

Diagnosis of the impact on wetland soils: Based on enzymatic parameters and heavy metals

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Highlights:

The La Chimba wetland sector had the highest concentration of heavy metals compared to the other sites studied.

A negative correlation was observed between heavy metal concentrations and enzyme activity in soils

The analysis of soil enzyme activity is a promising method for conducting studies on soil quality

1. Introduction

Urban wetlands are key ecosystems for the ecological resilience of cities, providing essential services such as water regulation, pollutant retention, and biodiversity support. In Chile, Law 21.202 (MMA, 2020) has promoted their legal protection; however, many of these systems remain exposed to severe anthropogenic pressures. Such is the case of the La Chimba Wetland, located in the city of Antofagasta, an arid area with a strong mining and industrial legacy, where high concentrations of geochemical pollutants have been reported in its soils. The recent declaration of the site as a "Nature Sanctuary" through Exempt Resolution No. 787 of the Ministry of the Environment (2022) represents a step forward in its protection, but scientific evidence is still needed to support its ecological restoration.

One of the least visible but most critical effects of soil contamination is the alteration of the edaphic microbiome and the inhibition of enzymatic processes associated with nutrient cycling. The activity of enzymes such as dehydrogenase, phosphatase, and glucosidases has been recognized as a sensitive indicator of the ecological status of the soil, as it reflects the system's ability to sustain key functions such as the mineralization of organic matter and the flow of essential nutrients. However, these functions can be severely compromised by the presence of heavy metals such as As, Cu, Pb, and Hg, which are common in degraded urban environments.

This study analyzes the relationship between heavy metal contamination and soil enzyme activity in the La Chimba Wetland, comparing its conditions with a controlled urban site (UA nursery) and an ecological reference site (Naltahua). Multivariate analysis techniques (PCA) and bivariate correlations were used to identify patterns of association and validate the hypothesis of edaphic dysfunction induced by geochemical contamination. The results allow baselines to be established for the planning of restoration strategies, such as phytoremediation and biostimulation, and offer a replicable model for other urban wetlands in northern Chile.

2.1 Urban wetlands

Urban wetlands are key ecosystems in the ecological balance of cities, acting as natural filters, reservoirs of biodiversity, water regulators, and sinks for atmospheric pollutants

(Iturriaga, 2024). In Chile, the recent enactment of Law 21.202 (MMA, 2020) has promoted their recognition and legal protection, although there are still gaps in their effective conservation, especially in regions such as Antofagasta, where the La Chimba Wetland is a unique case due to its location in a highly mining and desert city (Hiriart et al., 2018).

This wetland was officially declared a "Nature Sanctuary" by Exempt Resolution No. 787 of the Ministry of the Environment in 2022 and recognized as an "Urban Wetland" under Law 21.202, which reinforces its importance for local biodiversity and the urgent need for its protection (MMA, 2022).

Urban wetlands such as La Chimba are fragile and highly productive ecosystems that provide essential ecological services, including water cycle regulation, habitat for native wildlife, and carbon storage. However, their location in urban areas exposes them to multiple threats, including soil contamination, habitat fragmentation, and invasion by exotic species (Rojas-Quezada et al., 2022; Pizarro-Araya et al., 2022; Navarro et al., 2021; Hidalgo-Corrotea et al., 2023).

Despite being subject to intense anthropogenic pressure, these ecosystems represent unique opportunities for the conservation of essential ecological functions in densely populated urban environments. The multifunctionality of urban wetlands includes not only supporting ecosystem services (nutrient cycling, habitat), provisioning (water, food), regulating (air and water quality, flood mitigation), and cultural (environmental education, landscape), but also have a high capacity for resilience to disturbance (Alikhani et al., 2021).

The application of environmental monitoring tools based on enzymatic bioindicators can be strategic for these wetlands, particularly in the context of cities with arid climates where water resources are scarce. Thus, the conservation of the La Chimba Wetland represents a model for the adaptive and resilient management of urban socio-ecological systems in northern Chile.

2.2 Heavy metals and urban wetlands

The accumulation of heavy metals in urban soils is a critical environmental problem, especially in cities in northern Chile, where historical mining and industrial activities have left persistent environmental liabilities. Elements such as arsenic (As), lead (Pb), copper (Cu), zinc (Zn), and cadmium (Cd) are deposited in the soil through industrial waste, particulate matter, and runoff, posing a threat to human health and local biodiversity (Li et al., 2022; Tapia et al., 2018; Valdés et al., 2023)

One of the main threats to urban wetlands is contamination by heavy metals, such as Pb, Cd, Cu, Ni, Zn, and Cr, from urban, mining, and vehicular activities. These metals alter biodiversity and affect key biochemical processes in the soil (Yang et al., 2022). In this context, soil enzyme activity emerges as an effective bioindicator of ecological status and soil quality, allowing anthropogenic impacts to be detected before they manifest themselves at other levels of biological organization (Zerrari et al., 2023).

110

111 **2.3 Enzymatic Activity**

112 Soil enzymatic activity is a key indicator of its ecological health, as enzymes reflect the
 113 metabolic processes of the microbial biota responsible for nutrient recycling and organic
 114 matter decomposition (Aponte et al., 2020).

115 Soil enzymes, such as dehydrogenase, acid and alkaline phosphatase, urease, arylsulfatase,
 116 β -glucosidase, and β -galactosidase, play key roles in the carbon, nitrogen, phosphorus, and
 117 sulfur cycles (Nikolova et al., 2023). For example, dehydrogenase is related to microbial
 118 respiration and reflects the overall metabolic activity of the soil. β -glucosidase and β -
 119 galactosidase are involved in carbohydrate degradation, while arylsulfatase participates in
 120 the mineralization of sulfated compounds. Phosphatase contributes to the release of
 121 phosphorus available to plants, and urease catalyzes the hydrolysis of urea, which is
 122 essential in the nitrogen cycle.

123 **2.4 Enzymes and Heavy Metals**

124 Several studies have shown that chronic exposure to heavy metals can significantly reduce
 125 the activity of these enzymes or alter their relationship with the physicochemical properties
 126 of the soil, such as pH, organic matter, and texture (Zerrari et al., 2023; Nikolova et al.,
 127 2023). For example, Zerrari et al. found positive correlations between Cu, Pb, and Zn and
 128 phosphatase and urease activity, while Cr and Ni showed negative effects. In turn, Yang et al.
 129 (2022) demonstrated that enzyme activity can be modulated by land use, being higher in
 130 forest areas than in cropland or fallow land. For their part, Jaworska and Lemanowicz
 131 (2019) determined that dehydrogenase, catalase, and phosphatase activity correlates
 132 positively with the content of organic carbon and metals such as Pb, Zn, and Cd in soils near
 133 roads.

134 Additionally, meta-analytical studies have confirmed that heavy metals reduce soil enzyme
 135 activity by an average of 30% to 70%, with those involved in the carbon cycle (such as β -
 136 glucosidase) and sulfur cycle (such as arylsulfatase) being the most affected (Aponte et al.,
 137 2020). These intracellular enzymes are more susceptible to denaturation by metals such as
 138 As, Pb, Cu, or Cd, while extracellular enzymes stabilized on clay minerals are more resistant
 139 (Li et al., 2022).

140 On the other hand, the level of toxicity is closely linked to the chemical form of the metal
 141 and its bioavailability, rather than its total concentration. This implies that variables such as
 142 pH, clay content, and organic matter can modulate its impact on the soil. In arid
 143 environments such as Antofagasta, low vegetation cover and soils with low organic matter
 144 content hinder the immobilization of metals, increasing their ecological risk (Li et al., 2022).

145 The objectives of this study are to quantify physicochemical parameters and heavy metals
 146 and to evaluate the enzymatic activity associated with carbon cycles (β -glucosidases, β -
 147 galactosidase, PHE), nitrogen (urease, NAG), phosphorus (acid phosphatase), and general

microbial metabolism (dehydrogenase, arylsulfatase) in the soils adjacent to the "La Chimba" Urban Wetland, comparing the results with a semi-controlled site (Antofagasta University Nursery) and a reference site (Naltahua).

3. Methodology

3.1 Sampling Sites

The study area corresponds to a sector of the coastal plain in the northern coastal sector of the city of Antofagasta, together with an immediate catchment area located on the western slope of the Cordillera de la Costa.

The catchment area is dominated by outcrops of the La Negra Formation (Lower-Middle Jurassic), composed of a thick sequence of andesitic lavas and basalts, volcanic breccias, and tuffs interbedded with volcanoclastic sediments, interpreted as part of an ancient continental magmatic arc developed on an active margin . In the regional context, this sequence is underlain by Jurassic plutons of dioritic to granodioritic composition, with local occurrences of gabbros and tonalites exhibiting vein-type Cu, Au, and Ag mineralization and a degree of fracturing and hydrothermal alteration .

In the Aguada la Chimba sector, composite samples were taken from a total area of 2.24 ha (Fig. 1). A Falcon tube was used to collect the first 5 cm of soil from 10 points, each point was a composite of three samples of the La Chimba Wetland, with 5 points corresponding to the **El Rubio watershed** and 5 points to **Chimbanito**. The composite samples from the triplicate of each zone were stored in 50 mL Falcon tubes and then refrigerated at 10°C (EPA, 2020; EPA, 2023). The coordinates are found in Appendix A.

