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## Evaluation of the Biological Activity of Soil in a Gradient Concentration of Arsenic and Lead in Villa de la Paz, San Luis Potosi, Mexico

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### Abstract

The mining industry has generated different sources of pollution and effects in human and ecosystem health. The objective of this study was to evaluate the effect in gradient of the biological activity of the soil derived from the contamination by lead and arsenic. A linear transect was conducted from the tailings in Villa de la Paz to a reference site 10 km away against wind direction and runoff. The parameters that were analyzed in the soil samples included As, Pb, pH, organic matter, electrical conductivity, soil respiration, and soil enzymes. As concentrations ranged from 4.7 to 463.2 mg/kg, while those of lead ranged from 171.7 to 2319.0 mg/kg. The changes in the physicochemical parameters and in the biological activity of the soil were stronger within the first 100 m of distance from the tailings. The pattern of inhibition of biological activity was: urease> $\beta$ -glucosidase>arylsulfatase> dehydrogenase>phosphatase>respiration. Strong negative relationships were observed among biological activities and arsenic (from 86.5 to 96%). Metals and covariables jointly explain the 89.2% of variability of the effect in the biological activities. This study provides a field baseline that could be part of a long-term monitoring and remediation program.

**Keywords:** pollution gradient, soil enzymes, soil respiration, soil pollution, tailings

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## 1. Introduction

The soil is the upper layer of the solid surface of the planet, formed by weathering rocks, where plants can or may be rooted in and which is an ecological environment for certain types of living beings [1]. Soil is a living, dynamic and non-renewable resource, and its condition and functioning are vital for food production, and for the maintenance of local, regional and global environmental quality. The processes of decomposition and respiration that take place in it play a key role in maintaining the balance between the production and consumption of CO<sub>2</sub> in the biosphere [2]. The soil is composed of mineral particles of variable sizes, organic matter and numerous species of morphologically and physiologically distinct microorganisms. Currently, there is a growing concern about the sustainable use of land in terms of agriculture, environmental quality and human health, as well as its degradation. Soil health can be considered as *“the continuous ability of the soil to function as a living system, within the limits of the ecosystem and land use, to sustain biological productivity, to promote the quality of air and water environments, and maintain the health of plants, animals and humans”* [3].

The biological activity of the soil can be reflected in processes such as respiration and enzymatic activity [4]. Enzymatic activity in the soil is mainly of microbial origin that is derived from the intracellular enzymes associated with the cells or the physiological processes of the organisms. Enzymes are direct mediators for the catabolism of the biological components of the soil (organic and mineral). Therefore, these catalysts provide a meaningful evaluation of reaction rates of important processes that occur in the soil [5]. Breathing and activities of soil enzymes can be used as direct measures of microbial activity, soil productivity, and the effects of inhibition due to the presence of contaminants [6]. Microorganisms can react quickly to changes in the environment with alterations in metabolic activity, biomass and the structure of the community [7]. Due to the above, enzymes and respiration have been proposed as indicators for the monitoring of soil quality and the variation of microbial activity [8]. Respiration and enzymatic activity can be influenced in different ways by heavy metals. Dehydrogenase is present only in living organisms and represents active biomass, and it carries out a wide range of oxidation activities that are responsible for the degradation of soil organic matter [9]. Phosphatase is an extracellular enzyme; it hydrolyzes organic phosphorus compounds in different inorganic forms, which can be assimilated by plants [10]. Urease participates in the hydrolysis of urea into carbon dioxide and ammonia, which originates from microbes and shows extracellular activity [11]. Phosphatases are a group of enzymes that catalyze the hydrolysis of phosphoric acid esters and anhydrides. In this group, we can find the mono-ester-phosphate hydrolases, in which acid and alkaline phosphatase are found, which are nonspecific enzymes that catalyze the hydrolysis of glycerol phosphate [3]. Glycosidases ( $\beta$ -glucosidase and  $\beta$ -galactosidase) have the ability to intervene in the biogeochemical cycle of carbon and act on glycosides by catalyzing the release of sugars [12]. Overall, a general pattern of inhibition of enzyme activity and respiration due to heavy metal exposure in the soil has been observed in some studies [13].

Mining is considered one of the oldest and most fundamental activities of mankind [14]. Mexico has been characterized as a mining power. At present, it occupies the first places in the production of arsenic, cadmium, lead, mercury, fluorite, barium, among others, worldwide.

This large production is due to the fact that Mexican mining areas are located in large zones of the national territory. Thus, mining has become an important economic sector for states such as Guanajuato, Sonora, Chihuahua, Durango, Zacatecas, San Luis Potosi and Hidalgo [15]. Mining is distinguished by its different effects, magnitudes and environmental impacts, which can be of two types: the first includes the modification of the landscape, habitat, hydrological regime, changes in topography, among others; and the second involves pollution from the inadequate management of generated waste (e.g. tailings and tails) in the mineral extraction and processing stage, this type of waste usually comes with high concentrations of metals and metalloids [16]. The tailings are generated in the process of concentration of lead minerals, silver, zinc and copper; it usually contains residual metal sulfides such as pyrite, pyrrhotite, galena, sphalerite, chalcopyrite and arsenopyrite, which are sources of potentially toxic elements (PTE) such as arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), iron (Fe), and so on, which in high concentrations have toxic effects and are considered environmental pollutants capable of altering ecosystems [17–20]; furthermore, these elements are not biodegradable, they can bioaccumulate and some of them biomagnify [21].

The objective of this study was to evaluate the effects of As and Pb on the biological activity of soil in a distance gradient to the mining waste in Villa de la Paz, Matehuala, considering respiration and enzymatic activity as indicators of ecological integrity and soil health.

