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Research Article

Growth of Photosynthetic Biofilms and Fe, Pb, Cu, and Zn Speciation in Unsaturated Columns with Calcareous Mine Tailings from Arid Zones

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Mine tailing remediation aims to reduce the rate of sulfide mineral oxidation. Earlier studies showed that photosynthetic biofilms may act as a physical barrier against oxygen diffusion. Currently, a long-term assay (6 months) is required to evaluate the solid phase redistribution of the Pb, Fe, Cu, and Zn originally present in historic and calcareous mine tailing samples (in our case from a semiarid region in North-Central Mexico). The presence of biofilms may provide chemical gradients and physical conditions that shift the proportion of Fe, Cu, and Zn originally associated with oxides to carbonates and organic matter/sulfide fractions.

1. Introduction

The oxidation and dissolution of sulfide minerals (SMs) present in mine tailings (*vr. gr.* pyrite (FeS_2), arsenopyrite (FeAsS), galena (PbS), sphalerite (ZnS), chalcopyrite ($CuFeS_2$)) can produce acidic metal-rich waters known as acid mine drainage (AMD) within tailing deposits and at the receptors (soil and water sediments). Mine tailing remediation aims to reduce the rate of SM oxidation by avoiding or reducing contact between the SM and the oxidant agents such as atmospheric or dissolved oxygen (O_2) or ferric ions (Fe(III)). It has been proposed that water coverage could be used to reduce O_2 diffusion into tailing dams [1–3]. Other types of covering such as multilayer covers have also been proposed, and this consists of a profile of well structured layers (surface, drainage, moisture, retaining, and support layers) with different hydrogeological properties. Ideally, the surface layer must support vegetation and retain moisture. Bussière et al. [2] indicated that multiple covers with capillary barrier effects are useful for low-sulfide tailing from a wet or temperate climate region. However, designing efficient covers with different capillary barrier effects can be difficult because of variable climatic conditions and layer geometry as well as the complex behavior of unsaturated materials [4]. Furthermore, the multilayer cover might need considerable quantities of covering material. If sufficient cover material is not available in the vicinity of the tailing impoundments, then the material needs to be removed and transported to the site resulting in a significant increase in cost and the alteration of other environments. Therefore, the barrier of choice depends mainly on climate, geology, mineralogy, and economic factors. For abandoned mine tailing dams, the former is of special concern as no mining company assumes any responsibility for the remediation action.

García-Meza [5] showed that photosynthetic biofilms (composed mainly of cyanobacteria and green algae and some aerobic and anaerobic bacteria) successfully colonized the surface of mine tailing samples from Guanajuato (Mexico). The presence of biofilms on the surface enhanced trace element immobilization. Their results suggest that these biofilms may act as a physical barrier against oxygen diffusion preventing SM oxidation. In addition, the photosynthetic activity during the experiments resulted in a natural fertilization of the tailing samples. It has been suggested that photosynthetic biofilms may be useful as an initial step of mine tailing remediation. A biofilm-colonized substrate surface may possess the desired characteristics for the future development of vegetative species because of autotrophic microorganisms, which are the driving force for biogeochemical processes such as soil genesis, metal mineralization, and carbon and nitrogen fixation. Long-term bioassays using columns could provide more comprehensive information about the consequences of the colonization of photosynthetic biofilms on the chemical speciation of metals (Fe, Pb, Cu, and Zn). In this study, six columns (1 m high) were filled with samples of a recent tailing pile (*ca.* 7 years) to reproduce the profile that was observed *in situ*.

2. Materials and Methods

Mine tailing samples were taken from the Concepción del Oro mining district in Zacatecas, Mexico (24°37′ N and 101°25′ W, at 2080 masl) (Figure 1) where a semiarid climate dominates. Tailings from the mining operations were discharged in five separate tailing impoundments or piles (Figure 2). The tailing pile P2 was chosen for this study. P2 is slightly alkaline (pH 7.52–8.04) with a high acid neutralization potential (ANP; 153–493 meq CO_3^{2-}/ton) [6]. In a selected zone of the tailing P2, a pit 100 cm deep was dug and six different strata were recognized based on color change and textural variations. For each stratum up to 3 kg of tailing material was sampled. Because the investigation focused on the unaltered zones, the oxidized stratum was removed. The oxidized zone was recognized because of its reddish color.