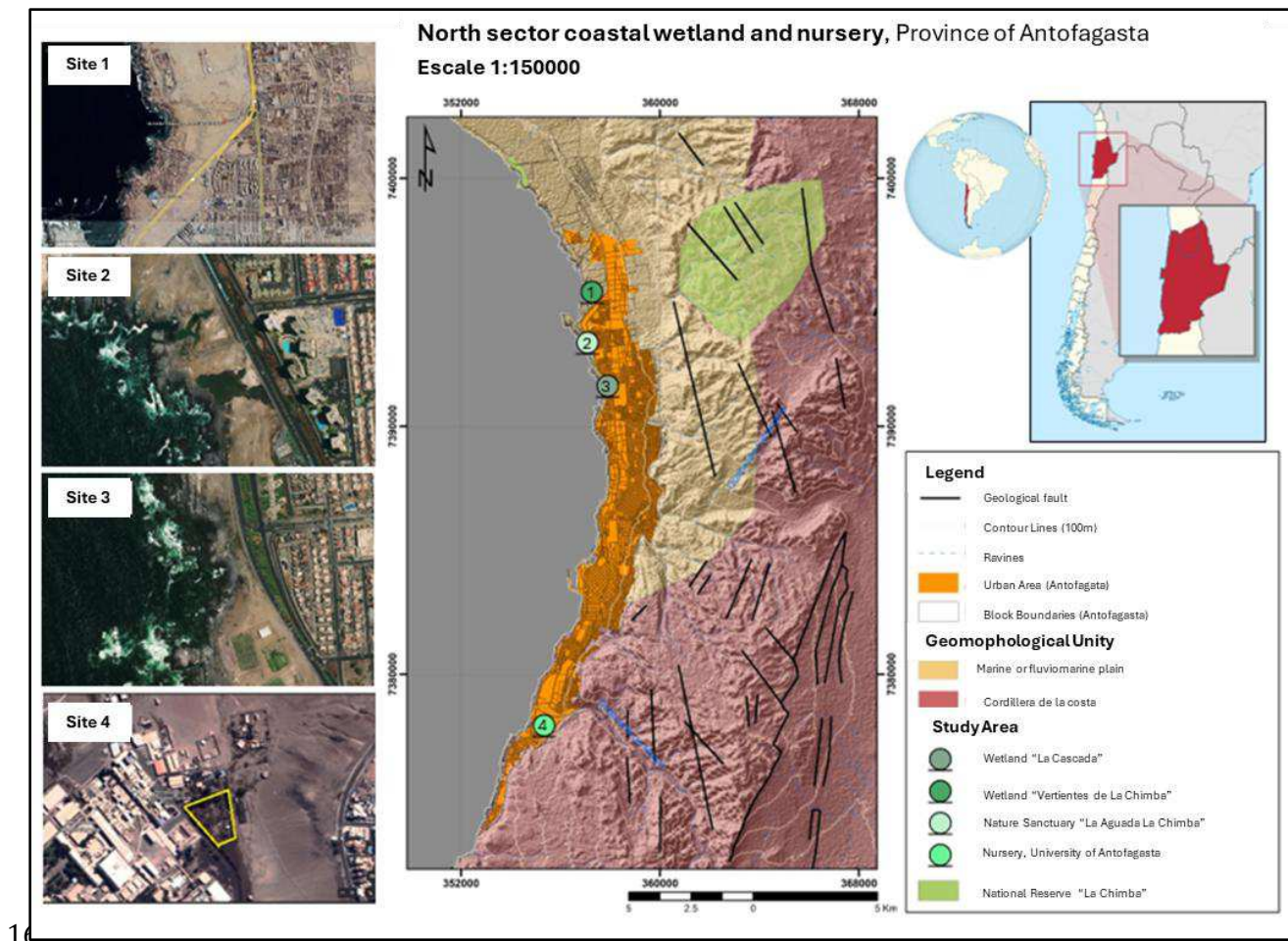


Fig. 1 Urban Wetland and Nursery sampling area, Antofagasta Commune. Site 1: La Cascada Wetland, Site 2: Vertientes de la Chimba Wetland, Site 3: La Aguada La Chimba Nature Sanctuary, and Site 4: Nursery, University of Antofagasta.

In the upper part of the Coloso Campus, in the midst of the aridity of the desert, hundreds of plant species find a perfect habitat to grow and reproduce in the University of Antofagasta Nursery (Fig. 1). This site was studied as a comparative site using the methodology described above, with 5 points instead of 10.

The community of Naltahua, located on the southern bank of the Maipo River and near the mouth of the Mapocho River (a tributary of the Maipo), was also studied as a reference site (Fig. 2) for active agricultural soils. It is located at the foot of Cerro Yervas Buenas, part of the Paine mountain range.

This territory has historically been used mainly for agriculture, although in 1908 a French company exploited the copper and minerals in the area, establishing a mining camp with more than 2,500 people, schools, a polyclinic, a general store, etc., which shaped what is now the town of Naltahua. When mining operations ended, the land was returned to its former owners, and due to the precarious conditions, many miners migrated to the city,

186 while others remained to work on farms under the supervision of a boss. Since then,
187 Naltahua has continued its tradition as an agricultural town.

188 In 2014, it was declared a saturated area due to daily MP 2.5 levels, and subsequently, the
189 Metropolitan Region's Air Pollution Prevention and Decontamination Plan was developed,
190 D.S. No. 31 of 2016 of the Ministry of the Environment.

191 Although the municipality does not have pollutant measurement stations, the Talagante
192 station is located nearby, which, according to current records and data from 2018, does not
193 show alert, emergency, or pre-emergency values. This situation is mainly due to its location
194 near areas with greater ventilation and, on the other hand, it does not have significant
195 sources of pollution.

196 According to the diagnosis of the Isla de Maipo Community Regulatory Plan, 2023
197 (Municipality of Isla de Maipo, 2023) update study, the main polluting activities are related
198 to the extraction of aggregates.

199 On the other hand, it is important to consider the use of firewood for heating in the winter
200 season, a situation discussed in participatory forums as a problem in Isla Centro, and the
201 increase in the vehicle fleet (926 additional vehicles between 2016 and 2018) due to the
202 arrival of new residents in the commune. Both factors can influence local air quality;
203 however, there is no air quality monitoring station in the municipality.

204 The samples methodology is the one described above but instead of composite samples
205 these were 5 points in triplicate.

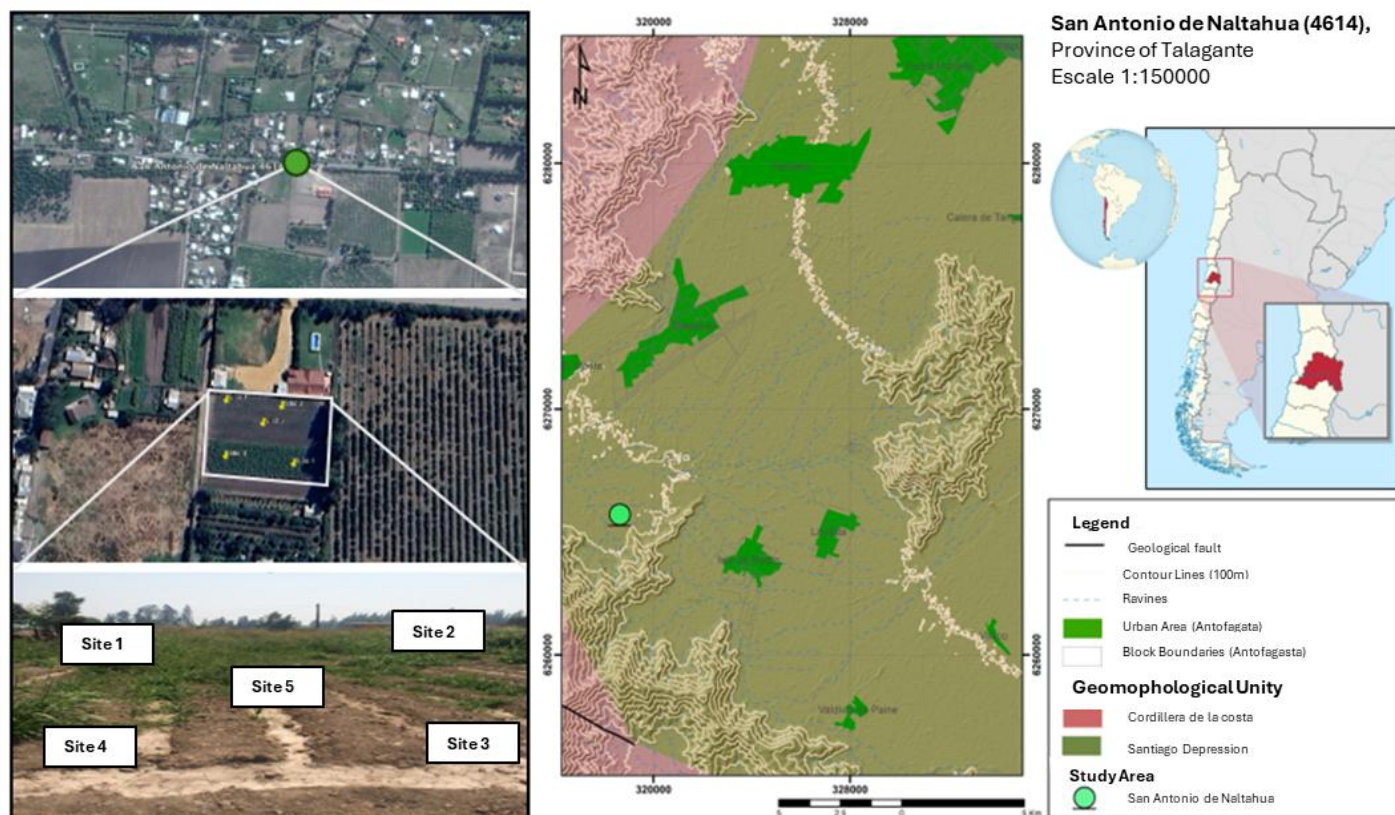


Fig. 2 Sampling area, reference site, San Antonio de Naltahua

3.2 Sample treatment

Once in the laboratory, the study and control samples are separated into two subsamples. For Hg analysis, they are dried at room temperature, and for As, Cu, Pb, Cd, Zn, Ni, and Co analysis, they are dried at 100°C in a WTC Binder forced-air oven for 24 hours.

The sample is prepared for physicochemical parameter (Total Organic Carbon (TOC), Ca⁺, Cl⁻, Mg⁺, pH, Conductivity, C, SO₄⁻², CO₃⁻²) analysis by leaching with distilled water. Ten grams of soil sample are weighed and added to a beaker, then 100 mL of distilled water is added, and leaching is carried out with agitation for 24 hours. Finally, the solution is filtered through a Nalgene vacuum system using a 0.45µm Milipore cellulose membrane filter.

3.2.1 Mineralization of the samples under study.

Approximately 0.5 g of sample is weighed into Teflon containers, which are wet-disintegrated with 6 mL of aqua regia (HCl: HNO₃), 2 mL of H₂O₂, and 2 mL of HClO₄. These are left to react for 24 hours, after which time they are placed in an oven and heated to 150°C. Once the containers have cooled, they are vacuum filtered through 0.45 µm cellulose membranes and a Nalgene system. They are measured with deionized water to 50 mL (EPA 3015A).

3.2.2 Pre-reduction As

In an Erlenmeyer flask, 10 mL of the original solution, broken down in the first step, is added, then 250 μL of HClO_4 and 500 μL of H_2SO_4 , it is placed on the hot plate and broken down in an open system, which consists of placing a short-stemmed funnel over the flask. Once nitrous vapors are observed, a watch glass is placed over the funnel in order to produce reflux and prevent the sample from drying out. Once a syrupy state is reached, the Erlenmeyer flask is removed from the hot plate and allowed to cool, filtered, and titrated with 0.5M HCl to 25 mL.

3.3.3 Hg Disintegration

The digestion process involves converting the analyte into chemical forms that remain stable in solution. The applicability of known digestion methods depends on the sample matrix.

In the disintegration of samples for Hg analysis, 0.5 g of each granulometric fraction per site is weighed and placed in Teflon pumps, followed by the addition of 6 mL of HNO_3 and 6 mL of H_2SO_4 .

3.4 Determination of total heavy metal concentration in soil.

The total concentration of heavy metals in the soil grain size fractions was determined using HG-AAS, FAAS, and HR-CSAAS, which allowed us to determine the concentrations with high analytical sensitivity.

For the analysis of total concentrations, calibration curves were made using the multiple standard addition methodology. For this purpose, a Titrisol Merck with a concentration of 1000mg/L of each element under study was used.

For the multi-element determination of Cr, Cu, Fe, Mn, Ni, Pb, and Zn, the equipment used was a ContrAA 700 (HR-CS AAS) Analytik Jena. Atomization was performed by flame, and a xenon lamp was used for the determination of the elements.

For the determination of As, a GBC 933plus Atomic Absorption Spectrometer was used, coupled with a GBC HG3000 hydride generator and a quartz cell. Air/acetylene and a hollow cathode lamp were used for As. In hydride generation, the concentration of NaBH_4 was 1% w/v and the concentration of HCl was 3 M.

The total concentration of Cd is determined using flame atomic absorption spectrometry, with a GBC 933plus atomic absorption spectrometer, an air (oxidizer)/acetylene (fuel) mixture, and a hollow cathode lamp for Cd. The determination of Hg is performed using the GBC HG3000 hydride generation technique coupled with a GCB 900Plus atomic absorption spectrometer. Using air/acetylene, a quartz cell with a thermal heating system, a Hg-specific hollow cathode lamp, and the concentration of NaBH_4 used corresponds to 1% w/v and 0.3 M HCl.

3.5 Analytical Validation

Analytical measurements are validated with Montana Soil-1 and Montana Soil-2 Certified Reference Materials and the recovery of added concentrations for those elements that are only reported and not certified in the reference material Appendix B.

