Environmental monitoring of La Concordia mine (Salta, Argentina): assessing heavy metal bioaccumulation and physiological responses of Parastrephia quadrangularis.

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Abstract

The Puna region is distinguished by its extreme environmental conditions and highly valuable mining resources. However, the unregulated management of mine tailings poses a significant threat to the ecological integrity of this region. This study presents a comprehensive investigation to assess the environmental impacts of mine tailings at La Concordia mine (Salta, Argentina) and examines the physiological and biochemical adaptations of *Parastrephia quadrangularis* that enable its survival under this extreme conditions. Our findings reveal that prolonged weathering of mine tailings results in the generation of acid mine drainage characterized by low pH levels (< 3.5) and elevated concentrations of As, Fe, Cu, Pb and Zn. These levels exceed drinking water standards by 5–10 times for As, 6–13 times for Zn, 80–120 times for Pb, 20–380 times for Fe and 4–10 times for Cu. Soil analyses highlights low pH, high salinity and elevated concentrations of Zn (310 mg kg^{-1}), Pb (153 mg kg^{-1}) and Cu (128 mg kg^{-1}). Despite these harsh environmental conditions, 7 plant species where identified, with *Parastrephia quadrangularis* being the only species present at the most polluted site. This species exhibits high heavy metal bioaccumulation and robust tolerance mechanisms against heavy metal-induced oxidative damage, as evidenced by stable total chlorophylls and malondialdehyde content, and increased levels of carotenoids, proline and phenolic compounds. These findings emphasize *Parastrephia quadrangularis* as a promising candidate for revegetation and phytostabilization for sustainable mine closure programs in La Puna region.

Introduction

The Puna region in Argentina encompasses an extensive high mountain plateau, with altitudes ranging from 3500 to 4700 meters above sea level (m.a.s.l.). This region is characterized by challenging environmental conditions, including extreme aridity, significant temperature variations, hypersalinity, acid hot springs, strong winds, low atmospheric humidity and elevated levels of ultraviolet (UV) radiation (Nieva et al., 2018). This area is sparsely inhabited, primarily due to its extreme environmental conditions and its distance from densely populated urban centers. Following Spanish colonization, medium-scale mining practices emerged, targeting copper (Cu), gold (Au), silver (Ag), lead (Pb) and zinc (Zn) (Gil Montero, 2018). Some of these mines ceased operations in the late twentieth century, leaving their wastes exposed to weathering processes for decades. The prolonged exposure of these wastes to environmental conditions has led to the contamination of soil and water, posing substantial negative impacts on both environmental quality and human health. This issue is not only a local concern but also a regional environmental problem affecting the Andean regions of Bolivia, Chile and Peru (Cacciuttolo & Cano, 2022). While the environmental impact of mine operations on nearby ecosystems is well-documented, only a limited number of studies have been conducted in the Northwest region of Argentina (NOA) (Nieva et al., 2016; Murray et al., 2021; Tapia et al., 2022).

Located in the central-west region of Salta, Argentina, at an altitude of 4200 m.a.s.l., La Concordia mine comprises abandoned mining facilities along with four successive tailing dams beside La Concordia stream, whose headwaters originate from the mine adit. Historically, this mine exploited Pb, Ag, Zn and

Cu until its closure in 1986, leaving the mine tailings untreated and abandoned on-site. This situation represents a hazardous reservoir and a long-term source of heavy metals (HMs) pollution (Nieva et al., 2018). The weathering process, exacerbated by large-scale disturbances characteristic of mining activities and the presence of iron-oxidizing microorganisms in the mine tailings, produces acid mine drainage (AMD), a low pH and HMs/metalloids-rich solution that pollutes water and soil (Tapia et al., 2019). Currently, fluvial erosion has partially destroyed the walls of the tailing dams, allowing La Concordia stream to seep through them. During the summer months, the stream experiences increased flow and eventually joins the San Antonio River, a vital source of water for San Antonio de los Cobres, the most populous town in the Argentine Puna and the closest populated center to the mine. Strong winds in the autumn and winter seasons also play a significant role in the dispersal of HMs in this arid region, sometimes observed to be more impactful than water-driven processes (Punia, 2021).

Currently, various physico-chemical techniques are available for mine tailings management. However, achieving sustainable reclamation of mining areas is feasible only through the incorporation of vegetation-based strategies for landscaping, stabilization and pollution control (Liu et al., 2018). To meet this objective, it is necessary to stabilize the surface soil to reduce water and wind erosion while also preventing the release of toxic contaminants into the surrounding environment (Xie & van Zyl, 2020). Usually, improving the vegetation establishment on top of the tailings is a common method for mine reclamation following its closure (Chen et al., 2018). This involves the full reconstruction of ecosystems, comparable to the earliest stages of primary succession following major land disturbances, but within a much shorter timeframe (Huang et al., 2012). This can be achieved through the implementation of complex technical restoration approaches, such as conserving native seed banks, improving soil through engineering techniques and designing landscapes tailored to specific local climatic conditions (Martins et al., 2020). One phytotechnology that can be used for revegetation is phytostabilization, which involves the establishment of tolerant vegetation capable of immobilizing HMs in the rhizosphere. In this process, HMs can precipitate and stabilize as stable minerals or be absorbed and accumulated in roots without transferring to aboveground parts. Phytostabilization also involves the use of native plant communities that can easily survive and prevent the spread of invasive species, thereby reducing the risk of biodiversity decline and local species replacement (Muthusamy et al., 2022).

However, establishing vegetation on mine tailings presents a formidable challenge due to the adverse conditions resulting from pollution (Xie & van Zyl, 2020). Careful selection of acid-tolerant and HM-tolerant plant species is crucial to ensure their survival and growth in such harsh conditions (Gastauer et al., 2020). Once established, these plants play a crucial role in reducing the mobility of HMs through various interactions, including bioaccumulation and bioadsorption facilitated by root exudates, which influence the physiochemical properties of the rhizosphere (Gil-Loaiza et al., 2016). The rhizosphere also acts as an efficient biogeochemical barrier, controlling the transport of HMs from tailings to groundwater (Venkateswarlu et al., 2016). Additionally, vegetation serves as protective ground cover, effectively preventing erosion and maintaining long-term soil stability (Zine et al., 2020).

