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Autotrophic Biofilm Development on Superficial Samples of the Gold–Silver Mine Tailings, Valenciana (Mexico): Pioneers in Tailings Remediation?

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Abstract We report the results of long term bio-assays on microorganism colonization of mine tailings samples, taken from the Valenciana mine tailings (Guanajuato, Mexico), under stable laboratory conditions (humidity, temperature, light exposure). In order to identify the main metabolic groups of the potentially colonizing microorganisms and the implications of their growth on the main tailing's characteristics related to biological succession, organic matter (OM) content, cationic exchange capacity (CEC), and pH values were measured as the colonization took place. We observe that photosynthetic biofilms (cyanobacteria, green algae, and diatoms) successfully colonize the mine tailings samples as pioneers; moreover, bacteria, yeast and fungi were also identified. Biofilm colonization significantly improved the OM contents, whereas the pH value is not modified during the entire observed colonization process. The results suggest that biofilms are useful during the first steps of the mine tailings remediation. This is the first report of microalgae and cyanobacteria grown of on tailings samples obtained from a semiarid region.

Keywords Biofilms · Autotrophic biofilm · Mine tailings · Remediation

The exploitation of mineral resources has resulted in the destruction of several areas of land and provoked serious environmental problems such as deforestation, erosion, and metal pollution of soil, water and groundwater. Particularly, the exploitation of ore deposits generates high amounts of non-economic by-products – the mine tailings, which are deposited in natural or artificial extended land depressions, known as tailing piles or tailing dams. These tailing deposits had been recognised as a main direct release of metals to the environment and generators of acid mine drainage (Allan 1995). The former indicates that mine tailings should be remediated using an environmentally and economically suitable approach. Cairns (1991) and Bradshaw (1997) expound that the reclamation involves the recreation of damaged and altered ecosystems, both in structure and function, with the purpose of re-establishing the pre-disturbance ecological attributes as natural succession processes. Therefore, it should be possible to use the ecological succession theories (i.e. colonisation through autotrophic pioneers) and apply it for the bioremediation of tailing piles (Gao et al. 1998).

The mines of the Guanajuato Mining District (21°01′–21°14′N and 101°15′–101°26′W) have been exploited for more than 450 years, and the District is still an important gold and silver producer in Mexico, with nearly 13 and 8% national production of gold and silver respectively; this exploitation has resulted in almost 150 million tonnes of waste material (Carrillo-Chávez et al. 2003; García-Meza et al. 2005). The Valenciana Mine Tailing (VMT) represents one of the largest tailing piles in Guanajuato; with a magnitude of about 20 million tonnes of material covering an area of 0.5 km^2. Because of its location on a steep hill along a creek just 4-km north of Guanajuato City, the local mine companies have considered remediation of mine tailing piles through vegetation to control the dispersion of fine particles (eolian pollution). However, previous investigations indicate that, under the present conditions, these tailings do not pose suitable conditions for healthy plant

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growth: the VMT has poor physical structure (coarse texture, poor water retention), an unsuitable nutrient capital (<1%) and a high proportion of bioavailable fraction of Cu, Cd and Pb (40, 46, and 55%, respectively) (García-Meza et al. 2005), which could severely limit plant growth (Ma and Rao 1997).

In fact, Ye et al. (2002) indicate that the high bioavailability of certain metals, the low organic matter (OM) content and the poor physical structure of most of the mine tailings are the major constraints for plant development. Additionally, the application of fertiliser is not only expensive, but ineffective due to the fertiliser runoff (Lan et al. 1998). Another approach implies the use of wetlands to remediate the mine tailings (Atkinson and Cairns 1994). However, the VMT is located in a semi-arid region (average precipitation 659–691 mm/year), like most of the major mine districts of Mexico. A low atmospheric moisture content and low water availability will be limiting factors for this biotechnology as such.

It is suggested that mine tailing remediation can be initiated using autotrophic, biofilm-forming and metal-tolerant microorganisms, since they may develop despite the low nutrient and water content and despite the presence of toxic metals; therefore, these biofilms may solve the limiting characteristics for the healthy development of plants, i.e. low OM content and poor substrate conditions. The aims of this study were to stimulate the biofilms' development on samples of the VMT under laboratory conditions (humidity, temperature and light exposure), and to identify the main metabolic groups and the community composition of these biofilms. The implications of biofilms' colonisation on the tailings' gross characteristics are discussed.