Once in the laboratory, 1.5–2.5 kg of each stratum was used to reconstruct the sampled profile in six acrylic columns (Figure 3); the rest (0.5 kg) of the stratum sample was used for preliminary characterization. The samples were dried at room temperature and sieved to <63 μm. For the analysis of particle size distribution, the dry samples were sieved to <250 μm. The physical and chemical characterization included (a) color on dry samples using the Munsell soil color chart [7], (b) particle size distribution by laser diffraction (Shimadzu SALD-1100) on each size-fractioned sample, (c) electric conductivity [8], (d) paste and rinse pH [9], (e) moisture content by loss of weight at 105°C, (f) acid neutralization potential (ANP) according to the Sobek method as dictated by Mexican legislation [10], (g) organic matter content by loss of weight at 550°C (see [11, 12]), and (h) Pb, Cu, Fe, and Zn content. The major mineralogical phases were determined by X-ray diffraction (XRD, Rigaku DMAX 2200) using Cu-Kα radiation.

For the analysis of metals, a 5-step sequential extraction procedure was carried out (see [13, 14]). The final operationally defined fractions were the following.

(i) Exchangeable (F1): tailing sample extracted with 8 mL of 1 M $MgCl_2$ (pH 7) for 30 min with continuous agitation at room temperature.

(ii) Bound to carbonates or specifically adsorbed (F2): the residue from (i) was extracted with 8 mL of 1 M NaOAc (pH 5) for 5 h with continuous agitation at room temperature.

(iii) Bound to crystalline Fe-Mn oxides (F3): the residue from (iii) was extracted with 20–25 mL of 4 M HCl for 30 min with occasional agitation at 94–96°C.

(iv) Bound to organic matter and sulfide (F4): the residue from (iv) was extracted with 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 (pH 2); the sample was heated progressively to 85°C and maintained at this temperature for 2 h with occasional agitation and then 3 mL of 30% H_2O_2 (pH 2) was added. The mixture was then heated to 85°C for 3 h with intermittent agitation. After cooling, 5 mL of 3.2 M CH_3COONH_4 in HNO_3 20% was added.

(v) Residual (FR): the metals present were scattered within the crystal lattice of the rocks and minerals: 0.5 g of the residue from (iv) was digested with 20 mL of a mixture 3 : 1 HNO_3 (66%) and HCl (37%) using a microwave oven (CEM-MARS-X).

Extractions were carried out on 1.0 g of sample; the analyses were performed in duplicate. The metal content of the extracted solutions was analyzed with atomic absorption spectrometry (AAS, Perkin Elmer 2380).

To evaluate the presence of neutrophile (pH 7±1.5) aerobic or anaerobic bacteria in the tailing samples, 1 g of a sample of the mine tailings from each stratum was transferred to a test tube with specific media, in duplicate. For aerobic microorganisms media number 69 of the DSMZ (2006) [15] were used as it is specific for sulfur oxidizing bacteria (SOB); these media are specific for *Starkeya novella* (formerly *Thiobacillus novellus*). For anaerobic bacteria we used a modified Postagate media [15], which are specific for sulfate-reducing bacteria (SRB). The test tubes were incubated for 7 days at 27°C and were placed in an anaerobic jar. Afterwards, an aliquot of 25 μL of culture media was stained and observed under a light microscope at 1000x magnification. To determine the relative abundance, the cell numbers per visual field were recorded a total of 10 visual fields per slide were examined and two slides per culture were done.

For the assay setup, six columns were constructed to perform the bioassay (Figure 3). Each column was filled with samples from the tailing pile P2, reproducing the sampled profile (six strata). After that, the photosynthetic biofilms previously obtained were disseminated onto the tailings surface of each column. The photosynthetic biofilms were obtained from the biological crusts sampled in the tailings pile P2 at three different sites. The three collected crusts were mixed in a sterile Petri dish to create a composite; the composite was spread in Erlenmeyer flasks (X6) containing sterile Woods Hole (WH) culture media, which are specific for microalgae [16]. Microalgae were grown under a 14 : 10 h light : dark cycle at 25°C. Microalgae colony growth started after 2 to 3 weeks.

The columns settled under environmental conditions (the roof of the laboratory). The bioassay was performed over seven months; the first three months corresponded to