To effectively manage species, it is crucial to investigate not only the uptake rate of HMs but also the mechanisms that regulate their response to this stress, as these could serve as potential bioindicators of HMs toxicity and pollution. Despite the significant environmental threat posed by HMs in the La Concordia mine and stream, which affects surrounding ecosystems and poses substantial risks to the population of San Antonio de los Cobres, there has been limited research into the biomonitoring and phytostabilization capacity of native plant species. However, previous studies have shown the predominance of the genus *Parastrephia*, which is not only one of the most important genera in this region, but also includes a species, *P. quadrangularis*, that often grows very close to mine tailings (Plaza-Cazon et al., 2021).

The objective of this study was to assess the ecological impact of mine tailings from La Concordia mine and to investigate the physiological and biochemical strategies of *P. quadrangularis* that enable its survival under extreme environmental and pollution conditions. These findings may contribute to the selection and management of suitable plant species for phytoremediation and mine closure programs in the Puna region.

Materials and methods

Study area and samples collection

The La Concordia mine is located within the Los Andes district of the Province of Salta, approximately 15 km WNW (west-northwest) of the town of San Antonio de los Cobres (24°13'32"S 66°19'09"W). The climate in the region, controlled by the South American Summer Monsoon system, is characterized by intense exposure to UV radiation and extreme aridity, with highly seasonal rainfall totaling 200 mm annually, of which approximately 70% occurs during late spring-summer months (November to February), followed by eight months of dry conditions. The region experiences an average annual temperature range of 3°C to 13°C, with summer temperatures reaching 40°C and winter night soften dropping to -25°C. South-easterly summer winds are generally weak, whereas westerly winter winds are substantially stronger during the dry season, with winds blowing at velocities ranging from7 km h^{-1} to 72 km h^{-1}, persisting for 10 to 18 hours daily (Gaiero et al., 2013). The mining site encompasses four tailing dams strategically positioned downstream on the right bank of La Concordia stream. Currently, the tailings deposits cover an area of 8537 m^2, with a calculated volume of 17074 m^3 (Plaza-Cazon et al., 2021).

Sampling was conducted during the first week of October. Four sites within the area influenced by the tailing dams, along with an additional control site, were subjected to analysis (Table 1). The control site was located north of the mine and upstream of La Concordia stream, deliberately positioned opposite to the dominant NW-SE wind direction, to ensure minimal influence from mine-related pollution (Fig. 1). At each designated site, a 25 m^2 plot was established for plant collection purposes. Simultaneously, soil samples were collected at a depth of 30 cm from the same points where plant samples were taken. Root samples were collected at depths of 30–50 cm. Liquid samples were collected from the section of La

Concordia stream that is affected by AMD, and the San Antonio River (Table 2). Geographical coordinates were measured and recorded using a Garmin GPS 73 (USA).

Table 1
Geographical coordinates of plants and soil sampling sites.

Sampling point identification	Height (m.a.s.l.)	Latitude (S)	Longitude (W)	Distance to the mine adit (m)
Site 0 (Control)	4154	24°11’47.79”	66°24’2.49”	418
Site 1	4112	24°12’0.39”	66°24’3.7”	76
Site 2	4104	24°12’2.9”	66°24’1.19”	179
Site 3	4112	24°12’6”	66°24’1.4”	267
Site 4	4106	24°12’7.3”	66°23’59.6”	347

Table 2
Geographical coordinates of acid mine drainage (AMD) sampling sites.

Samples identification	Height (m.a.s.l.)	Latitude (S)	Longitude (W)	Distance to the mine adit (m)*
AMDA	4117	24°12’1.8”	66°24’5.7”	35
AMD B	4115	24°12’2.1”	66°24’5.2”	48
AMD C	4109	24°12’2.5”	66°24’4.6”	66
AMD D	4103	24°12’2.5”	66°24’1.8”	145
AMD E	4114	24°12’4”	66°24’2”	151
AMD F	4110	24°12’4.5”	66°24’1.1”	180
AMD G	4102	24°12’6.7”	66°23’59.4”	255
AMD H	4100	24°12’10.6”	66°23´56.4”	396
San Antonio River	3784	24°13’51.8”	66°19’26.5”	8602

*Mine adit geographical coordinates: (24° 12' 2.05" (S); 66°24' 6.9" (W)).

Soil and liquid samples characterization

Soil samples from each site were dried at 60ºC for 48 hours, crushed to a fine powder and sieved to remove particles larger than 2 mm. Physicochemical analyses were conducted at the National Institute of Agricultural Technology (INTA) in Cerrillos, Salta, following methodologies established by the Methodological Support System for Laboratories Analyzing Soils, Water, Plants and Organic Amendments (SAMLA), the Argentine Institute for Standardization and Certification (IRAM) and the Secretariat of Agriculture, Livestock, and Fisheries (SAGPyA) (Azcarate et al., 2017). The analyses included the assessment of textural class, saturation capacity, pH, electrical conductivity, calcium and magnesium carbonate content, percentages of organic matter and organic carbon, total nitrogen content, carbon-nitrogen ratio, extractable phosphorus content and exchangeable sodium, potassium, calcium, magnesium and soluble chloride. To determine the presence of HMs, 1 g of dry soil samples were ground and digested using a mixture of HNO_3 and H_2SO_4 (4:1 ratio) following the method proposed by Bonfranceschi et al. (2009).

Liquid samples were characterized in situ by measuring pH, redox potential and temperature using a Hanna Instruments HI 2550 Bench pHmeter (USA). Subsequently, the samples were collected in sterile plastic bottles and transported under refrigeration for further analysis. Arsenic (As) content was determined using a commercial colorimetric kit (Merck MQuant™ Arsenic Test, Germany). Concentrations of Zn, Pb, Fe, Cu, Ni, Cr and Cd in soil and liquid samples were measured using a Shimadzu AA6650F Atomic Absorption Spectrophotometer (Japan).

