biological activity in these conditions could be due to the presence of certain groups of microorganisms that have acquired resistance characteristics with relevant importance. The damage done to the microbial component and the biological processes of the soil (e.g., mineralization and nutrient cycling) could trigger adverse changes in the flow of matter and energy in ecosystems. This study provides a baseline for the state of the enzymatic levels in soils from the mining region of San Luis Potosí; enzymatic determinations are quick, sensitive, and cheap methods that could become part of a long-term monitoring program for these locations.

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