## 2. Materials and methods

### 2.1. Study site

The mining district of Santa María de la Paz is located in the municipalities of Villa de la Paz and Matehuala in the state of San Luis Potosí (**Figure 1**). In this district, there is a skarn deposit of Pb-Zn-Ag (Cu-Au) (metamorphic rocks constituted by silicates of Ca, Fe and Mg derived from a protolith of limestones and dolomites in which large amounts of Si, Al, Fe and Mg have been introduced), which has been exploited continuously for a little more than two centuries. The municipal seat of Villa de la Paz is located on the following coordinates: 100°42'47" west longitude and 23°40'31" north latitude, at an altitude of 1837 m above sea level, with an extension of 131.33 km<sup>2</sup> and a population of 5350 inhabitants [22]. The municipality has a temperate dry climate. The annual average temperature fluctuates around 18°C; the hottest month is June with 28°C and the coldest month is January with 3°C. Rainfall is 486 mm per year [23]. In the region, there are no important fluvial currents, only intermittent streams, which are formed in the mountains and transport water only during torrential rain events, without any economic or social use are located, but the water they carry is captured on different boards that are used to provide cattle with a trough service and to a lesser extent for land irrigation [24]. Lithosol, xerosol and rendzina soils with petrocalcic and calcareous phases predominate in a remarkable way [25] (CEFIM, 2012). The predominant vegetation types are thorny desert scrub, microphyll, nopal, izotal, cardonal and grassland scrub. Fauna is characterized by species such as hare, rattlesnake, wild cat, wild birds and field mice. The productive activities of the area are mainly: (1) agricultural (tuna, pastures and meadows groomed, corn and beans), (2) livestock with activities of production of bovine meat (8 tons/year), swine (9 tons/year),

sheep (1 ton/year), goats (19 tons/year) and production of bovine milk (18,000 L/year) and goat milk (23,000 L/year) [26] and (3) mining industry, which occupies 38.66% of the total area of the municipal capital, therefore the site is a typical mining area [23].

During the time of operation of the mining activities in the area, contamination by heavy metals and arsenic has been generated in soil, water and sediment [24]. In recent years, several studies have been conducted in the area in which the presence of high concentrations of heavy metals and As in water, air and sediment has been demonstrated [24, 27–29]. In the study by Razo et al. [24], tailings, dams and slag deposits were identified as the main sources of pollution and dispersion of particles due to wind (**Figure 2**). On the other hand, it has been proven that there are health risks to human and ecological populations in the area, due to exposure to heavy metals and As [30–36].

## 2.2. Soil sampling

Twenty-five composite samples of surface soil were collected in a distance gradient located from the source of contamination (tailings); the gradient was established on a linear path at distances of 0, 10, 100, 1000 and 10,000 m (**Figure 1**); for the location of the route, the predominant direction of the winds and the intermittent water runoff in the area were considered. For each distance, five subsamples of 10 cm<sup>3</sup> of soil collected in an area of 1 m<sup>2</sup> were gathered. Sampling was carried out on October 2016. The samples were homogenized, sieved (2 mm), placed in 50 mL sterile conical tubes and placed at 4°C for transport to the laboratory, where they were stored for 7 days at –20°C for their analysis.

## 2.3. Analysis of arsenic and lead in soil

The samples were dried in an oven (at 30°C) until they reached a constant weight (from 24 to 36 h), and moisture percentage was determined. Samples (0.5 g of soil) were placed in teflon cups with 10 mL of 25% ultrapure HNO<sub>3</sub>. Acid digestion was performed in a closed system with a microwave oven (MDS-2000-CEM) at 100 W of power and 80 psi of pressure for 1 h with a TAP of 30 min. The digests were filtered and calibrated to 10 mL. The quantification was performed by atomic absorption spectrophotometry with a hydride generator in the case of As (PerkinElmer AAnalyst 100 Flame AA) and a graphite furnace for Pb (PerkinElmer PinAAcle 900 T). In order to calculate the concentrations, calibration curves were made with standards, and as a quality control, blanks and reference materials were used (Montana soil I-NIST-2710a). The percentages of recovery were from 90 to 110%. The samples were processed in duplicate, and the results expressed in mg/kg.

## 2.4. Physicochemical parameters

pH was determined using the water measurement method [37] in a 1:2 ratio. About 6 mL of deionized water were added to a sample of 3 g of soil and then were kept under agitation for three cycles of 5 min at 800 rpm. The electrical conductivity was measured using the method mentioned in [37] at a soil-water ratio of 1:5. About 15 mL of deionized water were added to a sample of 3 g of soil and then were kept under agitation for three cycles of 5 min at 800 rpm. The measurements of pH and electrical conductivity were calculated in the