Plant identification and physiological parameters determination

Fresh plants were collected in plastic bags and transported under refrigeration for further analysis. Taxonomic identification was conducted using a dichotomous key and specialized literature (Boelcke, 1992). For *P. quadrangularis*, the following physiological and biochemical parameters were measured. Total chlorophyll and carotenoid contents were measured from 100 mg of fresh leaves using the method described by Wellburn (1994), with absorbance readings at 647, 664 and 480 nm. Malondialdehyde (MDA) content was assessed from 200 mg of fresh leaves through reaction with thiobarbituric acid, following the method proposed by Heath and Packer (1968). Total phenolic compounds were quantified from 500 mg of fresh leaves using the Folin-Ciocalteu reagent, as described by Singleton and Rossi (1965). Proline content was measured from 100 mg of fresh leaves using the method outlined by Bates et al. (1973), with absorbance read at 520 nm to calculate proline content per unit of fresh weight. Absorbance measurements for all parameters were conducted using a Shimadzu UV-160 spectrophotometer (Kyoto, Japan).

Bioaccumulation of Zn, Pb, Cu and Fe was determined by digesting 500 mg of oven-dried (60°C) root and shoot samples with a mixture of HNO_3 and H_2SO_4 (4:1 ratio), following the method described by Bonfranceschi et al. (2009). Absorbance was measured using a Shimadzu AA6650F Atomic Absorption Spectrophotometer (Japan). The bioavailability factor (BAF), accumulation factor (AF), translocation factor (TF) and bioaccumulation factor (BCF) were calculated using the following equations:

- $BAF = [HM_{roots}]/[HM_{soil}]$
- $AF = [HM_{shoots}]/[HM_{soil}]$
- $TF = [HM_{shoots}]/[HM_{roots}]$
- $BCF = [HM_{biomass}]/[HM_{soil}]$

Where [HM_{shoots}], [HM_{roots}], [$HM_{biomass}$], and [HM_{soil}] represent the HMs concentrations in shoots, roots, total biomass and soil, respectively.

Data analysis

The data were analyzed using analysis of variance (ANOVA) and means were compared using the least significant difference (LSD) test at a significance level of 5% ($p < 0.05$) using InfoStat (2019) software. All results were expressed as mean values with corresponding standard deviations.

Results

Soil and liquid samples characterization results

Soil physicochemical analysis (Table 3) reveal a prevalent sandy textural class across the sampled sites, which is associated with low saturation water capacity percentages. Furthermore, the presence of $CaCO_3$ concretions in sites 0, 3 and 4 indicates dissolution and precipitation phenomena resulting from alternating water movement within the soil profile, coupled with subsequent dry periods. Across all sites, carbon and organic matter content, as well as essential nutrient levels, were observed to be low, as it typical in the Puna region. Site 3 stands out as the most affected by mine pollution, exhibiting high levels of acidity (pH 3), salinity (7 mmhos cm^{-1}) and elevated concentrations of Zn (310 mg kg^{-1}), Pb (153 mg kg^{-1}) and Cu (128 mg kg^{-1}) (Table 4). The highest Fe concentration (17130 mg kg^{-1}) was detected in site 2. Ni, Cr and Cd were not detectable at any of the sites probably due to the detection limit of the spectrophotometer (< 12.5 mg kg^{-1} for Ni, < 5 for Cr mg kg^{-1} and < 12.5 mg kg^{-1} for Cd).

Table 3
Soil physicochemical analysis of the different sites.

Parameter	Site 0	Site 1	Site 2	Site 3	Site 4
Sand (%)	68	78	86	76	80
Silt (%)	20	16	12	16	14
Clay (%)	12	6	2	8	6
Soil texture	Sandy loam	Loamy sand	Sand	Loamy sand	Loamy sand
Saturation capacity (%)	21	29	26	25	23
pH 1:2.5	6.2	6.2	7.2	3.0	5.8
EC ($mmhoscm^{-1}$)	0.20	6.48	0.92	7.00	3.00
Ca and Mg carbonate (%)	$CaCO_3$ concretions	0	2.2	$CaCO_3$ concretions	$CaCO_3$ concretions
Organic carbon (%)	0.67	0.51	0.53	0.13	0.41
Organic matter (%)	1.16	0.88	0.91	0.22	0.71
Total N (%)	0.06	0.06	0.04	0.02	0.04
C/N ratio	11	9	13	7	10
Extractable P (ppm)	14	11	3	18	21
Exchangeable Na (meq 100 g^{-1})	0.2	0.4	0.2	0.1	0.5
Exchangeable K (meq 100 g^{-1})	0.004	0.74	0.79	0.01	0.05
Exchangeable Ca (meq 100 g^{-1})	n.d.*	9.4	n.d.	n.d.	n.d.
Exchangeable Mg (meq 100 g^{-1})	n.d.*	8.1	n.d.	n.d.	n.d.

Note: n.d.* = not detected.

Table 4
Soil heavy metal concentration in the different sampling sites.

Heavy metal (mg kg^{-1})	Site 0	Site 1	Site 2	Site 3	Site 4
Zn	98.2 ± 8.4 b	80 ± 20 ab	67 ± 12 a	310 ± 20 c	90 ± 10 ab
Pb	15 ± 1.4 a	50 ± 10 c	27 ± 4 ab	153 ± 10 d	30 ± 10 b
Fe	12000 ± 200 c	14000 ± 500 d	17130 ± 100 e	8560 ± 200 b	3800 ± 300 a
Cu	37 ± 6 a	22 ± 4 a	27 ± 1 a	128 ± 26 b	41 ± 3 a
Ni	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*
Cr	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*
Cd	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*

Note: Data given in the table are the mean of 10 replications ± standard deviation. For each parameter, data followed by different letters indicate significant differences ($p < 0.05$). n.d.* = not detected.

The AMD samples exhibited low pH values (< 3.5), while the San Antonio River samples demonstrated alkaline pH (8.3). Elevated concentrations of arsenic (As) were identified in AMD samples A, B, C and D, as well as in the San Antonio River. None of the HMs evaluated were detected in the river samples. In contrast, the AMD samples displayed elevated values for all HMs, with the exception of Pb for samples AMD F, G and H and Cu for samples AMD A, B, C and E.

Table 5
Water physicochemical analysis of the different sampling sites (n.d. = not detected).