Materials and Methods

The VMT comes from the exploitation (floatation) of rich Au–Ag epithermal veins, which do not generate acid drainages due to their high carbonate/sulfide ratio (12:1) (García-Meza et al. 2005). The VMT is composed of fine mineral particles, where the major constituents are silicates (50–70%), carbonates and gypsum; the minor compounds are mainly toxic metals such as Cu, Zn and Pb. The VMT has low OM content and a mild alkalinity (García-Meza et al. 2005). Ten samples of VMT were taken from the surface material (less than 5 cm depth). All the samples were mixed thoroughly to obtain a single composite and homogeneous sample. The final sample was transferred to double sealed and sterilised plastic bags.

In order to increase the autotrophic species biomass, 1 g of the VMT samples was spread in a petri dish containing sterile agar-basal Bold culture media (BBM) (Stein 1973). Ten petri dishes were used. The daylight intensity was maintained constant following a 14/10 light/dark cycle. The room temperature was set at 25 ± 1°C. The microalgae colonies' growth started after 2 or 3 weeks in the petri dishes. Then the microalgae were transferred to BBM without agar and EDTA. These cultures were immediately spread over the mine tailings' surface at the beginning of the bioassays.

The VMT samples were distributed in acrylic cells (24 cm high, 20.5 cm wide and 40 cm length), previously acid cleaned and sterilized. The cells were filled with the material to 5 cm depth (final volume of 4,100 cm^3) and inoculated with the microalgae previously obtained. The cells were daily humidified with up to 150 mL of sterile tap water (Trial 1) or sterile synthetic wastewater (Trial 2; C:N:P at 15:1:0.1, pH 7). A control (no inoculum addition) was also carried out in duplicate. Each cell was illuminated with artificial daylight. The light intensity was maintained constant at 48 μmol m^{-2} s^{-1} following a 14/10 light/dark cycle. The room temperature was set at 25 ± 1°C. The bioassays were performed over 21 weeks. The identification of microorganisms (microalgae, bacteria, yeast and fungi) was initiated when the microorganisms became visible (week four).

Afterwards the bioassay samples were analysed for OM content by ignition at 550°C and by titration (Walkley 1947), cationic exchange capacity (CEC) according to Bower (1952) and paste pH. Bulk material samples were taken from each trial cell, homogenised and analysed in triplicate. Because the addition of water provoked leaching of metals, a comparison of the drainage rates of VMT material with and without biofilms (colonised and bare) was performed. The experiments were done in triplicate.

Microalgae determination was done by direct microscopic observations (1,000×) using in vivo material. Morphological and morphometric characters were considered for the genera or specie identification. The determination of the genera and the species were done using both specialised keys and descriptive literature. The identification of bacteria, fungi and yeasts was carried out using superficial samples (1 g) of the biofilm, which were transferred to test tubes with specific media for each microorganism. The tubes were incubated for 24 h at 35°C (bacteria) or during 3–4 days at 21°C (fungi and yeast). The isolation of the species was done using specific growth media. Once the monocultures were obtained, microorganism identification was performed using biochemical tests (MacFaddin 1990) and the API biochemical identification system (BioMeriux® biochemical tests); additionally, fungi and yeast identification was completed by direct microscopic observations using specific stains for the observations of the vegetative and reproductive structures.

Results and Discussion

Despite the relatively unsuitable characteristics of the tailings, microorganisms successfully colonised the surface samples of the VMT. After 4 weeks, the development of biofilms became evident in the trials previously inoculated and periodically irrigated. About 20 weeks later the biofilms covered up to 70% of the tailings' surface. The microorganisms colonised the VMT samples during the bioassays as autotrophic biofilms composed of 16 species of phototrophic microorganisms. Two unicellular green algae, *Chlorococcum* sp. and *Chlorella vulgaris*, and three species of the filamentous cyanobacteria *Phormidium* sp., *Pseudanabaena* sp. and *Anabaena* sp. (Table 1) were the predominant species. The term "biofilm" was given to the microbial community due to the colonies of green algae being well embedded into the cyanobacteria filaments network forming a biofilm structure (microscopic observations).

The presence of viable microalgae in such biofilms is a promising result, since it indicates a continuous performance of photosynthesis (release of both oxygen and organic carbon) and a natural fertilisation of the VMT sample. The occurrence of *Anabaena* sp. (Table 1) specifically implies nitrogen fixation. Also, the ecological role played by phototrophic communities in soils is the starting point for a primary succession process. Additionally heterotrophic aerobic bacteria (eight species), anaerobic bacteria (two), and some fungi (five) and yeast (three) were also observed (Tables 2 and 3). These groups of microorganisms were closely linked with microalgae, since they were directly isolated from the autotrophic biofilm.