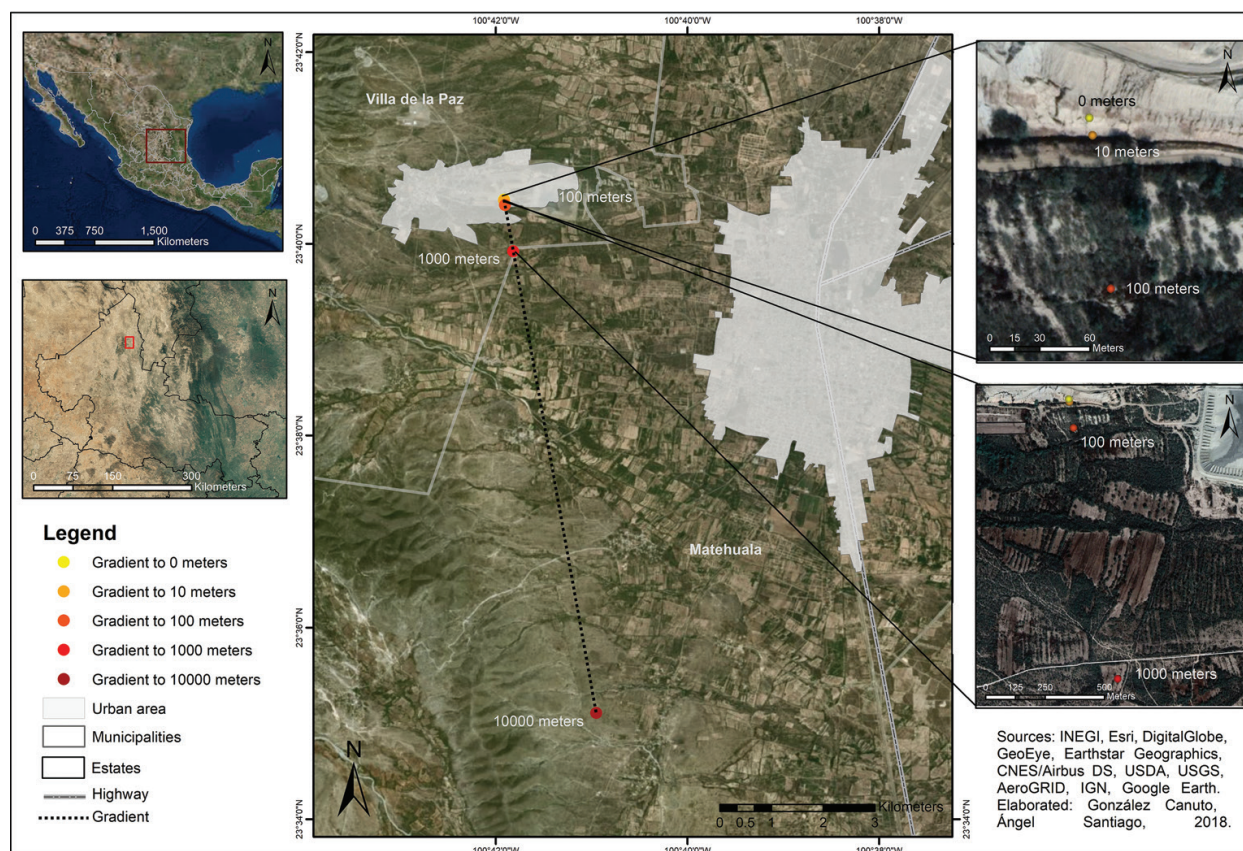


Figure 1. Study area and location of sampling sites.



Figure 2. Mine tailing deposits and acid drainage.

supernatant after the sample had been resting for 15 min in a benchtop multiparameter meter (HANNA-HI2550). Organic matter was determined using the wet digestion method [38]. In addition, 2.5 ml of  $\text{Na}_2\text{Cr}_2\text{O}_7$  solution (0.5 M) and 5 mL of concentrated  $\text{H}_2\text{SO}_4$  were added to a sample of 0.250 g of soil. The mixture was allowed to stand for 5 min after which 17.5 mL of deionized water were added, and it was stirred at 150 rpm for 30 min. The quantification was performed after 24 h using a UV-visible spectrophotometer (Biomate 3 s Thermo Fisher Scientific) at 600 nm in 2 mL of supernatant. All samples were made in duplicate.

## 2.5. Respiration and enzyme assays

Soil respiration was determined using the MicroResp method [39], which consists of filling a 96-well plate microplate (1.2 mL per well) with soil, a carbon source (25  $\mu\text{L}$  of glucose at 25%) and water (375  $\mu\text{L}$ ). The plate was subsequently sealed (silicone rubber gasket with interconnection holes) with a  $\text{CO}_2$  colorimetric detection trap consisting of a microplate (300  $\mu\text{L}$  per well) with 150  $\mu\text{L}$  of agar enriched with  $\text{KCl}$  (0.15  $\text{mol L}^{-1}$ ) and cresol red dye (32.7  $\mu\text{mol L}^{-1}$ ). Incubation was carried out at 25°C for 6 h in dark conditions. The absorbance in the detection plate was measured at 570 nm. The amount of  $\text{CO}_2$  released was calculated using a calibration curve. The results were expressed in  $\mu\text{g C-CO}_2 \text{ g}^{-1} \text{ soil h}^{-1}$ .

Arylsulfatase was quantified according to the method of Tabatabai and Bremner [40]. The method is based on the hydrolytic capacity of the enzyme on an artificial substrate (p-nitrophenyl sulfate) whose product p-nitrophenol is evaluated spectrophotometrically at a wavelength of 420 nm. The activity of arylsulfatase was expressed in  $\mu\text{g}$  of p-nitrophenol  $\text{g}^{-1} \text{ h}^{-1}$ .

The  $\beta$ -glucosidase was determined according to the method of [41]. The method is based on the quantification of p-nitrophenol obtained by the action of glycosides after incubation of the soil with the substrate  $\beta$ -D-glucopyranoside in medium at pH 6. The incubation was carried out at 37°C for 1 h; the released p-nitrophenol was removed by filtration after having added  $\text{CaCl}_2$  and adjusted with THAM buffer at pH 12. The absorbance was recorded at 405 nm. The activity of  $\beta$ -glucosidase was expressed in  $\mu\text{g}$  of p-nitrophenol  $\text{g}^{-1} \text{ h}^{-1}$ .

The activity of the enzyme dehydrogenase was determined by the method of [42–44]. The method is based on the measurement of idonitrotetrazolium formazan (INTF) produced by the reduction of 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium (INT) in soil incubated with INT in buffered medium under dark conditions for 1 h at 40°C. The absorbance was measured at 464 nm. The activity of the dehydrogenase was expressed in  $\mu\text{g}$  of INTF  $\text{g}^{-1} \text{ h}^{-1}$ .

The enzymatic activity of phosphatase was estimated according to the method of [45, 46]. The method is based on the spectrophotometric determination of p-nitrophenol released when the soil is incubated at 37°C for 1 h with p-nitrophenyl phosphate buffered solution; the alkaline solutions of this compound have a yellow color. Absorbance was measured at 405 nm. The activity of the phosphatase was expressed in  $\mu\text{g}$  of p-nitrophenol  $\text{g}^{-1} \text{ h}^{-1}$ .

Urease was quantified by the technique mentioned in [47]. This technique is based on the determination of the ammonium released in the incubation of a soil solution at 37°C for 2 h. The determination of ammonium is carried out through the Berthelot reaction with certain modifications. The Berthelot reaction consists of the reaction of ammonium in an alkaline medium with a coloring agent giving monochloramine, which is transformed into 2,2-isopropyl-5,5-methyl-indophenol when thymol is added. In this method, the ammonia produced by the urease activity reacts with salicylate and dichloro isocyanate giving a bluish green color. Absorbance was measured at 610 nm. The activity of urease was expressed in  $\mu\text{g}$  of  $\text{NH}_4\text{-N g}^{-1} \text{ 2 h}^{-1}$ .