3.6 Determination of enzyme activity in soil

The enzymatic activities of arylsulfatase (ARYLS), β -galactosidase (β -GAL), α -glucosidase (α -GLU), β -glucosidase (β -GLU), N-acetyl- β -D-glucosaminidase (NAG), acid phosphatase (PHOS), acid phosphatase (PAC), alkaline phosphatase (PAK), leucine aminopeptidase (ARYLN), and urease (URE) were determined using colorimetric microplate assays. (Vélez Azeraño et al., 2024; Ochoa et al., 2007)

As a standard solution for the enzymes ARYLS, β -GAL, α -GLU, β -GLU, NAG, PHOS, 0.36 mM p-nitrophenol (PNP) was used from a 3.6 mM stock solution; for ARLYS, a 1 mM β -naphthylamine stock solution was used, and for URE, a 0.62 mM ammonium chloride standard solution was used from a 62 mM stock standard.

Each enzyme was evaluated with a specific substrate prepared in ultrapure water and stored at -20°C . Potassium 4-nitrophenyl sulfate (0.025 M) was used for ARYLS, p-nitrophenyl β -D-galactopyranoside (0.020 M) for β -GAL, 4-nitrophenyl α -D-glucopyranoside (0.025 M) for α -GLU, and β -GLU 4-nitrophenyl β -D-glucopyranoside (0.050 M), for NAG p-nitrophenyl N-acetyl β -D-glucopyranoside (0.010 M), and for PHOS/PAC/PAK 4-nitrophenyl-phosphate disodium hexahydrate (0.050 M). ARYLN analysis was performed with 0.008 M L-leucine β -naphthylamide HCl dissolved in 50 mM Trizma base buffer pH 7.5, while URE activity was assessed with 0.4 M urea prepared fresh weekly and stored at 4°C .

The reactions were performed by mixing sample, substrate, and buffer/ultrapure water, incubating at room temperature and in the dark for 20 minutes for PNP and β -naphthylamine and 30 minutes for urease. The reaction was stopped with specific reagents: 0.5 mM CaCl_2 and Trizma pH 12 for PNP, acidified ethanol and DMCA for β -naphthylamine, and salicylate/cyanurate reagents for urease, with centrifugation prior to reading in the latter case.

Finally, 150 μL of β -naphthylamide and 200 μL of PNP and urease were transferred to a plate for absorbance measurement at 405 nm (PNP), 540 nm (β -naphthylamine), or 650 nm (urease). Enzyme concentrations were calculated from the corresponding calibration curves.

3.7 Determination of Microbial Biomass in Soils

The substrate-induced respiration (SIR) methodology (Bruni et al., 2025; Anderson and Domsch, 1978) was used to measure the microbial biomass of soils. Based on short-term respiration rates (CO_2 production). The higher the respiration rate, the greater the microbial biomass in a given soil.

3.8 Determination of Nitrification Potential

To measure nitrification potential, the rates of nitrification potentials in the soil are measured in order to infer the size of the nitrifying population in a given soil. To do this, a solution containing NH_4^+ and PO_4^{3-} suitable for maximizing nitrification rates is added to the sample along with chlorate to block the reduction of nitrite to nitrate. Measurements are taken at 543 nm and the slope of the line relating NO_2^- concentration to time is calculated (Belser and Mays, 1980).

3.9 Statistical procedure.

For the Statistical procedure and generation of graphs for the analysis of the data, the software used was Rstudio Version 2024.09.1.

2.6.2. Environmental Index

To evaluate the environmental condition of the study areas, different environmental indices were used. Since Chile does not have regulations that establish background values, four different baselines were applied — two general (Hakanson, 1980; Turekian and Wedepohl, 1961) and two local (CENMA, 2014). Using these reference values, the environmental indices were calculated, such as the Contamination Factor (Cf) for each heavy metal:

$$Cfi = \frac{Ci}{Ci \text{ background}}$$

where Cfi is the contamination level of heavy metal i, Ci (mg/kg) is the measured concentration of metal i, and Ci background is the baseline concentration of that metal. The contamination levels of heavy metals were categorized as low, moderate, considerable, and very high when $Cf < 1$, $1 \leq Cf < 3$, $3 \leq Cf < 6$, and $Cf \geq 6$, respectively (Hakanson, 1980).

The Pollution Load Index (PLI) was also used as an overall estimator of the contamination status of the different zones:

$$PLI = (Cf1 \times Cf2 \times Cf3 \times Cf4 \dots)(1/n)$$

where Cfn corresponds to the contamination factor of each metal, and n is the total number of metals considered in the calculation. The overall contamination status was classified as moderate, high, or extreme when $PLI < 1$, $1 \leq PLI < 2$, $2 \leq PLI < 3$, and $PLI \geq 3$, respectively (Tomlinson et al., 1980).

4. Results and Discussion

4.1 Heavy metals by site

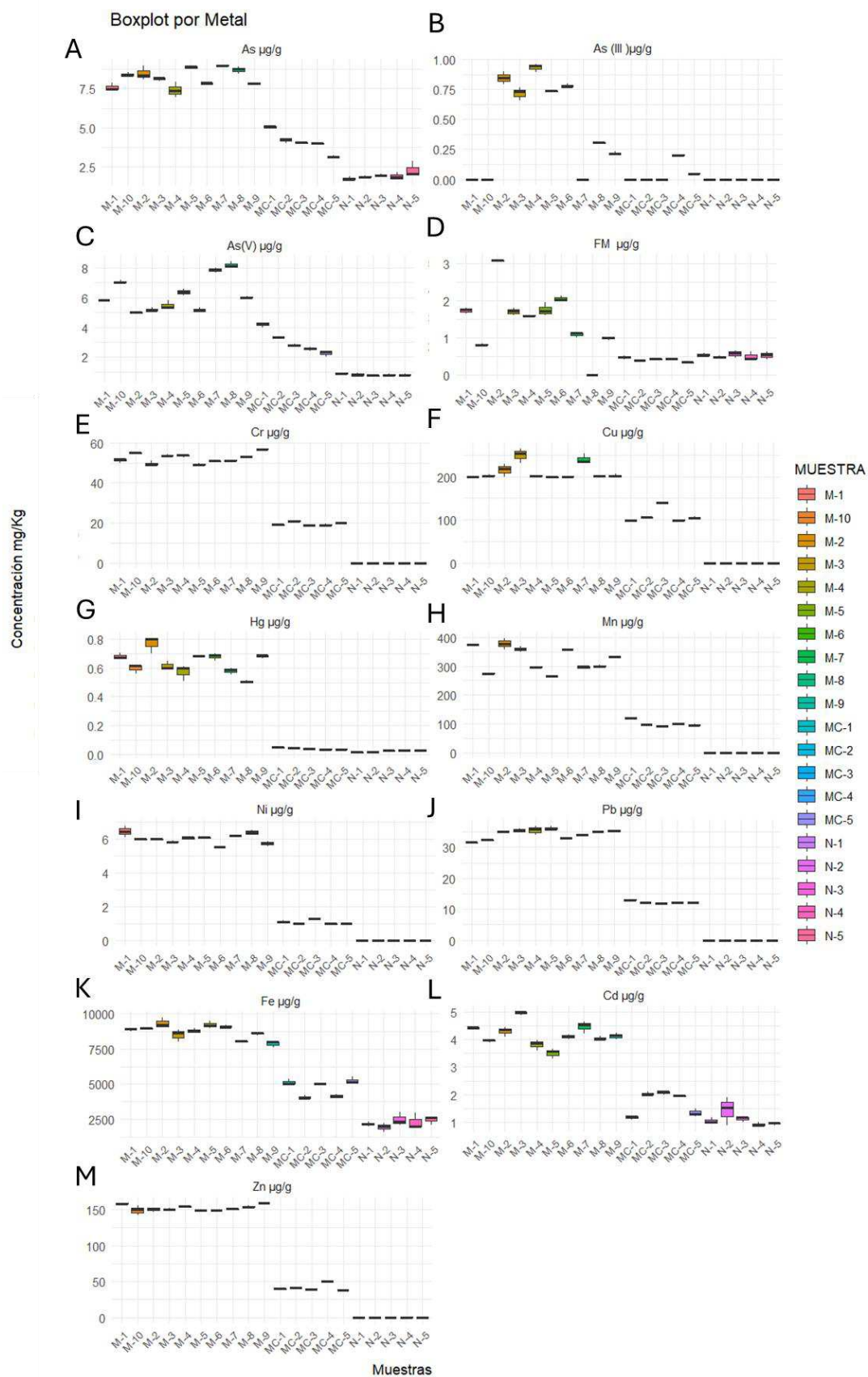


Fig.3. Boxplot of heavy metals concentration (mg/Kg) by site soil samples. A. Arsenic Concentration, B, Arsenic (III) concentration, C. Arsenic (V) concentration, D. Methylated Forms of Arsenic concentration, E. Chromium concentration, F. Copper Concentration. G. Mercury concentration, H. Manganese, I. Nickel concentration, J. Lead concentration, K. Iron concentration, L. Cadmium concentration, M. Zinc concentration.

The analysis of heavy metal concentrations in soil samples revealed marked and consistent differences between the three sites evaluated: La Chimba Wetland (M), the controlled urban nursery (MC), and the ecological reference site (Naltahua, N). In general, La Chimba had the highest levels of contamination, with values well above those recorded at the other sites and, in several cases, exceeding international soil quality thresholds for agricultural or ecological use (CCME, 2007).

Total arsenic (As) (Fig. 5.A) reached concentrations of up to **8.6 µg/g** in samples from the La Chimba wetland, distributed in its trivalent (As(III)) (Fig. 5.B) and pentavalent (As(V)) (Fig. 5.C) forms. The highest concentrations of FM (up to ~3 µg/g) (Fig. 5.D) were found in samples from the La Chimba wetland. These samples could correspond to areas with greater anthropogenic influence, such as areas close to sources of contamination. As(III) ranged from **0.53 to 0.81 µg/g**, while As(V) remained between **5.5 and 8.4 µg/g** in La Chimba, decreasing to less than **1.5 µg/g** in the Naltahua. This suggests an oxidizing environment, conducive to the stabilization of the pentavalent form, and a continuous source of arsenic input, possibly from industrial runoff or urban deposits.

Chromium (Cr) (Fig. 3.E) showed high values in La Chimba, with a median close to **47 µg/g**, decreasing to **20 µg/g** in the nursery and being practically undetectable in the Naltahua samples. The persistence of Cr in soils may be associated with industrial waste, pigments, or the presence of minerals rich in metal oxides.

Copper (Cu) (Fig. 3.F) recorded its highest values in La Chimba, with a median of **227.5 µg/g**, decreasing to ranges of **110–125 µg/g** in MC and values below **5 µg/g** in Naltahua. This pattern reflects an obvious anthropogenic origin and is consistent with the presence of Cu in urban metal waste.

As for mercury (Hg) (Fig. 3.G), abnormally high levels were observed in La Chimba, with average concentrations close to 0.65 µg/g, in contrast to values <0.02 µg/g at the control sites. This notable difference suggests a localized source of Hg, probably related to former industrial activities or unregulated urban discharges.

The accumulation of **Mn (Fig. H) (380–410 µg/g** in La Chimba vs. <40 µg/g in Naltahua) and extremely high levels of **Fe (Fig. 3.K) (>750,000 µg/g)** could be linked to natural concentration processes associated with the wetland's water regime, but also to the sedimentation of atmospheric industrial particles.

Nickel (Ni) (Fig. 3.I) was distributed consistently with the other metals, with values ranging from **5.7 to 6.2 µg/g** in La Chimba and **<2 µg/g** in MC and Naltahua. Ni accumulation is often linked to industrial activities or the use of contaminated phosphate fertilizers.