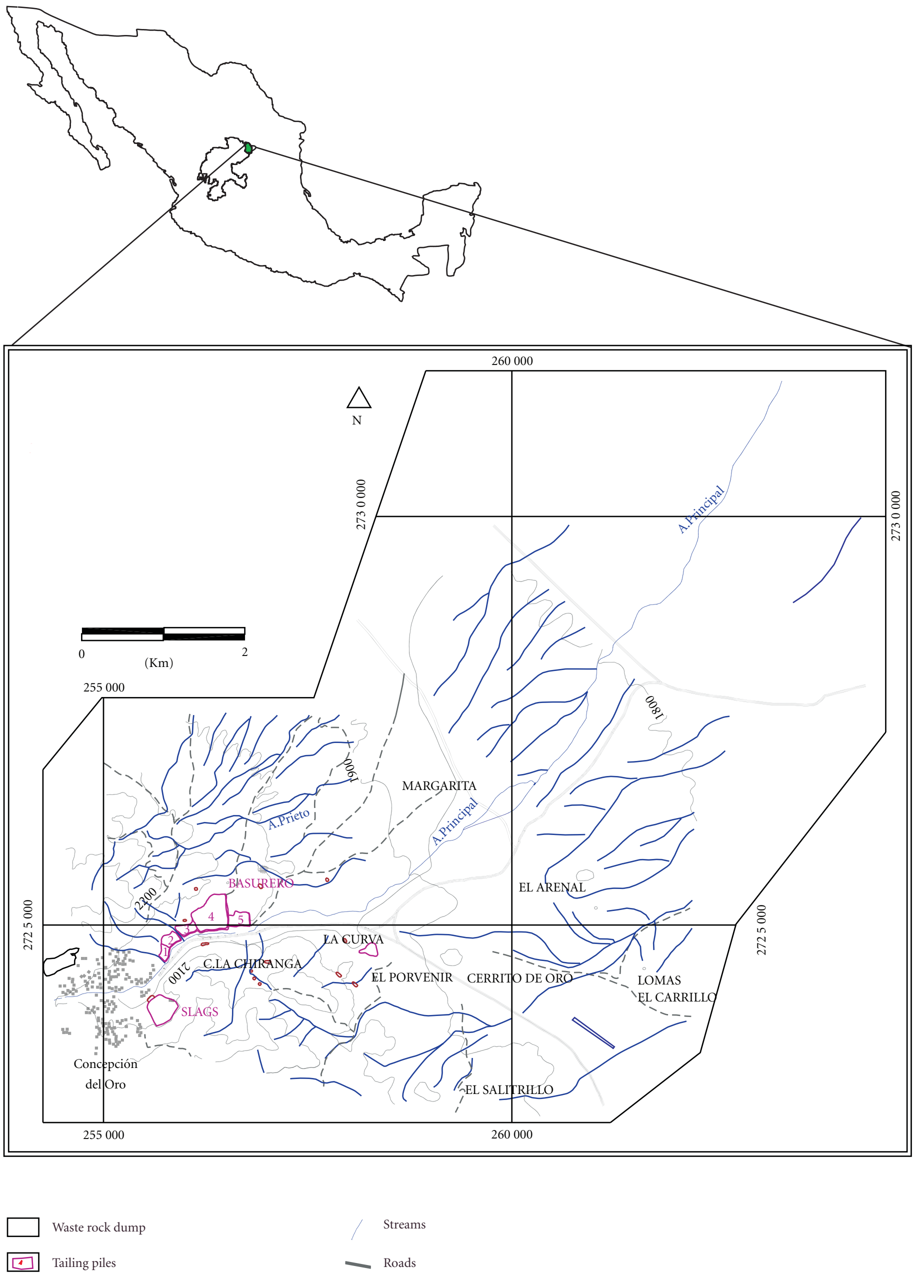

Figure 1: Location of the Concepción del Oro mining area (Zacatecas, North-Central Mexico).

the hot and wet (t_w) season (July 21–October 26, 2006; monthly average temperature was 19°C with a maximum precipitation of 109.9 mm during September) while the last four months corresponded to the cold-dry season (t_d) (November 1st 2006–February 13, 2007; monthly media temperature of 13°C and precipitation of 0 to 14 mm). During the wet season, the columns were irrigated daily by spraying 10–20 mL of WH over two weeks and then every 3rd day with sterile tap water. During the dry season the biofilms were moistened every 4th day by spraying with sterile tap water. At the end of wet (t_w) and dry (t_d) seasons, the mine tailing samples underwent chemical analysis in triplicate as previously described. The samples were taken from the previously defined strata. Finally, the samples were analyzed to determine the relative abundance of aerobic sulfur-oxidizing bacteria (SOB) and anaerobic sulfate-reducing bacteria (SRB). The photosynthetic biofilm was maintained over the mine tailing surface in each column during the bioassay.

3. Results and Discussion

All the strata have the same gross mineralogy, which consists of quartz (SiO_2), calcite ($CaCO_3$), pyrite (FeS_2), covellite (CuS), and andradite ($Ca_3Fe_2(SiO_4)_3$) (Figure 4). The physicochemical results (Table 1) showed that the strata also share

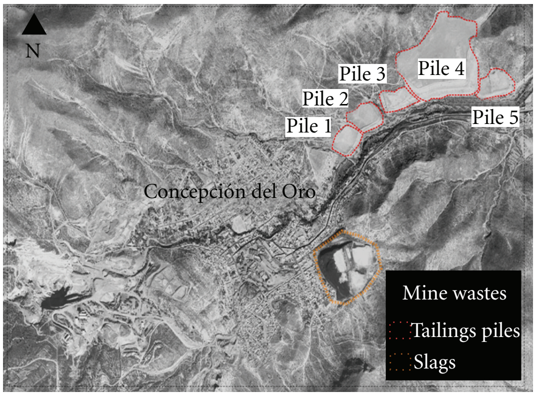

FIGURE 2: Mine tailings piles at Concepción del Oro. Location of the tailings piles at the Concepción del Oro mining area (North-Central Mexico) and Pile 2 from where the sample was taken.