The respiration and the concentration of the enzymes in the soil were determined 7 days after their collection. The concentrations were adjusted according to the moisture content. All determinations were made in triplicate in a spectrophotometer (BioTek Synergy H1). In our experiments, all the reagents were analytical grade.



## 2.6. Statistical analysis

The median and the interquartile range are reported. To compare the concentrations of As, Pb, physicochemical parameters and biological activity (respiration and enzymatic activity) by the distance to the source, the Kruskal-Wallis test was used in logarithmically transformed data. Linear models based on distance (DistLM, models based on Euclidean distances with “step-wise” procedure obtained by 9999 permutations and selected under the criterion of best  $r^2$ ) were performed to evaluate the association between biological activity and physicochemical parameters, as well as polluting elements. The DistLM model was calculated with logarithmically transformed and normalized data, excluding electrical conductivity due to its high association with the rest of the co-variables (As, Pb, pH and OM). In order to visualize the ordering patterns of the samples and the relationships between the variables, a redundancy analysis (dbRDA) was performed, representing the axes of greatest variation and the correlation between the covariates. The univariate analysis was performed using the GraphPad Prism Version 6 software, and the multivariate was performed using the software PRIMER 6 Version 6.1.18 & PERMANOVA Version 1.0.8 of PRIMER-E Ltd. Statistical significance was determined at 5%.

## 3. Results and discussion

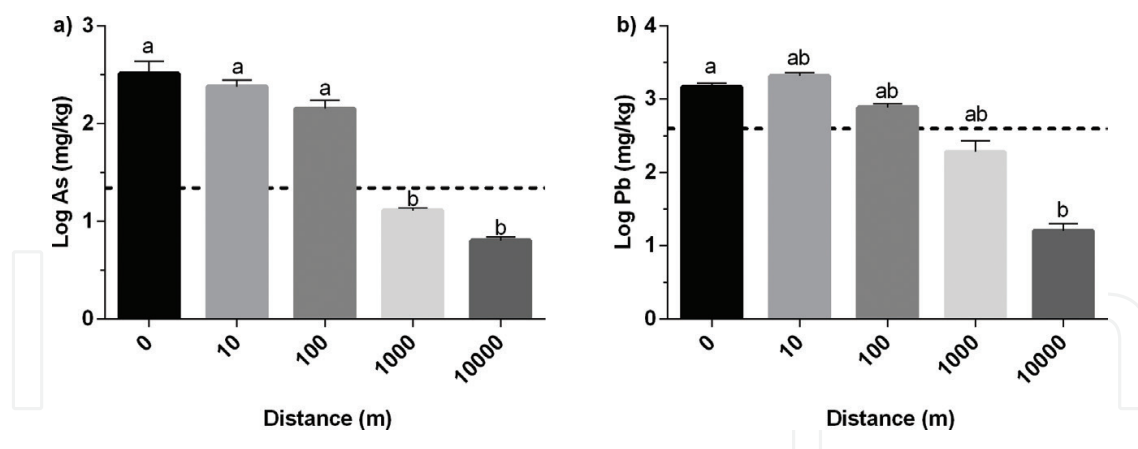
### 3.1. Arsenic and lead in soil

The average content of As in the Earth’s crust is 1.8 mg/kg, and, that of the soils has been found to be at 6.83 mg/kg. The most mobile forms of As are absorbed in a pH range of 7–9. The ions of As are known to be easily fixed in soils by Fe and Al hydroxides, the clay fraction, P and Ca compounds and organic matter [48]. The arsenic concentrations in this study ranged from 4.7 to 463.2 mg/kg. Changes in arsenic concentration were found mainly between the distances of 0–100 m and 1000–10,000 m ( $kW-H_{4,25} = 21.71$   $p = 0.0002$ , **Figure 3a**); with the highest concentrations being from the samples located in the first 100 m from the source.

Pb is a toxic metal naturally present in the Earth’s crust (15 mg/kg). Mining, metallurgy, manufacturing and recycling activities, and, in some countries, the use of lead paints and gasolines, are among the main sources of environmental pollution [49]. The average level of Pb in soils has been measured at 27 mg/kg [48]. In our study, lead concentrations (mg/kg) ranged from 171.7 to 2319.0; the zones located between distances of 0 and 10,000 m showed the greatest difference ( $kW-H_{4,25} = 13.61$   $p = 0.0086$ , **Figure 3b**). Arsenic and lead concentrations of the sites located from 0 to 100 m from the source are above the levels established for the remediation of contaminated soils dictated by Mexican regulations (NOM-147-SEMARNAT/SSA1–2004), which establishes the limits at 22 mg/kg of As and 400 mg/kg of Pb.

In Villa de la Paz, Martínez-Toledo et al. [50] found values (mg/kg) of As from 1461.9 to 28274.0 and of Pb from 466.1 to 3486.4 in a site close to the source of contamination. In another study, Márquez-Reyes [51] determined the average total concentrations (mg/kg) of 13,443.4 of As and 1301.7 of Pb. González-Mille [52] reported average concentrations (mg/kg), for the site, of 222.1 of As and 204.3 of Pb. Chipres [53] found the presence of heavy metals of environmental