The main bacteria genera present in the biofilms were *Bacillus*, *Pseudomonas* and *Corynebacterium* (Table 2). These genera had been reported in the old Au-mine tailings of Otago, New Zealand (Chappell and Craw 2002) and as residents of soils affected by mine drainages (Babich and Stotzky 1985).

Chappell and Craw (2002) specifically suggested that the organic carbon and carbonates present (up to 1 and 15% respectively) in the tailings of Otago were in high-enough

Table 1 Main classes and the corresponding species of the identified phototrophs developed as biofilms on the surface of samples from Valenciana mine tailings

Cyanobacteria	*Aphanothece* sp., *Synechococcus* sp., Cfr. *Cyanobium* sp., Cfr. *Cyanothece* sp., *Aphanocapsa* sp., *Pseudanabaena* sp., *Phormidium* sp., *Planktolygnby* sp., *Anabaena* sp.
Chlorophyta	*Chlorosarcinopsis* sp., *Chlorella vulgaris*, *Chlamydomonas* sp., *Cholorococcum* sp.
Bacillariophyta	*Fragilaria construens* fo. *construens*, *Cocconeis placentula*

Table 2 Identified aerobic and anaerobic bacteria isolated from the surface of samples from Valenciana mine tailings

Aerobics	*Actinomyces naesiundii*, *Bacillus brevis*, *B. cereus*, *B. pumillus*, *B. subtillis*, *Acinetobacter* sp., *Klebsiella* sp., *Plesiomonas shigelloides*, *Pseudomonas aeruginosa*, *P. fluorescens*. *Corynebacterium* sp. 1, *Corynebacterium* sp. 2, *C. aquaticum*, *Rhodococcus* sp.
Anaerobics	*Staphylococcus* sp., *S. saprophyticus*, *S. xilosus*, *Clostridium* sp., *Peptococcus* sp.

Table 3 Identified fungi and yeast isolated from the surface of samples from Valenciana mine tailings

Fungi	*Aspergillus niger*, *Aspergillus rubber*, *Cephalosporium* sp., *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp.
Yeast	*Candida albicans*, *Candida* sp., *Rhodotorula rubra*

concentration for the development of those heterotrophic bacteria. Similar observations were done by Villaseñor et al. (2005), who describe *Bacillus* and *Pseudomonas* as well as the fungi *Penicillium* sp., *Aspergillus niger*, *Cladosporium* sp., and *Fusarium* sp., among other genera isolated from tailings with less than 1% of OM and high carbonate contents.

However, the observations in the present work suggest that the autotrophic biofilms are the primary carbon supply for the heterotrophic bacteria and fungi, since the microalgae were the initial colonizers in the bioassays. Jahnke and Priefer (2002) also found that the significant increase of OM was produced by photosynthetic activity that enhanced the development of bacteria and fungi. In fact, the most relevant result in the present study is that the OM content was significantly higher ($p < 0.05$) in trials where the biofilms were developed (up to 3.5 + 0.4%) than in the controls (0.8 + 0.4%) (Fig. 1). This OM increase results in more organic colloids, which on the other hand provide more chemically active surfaces and the concomitant increase of the CEC (Arias et al. 2002) (Fig. 1). There are no significant changes in pH between the colonised trails and the controls (7.8 + 0.3). During the trial the pH stays slightly alkaline because of the high buffer capacity of the VMT that contains a high percentage of carbonates (Carrillo-Chávez et al. 2003) and because of the CO_2 consumption during the photosynthesis (Liehr et al. 1995).

Both the OM increment and the negligible pH variation after 20 weeks of biofilm colonisation are promising conditions for mine tailing remediation. The first implies a natural fertilisation process, and the latter may ensure immobilisation of trace elements (Stark et al. 1996). Singh (1961) noticed that the OM produced by microalgae binds soil particles and thus improves soil permeability and aeration, while Hu et al. (2002) showed that four