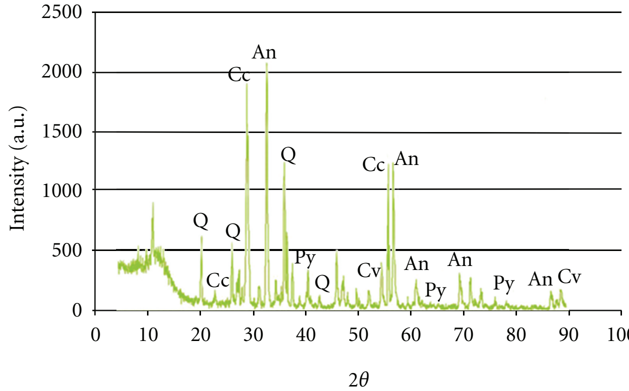

FIGURE 4: Major mineralogical phases obtained by X-ray diffraction (XRD). An: andradite ($Ca_3Fe_2(SiO_4)_3$); Cc: calcite ($CaCO_3$); Cv: covellite (CuS); Q: quartz (SiO_2); Py: pyrite (FeS_2).

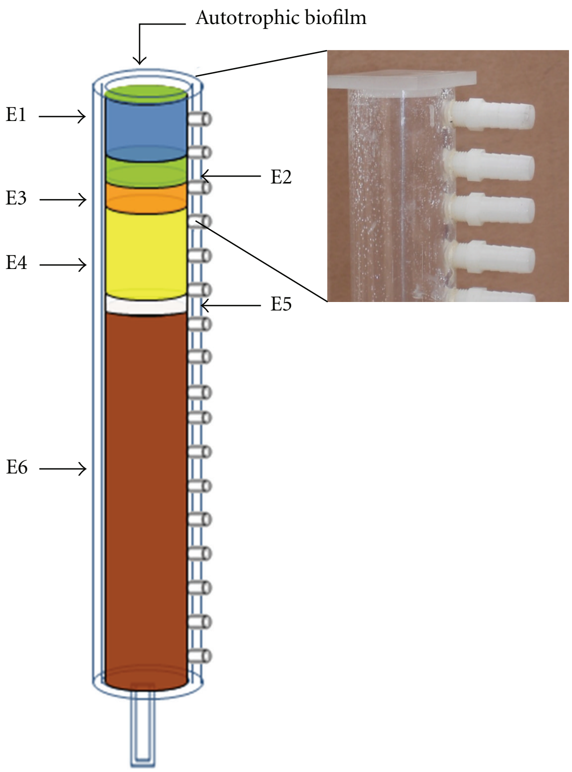

FIGURE 3: Columns constructed for the bioassays.

some common characteristics; that is, brown color (except stratum E5, with a light gray color), sandy to loam texture (except E4, with a silt loam texture), slightly alkaline, pH 7.82, low conductivity, high ANP and low organic matter content, clay, and humidity (Table 1). The conductivity and the pH increased with depth, being higher in the lower strata (E5 and E6). The moderate content of carbonates (16.35 ± 0.89%) results in a relatively high ANP from 71 to 173 ton/eq $CaCO_3$.

Figure 5 showed the absolute data for the concentration of the extracted metals in the six strata of the selected profile from Pile 2, before the assay (t_0) and after the wet (t_w) and dry (t_d) seasons. The relative abundance of the metals in each stratum was similar throughout the profile (Fe > Cu > Zn > Pb). The highest concentration of the metals was recorded in the lowermost strata, E5 and E6. The total Fe and Cu concentrations were high (up to 135 and 1.11 g/kg, resp.), and this reflects the mineralogy of the exploited ore body, which consists mainly of Cu and Fe sulfides or oxides [17]. Fe and Pb were mainly associated to refractory minerals of the residual fraction (FR); on the other hand, Cu and Zn were mainly associated with the Fe-Mn-Oxide fraction (F3). The carbonate fraction (F2) contributed more than 15% of the Cu and Zn, and this represents the second major fraction for these metals in stratum E6. The exchangeable fraction (F1) of the sequentially extracted elements was less than 3% for all strata of the profiles indicating that sorption occurred to a lesser extent by nonspecific electrostatic mechanisms. The developed biofilm does not increase the bioavailable forms, and practically no metals were leached during the assay (Table 2). In general, under neutral to alkaline conditions, the metals associated with the exchangeable fraction (F1) are a minor proportion compared to those in the acidic soils [18] and are associated with secondary minerals resulting in metal fixation in the soils [19] and in mine tailings [20].