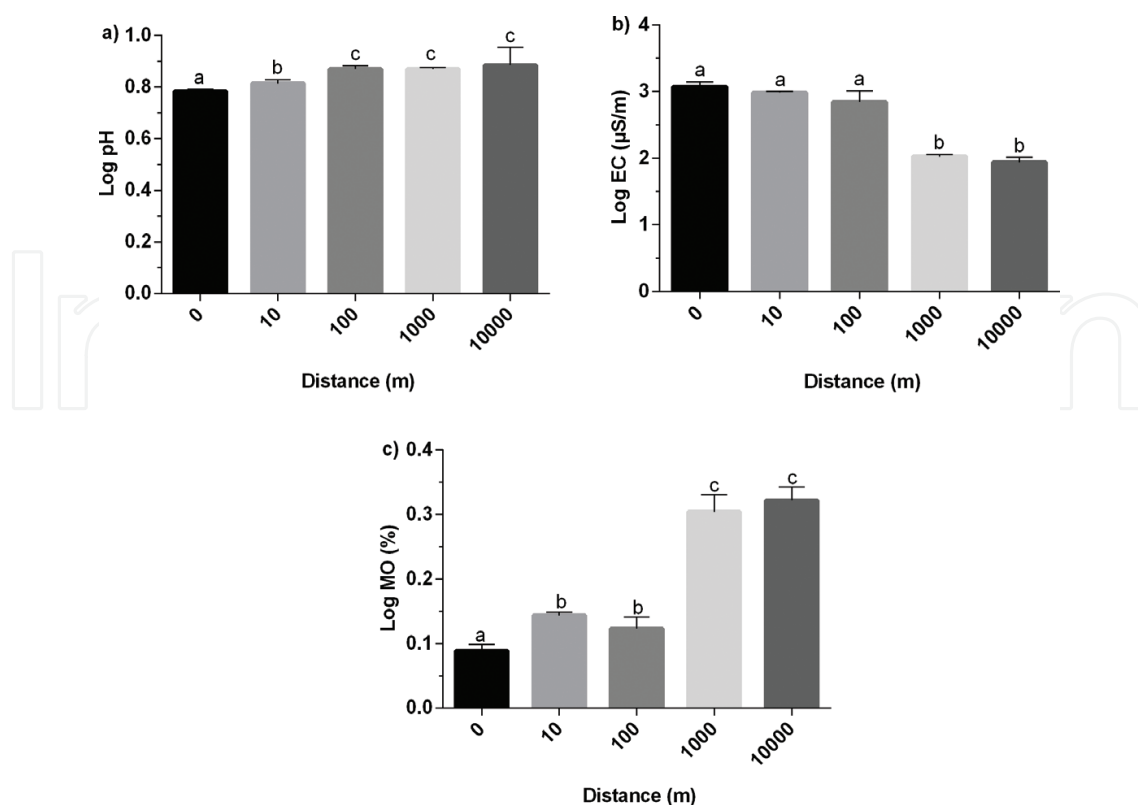
**Figure 3.** Comparison of As (a) and Pb (b) in distance gradient. The bars indicate the median, and the error bars the interquartile range. Different letters indicate the significant differences ( $p < 0.01$ ) by the distance to the source. The dotted line represents the Mexican normative.

interest related to the geochemical state of the Altiplano showing ranges (mg/kg) from 4 to 324 for As. Due to the above, the values found in this study are similar and even lower than those found in previous studies for the area. In a study similar to ours, in this regard, Puga et al. [54] evaluated soil contamination by Pb, Cd, Zn and As at different distances (300, 600, 900, 1200 and 1500 m) and soil depth levels (0–40, 40–60, 60–80 cm), in an area of influence of mining waste in San Francisco del Oro, Chihuahua; the results showed that at a greater distance from the tailings, the concentrations of As and Pb decreased in a similar way to the one found in this study.

### 3.2. Physicochemical parameters

The pH levels in this study ranged from 3.9 to 8.2. A pH decrease was found in the distances of 0 and 10 with respect to the distances of 1000–10,000 m ( $kW-H_{4.25} = 19.61$ ,  $p = 0.0006$ , **Figure 4a**). According to Mexican regulations (NOM-021-SEMARNAT-2000), pH levels in soils in zones 0 and 10 m can be considered as strongly acidic and moderately acidic, respectively; while for distances from 1000 to 10,000 m, soil can be considered neutral. The pH values found were lower than those in other studies [50, 55, 56] for the area ranging from 7.1 to 8.2%, which may be due to the technique of pH determination or the selection of sampling points. pH is one of the most important parameters that control the change of the chemical forms of the elements in soil [57]. According to Rieuwerts et al. [58], the acid pH influences the absorption of heavy metals by plants and other organisms because the solubility of metals tends to increase at low pH values and decreases when pH is high. Near a neutral pH, the formation of complexes can become a metal immobilization mechanism, meaning that they have a low bioavailability [59]. According to what has been described above, it can be assumed that in the samples near the source of contamination (0 and 10 m), there is a high bioavailability of metals due to the low pH reported.

The EC varied from 82.8 to 1660 ( $\mu\text{S}/\text{m}$ ). An increase of the EC was found mainly between the distances of 0–100 and the distances of 1000–10,000 m ( $kW-H_{4.25} = 21.06$ ,  $p = 0.0003$ , **Figure 4b**). EC levels can be considered as negligible effects of salinity for all sites in accordance with



**Figure 4.** Comparison of elements and physicochemical parameters in distance gradient. a) pH, b) electric conductivity and c) Organic matter. The bars indicate the median and the error bars the interquartile range. Different letters indicate the significant differences ( $p < 0.01$ ) by the distance to the source.

Mexican regulations (NOM-021-SEMARNAT-2000). The EC values found in this study were low with respect to other studies [50, 55, 56] done in the area ranging from 2200 to 12,200  $\mu\text{S}/\text{m}$ ; the differences could be due to the technique of determining EC or the selection of sampling points. A high EC could facilitate the mobility and bioavailability of metals in the soil through two possible means: (1) positively charged ions associated with salts (Na and K) that can replace heavy metals in the absorption sites and (2) negatively charged ions (for example chlorides) which can form stable soluble compounds with heavy metals (Cd, Zn and Hg), and have a tendency to generate acidic soils [59], as could be the case of the first 100 gradient meters.

OM varied from 0.8 to 1.2%. A significant reduction of OM was found mainly between the distances of 0 and 10–100 m and the distances of 1000–10,000 m ( $kW-H_{4,25} = 21.49$ ,  $p = 0.0003$ , **Figure 4c**). OM is very low in the distances from 0 to 100 m and low in the distances of 1000–10,000 m in accordance with Mexican regulations (NOM-021-SEMARNAT-2000). OM values found were comparable with other studies [50, 55, 56] done in the area ranging from 1.2 to 11.6%. Organic matter is formed by humic acids that provide a broad cation exchange capacity [60]; the interaction between organic matter and clays can improve soil adsorption capacity of inorganic contaminants [61]. It has been shown through various studies that the addition of organic matter to the soil (compost, waste, leaf litter) can help to reduce the toxicity of heavy metals regarding the enzymatic activity of the soil, as well as their bioavailability [48, 62].