Sample	pH	As (mg L^{-1})	Zn (mg L^{-1})	Pb (mg L^{-1})	Fe (mg L^{-1})	Cu (mg L^{-1})
AMD A	3.34	0.05	20 ± 2 a	0.8 ± 0.1 a	120 ± 14 e	n.d.*
AMD B	3.36	0.05	34 ± 4bc	1.2 ± 0.1 b	180 ± 16 f	n.d.*
AMD C	3.25	0.1–0.2	38 ± 3 cd	1 ± 0.2 ab	190 ± 12 f	n.d.*
AMD D	3.52	0.2–0.5	35 ± 5bcd	n.d.*	10 ± 0.2 a	9.8 ± 0.3 b
AMD E	2.80	< 0.05	35 ± 4bcd	0.8 ± 0.1 a	70 ± 9 d	n.d.*
AMD F	3.00	< 0.05	40 ± 3 d	n.d.*	40 ± 4 c	3.8 ± 0.5 a
AMD G	3.25	< 0.05	30 ± 4 b	n.d.*	25 ± 6bc	4.5 ± 0.8 a
AMD H	3.31	< 0.05	30 ± 1 b	n.d.*	20 ± 4 b	4.2 ± 0.7 a
San Antonio River	8.30	0.05	n.d.*	n.d.*	n.d.*	n.d.*

Note: Data given in the table are the mean of 10 replications ± standard deviation. For each parameter, data followed by different letters indicate significant differences ($p < 0.05$). n.d.* = not detected.

Plant identification, physiological parameters and HMs bioaccumulation

A total of 7 different plant species were identified (Table 6). It was observed that 71% of the identified species belong to the Asteraceae family, while 14% are classified under the Fabaceae family and 14% under the Poaceae family. Moreover, within the Fabaceae family, *Adesmia horrida* stands as the sole representative, while *Festuca orthophylla* exclusively represents the Poaceae family. Notably, *P quadrangularis* (Asteraceae) was present in all sites and the only species found in site 3, the most severely impacted by mining activities, suggesting a possible tolerance to environmental pollution.

Table 6
Identification and distribution of plant species in the sampling sites.

Scientific name	Family	Site 0	Site 1	Site 2	Site 3	Site 4
Adesmia horrida	Fabaceae	X	X	X		X
Baccharis alpina	Asteraceae					X
Baccharis tola	Asteraceae	X	X			
Chuquiraga acanthophylla	Asteraceae	X		X		
Festuca orthophylla	Poaceae	X	X	X		
Parastrephia quadrangularis	Asteraceae	X	X	X	X	X
Senecio nutants	Asteraceae			X		X

Note: “X marks” indicate their presence in the site.

P. quadrangularis exhibited higher accumulations of Zn and Cu in sites 2, 3 and 4, while Fe accumulation was notably higher in sites 1, 3 and 4 compared to the control site (Fig. 2). The distribution of HMs indicated that a higher accumulation occurred in roots compared to shoots for all studied metals, with root accumulation being approximately twice as much as in shoots. Translocation factor (TF) and accumulation factor (AF) values were less than 1 for all HMs, except for Zn in Site 2 and 4. Bioaccumulation factor (BAF) and bioconcentration factor (BCF) values were higher than 1 only for Zn and Cu (Table 7).

Table 7
Bioaccumulation factor (BAF), accumulation factor (AF), translocation factor (TF) and bioconcentration factor (BCF) of *P. quadrangularis* for Zn, Cu and Pb for each sampling site.

HM	Site	BAF	AF	TF	BCF
Zn	Site 0	1.01 ± 0.3 a	0.50 ± 0.1 a	0.50 ± 0.1 a	1.51 ± 0.3 a
	Site 1	0.91 ± 0.1 a	0.39 ± 0.1 a	0.43 ± 0.1 a	1.30 ± 0.1 a
	Site 2	5.73 ± 0.6 c	2.88 ± 0.5 b	0.50 ± 0.2 a	8.61 ± 2.1 b
	Site 3	0.98 ± 0.2 a	0.43 ± 0.1 a	0.43 ± 0.1 a	1.41 ± 0.9 a
	Site 4	4.91 ± 0.6 b	2.44 ± 0.3 b	0.50 ± 0.2 a	7.35 ± 1.8 b
Cu	Site 0	0.34 ± 0.1 a	0.13 ± 0.1 a	0.39 ± 0.1 a	0.47 ± 0.1 a
	Site 1	0.55 ± 0.1 ab	0.23 ± 0.2 a	0.43 ± 0.2 a	0.78 ± 0.2 a
	Site 2	0.79 ± 0.2 b	0.41 ± 0.2 a	0.52 ± 0.2 a	1.20 ± 0.7 a
	Site 3	0.76 ± 0.3 b	0.39 ± 0.3 a	0.52 ± 0.2 a	1.15 ± 0.6 a
	Site 4	0.68 ± 0.2 ab	0.35 ± 0.2 a	0.50 ± 0.1 a	1.03 ± 0.4 a
Fe	Site 0	0.03 ± 0.01 a	0.02 ± 0.01 a	0.47 ± 0.1 a	0.05 ± 0.01 ab
	Site 1	0.09 ± 0.02 b	0.05 ± 0.02 a	0.51 ± 0.2 a	0.14 ± 0.04 ab
	Site 2	0.02 ± 0.01 a	0.01 ± 0.01 a	0.55 ± 0.2 a	0.03 ± 0.01 a
	Site 3	0.52 ± 0.01 d	0.24 ± 0.09 b	0.46 ± 0.1 a	0.76 ± 0.2 c
	Site 4	0.14 ± 0.03 c	0.07 ± 0.02 a	0.49 ± 0.1 a	0.22 ± 0.1 b

Note: data given in the table are the mean of 10 replications ± standard deviation. For each parameter, data followed by different letters indicate significant differences ($p < 0.05$).

MDA and total chlorophyll concentrations showed no significant differences between the control and polluted sites. However, differences in total chlorophyll concentrations were observed between site 4 and sites 1 and 3, both of which exhibited higher values. Carotenoid content was 15% higher in sites 1 and 3 compared to the control. Phenolic compounds content was higher in all sites compared to the control, reaching values 44% higher in site 1. Similarly, proline content was higher in all sites, reaching values 280% higher in site 3.