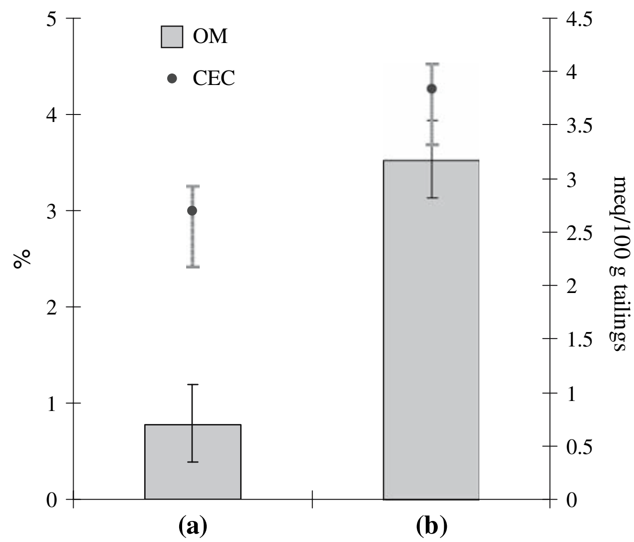

Fig. 1 Organic matter (OM) contents (%) and cationic exchange capacity (CEC) (meq·100g^{-1} mine tailings) of mine tailing samples. (**a**) control; (**b**) colonised by biofilms. Data: average (n = 3) and standard deviation (error bars)

Table 4 Rate of drainage water (mL min^{-1}) in samples of Valenciana tailings with and without biofilms

With biofilms	Without biofilms
1.3 ± 0.2 (13%)	7.1 ± 0.1 (71%)

(n = 3 ± standard deviation)

filamentous cyanobacteria (such as *Phormidium tenue* and *Nostoc* sp.) maintain biological crust cohesion, reducing eolian erosion of the particles. So, the whole biofilm structure and the formation of organic colloids, especially those derived from the extracellular polymeric substances (EPS) of the biofilm, lead to the substrate consolidation (Mazor et al. 1996). Moreover, in carbonate-rich sediments such as the VMT, the calcification of microbial filaments and the organic mucus may be the main process of sediment stabilisation (Hillgärtner et al. 2001). The later could explain why the biofilm colonisation contributes to a lower rate of water diffusion (Table 4), since the high hydric capacity of EPS reduces water infiltration (Wolfaardt et al. 1999).

The reduction of water infiltration has also practical applications in mine tailing remediation, since it increases the possibility of plant growth and the amelioration of extreme soil temperatures (Mazor et al. 1996) and may decrease the leaching of potentially soluble metals.

The species of the biofilms come already from highly metal-polluted sediments, which mean that species are likely to be already adapted to polluted conditions. In fact, some of the biofilm-forming species identified in this work had been reported as metal-tolerant of mine tailings (Foster 1982; Motohiro et al. 1983; Mullen et al. 1992; Chappell and Craw 2002). Nevertheless, the reason why biofilms survive is not well known yet. An earlier study on metal-exposed microalgae-forming biofilms has shown that the EPS could be partly responsible for an increased biofilm tolerance (García-Meza et al. 2005) acting as metal-binding sites (Kaplan et al. 1987).

The results of this research offer corroboration that the photosynthetic microorganisms are pioneer colonisers of oligotrophic environments. Eventually, heterotrophic aerobic and anaerobic bacteria, fungi and yeast were also present. The results discussed above are relevant from an ecological perspective, because the biodiversity maximises the metabolic capacities and optimises resource exploitation through complementary enzymes and metabolic pathways. The presence of active photosynthetic species within a biofilm gel (that provides a suitable micro-environment) can become an instrument for the remediation of mine tailings. Biofilms favour reduction of water infiltration and sediment erosion and a reduction of leaching of soluble metals. The biofilm-colonised substrate may possess the desired characteristics for the future development of vegetal species. This is a consequence of microorganisms that are the driving force of biogeochemical processes such as soil genesis, nitrogen fixation, and metal mineralization. Further investigation should focus on the microbial ecology of the biofilms under real climatic conditions.

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References

Allan RJ (1995) Impact of mining activities on the terrestrial and aquatic environments. In: Salmons W, Förstner U, Mader P (eds) Heavy metals, problems and solutions. Springer-Verlag, Berlin, p 120

Arias A, Barral MT, Mejuto JC (2002) Enhancement of copper and cadmium adsorption on kaolin by the presence of humic acids. Chemosphere 48:1081–1088

Atkinson RB, Cairns J Jr (1994) Possible use of wetlands in ecological restoration of surface mine land. J Aquat Ecosys Health 3:139–144

Babich H, Stotzky G (1985) Heavy metal toxicity to microbe-mediated ecologic processes. Environ Res 36:111–137

Bower CA (1952) Exchangeable cation analysis of saline and alkali soils. Soil Sci 73:252–261

Bradshaw AD (1997) Restoration of mine land using natural processes. Ecol Eng 8:255–269

Cairns J (1991) The status of the theoretical and applied science of restoration ecology. Environ Prof 13:186–194