Lead (Pb) (Fig. 3.J) showed similar behavior, with values between **32.8 and 35.5 µg/g** in La Chimba, intermediate levels of **12–13 µg/g** in MC, and concentrations below **1 µg/g** in Naltahua. The homogeneous distribution at the impacted site suggests a diffuse and persistent source of this contaminant, probably associated with vehicular traffic, deteriorated paint, or old waste.

Cadmium (Cd) (Fig. 3.L), concentrations of up to **4.8 µg/g** were observed in La Chimba, decreasing to a range of **2.0–2.5 µg/g** at the control site and **0.9–1.2 µg/g** in Naltahua. This element is particularly relevant due to its high mobility and toxicity at low concentrations, making it a sensitive marker of soil contamination.

As for **Zinc (Zn)** (Fig. 3.M) showed a marked accumulation in La Chimba, reaching between **148 and 157 µg/g**, with values of **50 µg/g** in MC and **<10 µg/g** at the reference site. Zn, although essential as a micronutrient, can interfere with enzymatic and microbial processes in the soil when present in excess.

These results reflect a clear **geochemical disturbance gradient**, in which La Chimba wetland is the most impacted site, with the simultaneous and elevated presence of multiple metals. This contaminant profile coincides with the urban and industrial nature of the wetland's surroundings and suggests a cumulative history of diffuse contamination. The homogeneity of levels within the impacted site reinforces the hypothesis of a chronic rather than episodic accumulation process. In contrast, Naltahua acts as an ecological control with baseline values for regional comparison

Anova analysis for heavy metals in each site shows that there is a p value **< 0,05** for all the sites in study (Appendix C).

399 4.2 Enzymatic activity in soils from the three study sites

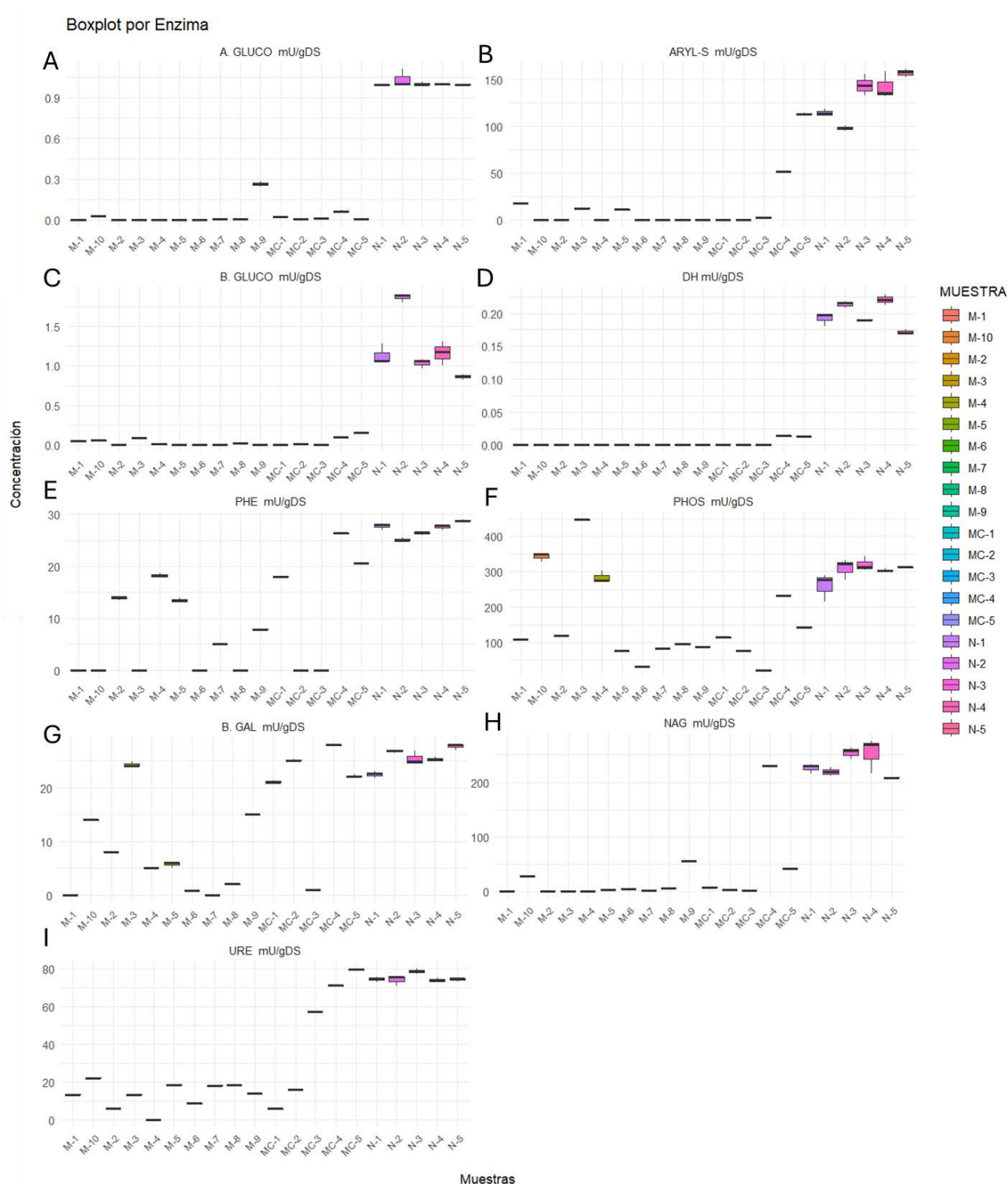


Fig.4. Boxplot of heavy metals concentration (mU/gDS) (milliunits per gram of dry soil) by site soil samples. A. α -glucosidase, B, Arylsulfatase concentration, C. β -glucosidase concentration, D. Deshydrogenase concentration, E. Acid/alkaline

phosphatase concentration, F. Acid phosphatase Concentration. G. β -galactosidase concentration, H. N-acetyl- β -D-glucosaminidase concentration, I. Urease concentration.

Analysis of soil enzyme activity at the three study sites—the La Chimba urban wetland (M), the University of Antofagasta nursery (MC), and the Naltahua ecological reference site (N)—revealed marked differences in soil biological functionality, reflected in the levels of nine key enzymes associated with the biogeochemical cycles of carbon, nitrogen, phosphorus, and sulfur (Figs. 3 and 4).

At site M (La Chimba), a generalized inhibition of enzymatic activity was observed. In particular, hydrolytic enzymes of the carbon cycle, such as α -glucosidase (A. GLUCO) (Fig. 3.A) and β -glucosidase (B. GLUCO) (Fig. 3.C), which are key to the decomposition of polysaccharides, recorded levels below 0.2 and 0.5 mU/gDS (milliunits per gram of dry soil), respectively. Aryl-sulfatase (ARYL-S) (Fig. 3.B), associated with the sulfur cycle, remained at very low levels (5–20 mU/gDS), while dehydrogenase (DH) (Fig. 3.D)—an indicator of general microbial activity—had values below **0.03 mU/ μ gDS**, practically zero in most samples. Phenol oxidase (PHE) (Fig. 3. E), involved in the degradation of lignocellulosic compounds, showed low concentrations (<10 mU/gDS), suggesting a reduction in the decomposition of recalcitrant organic matter. In the case of acid phosphatase (PHOS) (Fig. 3.F), associated with the phosphorus cycle, values did not exceed 70 mU/gDM, being considerably lower than those recorded at the other sites, while β -galactosidase (B. GAL) (Fig. 4.G) did not exceed **8 mU/gDS**. As for nitrogen cycle enzymes, N-acetylglucosaminidase (NAG) (Fig. 4.H), essential for chitin mineralization, remained below 100 mU/gDS, and urease (URE) (Fig. 4.I) showed values between 10 and 25 mU/gDS. This ultimately indicates a cross-sectional deterioration in soil biogeochemical processes.

In contrast, site MC (Nursery) showed a partial recovery of enzymatic activity. At this site, enzymes such as PHOS, B. GAL, URE, and PHE showed average values ranging between 70 and 150 mU/gDS, suggesting a relative improvement in soil functionality possibly linked to its status as a managed green area, although still influenced by the urban environment.

The Naltahua (N) reference site showed the highest ecological functionality of the soil. All enzyme parameters showed high and stable levels, characteristic of healthy soils: DH exceeded 0.2 mU/gDS, PHOS exceeded 400 mU/gDS, B. GLUCO and A. GLUCO reached 1.2 and 0.9 mU/gDS respectively, NAG remained above 220 mU/gDS, and URE around 80 mU/gDS. Likewise, PHE, B. GAL, and ARYL-S presented values of 35, 25, and 150 mU/gDS, respectively, evidencing intense microbial activity and efficiency in the transformation of organic matter and nutrients.

Anova analysis for enzymes in each site shows that there is a p value < 0,05 for all the sites in study (Appendix C).

4.3 Relation between enzymes and soil

The impact of As on soils depends critically on whether it is present as arsenite [As(III)] or arsenate [As(V)].

The results of enzymatic activity in contaminated soils for the different samples under study (M-1 to M-10), measured in mU/gDS (milliunits per gram of dry soil), show that enzymes act as bioindicators; low or zero values are usually correlated with high levels of toxicity from this metalloid. The presence of these arsenic species generates oxidative stress in the soil microbiota, which decreases the total enzyme reserve and its catalytic efficiency. This means that even if the soil has nutrients, microorganisms cannot process them because the enzymes are blocked by arsenic.

We observed (Table.1) that M-4 and M-3 have a more critical and alarming biological toxicity profile compared to the other enzymes under study, due to the total inhibition of multiple essential metabolic processes in the soil.

Table 1. Risk interpretation of indicators in samples M-3 and M-4.

Indicator	Sample M-3 mU/gDS	Sample M-4 mU/gDS	Risk Interpretation
α GLUCO	nd	0,0018	M-4 shows signs of activity, but it remains extremely low.
β GLUCO	0,0032	0,0018	M-4 has almost half the activity of M-3-1, indicating greater inhibition of the carbon cycle.
PHOS	100,53	445,95	The high value in M-4 suggests severe competition with As(V). The enzyme tends to have an affinity for arsenate.
ARYL-S	13,308	nd	Critical Point: M-4 has no activity in the sulfur cycle, while M-3-1 is still functioning.
URE	13,308	nd	Critical Point: M-4 has completely lost its ability to process nitrogen (urea), unlike M-3.

DH	0,0005	0,0005	Both are in “biological death,” with almost no microbial respiration.
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Although M-3 shows undetectable values for α Gluco and DH, M-4 shows systemic collapse. The total absence of urease (URE) and arylsulfatase (ARYL-S) in M-4 indicates that arsenic toxicity has blocked not only energy, but also the nitrogen and sulfur cycles, which are fundamental to any form of plant or microbial life (Trasar-Cepeda et al., 2000).

In addition, the PHOS value in M-4 is four times higher than in M-3. In soil ecotoxicology, an excessive increase in phosphatase in the presence of arsenic is usually a stress response to the “deception” of arsenate [As(V)], which displaces real phosphorus (Dixit and Hering, 2003)

Carbonate and pH levels are parameters that influence the mobility and toxicity of arsenic. In M-4, the pH is 6.97 (almost neutral). At a pH close to 7, arsenate [As(V)] is in its most mobile ionic forms. Arsenic is released from iron and clay particles in the soil and passes into the liquid phase, where it can directly attack enzymes such as ARYL-S and URE, whose concentrations were not detected in this study (Aponte et al., 2020; Trasar-Cepeda et al., 2000).