Table 8
Physiological and biochemical parameters of *P. quadrangularis* for each tested site.

Site	Total chlorophyll ($\mu g\ cm^{-2}$)	Carotenoid ($\mu g\ cm^{-2}$)	Shoot MDA (nmol MDA g FW^{-1})	Shoot phenolics (μg GAE g FW^{-1})	Shoot proline (μmol g FW^{-1})
Site 0	53.47 ± 7.35 ab	10.89 ± 1.48 a	11.67 ± 0.74 a	16990 ± 2740 a	69.61 ± 13.47 a
Site 1	59.26 ± 7.83 b	12.4 ± 1.24 b	10.19 ± 1.1 a	24550 ± 2840 b	172.71 ± 26.78 bc
Site 2	51.34 ± 1.7ab	10.21 ± 0.3 a	10.98 ± 1.69 a	23920 ± 1290 b	144.9 ± 5.01 b
Site 3	64.416 ± 2.75 b	12.56 ± 1.11 b	11.23 ± 1.57 a	22640 ± 1550 b	195.01 ± 25.37 c
Site 4	44.88 ± 4.9 a	11.18 ± 0.3 ab	11.62 ± 0.76 a	23790 ± 2810 b	146.41 ± 18.63 b

Data given in the table are the mean of 10 replications ± standard deviation. For each parameter. data followed by different letters indicate significant differences ($p < 0.05$).

Discussion

Although water from La Concordia stream (AMD samples) are not used directly for drinking, this stream flows into the San Antonio River, which is the main source of water supply for San Antonio de los Cobres. Therefore, metal concentrations are compared against national drinking water guidelines. Our study showed concentrations of HMs and metalloids that exceeded the limits established by Argentine legislation on hazardous waste (Decree 831/93, Law No. 24051) which are 0.01 mg L^{-1} for As, 2 mg L^{-1} for Zn, 0.05 mg L^{-1} for Pb, 0.3 mg L^{-1} for Fe and 5 mg L^{-1} for Cu. Our findings exceeded the established limits by 5–10 times for As, 6–13 times for Zn, 80–120 times for Pb, 20–380 times for Fe and 4–10 times for Cu. Liquid samples collected from the San Antonio River did not show detectable levels of HMs but did show elevated As concentrations (0.05 mg L^{-1}). This may be due to the alkaline pH of the river (8.3), contrasting with the acidic pH (~ 3) of AMD samples near the tailing dams. At higher pH levels, HMs may precipitate and adsorb onto sediments, as suggested by Duncan et al., (2018). In addition, this environment is affected by high natural background levels of As in water (Nicolli et al., 2012). Natural sources of As include mineral deposits, brines, hot springs and volcanic rocks, while anthropogenic sources are related to mining activities and the release of AMD. As is found over a wide range of concentrations (0.01 mg·L^{-1} to 10 mg·L^{-1}), with the highest concentrations attributed to anthropogenic sources (Tapia et al., 2019).

Concha et al., (2010) found that San Antonio de Los Cobres has elevated concentrations of As in their drinking water (0.21 mg·L^{-1}) and in the urine of its habitants (0.27 mg·L^{-1}). Consistent with our findings, Kirschbaum et al., (2012) also reported low pH (3–4) and high concentrations of As (8.7 mg L^{-1}), Pb (1 mg L^{-1}) and Zn (32 mg L^{-1}) in La Concordia stream. Similarly, Nieva et al., (2018) found low pH (3.15–3.7) with high concentrations of Fe (245.8 mg L^{-1}), Pb (1.58 mg L^{-1}), Cu (3.85 mg L^{-1}) and Zn (32.03 mg L^{-1}), showing a downstream decrease. Their study, conducted in April and June, found higher HMs concentrations during the dry period (June) compared to the end of the wet season. The variation in temperature, dissolved oxygen concentration and water turbulence can influence HMs solubility and bioavailability (Osakwe et al., 2014), suggesting seasonal fluctuations in HMs concentrations. To better assess the risk posed by HMs in the San Antonio River, comprehensive sampling of sediment and water throughout the year is necessary.

The soil physicochemical characteristics reveal a prevalent sandy texture, low organic matter, nutrient content and saturation water capacity across all sites. This suggests a soil structure prone to erosion and limited water retention, further exacerbated by strong winds. The presence of $CaCO_3$ concretions at specific sites indicates alternating water movement in soil influencing mineral precipitation and dissolution (Punia & Siddaiah, 2019). According to Punia (2021), these processes hinder vegetation establishment and exacerbate soil erosion and HMs dispersion, particularly in arid and semiarid regions at various mining sites worldwide. Site 3 is the most severely affected by mine pollution, as evidenced by high acidity, salinity and concentrations of Zn, Pb and Cu. Although, these concentrations do not exceed the soil pollution limits established by Argentine legislation on hazardous waste (Decree 831/93, Law No. 24051), which are 600 mg kg^{-1} for Zn, 375 mg kg^{-1} for Pb and 150 mg kg^{-1} for Cu, they exceeded the international thresholds set by the WHO/FAO for soils which are 300 mg kg^{-1} for Zn, 50 mg $kg^{-1}$for Pb and 100 mg $kg^{-1}$for Cu. This highlights the importance of considering both national and international standards in environmental assessments. The highest Fe concentration (17130 mg kg^{-1}) detected in site 2 remains within the permissible limits for soils established by the WHO/FAO (50000 mg kg^{-1}); notably Fe does not have a specified limit in Argentine legislation.