Carrillo-Chávez A, Morton BO, González PE, Rivas SH, Oesler G, García-Meza JV, Hernández E, Morales P, Cienfuegos E (2003) Environmental geochemistry of the Guanajuato mining district, Mexico. Ore Geol Rev 23:277–297

Chappell DA, Craw D (2002) Geochemical analogue for circumneutral pH mine tailings: implication for long-term storage, Macraes Mine, Otago, New Zealand. Appl Geochem 17:1105–1114

Foster PL (1982) Metal resistant *Chlorophyta* from rivers polluted by heavy metals. Freshwat Biol 12:41–61

Gao L, Miao Z, Bai Z, Zhou X, Zhao J, Zhu Y (1998) A case study of ecological restoration at the bauxite mine, Shanxi Province, China. Ecol Eng 11:221–229

García-Meza JV, Ramos E, Carrillo-Chávez A, Duran C (2004) Mineralogical and chemical characterization of historical mine tailings from the Valenciana mine, Guanajuato, Mexico: environmental implications. Bull Environ Contam Toxicol 72:170–177

García-Meza JV, Barrangue C, Admiraal W (2005) Biofilm formation by algae as a mechanism for surviving on mine tailing. Environ Toxicol Chem 24:573–581

Hillgärtner H, Dupraz C, Hug W (2001) Microbially induced cementation of carbonate sands: are mictric meniscus cement good indicators of vadose diagenesis? Sedimentology 40:117–131

Hu C, Liu Y, Song L, Zhang D (2002) Effect of desert soil algae on the stabilization of fine sands. J Appl Phycol 14:281–292

Jahnke J, Priefer UB (2002) Phototrophic biofilms of reforested fields in the Rhenish lignite mining area: development of soil algal, bacterial, and fungal biomasses. Soil Biol Biochem 34:1157–1165

Kaplan D, Christiaen D, Arad S (1987) Binding of heavy metals by algal polysaccharides. In: Stadler T, Mollion J, Verdus MC, Karamanos Y, Moran H (eds) Algal biotechnology. Elsevier Applied Sciences, London, pp 179–187

Lan CY, Shu WS, Wong MH (1998) Reclamation of Pb/Zn mine tailings at Shaoguan, Guangdong Province, People's Republic of China: the role of river sediments and domestic refuse. Biores Technol 65:117–124

Liehr SK, Chen HJ, Lin SH (1995) Metal removal by algal biofilms. Wat Sci Technol 30:59–68

Ma LQ, Rao GN (1997) Chemical fraction of cadmium, copper, nickel, and zinc in contaminated soils. J Environ Qual 26:259–264

MacFaddin JF (1990) Pruebas bioquímicas para identificación de bacterias. Panamericana, México DF

Mazor G, Kidron GJ, Vonshak A, Abeliovich A (1996) The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts. FEMS Microbiol Ecol 21:121–130

Motohiro F, Sunao Y, Shozo T (1983) Distribution of cupper in the cells of the heavy metals tolerant fungus Penicillium achrochloron cultured in concentrated copper medium. Agr Biol Chem 47:1367–1369

Mullen MD, Wolf DC, Beveridge TJ, Bailey GW (1992) Sorption of heavy metals by the soil fungi *Aspergillus niger* y *Mucor rouxii*. Soil Biol Biochem 24:129–135

Singh RN (1961) Role of blue-green algae in nitrogen economy of Indian agriculture. ICAR, New Delhi

Stark LR, Williams FM, Wenerick WR, Wuest PJ, Urban C (1996) The effects of substrate type, surface water depth and flow rate on manganese retention in mesocosmos wetlands. J Environ Qual 25:97–106

Stein JR (1973) Handbook of phycological methods: culture methods and growth measurements. Cambridge University Press, Cambridge

Villaseñor B, Moctezuma G, Acosta I, García-Meza JV (2005) Inmovilización de arsénico y metales pesados mediada por hongos. In: Rodríguez RR, López-Doncel R, Castro-Larragoitia J (eds) Proceedings of the XV national congress of geochemistry (INAGEQ), San Luis Potosí, Mexico, September 19–23, p 43

Walkley A (1947) A critical examination on a rapid method for determining organic carbon in soil. Soil Sci 63:451–464

Wolfaardt GM, Lawrence JR, Korber D (1999) Function of EPS. In: Wingender J, Neu TR, Fleming HC (eds) Microbial extracellular polymeric substances. Springer-Verlag, Berlin, p 171

Ye ZE, Shu WS, Zhang ZQ, Lan CY, Wong MH (2002) Evaluation of major constraints to revegetation of lead/zinc mine tailings using bioassay techniques. Chemosphere 47:1103–1111