Sample M-4 has a value of 8.179 mg/kg of carbonates that compete for the same adsorption sites as arsenic on the surface of soil minerals. When carbonates are present, as in M-4, they displace arsenic from the minerals, increasing its bioavailability (Puglisi et al., 2005).

This explains why, despite having very high PHOS activity (445.95 mU/gDS), the other enzymes are completely inactive; there is so much free arsenic due to the carbonates that the biological system is saturated with the toxin (Trasar-Cepeda et al., 2000). The arsenic is not “trapped” in the soil; it is free and active. The pH close to 7 and the carbonate levels are acting as a vehicle that delivers the arsenic directly to the enzymes, completely blocking the nitrogen and sulfur cycles. When compared to other samples such as M-1 (pH 7.14 and carbonates of 7.454 mg/kg), it can be deduced that M-4 has a chemical balance that is more conducive to the release of the metalloid.

The ratio between As(III) 0.722 mg/kg and As(V) 5.328 mg/kg in M-4 confirms that the most mobile form is As(V), approximately 7 times more than As(III) (Hughes, 2002).

Although As(V) predominates in quantity, the As(III) present is sufficient to cause the collapse of the other enzymes, which have undetectable values for ARYL-S and URE. As(III)

is much more potent in blocking the sulfur groups of these enzymes, so even in smaller amounts, it is toxic to the biological health of the soil (Hughes, 2002).

The combination of neutral pH, high carbonates, and a predominance of As(V) creates a perfect synergy. Arsenic is not only present, but it is in its most mobile form, competing with phosphorus and nullifying microbial life (Dixit and Hering, 2003).

When comparing sample M-4 with M-9 (Table.2), a stark contrast is observed: while M-4 is a “biologically dead” soil due to arsenic toxicity, M-9 shows remarkable biological resilience, with active and healthy nutrient cycles (nitrogen and carbon).

Table 2. Biological Meaning of the enzyme results in samples M-4 and M-9

Enzyme	M-4 (Toxic Profile) mU/gDS	M-9 (Healthy Profile) mU/gDS	Biological Meaning
NAG	nd	16,481	M-9 has the ability to degrade chitin and recycle nitrogen, a process that is not possible in M-4.
URE	nd	14,211	Urea is processed perfectly in M-9, while in M-4 the nitrogen cycle is blocked..
β GLUCO	0,0018	0,0032	Carbohydrate degradation is almost twice as efficient in M-9-1.
PHOS	445,95	91,105	In M-9, phosphatase has a balanced value, indicating that phosphorus does not compete for arsenic as in M-4.

When observing the arsenic species in both samples, M-4 has 5.328 mg/kg of As(V). This is a very high and mobile toxic load (Abbas et al., 2018). M-9-1 has 4.888 mg/kg of As(V). Although the concentration is similar, its impact is much lower and it has a pH of 7.55 (more alkaline) compared to 6.97 for M-4.

In more alkaline soils, arsenic may be interacting differently with minerals, allowing the microbial community to develop resistance mechanisms or making the metal less “available” to attack enzymes (Dixit and Hering, 2003). These results are reflected in the high concentrations of NAG (16,481 mU/gDS) and URE (14,211 mU/gDS). Despite the presence of arsenic, their enzymes function at full capacity. This suggests that at this point the soil has a higher quality of organic matter or an adapted microbiota that “sequesters” the arsenic, preventing it from blocking the active sites of the enzymes (Guan et al., 2024).

In contrast, M-4 showed severe impairment of soil biological functioning, characterized by very low dehydrogenase activity and the loss of urease and arylsulfatase activity, consistent with the inhibitory effects of metal(loid) contamination on soil enzymatic processes (Trasar-Cepeda et al., 2000; Aponte et al., 2020)

By analyzing the TOC (Total Organic Carbon) and Conductivity values, we find the explanation for why sample M-9 is so resistant and healthy, while M-4 has collapsed. The difference in organic content is the main key to protection. M-4 has a TOC concentration of 22,917.25 mg/kg, while M-9 has a TOC of 27,000.00 mg/kg (approximately 18% more) (Guan et al., 2024). Organic matter acts as a chemical “sponge.” The functional groups of TOC trap [As(V)], preventing it from circulating freely in the soil water. In M-9-1, there is greater immobilization capacity to sequester As, leaving the NAG, URE, and β GLUCO enzymes free (Aftabtalab et al., 2022).

Comparison between the M study samples and the MC control. The fundamental difference lies in how the presence of arsenic has fractured biological health in the M samples compared to the stability of the MC.

The contrast in enzymatic activity reveals a collapse in the vital functions of the contaminated soil:

- Cycle Cancellation (N and S): While MC samples maintain functional levels of NAG (14.19 mU/gDS), URE (13.30 mU/gDS), and ARYL-S (14.58 mU/gDS), samples such as M-4 show an undetectable value. This indicates that arsenic has interfered with the soil's ability to recycle N and S (Trasar-Cepeda et al., 2000).

- Carbon Resilience: The β GLUCO enzyme is the most resistant; in the Study Samples, it retains about 43.9% of the activity of the Control Samples, suggesting that it is the last metabolic cycle to fail in the face of toxicity (Wang and Mulligan, 2006).

The comparison explains why the control samples are generally healthy and why some study samples, such as M-9, manage to survive:

- The control has a mineral balance that immobilizes metals. M-9 matches this health because it has 27,000 mg/kg of Fe, the same level as the control, acting as a magnet that traps arsenic (Aponte et al., 2020; Trasar-Cepeda et al., 2000)

- M-4 has a deficit of organic matter compared to the control samples (22,917 vs. 27,000 mg/kg). Without this “buffer,” arsenic is free to attack enzymes, unlike in the control sample, where TOC protects the system (Wang and Mulligan, 2006).

- The Study Samples show severe degradation, where As has interfered with the soil chemistry. Only those samples with high levels of Fe and TOC come close to the performance of the Control Samples.

4.4 Enrichment Factor (EF)

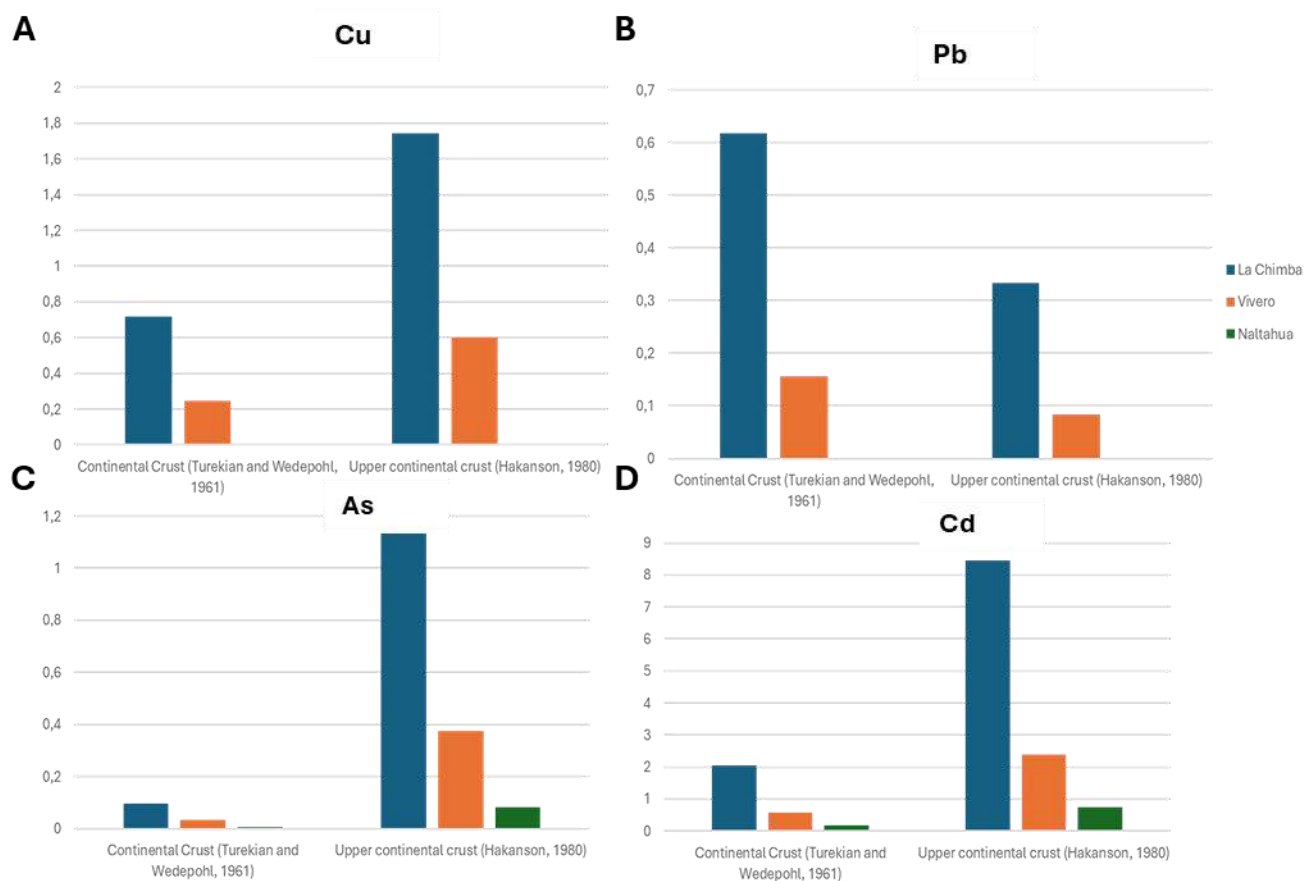


Fig.5 Enrichment Factor of samples applied to soil international references

It can be observed that for the element Cu, when comparing with levels presented as possible local background levels (Valdés and Tapia, 2019), the EF indicates that the La Chimba sector has a value of 1.7, which indicates a Minor Enrichment. However, when comparing the background levels presented by CENMA, 2014. All sectors have values less than 1, indicating no enrichment of this metal.

The enrichment factor for the element Pb using both background levels indicates that there is no enrichment of this metal in the soils of the different areas.

In the case of the element As, the La Chimba sector showed minor enrichment with a value of 1.13 when using the values referenced by Upper Continental Crust. In the case of Turekian and Wedepohl, 1961, the sectors show no enrichment.

Cd showed severely moderate enrichment in the La Chimba sector and minor enrichment in the Viverra sector when using the values presented by Upper Continental Crust. However, when using the levels of Turekian and Wedepohl 1961, only the La Chimba sector shows enrichment, which is minor.

The Enrichment Factor (EF) analysis shows that the interpretation of the degree of contamination depends significantly on the selected background value, whether local or global. This variability highlights the importance of using geochemical references that are representative of the geological context of the study area.

From a spatial perspective, the La Chimba sector consistently presents the highest EF values for most of the elements evaluated, which could indicate greater relative anthropogenic pressure compared to the other sectors. However, in general terms, most of the metals analyzed show low or non-existent enrichment.