These conditions are consistent with other mining sites in the Altiplano-Puna plateau of Argentina, Bolivia, Chile and Peru, where AMD generation from tailing dams lowers soil pH and increases the concentration and bioavailability of HMs(Tapia et al., 2022).Nieva et al., (2018) reported high concentrations of Zn, Cu, Pb, As and Fe in the first 40 cm of tailing dam 1of La Concordia mine (the closest to the mine adit), with values of 2826 mg kg^{-1}, 3405 mg kg^{-1}, 5324 mg kg^{-1}, 1657 mg kg^{-1} and 25000 mg kg^{-1}, respectively. They also reported low pH values (1.4–2.34), similar to those found in our study. Although HMs values were much higher than those we found, it is important to note that these authors sampled the tailing dams, while we sampled the surroundings areas, where plants were growing. We observed no vegetation growing on the tailing dams, which we attribute to excessive HMs concentrations and low pH as reported by these authors. Additionally, sampling times differed, as we collected samples in October, while they sampled in June. This suggests that the tailing dams at La

Concordia mine still have high concentrations of HMs, which continue to pollute the surrounding areas and prevent vegetation from growing on them. Furthermore, similar to water, soil pH, as well as HMs concentrations and dispersion could vary seasonally; however, further studies are required to confirm this.

Regarding plant species identification, we identified 7 species in this environment, representing the Asteraceae, Fabaceae and Poaceae families, with 5 out of the 7 species belonging to the Asteraceae family. Oxman and Lupo (2024) reported that plant species distribution exhibit altitudinal variations, defining three vegetation belts: the Puna, the lower High-Andean belt, and the upper High-Andean belts. The Puna belt occupies the lower altitudinal level (3500–4200 m.a.s.l.) and features a shrub steppe with various species of *Baccharis*, *Fabiana* and *Parastrephia* genera. The lower High-Andean belt, situated between 4200 and 4500 m.a.s.l., is characterized by Poaceae clumps, xerophytic shrubs like *A. horrida* and diverse *Cactaceae* species. The upper High-Andean belt, above 4500 m.a.s.l., is dominated by circular and semicircular clumps of *F. orthophylla*. In our study, we found representatives of the previously described genera and species, such as *A. horrida*, *B. alpina*, *B. tola*, *F. orthophylla* and *P. quadrangularis*, with *P. quadrangularis, A. horrida* and *F. orthophylla* being present at most sites. Notably, *P. quadrangularis* (Asteraceae) was the only species present at all sites, including site 3, which is considered the most contaminated, suggesting potential resilience to mine pollution-induced stress.

Several studies have highlighted the growth of plants of the genus *Parastrephia* in extreme conditions such as the Atacama Desert (Chile), the pre-cordillera and Altiplano in the North of Chile and Argentina (Echiburu-Chau et al., 2017; Razmilic et al., 2023). Acuña et al., (2019) have also noted their ability to thrive in high salinity conditions. However, few reports exist regarding their capacity to grow and bioaccumulate HMs in polluted environments. Previous studies reported *P. quadrangularis* growing in the Pan de Azúcar mine in northwest Argentina (Jujuy province) at 3700 m.a.s.l, where high concentrations of Zn (480 mg kg^{-1}) and Pb (410 mg kg^{-1}) were found (Plaza Cazón et al., 2013). Additionally, this species was found to accumulate 260 mg kg^{-1} of Zn in leaves. Benítez et al. (2014) also found *P. lepidophylla* (= *P. quadrangularis*) and *P. lucida* at the same site, with *P. lucida* bioaccumulating up to 600 mg Zn kg^{-1} DW. In our study, we observed similar Zn bioaccumulation in total biomass, reaching maximum values of 690 mg Zn kg^{-1} DW.

While Zn, Cu and Fe are essential micronutrients for plant growth, elevated concentrations can become phytotoxic (Anjum et al., 2015). Phytotoxic limits, often based on experiments conducted on crops, may not be universally accurate as tolerance to HMs varies among species (Sytar et al., 2021). Nonetheless, these limits are commonly used as reference thresholds for phytotoxic levels. The optimal Zn levels for most crops range between 30 and 200 mg Zn kg^{-1} leaves dry weight (LDW), with phytotoxicity occurring above 400 mg Zn kg^{-1} LDW (Kaur & Garg, 2021). For Cu, optimal concentrations are 5–10 mg Cu kg^{-1} LDW, while toxicity occurs above 20–30 mg Cu kg^{-1} LDW (Kumar et al., 2021). Normal Fe contents in plant leaf tissues range between 120–250 mg Fe kg^{-1} LDW, with toxicity occurring above 500 mg Fe kg^{-1} LDW (Lapaz et al., 2022). In our study, *P. quadrangularis* accumulated values surpassing the phytotoxic thresholds for Cu at site 3 and Fe at sites 1 and 3, reaching maximum leaf values of 52 mg Cu kg^{-1} LDW and 6559 mg Fe kg^{-1} LDW at site 3. The higher metal accumulation in roots compared to shoots, indicated by translocation factors values (TF) less than 1, suggests limited translocation capacity to aerial parts, contributing to overall tolerance to HMs. Plant species with low TFs (< 1) are considered potential candidates for phytostabilization (Shackira & Puthur, 2019).

The physiological analysis of *P. quadrangularis* offers insights into adaptive strategies to challenging environmental conditions and HMs stress. Although there are no reports on how HM pollution alters the physiological and biochemical aspects of *P. quadrangularis*, these measurements are commonly used to assess abiotic stress in plants and can often serve as bioindicators of HM stress and pollution (Cakaj et al., 2024). Specifically, MDA serves as an indicator of lipid peroxidation and higher concentrations suggest HMs-induced oxidative damage (Mao et al., 2018). Liu et al., (2019) found that MDA levels increased in *Arundo donax* when it was growing in a mining area with high concentration of Cd and Pb. However, the absence of differences in *P. quadrangularis* between sites in our study may indicate that there was no enhanced oxidative damage. This could correlate with the increase in proline and phenolic compounds content found in *P. quadrangularis* in mine-polluted sites compared the control site, as this response is considered a non-enzymatic antioxidative plant defense mechanism to cope with HM stress (Lajayer et al., 2017). Gao et al., (2022) also found increased proline concentration in leaves of *Chenopodium glaucum* growing in Cr, Cu, Pb and Zn mine-polluted soils. Similarly, Zafari et al., (2016) reported an enhancement of phenolic compound concentration in *Prosopis farcta* treated with increased concentrations of Pb.