### 3.3. Respiration and enzyme activities

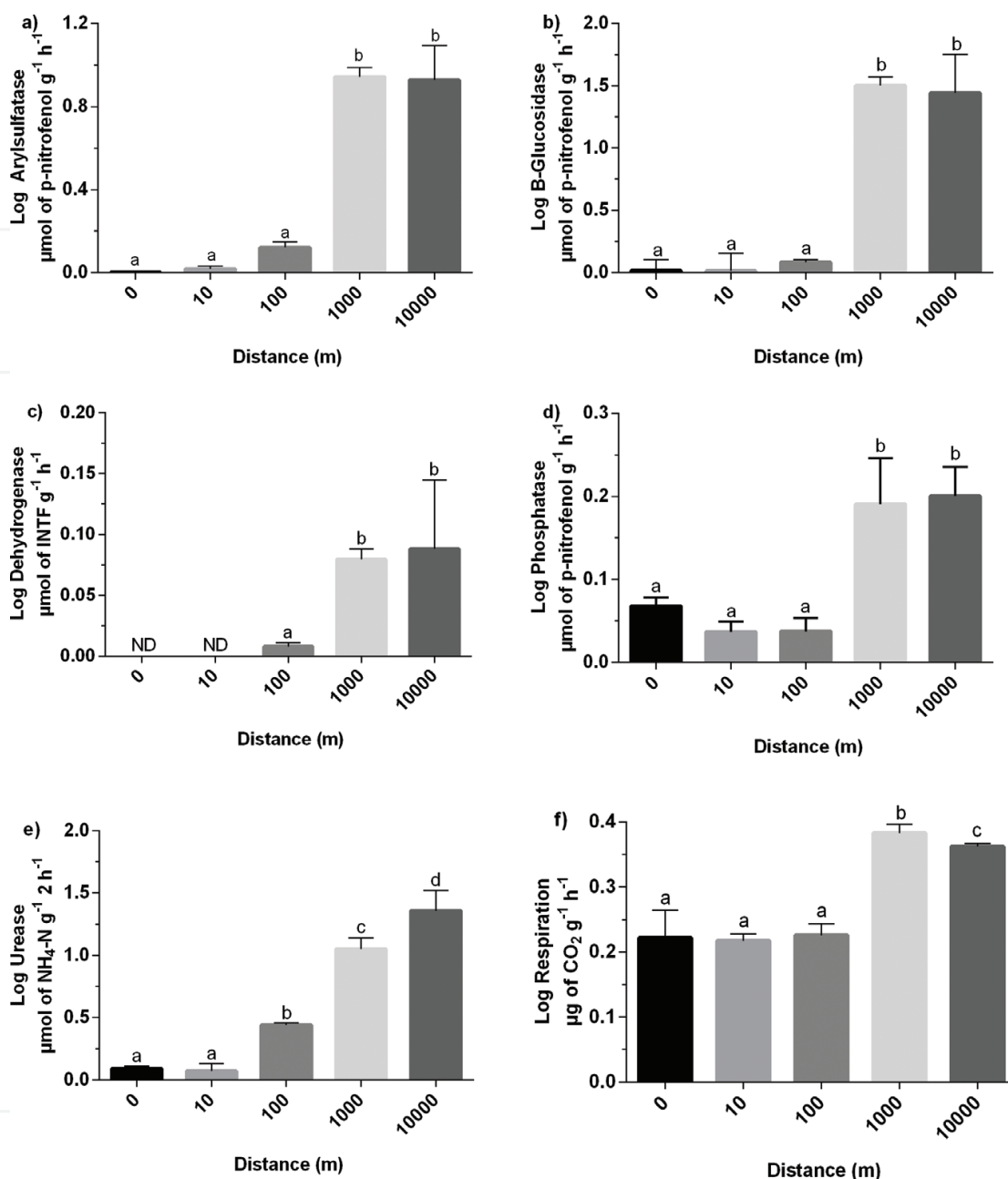
The activity of arylsulfatase varied from ND to 11.6 ( $\mu\text{mol}$  of p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ). A significant decrease in the activity of the enzyme was found in the distances of 0–100 m and the distances of 1000–10,000 m ( $\text{kW-H}_{4,25} = 21.86$ ,  $p = 0.0002$ , **Figure 5a**), which represents an inhibition of 98.3%. The activity of Arylsulfatase was higher than that found by Montes-Rocha [55] for the area (ND at 0.68  $\mu\text{mol}$  of p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ); but the inhibition pattern in the enzyme was similar (98.8%). Borowik et al. [63] determined that arylsulfatase can be inhibited (85.7%) by high concentrations of Zn (2400 mg/kg). Hernández et al. [64] found an inhibition (64.3%) in the activity of arylsulfatase in a highly contaminated soil (mg/kg) by Zn (18900), Pb: 4930, Cd (15.10) and Cu (11.90).

The activity of  $\beta$ -Glucosidase ranged from 0.02 to 63.9 ( $\mu\text{mol}$  of p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ). A significant decrease in the activity of the enzyme was found in the distances of 0–100 m with respect to the distances of 1000–10,000 m ( $\text{kW-H}_{4,25} = 18.60$ ,  $p = 0.0009$ , **Figure 5b**), which represents an inhibition of 99.4%. The activity of  $\beta$ -Glucosidase was higher than that found by Montes-Rocha [55] for the area (0.002–0.132  $\mu\text{mol}$  of p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ), and; the inhibition pattern in the enzyme was lower (86.4%). Experimental studies in soil contaminated mainly with Cu (450 mg/kg [65]), Zn (2400 mg/kg [63]) and mixtures of elements (Zn: 18,900, Pb: 4930, Cd: 15.10, Cu: 11.90 mg/kg), showed inhibitions of  $\beta$ -Glucosidase with respect to the control sample from 36.2 to 89.0%.

The activity of the dehydrogenase varied from ND to 0.44 ( $\mu\text{mol}$  of INTF  $\text{g}^{-1} \text{h}^{-1}$ ). A significant decrease in the activity of the enzyme was found in the distances of 0–100 m with respect to the distances of 1000–10,000 m ( $\text{kW-H}_{4,25} = 2.19$ ,  $p = 0.0002$ , **Figure 5c**), which represented an inhibition of 97.4%. The activity of dehydrogenase was higher than that found by Montes-Rocha [55] for the area (0.001–0.003  $\mu\text{mol}$  of p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ), and the inhibition pattern in the enzyme was considerably lower (18.4%). Kucharski et al. [66] found an inhibition (34.3%) in dehydrogenase in laboratory studies due to exposure to Ni.