4.5 Geological Index (Igeo)

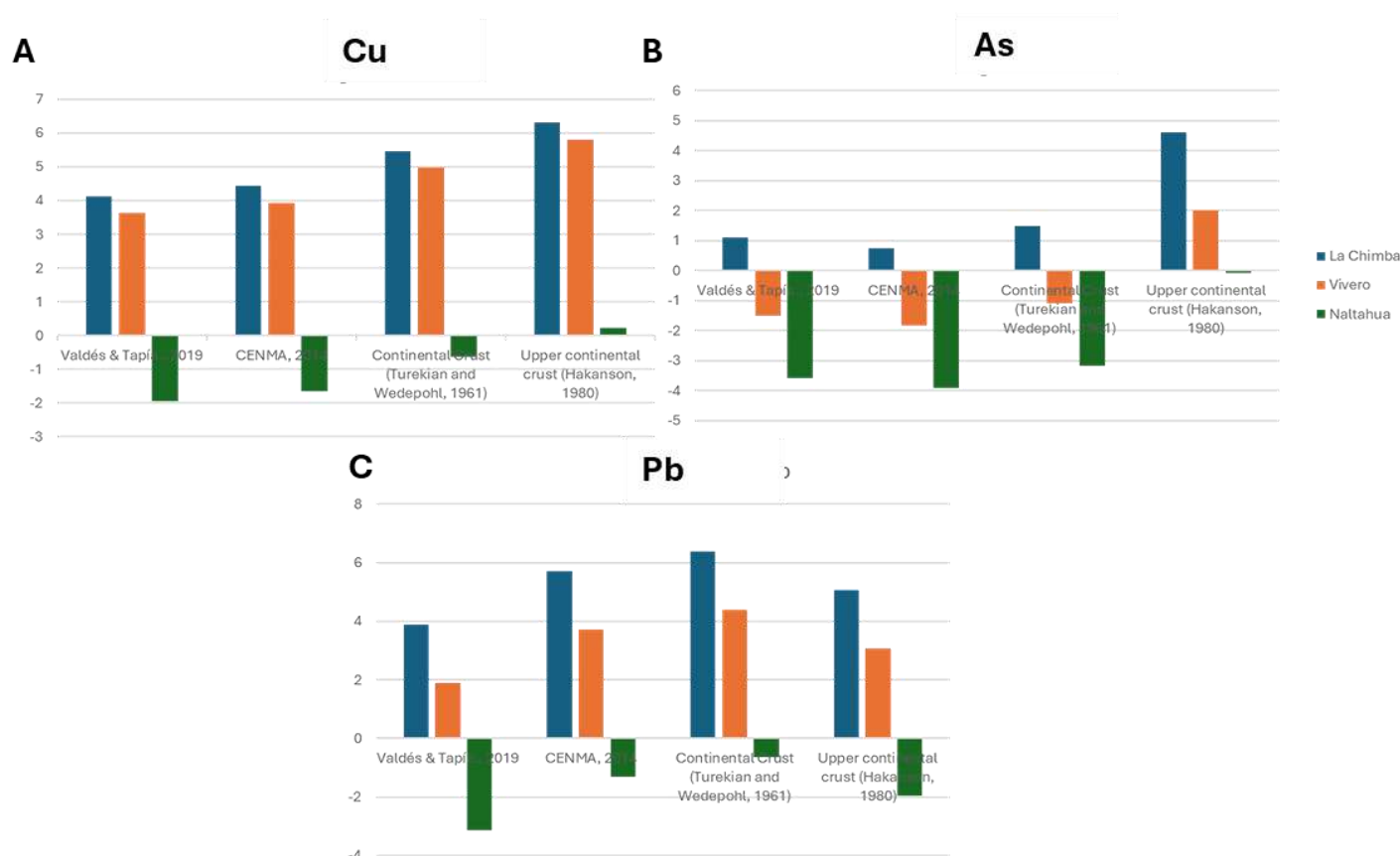


Fig. 6 Geological Index of samples applied to local and international references concentration.

In the case of the Geological Index (Fig. 6), local and foreign base levels were used, with the La Chimba sector presenting the highest values when compared to local values (Valdés and Tapía, 2019; CENMA, 2014). The La Chimba sector showed strong to extreme contamination, while the Vivero sector showed strong contamination. Metal concentrations in Naltahua are below the concentrations presented in the local values and those of Turekian and Wedepohl, 1961.

In the case of Cu (Fig 6.A) When compared with international values, the La Chimba sector shows extreme contamination, as it is greater than 5, as does the Vivero sector, which shows values close to and above 5.

This indicates that there is contamination in these areas, which highlights the need to characterize the soils in the region in order to generate comparisons that are more in line with the reality of the location.

The results obtained for As (Fig. 6.B) using local values and Turekian and Wedepohl, 1961, indicate no contamination in general to moderately contaminated for the La Chimba sector.

When compared with the Upper Continental Crust, the La Chimba sector shows strong to extreme contamination of As and the Vivero sector as moderately contaminated.

For Pb (Fig. 6.C), we can observe that the La Chimba sector shows strong to extreme contamination when compared to local and international baselines, while the Vivero sector shows moderate to strong contamination, with values mainly between 2 and 4.

Overall, the results demonstrate that contamination classification is highly sensitive to the selected reference baseline. In metallogenic regions such as northern Chile, the establishment of robust, site-specific geochemical background values is essential to avoid misinterpretation of contamination levels.

The data support the interpretation that La Chimba and, to a lesser extent, Vivero are affected by anthropogenic metal enrichment, while Naltahua represents background geochemical conditions. These findings reinforce the importance of integrating local baselines, spatial analysis, and geochemical behavior in environmental contamination assessments.

4.6 Contamination Factor

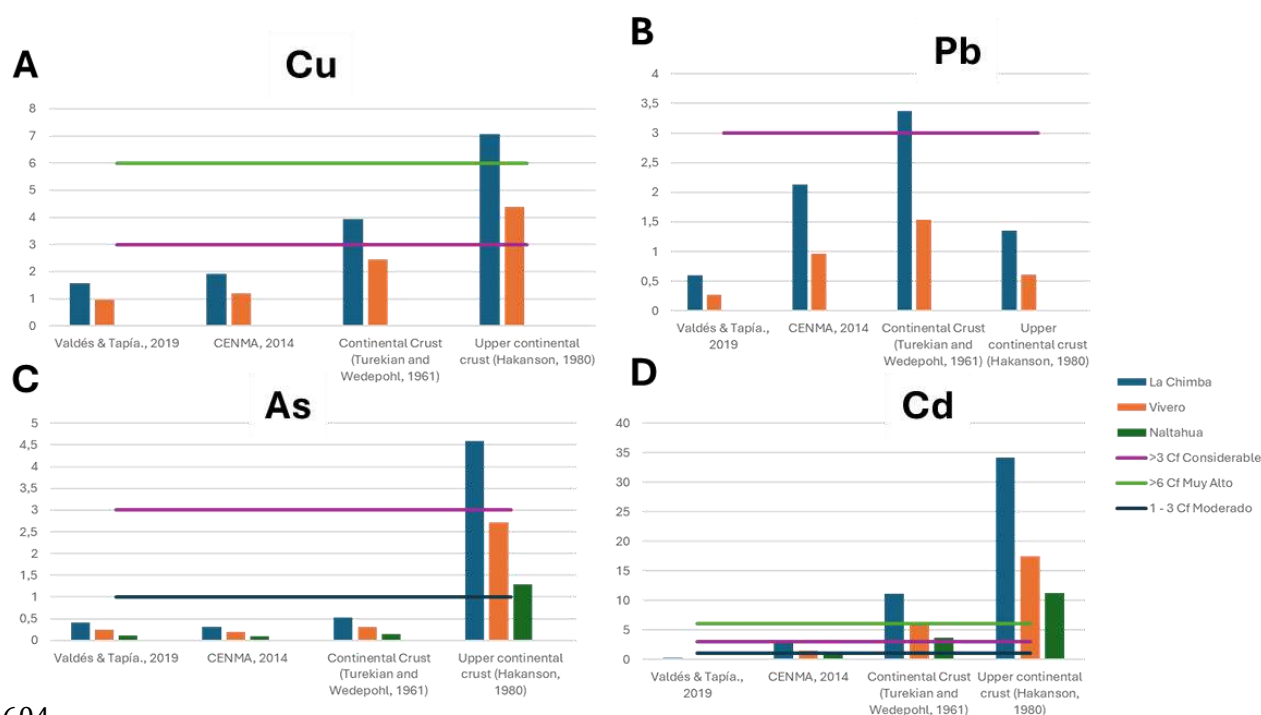


Fig.7. Contamination Factor of samples applied to local and international concentration references in soil.

The Contamination Factor (CF) (Fig. 7) results show marked variability depending on the selected reference baseline, reinforcing the sensitivity of contamination indices to background definitions.

For **Cu** (Fig. 7.A), moderate CF values were observed in La Chimba and Vivero when local baselines (Valdés and Tapía, 2019; CENMA, 2014) were applied. However, when international reference values such as Turekian and Wedepohl (1961) and the Upper Continental Crust (UCC) were used, CF values increased substantially, reaching considerable to very high contamination levels in La Chimba. This trend indicates that Cu enrichment is pronounced relative to global crustal averages but less extreme when evaluated against regionally representative baselines. Such differences likely reflect both anthropogenic inputs and the naturally elevated Cu background characteristic of metallogenic terrains in northern Chile.

For **Pb** (Fig. 7.B), CF values were low for all sectors when compared to the local baseline proposed by Valdés and Tapía (2019), suggesting that Pb concentrations in the study area are comparable to regional background conditions. However, when compared with CENMA (2014) and international values, CF values increased to moderate levels, and in some cases considerable contamination was observed (particularly when compared with Turekian and Wedepohl, 1961). This pattern suggests that although Pb may not exceed locally established background thresholds, it is enriched relative to global averages, potentially reflecting cumulative anthropogenic deposition.

In the case of **As** (Fig 7.C) CF values remained low across the three first comparisons (local baselines and Turekian and Wedepohl, 1961). Nevertheless, when UCC values were applied, La Chimba reached considerable contamination levels, while Vivero and Naltahua exhibited moderate contamination. This discrepancy suggests that arsenic concentrations are not anomalous within a regional context but may exceed global crustal averages. Given the redox-sensitive nature of As and its association with Fe and Mn oxides, its distribution likely reflects both lithogenic background and localized anthropogenic influence.

The most pronounced variations were observed for **Cd** (Fig. 7.D). When compared with Valdés and Tapía (2019), CF values were generally low. However, using CENMA (2014), La Chimba exhibited moderate to considerable contamination. Under international reference baselines (Turekian and Wedepohl, 1961; UCC), Cd showed very high CF values across the study area, particularly in La Chimba. This suggests that Cd enrichment is significant relative to global crustal concentrations and may represent a strong anthropogenic signal, given the typically low natural abundance of Cd in soils.

4.7 Pollution Load Index

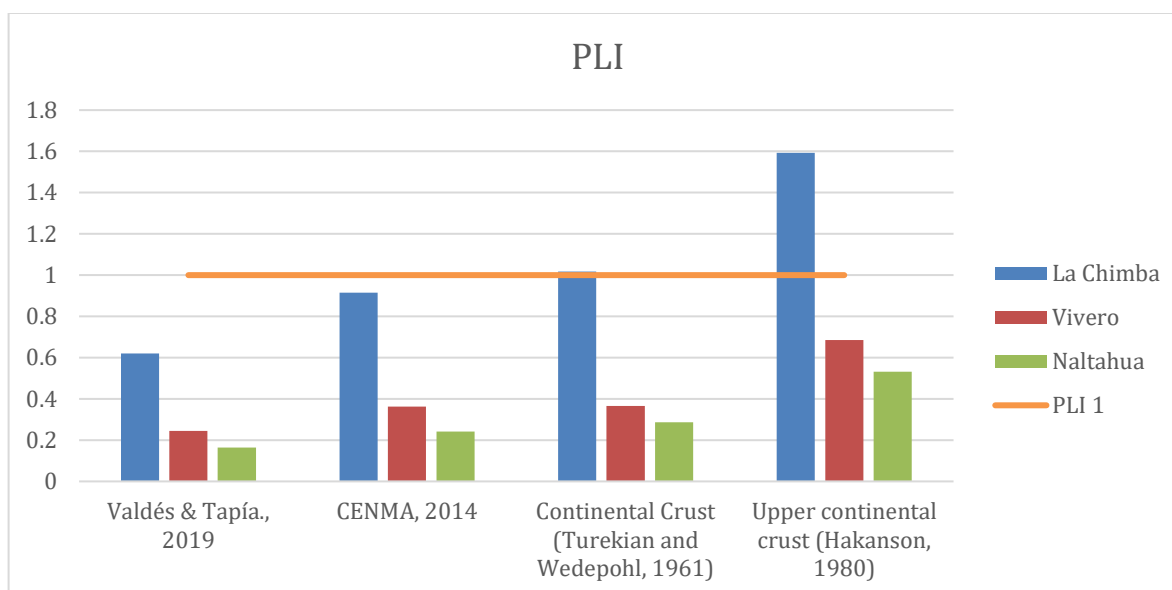


Fig.8. Pollution Load Index of samples applied to local and international concentration references in soil.