Microalgae (cyanobacteria and green algae or chlorophyceae) successfully colonized the surface of the mine tailing samples from Pile P2. Colonization by cyanobacteria and chlorophytes has been reported as useful during the first stage of mine tailing remediation and other degraded terrestrial environments [5, 21]. The presence of viable microalgae performing the photosynthesis resulted in a significant ($P < .05$) increase in the organic matter content in the uppermost strata (E1 and E2) from 0.9–0.11% to 3.5% at the end of the t_w season (Figure 5). In 1961 Singh [22] noticed that the organic matter produced by microalgae binds soil particles together, which reduces soil permeability and aeration. Hu et al. [23] showed that some filamentous cyanobacteria maintain crust cohesion and reduce the eolian erosion of the particles leading to substrate consolidation. Thus, active microalgae form a biofilm and increase the organic matter content, and this may biostabilize the mine

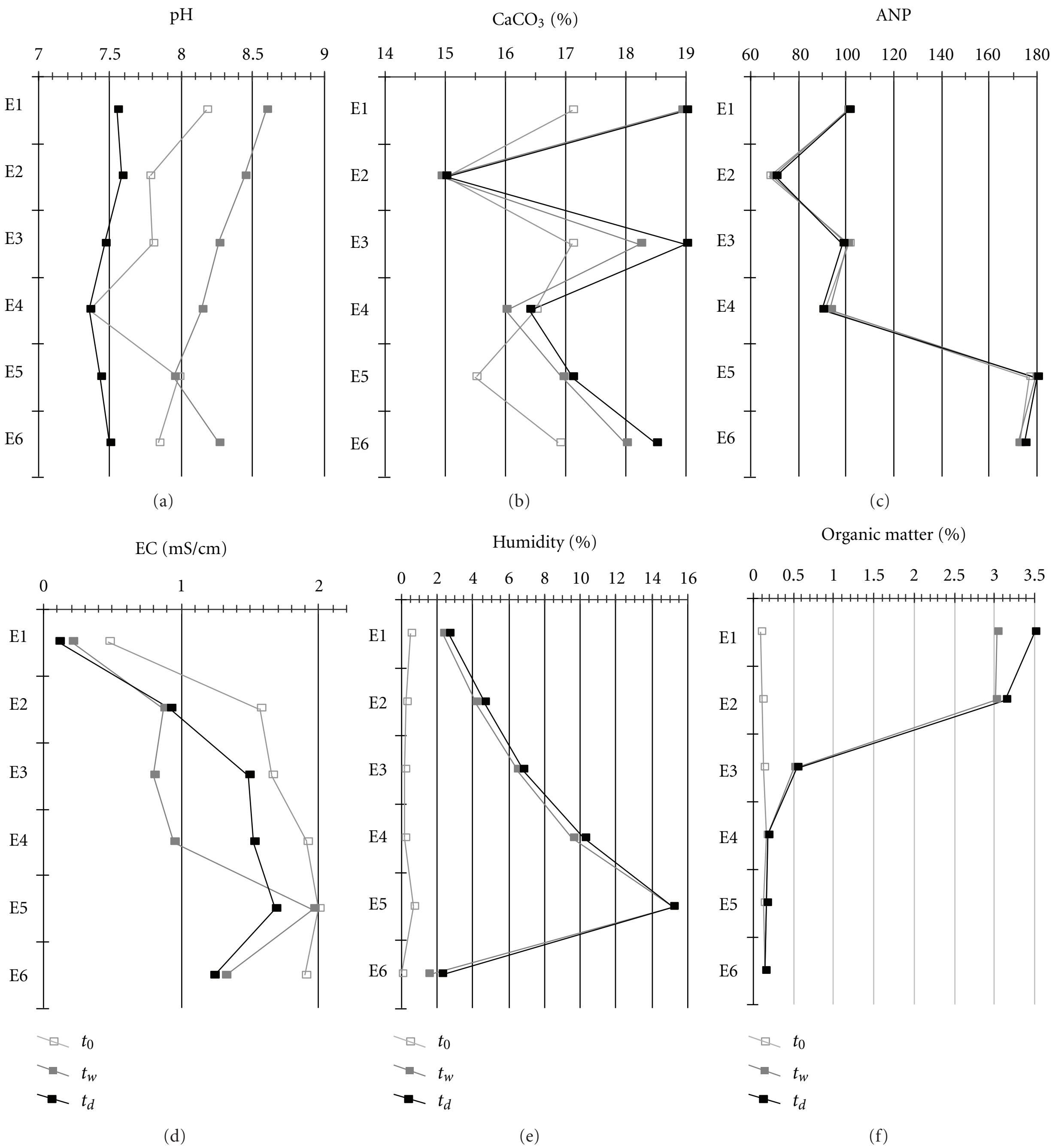

Figure 5: pH, % calcium carbonate ($CaCO_3$), acid-neutralization potential (ANP, in CO_3^{2-}/ton), electric conductivity (EC, in mS/cm), % humidity, and % organic matter of strata E1 to E6 before the assay (t_0; light gray and open squares) and after the wet (t_w; dark gray) and dry (t_d; black) seasons. Data: average, $n = 3$.

tailings, reduce the potential spread of toxic elements from tailings piles, and fertilize the tailings in addition to building up an appropriate substratum for the further establishment of plants. The stabilization effect of the biofilms is due to the secretion of extracellular polymeric substances [24], mostly proteins and carbohydrates [25]. Furthermore, it has been reported that the development of algal communities in mine tailings improves the habitat's quality with time [26] and that the large particle-binding capacity due to extracellular substances might improve sediment transport in water flows [25]. In addition, the pH remains neutral during the assays performed in this work (Figure 5) because of the high buffer capacity of the tailings that contain a high percentage of carbonates and because of CO_2 consumption during photosynthesis [27].

The initial three-month irrigation (wet season, t_w) ensured that biofilm growth occurred. After the t_w season the biofilm completely covered the surface of the tailing profiles

Table 1: Certain physical and chemical characteristics of the strata E1 to E6.

Stratum	E1	E2	E3	E4	E5	E6
Depth (cm)	4.50	3.50	3.50	14.00	1.00	55.00
Color (dry)	Light brown	Light brown	Light brown	Light brown	Light grey	Brown
Sand (%)	77	53	66	21	56	92
Silt (%)	15	38	27	71	42	6
Clays (%)	8	9	7	8	2	2