The activity of the phosphatase ranged from 0.04 to 0.82 ( $\mu\text{mol}$  of p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ). A significant decrease in the activity of the enzyme was found in the distances of 0–100 m with respect to the distances of 1000–10,000 m ( $\text{kW-H}_{4,25} = 18.46$ ,  $p = 0.001$ , **Figure 5d**), which represented an inhibition of 79.6%. The activity of the phosphatase was similar with respect to that found by Montes-Rocha [55] for the area (0.017 to 0.103  $\mu\text{mol}$  of p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ), and the inhibition pattern in the enzyme was considerably lower (1.7%). Forty percent of inhibition of phosphatase is being found by exposure to Cd [67].

The activity of urease varied from 0.109 to 39.53 ( $\mu\text{mol}$  of  $\text{NH}_4\text{-N}$   $\text{g}^{-1} 2 \text{h}^{-1}$ ). The concentration gradient in the activity of the enzyme was found ( $\text{kW-H}_{4,25} = 21.92$ ,  $p = 0.0002$ , **Figure 5e**) in the following order: 0–100 < 100 < 1000 < 10,000 m. The inhibition pattern of the distances of 0–100 and 100 m with respect to 10,000 m was 99.9 and 96.7%, respectively. The activity of urease was lower than that found by Montes-Rocha [55] for the area (ND at 0.63  $\mu\text{mol}$  of  $\text{NH}_4\text{-N}$   $\text{g}^{-1} 2 \text{h}^{-1}$ ), and the inhibition pattern in the enzyme was similar (100%). There are numerous studies of the inhibition of urease by different metals. In this regard, Belyaeva et al. [68] found urease inhibitions of 82.3 and 93.6% by Zn (8100 mg/kg). Yang et al. [69] showed that urea was inhibited by 75.1% with the presence of Cd (100 mg/kg) and Pb (500 mg/kg) in a controlled experiment. Kim et al. [70] found inhibitions from 21.5



**Figure 5.** Comparison of enzymes and soil respiration in distance gradient. a) arylsulfatase, b)  $\beta$ -glucosidase, c) dehydrogenase, d) phosphatase, e) urease and f) respiration. The bars indicate the median, and the error bars the interquartile range. Different letters indicate the significant differences ( $p < 0.01$ ) by the distance to the source. ND: Not detectable.

to 37.7% caused by Cu (200 mg/kg) in two types of soil. Researchers observed an inhibition of urease (65.3%) caused by Cu (800 mg/kg) in agricultural soils [71]. Borowik et al. [63] determined that urease can be inhibited (74.7%) by high concentrations of Zn (2400 mg/kg). Wyszowska et al. [72] found that urea activity in agricultural soil can be inhibited (34.6–78.3%) by different mixtures and concentrations of heavy metals.

The production of CO<sub>2</sub> in soil varied from 0.62 to 1.5 (μg of CO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>). A significant decrease in respiration was found mainly between the distances of 0–100 and the distances



of 1000–10,000 m ( $kW-H_{4,25} = 18.64$ ,  $p = 0.0009$ , **Figure 5f**), which represents an inhibition of 47.7%. There are no respiration data in soil samples from the area. Speir et al. [73] found inhibitions in basal soil respiration due to addition of As.

### 3.4. Relationship between toxic elements and biological activities

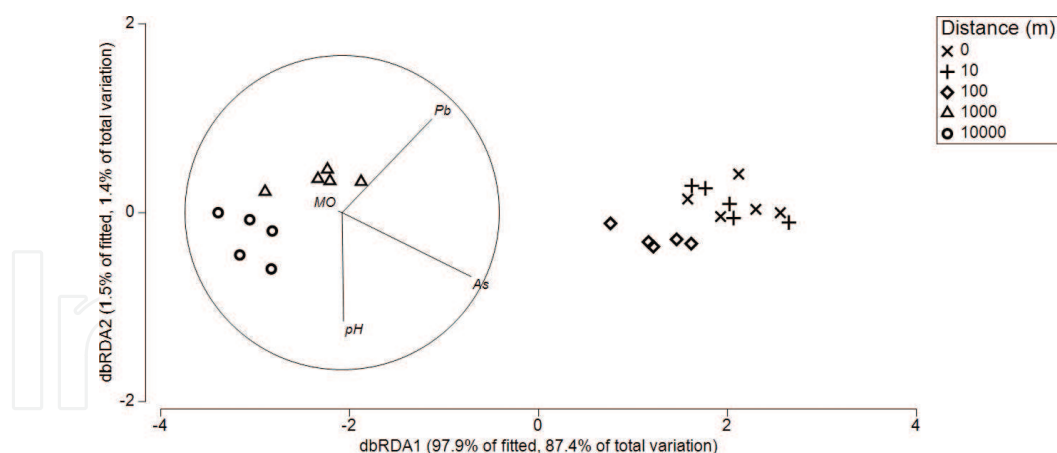
The activity arylsulfatase (93.6%),  $\beta$ -glucosidase (92.5%), dehydrogenase (85.2%), phosphatase (69.9%), urease (96.0%) and respiration (81.8%) was mainly explained by the variations in As concentrations (**Table 1**). Few studies have been done on the effects of As in soil biological activity; in this regard, Speir et al. [73] found inhibitions in sulfatase, phosphatase, urease and basal respiration of soil due to addition of As (concentrations of 0–50 ( $\mu\text{mol As [V] g}^{-1}$  soil)). Researchers conducted studies in soils of the USA contaminated by mixtures of toxic elements (among them 5.64 mg/kg of As and 250 mg/kg of Pb) in which they found a strong inhibition of  $\beta$ -glucosidase and phosphatase [4]. A slight negative effect of Pb (1.2%) is present in urease. Speir et al. [73] showed that urease was inhibited by 75.1% with the presence of Cd (100 mg/kg) and Pb (500 mg/kg) in agricultural soil in a controlled experiment. A 3.6% decrease in soil respiration can be associated with the change of pH of the soil, as mentioned above acidity increases mobility and bioavailability of the toxic elements and has a direct effect on microorganisms.