In the case of the PLI (Fig. 8), when comparing our results with the referenced local baseline values, the PLI value is less than 1, indicating that these sites are not contaminated, at least in terms of the elements as a whole. In the case of international baseline values, these do indicate that there is contamination in the La Chimba sector in Antofagasta. Due to these differences, it is necessary to create national and even regional environmental regulations. In this way, it will be possible to accurately represent the reality of the situation in northern Chile in this case.

4.8 Environmental Risk Assessment

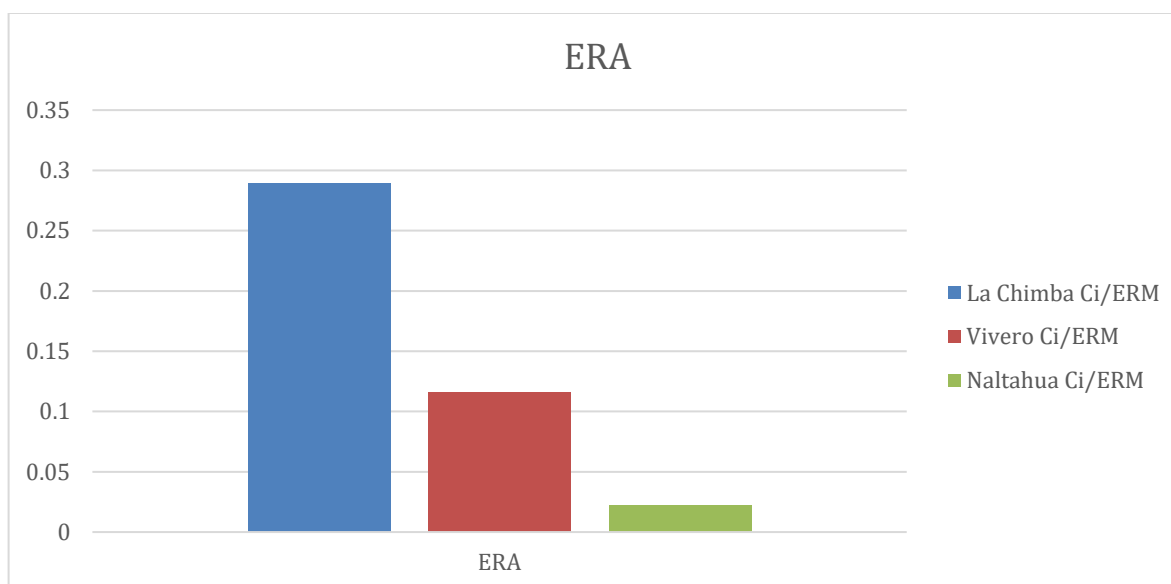


Fig.9. Environmental Risk Assessment by sites in study.

Finally, by comparing the ERA (Fig. 9) of each site, we can determine which has the highest probability of toxicity, which indicates a 21% probability of toxicity with a value close to 0.3. In the case of the Vivero sector, it has a value slightly above 0.1, which reveals a 9% probability of toxicity, and the Naltahua sector is not within the range, thus presenting no probability of toxicity when using this risk assessment.

Importantly, the observed discrepancies between local and international baseline comparisons highlight the strong influence of background selection on contamination classification. In metallogenic regions such as northern Chile, naturally elevated metal concentrations may coexist with anthropogenic inputs, emphasizing the need for region-specific geochemical baselines and regulatory thresholds. The integration of multiple indices therefore provides a more robust and realistic assessment of environmental quality, allowing discrimination between natural enrichment and anthropogenic contamination.

Overall, the results indicate that urban soils in La Chimba are affected by cumulative metal enrichment associated with anthropogenic activity, whereas Vivero shows moderate influence and Naltahua reflects background geochemical conditions. This integrated approach demonstrates the importance of combining contamination indices with ecological risk metrics to achieve a comprehensive environmental assessment in arid, mining-influenced regions.

5. Statistical treatment

5.1 Principal Component Analysis (PCA)

For the PCA the variables included were:

Heavy metals: As, FM, As(III), As (IV), Cd, Cu, Fe, Mn, Ni, Pb, Hg, Cr

679 **Enzymes:** A. GLUCO, B. GLUCO, B. GAL, PHOS, NAG, ARYL-S, URE, DH, PHE.

680 **Physicochemical parameters:** C, TOC, pH, Conductivity, Cl⁻, SO₄⁻², Mg⁺², Ca⁺², CO₃⁻²,
681 NO₂⁻, Humidity.

682 The standardization method used was Z-Score due to the difference in units of the different
683 variables in the data. This was obtained from the basic R package with the code:

684 data_scaled <- scale(data_clean %>% select(-MUESTRA, -Grupo))

685 The criteria for selecting components were based on the Kaiser Criterion and the Scree Plot:
686 The Kaiser Criterion showed that the first four components had values greater than 1,
687 indicating a significant contribution to the variance. The Scree Plot was then used to
688 determine which components explain the highest percentage of variance, with PC1 at 72.9%
689 and PC2 at 8.8% being the highest. Therefore, these dimensions were used to perform the
690 PCA (Appex).

691

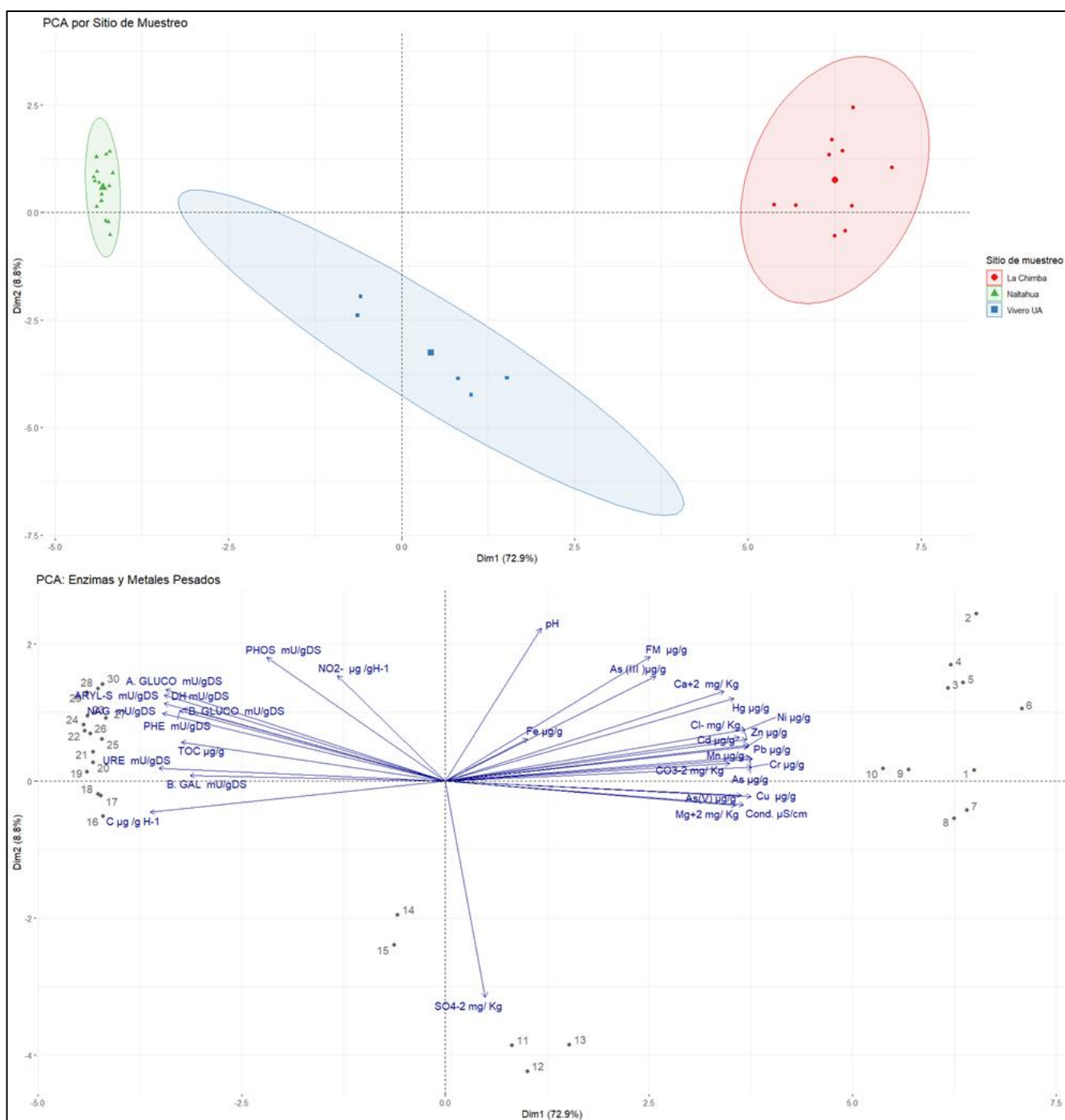


Fig. 10. PCA: Sampling Sites and Enzymes with heavy metals

Principal component analysis (PCA) (Fig. 10) allowed us to visualize the multivariate differentiation between sampling sites. Dimension 1 explained 72.9% of the total variability, while Dimension 2 contributed 8.8%, indicating that the model adequately represents the main trends in the data. Clear, non-overlapping clusters were observed among the three sites studied: La Chimba, Vivero UA, and Naltahua.

The Naltahua site showed a highly compact grouping towards positive values of Dim1, suggesting internal homogeneity and a distinctive compositional profile, possibly associated with its status as an ecological reference site. In contrast, the La Chimba samples were grouped towards negative values of Dim1, showing a relatively homogeneous structure, but clearly differentiated from Naltahua. Finally, UA Nursery was located in an intermediate position, although with greater dispersion, reflecting greater heterogeneity among its samples.

The clear spatial separation between the groups indicates marked differences in the variables measured between sites. Taken together, these results suggest that the environmental condition of the Naltahua site differs significantly from the urban sites of La Chimba and Vivero UA, which may be related to differential levels of anthropogenic disturbance, pollution, or soil management.

On the factorial plane, **two opposing clusters of variables** were observed. On the one hand, **soil enzymes** (PHOS, DH, ARYL-S, PHE, B. GAL, URE, NAG, etc.) were grouped in the **right quadrant**, together with variables such as **pH** and **NO₂⁻**, which were also positively associated with soil biological activity. On the other hand, **heavy metal concentrations** (Cu, Pb, Cd, Hg, As, and their species), as well as other elements such as Cr, Ni, and FM, were located in the **left quadrant**, indicating a clear functional separation between contaminant variables and enzymatic bioindicators.