Proline is an important amino acid that accumulates in plants in response to various environmental stresses, typically associated with salt and HM stress tolerance (Aslam et al., 2017). It acts as a signaling molecule, regulates osmotic pressure, maintains cytosolic pH, stabilizes and protects protein structures, prevents membrane disruption and scavenges ROS (reactive oxygen species) produced by HMs stress (Badiaa et al., 2020). Phenolic compounds also serve as protective agents against stress, demonstrating antioxidant properties due to their high tendency to chelate metals through hydroxyl and carboxyl groups, inhibiting lipid peroxidation by trapping the alkoxyl radicals generated during oxidative damage (Kulbat, 2016). Notably, *P. lucida* and *P. quadrangularis* have been documented to contain naturally high concentrations of various phenolic compounds known for their medicinal, antioxidant, antibacterial and antifungal properties (Echiburu-Chau et al., 2017; D'almeida et al., 2020). However, there are no previous reports on their modifications or possible roles against HMs phytotoxicity in these species.

HM pollution usually disrupts the biosynthesis of photosynthetic pigments, leading to alterations in carbon assimilation and chloroplast damage (Asati et al., 2016). These alterations include decreased Rubisco activity, inhibition or alteration of both photosystems I and II, impairment of the photosynthetic electron transport chain, changes in lipid composition of thylakoid membranes, distortion of thylakoid membranes architecture and overall disruption of chloroplast ultrastructure, which is ultimately observed as a reduction in photosynthetic pigments content (Rai et al., 2016). However, no difference in total chlorophyll content in *P. quadrangularis* was observed between the control site and sites influenced by mine pollution. Additionally, carotenoid content exhibited an increase at sites 1 and 3 compared to the control site. Pistelli et al., (2017) also found no significant differences in total chlorophyll content in *Dittrichia viscosa* growing in mine-polluted soil with high concentrations of As, Cu, Pb and Zn. Additionally, Houri et al. (2020) found higher carotenoid contents in *Urginea maritima* growing in soil with high concentrations of Cr, Al, Cd and Pb. Carotenoids not only expand the light-harvest spectrum but also act as a defense mechanism against ROS and oxidative stress as part of the non-enzymatic antioxidative plant defense system (Sharma et al., 2021).

P. quadrangularis, although it accumulated concentrations of HMs which exceeded phytotoxic thresholds, showed no stress symptoms as indicated by stable total chlorophyll content and MDA levels. This could be related to the activation of non-enzymatic antioxidant mechanisms like carotenoid, proline and phenolic compound accumulation.

Conclusion

The Puna region, characterized by its environmental fragility and significant economic resources, faces substantial threats from widespread untreated mine tailings and ongoing mining operations across South America. These activities contribute to the release of HMs into the environment. In our study we found that in La Concordia mine, the extensive weathering of mine tailings has resulted in the generation of AMD rich in As, Fe, Cu, Pb, and Zn. Consequently, La Concordia creek has experienced a marked deterioration in water quality, evidenced by low pH levels (< 3.5) and HMs concentrations exceeding drinking water guidelines. Soil analysis further reveals adverse effects, including low pH, high salinity and elevated HMs concentrations.

Despite the challenging natural environmental and mine pollution conditions, the plants species we identified in the area demonstrate notable adaptability, especially *P. quadrangularis*. This was the only species found across all sampling sites, including those with high pollution levels *P. quadrangularis* exhibited no stress symptoms, as indicated by stable total chlorophyll content and MDA levels and robust tolerance mechanisms against HM-induced stress, reflected in increased levels of carotenoid, proline and phenolic compounds content. These adaptive responses are crucial for the survival of *P. quadrangularis* in polluted environments and highlight its potential for use in revegetation and phytostabilization programs. Such applications could contribute significantly to reducing soil erosion, as well as mitigating the dispersion and bioavailability of HMs in La Concordia mine and other mining-affected areas of the Puna region.

Future research will aim to elucidate the precise mechanisms by which *P. quadrangularis* and other native tolerant plant species in La Concordia mine manage HM stress. The unique environmental challenges of this region restrict the applicability of reclamation strategies to native species of La Puna, for which research remains limited. Gaining a deeper understanding of these mechanisms will be crucial for optimizing their use in mine closure programs, environmental restoration and sustainable land management practices within the extreme conditions of the Puna region.

Declarations

Ethics Approval: Not applicable (no animal or human is used)

Consent to Participate: Not applicable (no animal or client participants)

Consent for Publication: Not applicable (no person’s data in any form)

Data Availability: The authors confirm that the data supporting the findings of this study are available within the article.

Competing Interests: The authors declare no competing interests.