Regarding the total biological activity (dbRDA, **Figure 6, Table 1**), the grouping of the total samples into two large clusters corresponding to the distances 0–100 and 1000–10000 is shown as well as a greater dispersion in the information between the group of samples closest to the source of contamination. The direction of the vectors shows the influence of the variables toward the samples and the angles of the vectors represent the correlation between the variables. The DistLM model explained 89.2% of the total biological activity (**Table 1**).

Source	R <sup>2</sup>	RSS	No	Variables	Pseudo-F	Prop	Cumu	Effect
Arylsulfatase	0.936	1.515	1	As	341.36	0.936	0.936	-
B-Glucosidase	0.925	1.799	1	As	238.8	0.925	0.925	-
Dehydrogenase	0.852	20.47	1	As	133.38	0.852	0.852	-
Phosphatase	0.699	7.20	1	As	53.56	0.699	0.699	-
Urease	0.973	0.647	2	As	554.67	0.960	0.960	-
Respiration	0.855	3.46	2	As	103.97	0.818	0.818	-
				pH	5.60	0.036	0.855	+
All	0.892	15.432	4	As	148.11	0.865	0.687	-
				pH	2.137	0.011	0.877	+
				OM	1.65	0.008	0.886	+
				Pb	1.19	0.006	0.892	-

RSS: Residual sum of squared errors. Prop: Proportional  $r^2$ . Cummu: Cumulative proportional. (-) inhibitory effect. (+) inductive effect.

**Table 1.** Results of the distance-based multivariate model (DistLM) for enzyme activity.



**Figure 6.** Distance-based redundancy analysis (dbRDA) ordination plot for biological activities (soil respiration and enzymes) with elements and covariables.

The predominant variables that negatively affect the total biological activity were electrical conductivity As, pH and Pb (88.4%) while OM (0.8%) has a positive effect.

Different studies show that enzymatic activities of the soil are inhibited by trace elements, for example,  $\alpha$  and  $\beta$ -glucosidases,  $\alpha$  and  $\beta$ -galactosidases, urease, phosphatases, arylsulfatase, nitrate reductase, l-glutaminase, l-asparaginase, L-aspartase and cellulase [74]. Toxic elements can modify the biological properties of the soil such as microbial activity, enzymatic activity and respiration [75]. Metals and metalloids are a fundamental part for the functioning of an ecosystem; however, when the concentration of these elements rises, they can cause great alterations at different scales. The biological activity of the soil can be seriously affected due to concentrations of certain heavy metals such as Cu, Ni, Cd, Zn, Cr, As and Pb. Several authors have shown that under certain concentrations and conditions, heavy metals and metalloids can cause harmful effects in the soil ecosystem [62]. These can inhibit enzymatic activity in an irreversible or reversible manner occupying the active place of the enzyme that the substrate would occupy preventing in this way the union with the substrate avoiding the formation of the products. Different authors have documented that the inhibition takes place at the level of the SH group. This mechanism occurs in each of the enzymes [76, 77].

Several authors have mentioned that the enzymatic inhibition mechanisms are as follows: (1) by interacting with the enzyme-substrate complex, (2) by denaturing the enzyme protein and (3) by interacting with active protein groups [3, 76]. In places like Villa de la Paz, these processes can be performed chronically for more than 200 years.

The inhibition of enzymatic activity cannot be attributed only to heavy metals since there are other variables that can influence this activity such as pH. Acosta-Martínez and Tabatabai [74] found that  $\beta$ -Glucosidase is sensitive to changes in soil pH and soil management practices, as well as Sherene [59] found that pH can affect the biological activity of the soil as well as the availability of nutrients. Another property that must be considered is organic matter because they can form complexes with heavy metals and enzymes and in this way reduce their toxicity. Martínez-Toledo et al. [50] found an increase in several enzymes directly associated with pH and OM content in mining sites of San Luis Potosí.

## 4. Conclusion

The amount of As and Pb detected in this study showed levels that were above the national criteria (NOM-147-SEMARNAT/SSA1-2004) mainly for the distances of 0–100 m, which reflects the danger of these elements for the environment. The historical mining activity in this site has left large amounts of mine tailings rich in heavy metals exposed to the elements, which are currently causing a problem of contamination and health impacts of soil, plants, animals and humans. The levels identified in this study justify immediate intervention in the area close to the tailings, followed by long-term monitoring for the protection of human and ecological receptors, as well as avoiding the mobility of contaminants to different environmental matrices (water and sediments).

The contamination by heavy metals and the effect of the mining activities in Villa de la Paz over time have caused for the physical–chemical properties (organic matter, electrical conductivity and pH), of the soils developed on the mining residues, to be significantly different to those of soils without the influence of pollution. These properties are involved in the interactions between microorganisms, enzymes and roots of plants, which are responsible for maintaining adequate soil health. It is recommended to carry out tests to evaluate the degree of degradation in at least 1 km around the mining waste to identify the alterations caused by the mining activity punctually and thus propose methods of soil recovery.

The biological activity of the soils presented a strong affectation due to the presence of heavy metals, which proved its usefulness as an indicator for rapid, reliable and low-cost evaluation. Levels such as those registered in the evaluated sites reduce the biological activity of soil in an alarming way, affecting their health, as well as the biogeochemical cycles and the environmental services that this provides. It was determined that metals are not the only ones who can negatively affect enzymatic activity, but also electrical conductivity and pH can interact in the phenomenon of inhibition. The organic matter with its capacity of immobilization of heavy metals promotes the increase of enzymatic activity.

The general pattern of inhibition for the area was urease>glucosidase>arylsulfatase>dehydrogenase>phosphatase>respiration; the latter being the least sensitive to inhibition. The continuous monitoring of parameters in this area is recommended. The alteration of these enzymes could be interrupting the biogeochemical cycles, as well as the material and energy flows of the ecosystems, making it more difficult to recover key processes for the maintenance of life such as the fixation of nitrogen and CO<sub>2</sub>.

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## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this chapter.

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