Texture	Sandy loam	Loam	Sandy loam	Silt loam	Sandy loam	Sand
Moisture (%)	0.51	0.27	0.18	0.19	0.66	0.03
Conductivity (mS/cm)	0.48	1.57	1.66	1.92	2.0	1.9
pH	8.17	7.77	7.86	7.98	7.98	7.84
Carbonates (%)	17.10	15.00	17.10	16.50	15.50	16.90
ANP (meq CO_3^{2-}/ton)	104.80	71.30	102.70	90.00	178.10	173.30
Organic matter (%)	0.09	0.11	0.13	0.16	0.12	0.14

ANP: acid-neutralization potential.

Table 2: Concentration ($mg \cdot mL^{-1}$) of Pb, Fe, Cu and Zn in the leachate after the assay. Data: average ± standard deviation, $n = 3$.

Column	Pb	Fe	Cu	Zn
t_w	—	0.38 ± 0.01	0.17	0.10 ± 0.01
t_d	—	1.93 ± 0.02	0.17	0.12 ± 0.01

in the columns and remained there during the subsequent three months or the dry season (t_d). This initial and periodic irrigation of the columns resulted in an increase in the moisture content of all the strata (Figure 5); however, it did not affect the thickness of the oxidized zone. A shallow water covering or a thick superficial fine-grain layer may reduce oxygen diffusion through the tailings by more than three orders of magnitude [28]. If photosynthetic biofilms develop on the surface of the tailings they also act as a physical barrier against oxygen diffusion upon which an anaerobic microbial community inhabits the deepest strata. The results of this study confirm the former: the anaerobic SRB were twice as abundant after the assay (t_d) compare to before (t_0). Additionally, the biomass of SRB was more abundant than the biomass of SOB during the study period (Table 3). The relative abundance of SOB increased slightly after the wet season (t_w) but at the end of the assay the abundance of SOB decreased.

Neutrophilic SOB like *Starkeya novella*, *Halothiobacillus neapolitanus* (formerly *Thiobacillus neapolitanus*, pH 6.6–7), *Thiobacillus thioparus* (6.5–7.5), *and Thiomonas intermedia* (5–8) [29], are not able to oxidize sulfide minerals, but other reduced sulfur compounds as elemental sulfur, thiosulfate, and polythionates. Accordingly, the sulfide mineral as pyrite and covellite may be previously and partially oxidized by aqueous oxidants (commonly Fe^{3+} or O_2). Bulk sulfurs that are directly exposed to atmospheric gases undergo oxidation to mainly forms of electron deficient disulphide [30]; when O_2 is combined with H_2O, there is a more aggressive oxidation of the surface and the sulfur atoms must pass through several oxidation states during the oxidation process, so many different sulfur compounds might be involved [31], which neutrophilic SOB may oxidize:

$$\begin{gathered}
2S^0 + O_2 + 2OH^- \longrightarrow S_2O_3{}^{2-} + H_2O \\
S_2O_3{}^{2-} + 2O_2 + 2OH^- \longrightarrow 2SO_4{}^{2-} + H_2O \qquad (1) \\
SO_3{}^{2-} + H_2O \longrightarrow SO_4{}^{2-} + 2e^- + 2H^+
\end{gathered}$$