This pattern suggests a **generalized inverse relationship** between metal contamination levels and soil functionality. The distribution of samples in PCA space also shows a **clear spatial separation between sites**: samples from La Chimba (M) are clustered in the metal-dominated zone, while those from Naltahua (N) are located toward the enzyme side, reflecting a functional gradient at the edaphic microbial community level.

Overall, the PCA statistically validates that the presence of heavy metals in the La Chimba wetland is associated with a significant reduction in soil enzyme activity and confirms the usefulness of these variables as key interpretive axes in the ecological monitoring of contaminated urban soils.

5.2 HEATMAP. Correlation between enzymatic activity, heavy metals, and physicochemical parameters

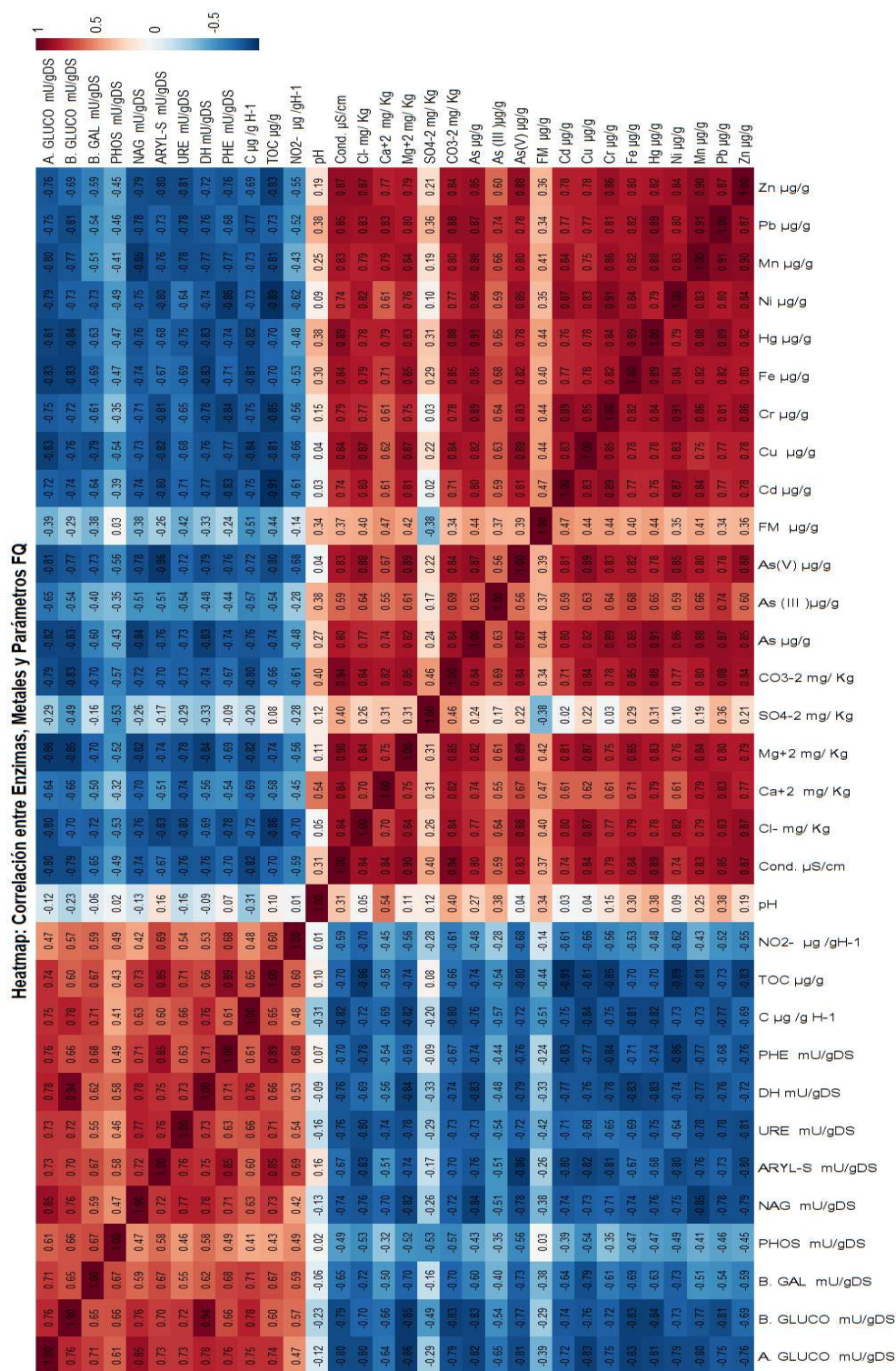


Fig. 11.- Heatmap Correlation between enzymes, metals, and physicochemical parameters

Due to the skewness of the data, the Spearman correlation was used for the analysis between biological (enzymatic activity), geochemical (heavy metals), and physicochemical soil variables which revealed consistent and significant patterns (Fig. 11). In general, a marked **negative correlation** was observed **between enzyme activity and heavy metal concentration**, suggesting functional inhibition of the edaphic microbiome in the presence of metal contamination.

The metals **Cu, Pb, Hg, Cd, and As** (in their total, As(III), and As(V) forms) showed strongly negative correlation coefficients ($r < -0.80$) with multiple enzymes. For example, **dehydrogenase (DH)** showed $r = -0.93$ with Cu and $r = -0.94$ with Pb, while **acid phosphatase (PHOS)** showed $r = -0.90$ with Cu and $r = -0.92$ with Hg. Similarly, enzymes involved in the carbon and nitrogen cycles, such as **NAG** and **URE**, recorded negative correlations greater than -0.85 with at least five different metals, reinforcing their role as sensitive bioindicators of soil toxicity.

In turn, **high positive correlations** were observed **between heavy metals** ($r > 0.85$), especially between Cu, Pb, Hg, Ni, Cr, and As, suggesting a possible **common source of contamination** and synergistic distribution in soil matrices.

On the other hand, **enzyme activity was positively correlated with favorable soil conditions**, such as **soil pH** ($r > 0.65$ for most enzymes), **total organic carbon content (TOC, $r \approx 0.60-0.70$)**, and **nitrite concentration (NO_2^-)**, with values up to $r = 0.76$ in the case of NAG and B. GAL. This indicates that soils with better chemical quality and greater nutrient availability have more active and resilient biological functionality.

This set of biochemical alterations suggests a loss of edaphic functionality, reflected in the decline of essential processes such as nutrient mineralization, organic matter decomposition, and microbial respiration. Such inhibition is probably due to the presence of high concentrations of heavy metals in the soil, as evidenced in geochemical analyses, where elements such as **As, Cu, Pb, Cd, Zn, and Ni** greatly exceeded background levels.

The strength of inhibition depends on the affinity of the metal for the enzyme. Inhibition of β -glucosidase follows the sequence: $\text{Cd} > \text{Zn} > \text{Cu} > \text{Co}$ (Kandziora-Ciupa et al., 2021). Urease inhibition follows the sequence: $\text{Cr} > \text{Cd} > \text{Zn} > \text{Mn} > \text{Pb}$ (Opande et al., 2025). The combined presence of metals such as Cd and Zn produces a negative interaction due to competition for binding to sorption sites (Sethi and Gupta, 2015). The same occurs with the combined presence of Cu and Cd (Liu et al., 2024). Metals, including heavy metals, are present in high concentrations in soils. Some of the metallic elements are essential micronutrients for microorganisms. Zn, for example, is necessary for the functioning of many enzymes such as carbonic anhydrase, some dehydrogenases, DNA and RNA polymerase, t-RNA transferase, etc., but at high concentrations it causes enzyme inhibition.

This evidence is consistent with previous studies (e.g., Zerrari et al., 2023; Nikolova et al., 2023) that have documented the sensitivity of these enzymes to metals such as As, Pb, Cu, and Cd. It has been proposed that the toxicity of these elements inhibits the synthesis of

intracellular enzymes and alters the structure of the microbial community, especially in soils with low- r organic matter retention capacity and high abiotic stress, as is characteristic of the Atacama Desert.

Our results show a marked inhibition of key enzymes such as dehydrogenase, phosphatase, and urease in the La Chimba wetland, which is consistent with the global meta-analysis by Aponte et al. (2020), which documents a generalized reduction of between 30% and 70% in enzymatic activity in soils contaminated with heavy metals. However, unlike what has been reported in urban soils near roads, where surface enzyme activity remains relatively high due to the greater presence of organic carbon (Jaworska and Lemanowicz, 2019), in La Chimba, uniform inhibition is observed even in the most superficial horizons, reflecting more severe edaphic stress. Likewise, the negative correlation found between urease and N-acetylglucosaminidase activity with Cu and Pb is consistent with that reported by Bartkowiak et al. (2020), who demonstrated that the bioavailability of these metals, rather than their total concentration, explains the functional loss of the edaphic microbiome. Complementarily, Yang et al. (2017) showed that in contaminated soils, the rhizosphere maintains higher enzymatic activity than the surrounding soil, highlighting the role of vegetation in mitigating the impact of metals, an aspect that could be explored in restoration plans for La Chimba. Finally, experimental studies with cadmium (Cd) in agricultural soils confirm a rapid and significant inhibition of catalase, urease, and dehydrogenase (Yeboah et al., 2021), a pattern that is also observed in La Chimba, but under a scenario of chronic and multi-element contamination.

Taken together, these results support the hypothesis that geochemical contamination directly affects soil functional integrity and position enzymes such as **DH, PHOS, NAG, and URE** as integrative indicators of the ecological status of the edaphic system in contaminated urban environments.

6. Conclusion

The results of this study show significant geochemical and biological alteration in the soils of the La Chimba Urban Wetland, attributable to the chronic accumulation of heavy metals from urban and industrial activities. The presence of high concentrations of **arsenic, copper, lead, cadmium, mercury, chromium, and zinc**, especially in La Chimba, was significantly associated with **a generalized reduction in the activity of key enzymes** involved in carbon, nitrogen, phosphorus, and sulfur cycling.

The analysis of soil enzyme activity is a very promising method for conducting studies on soil quality, as these are closely related to the biological activity present.

In particular, **dehydrogenase, acid phosphatase, urease, N-acetylglucosaminidase, and β -galactosidase** showed critically low levels in soils with higher metal loads, while at the ecological reference site (Naltahua), where metal levels were low or zero, high and diversified enzyme activity was maintained.

Enzymatic activity reflects heavy metal contamination. However, inhibition does not depend solely on the metal. There are many factors that influence metal-enzyme interaction, making it virtually impossible to say which of them affects each one more or less. Continuing to study enzyme activity under different conditions will be crucial to gaining an in-depth understanding of all the variables that define metal-enzyme interaction. This will enable the creation of a simple yet powerful tool that can be integrated into risk analysis in the future.

Finally, this work contributes to applied knowledge about the functional degradation of urban soils in arid areas and reinforces the importance of actively managing ecosystems declared as urban wetlands and nature sanctuaries, such as La Chimba, based on scientific evidence.

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1040 **Declaration of Competing Interest**

1041 The authors declare that they have no known competing financial interests or personal
1042 relationships that could have appeared to influence the work reported in this paper.

1043 **Data availability:**

1044 The authors declare that the data supporting the findings of this study are available
1045 within the paper and its Supplementary Information files. Should any raw data files
1046 be needed in another format they are available from the corresponding author upon
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1048 **Supplementary Data:**

1049 Appendix A

1050 Appendix B

1051 Appendix C

1052 **Declaration of generative AI and AI-assisted technologies in the writing**
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1054 During the preparation of this work the author(s) used "ChatGPT 5o" in order to re-
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1056 the content as needed and take(s) full responsibility for the content of the published
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