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Figures

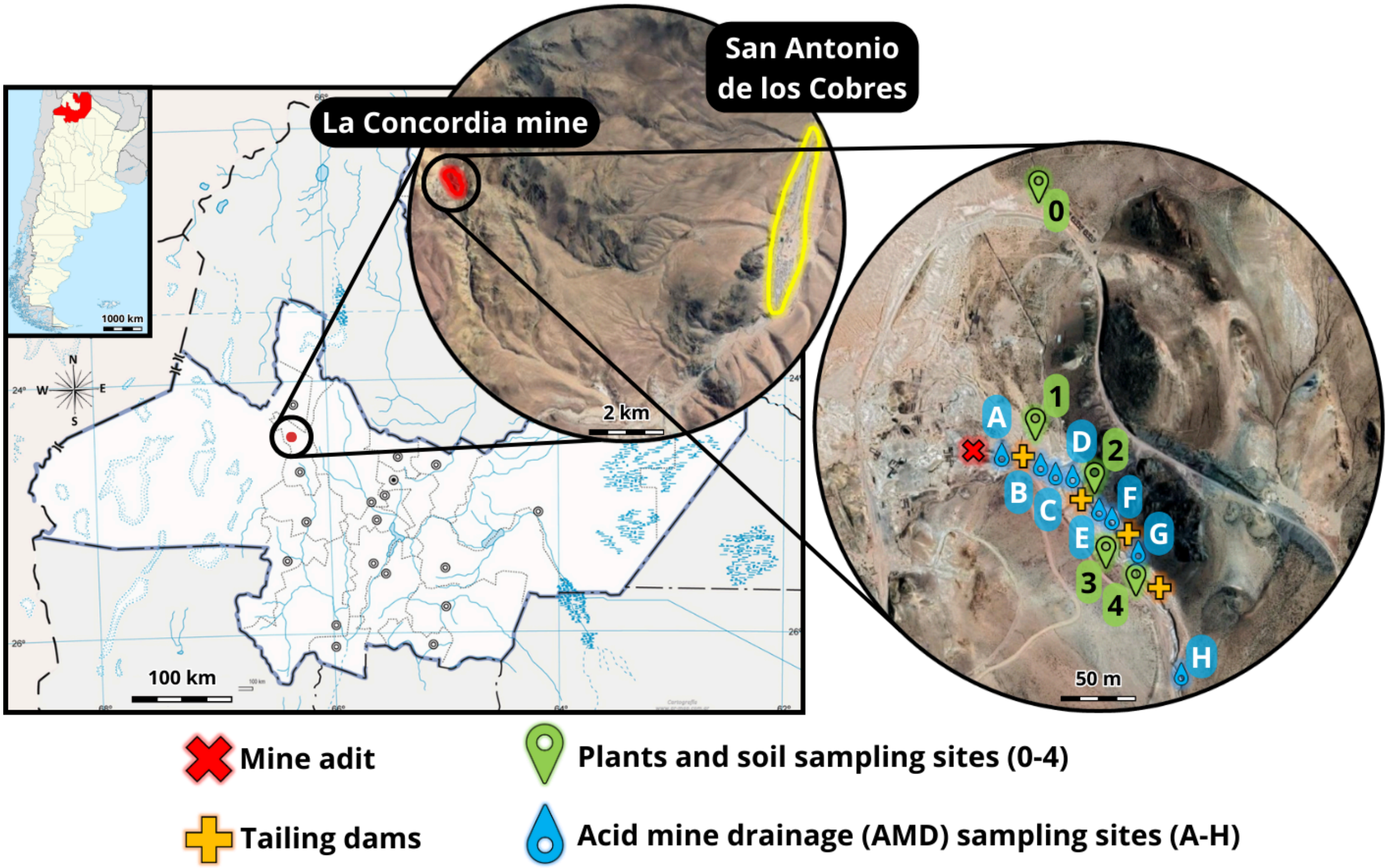

Figure 1

Map of the study area and sampling points in La Concordia mine (Salta province, Argentina).

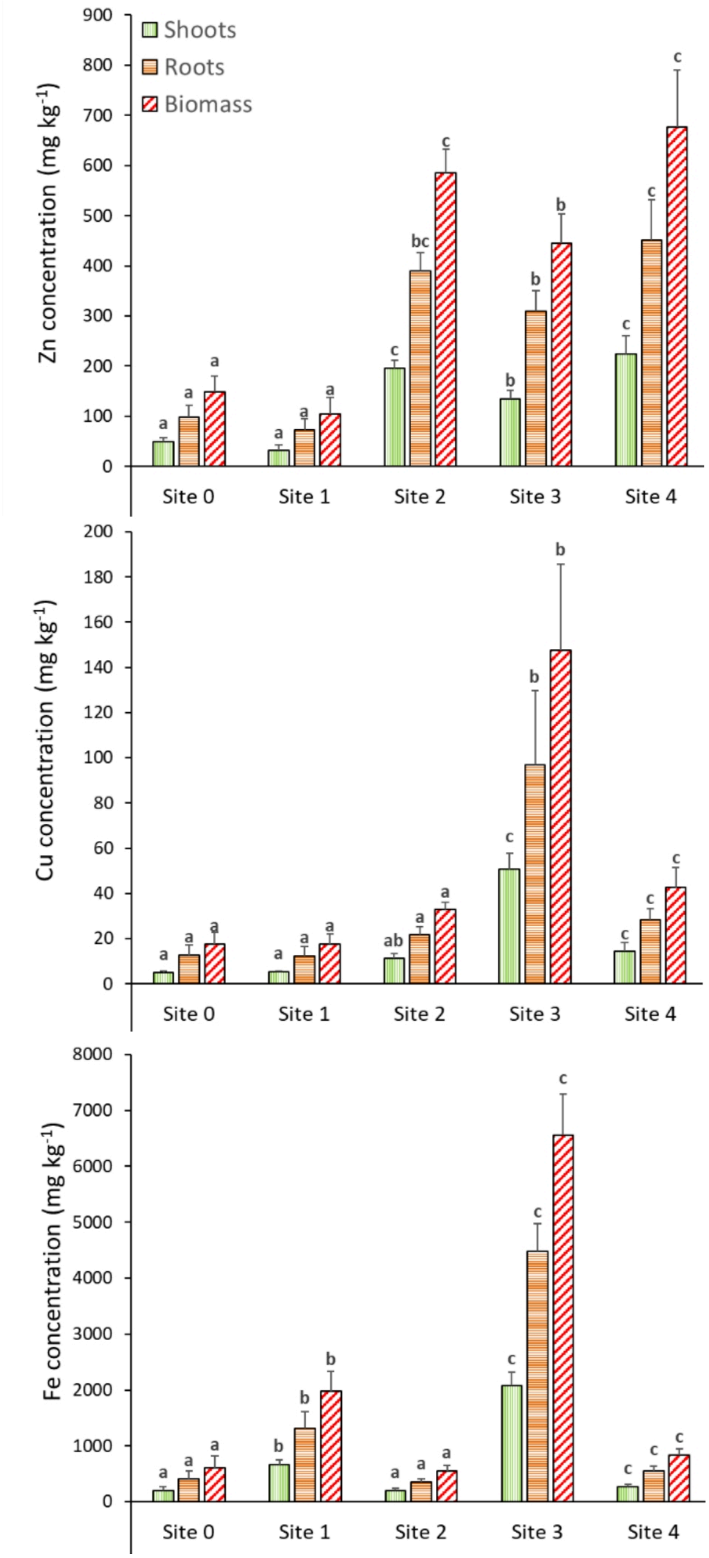

Figure 2

Zn, Cu and Fe accumulation in shoots, roots and biomass of *P. quadrangularis*. Columns represent the mean values of 10 replications, and vertical bars show the standard deviation. Columns sharing different letters indicate significant differences (p <0.05).