Actually, SOB like *Thiobacillus thioparus* may generate elemental sulfur from sulfides, under low O_2 conditions. Maybe, SOB like *T. thioparus* were presented in the deeper strata, during the wet season (t_w). The absence of SOB in the lowest stratum E6 at the dry season (t_d) may indicate that the oxidation of sulfide minerals (SMs) had stopped [32] but also that E4 (the only stratum with more silt than sand, Table 1) and E5 (the thinner and more saturated stratum) had significantly reduced oxygen fluxes [28, 33]. The largest fraction of Fe, Cu, and Zn was the fraction F3 (bound to Fe-Mn oxide, Figure 6). The former indicated that stratum E6 was slightly more reducing than the layers above it (see below).

If microaerophilic conditions were settled in the profile, the metals may shift from an unstable fraction (oxides, F3) to a more stable fraction (the organic matter/sulfur fraction, F4) under these suboxic conditions. In fact, the relative abundance of Fe, Cu, and Zn associated with oxides was significantly lower ($P < .05$) at the end of the assay (t_d) compared to before (t_0) the assay (except in E6). Additionally, the Cu and Zn associated with the oxides (F3) shifted from this fraction before the assay to the organic matter/sulfide fraction, F4, after the assay (Figure 6) because of the suboxic

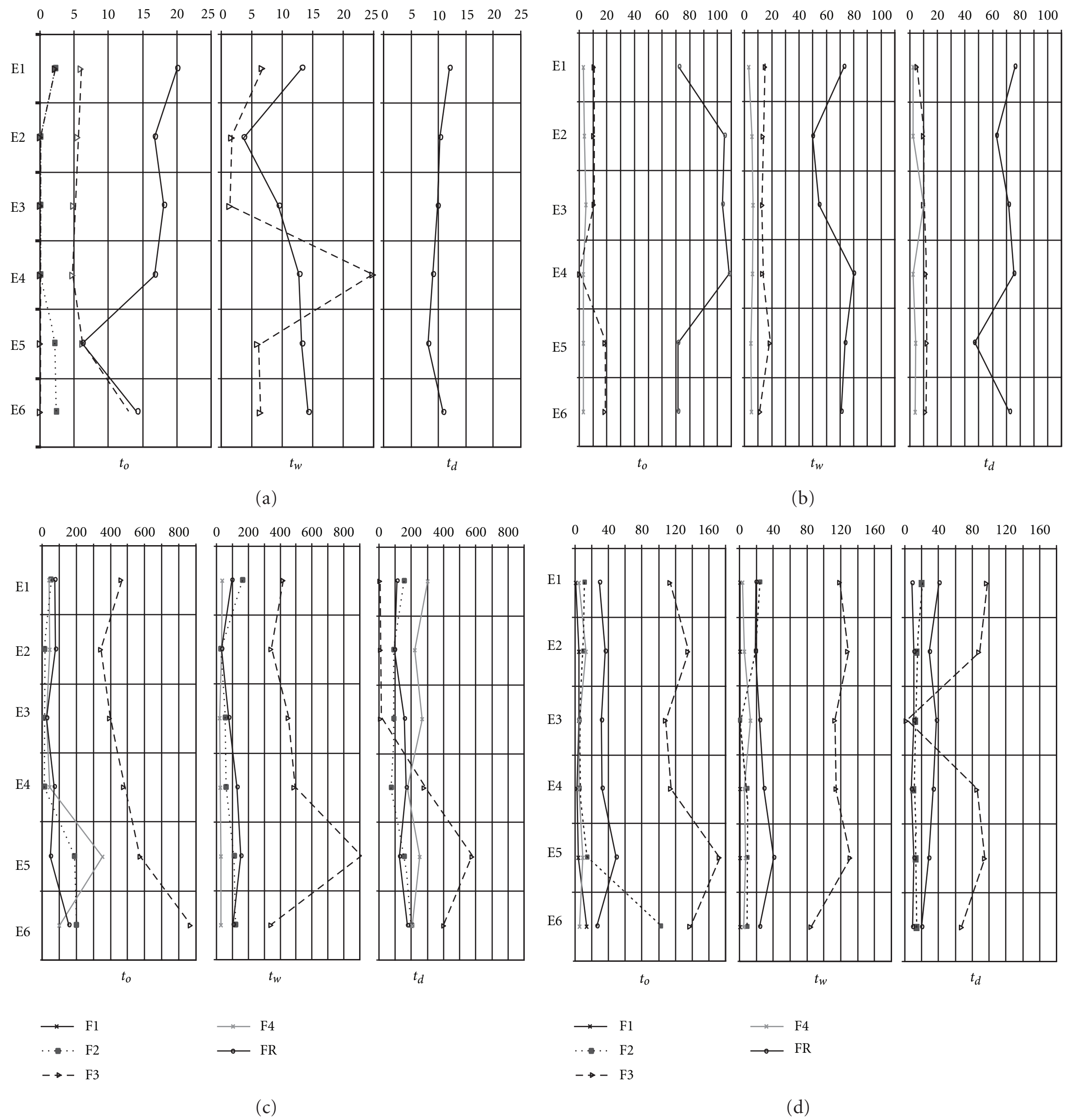

Figure 6: Absolute data for the concentration ($mg \cdot kg^{-1}$) of the extracted Pb (a), Fe (b), Cu (c), and Zn (d) in the six strata of the selected profile from Pile 2 before the assay (t_0) and after the wet (t_w) and dry (t_d) seasons. Data: average, $n = 3$.

Table 3: Relative abundance of the sulfur-oxidizing bacteria (SOB) and of the sulfate reducing bacteria (SRB) before the assay (t_0) and in the columns after the wet (t_w) and dry (t_d) seasons, per stratum.

	Aerobic SOB (pH 8.5)			Anaerobic SRB (pH 8.0)		
	t_0	t_w	t_d	t_0	t_w	t_d
E1	+	++	++	++	+++	+++
E2	+	+++	++	+++	+++	+++
E3	−	+	++	++	++	++
E4	++	+++	++	++	++	+++
E5	+	++	++	++	+++	+++
E6	−	+	−	+	++	+++

Note: number of cells in each of the 10 visual fields: − : 0; +: ≤10; ++: ≤20; +++: ≤40.

conditions. Cu and Zn can interact chemically with sulfide minerals (F4) and the covellite under nonoxidizing conditions [34]. Covellite was determined to be present using XRD in this work (Figure 4).

Under microaerophilic to suboxic, low saturation and high carbonate content soil conditions, the relative mobility of the metals is low [35]. Since the metals are released from oxides they are readsorbed or precipitated as carbonates or other stable phases that do not dissolve under suboxic conditions while the pH remains circumneutral [19, 29, 36–40]:

$$Cu^{2+} + CaCO_3 \longleftrightarrow Ca^{2+} + CuCO_3 \quad (2)$$

Under high carbonates content, photosynthetic activity leads to bicarbonate, HCO_3^- [36]:

$$CaCO_3 + H^+ \longrightarrow Ca^{2+} + HCO_3^- \quad (3)$$

The HCO_3^- may cause the precipitation of some phases such as $Cu_2(OH_2)CO_3$ [41] or smithsonite ($ZnCO_3$) [37]:

$$Zn^{2+} + HCO_3^- \longrightarrow H^+ + ZnCO_3 \quad (4)$$

Because the photosynthetic biofilms cover the surface of the carbonaceous tailings, the formation of metal-carbonate complexes increases. The carbonate may be stable under unsaturated O_2 conditions [42], and carbonates are known to be the soil component, mostly responsible for Cu retention [32]. Cu retention by carbonates from calcite is about 99% in calcareous soils [40]. Therefore, the presence of biofilms may provide chemical gradients and physical conditions that shift the proportions of Fe, Cu, and Zn from the oxides fraction (F3) to the carbonate/specifically adsorbed fraction (F2) and the organic matter/sulfide fraction (F4). The metals specifically adsorbed onto carbonates can be transferred to exchangeable forms only if the environmental conditions change (i.e., lower pH, redox potential). However, the pH may be stable because of the high ANP of the tailings material.

4. Conclusions

Mine tailing remediation here studied provided experimental evidence aiming to reduce the rate of sulfide mineral oxidation in calcareous tailings by photosynthetic biofilms; such mineral environment is characterized by highly positive net neutralization potentials. We confirmed that photosynthetic biofilms act as a physical barrier against oxygen diffusion, as described by a long-term assay (6 months) resulting in solid phase redistribution of the Pb, Fe, Cu, and Zn under high carbonates content conditions. Consequently, the photosynthetic biofilms represent an excellent opportunity for mine tailing remediation and stabilization of the Concepción del Oro mine tailings by applying ecological succession theories, that is, colonization through autotrophic pioneers [43], that also could be applied to the pollution and remediation of alkaline soil.

Acknowledgments

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