*Advanced Materials Research Vol. 825 (2013) pp 88-91*
*© (2013) Trans Tech Publications, Switzerland*
*doi:10.4028/www.scientific.net/AMR.825.88*

# Environmental impact on soil, water and plants from the abandoned Pan de Azúcar Mine

Josefina Plaza Cazón[1 a], Leonardo Benítez[1 b], Jesica Murray[2,3 c], Alicia Kirschbaum[2,3 d], Pablo Kirschbaum[2 e], Edgardo Donati[1 f]

[1] CINDEFI (CCT La Plata, CONICET - UNLP), Facultad de Ciencias Exactas, 47 y 115, (1900) La Plata, Argentina.

[2] Universidad Nacional de Salta, Av. Bolivia N° 5150 (4400) Salta, Argentina.

[3] Instituto de Bio y Geociencias del NOA-IBIGEO, Av. Bolivia N° 5150 (4400) Salta, Argentina.

[a] joplaca@hotmail.com, [b] leonardo_benitez26@yahoo.com.ar, [c] jesimurray@yahoo.com.ar,

[d] alikir2003@yahoo.com.ar, [e] pkirschbaum@gmail.com, [f] donati@quimica.unlp.edu.ar

**Keywords**: tailings, acid mine drainage, zinc, lead, vegetation, soils.

**Abstract.** Pan de Azúcar abandoned mine is located in Jujuy province, Argentina. The impact of that pollution was evaluated determining the density and the composition of the vegetation around the tailings. Also soil and plants samples were collected and their metal contents were determined. *Deyeuxia breviaristata* was the main native plant in the most polluted soils and its biomass had the highest concentration of zinc and lead. Acid mine drainage (AMD) samples were collected and their toxic effects were analyzed by ecotoxicology assays employed *Lactuca sativa* L. seeds; $CI50_{120}$ of 0.03 % v/v was determined. From mineral and AMD samples some iron- and sulphur-oxidizing microorganisms were isolated and also characterised by molecular techniques. AMD potential was evaluated for different tailing samples inoculating native microorganisms; deeper samples produced drainage with pH close to 1 and released iron above of 1000 ppm. AMD can reach Cincel River which is the main tributary of Laguna Pozuelos (just 25 km from the mine). Laguna Pozuelos is a Natural Monument, Biosphere Reserve and Ramsar site.

## Introduction

Explotation of mineral resources containing metals is one of the essential activities that contribute to the development of countries. However, mining has a negative reputation due to the large amounts of wastes accumulated in tailings impoundments which have caused serious impact on to the surrounding mining areas through soil, water and air pollution. Acid mine drainage (AMD) is one of the environmental impacts directly associated to metal mining. AMD is formed when sulphide minerals are exposed to oxygen, water and chemoautotrophic bacteria; the sulphide oxidation generates sulphuric acid that produces acidification, high iron and sulphate concentrations, and elevated levels of soluble toxic metals [1]. AMD poses a serious hazard to the environment contributing to the contamination of soil substrates, destruction of soil texture, destruction of ecological landscape, groundwater pollution, and decrease the biological diversity [2].

In the north of Argentina there are several abandoned mines; one of them is Pan de Azúcar Mine, located in the northwest of Argentina (Jujuy province), at 3700 m above sea level (22° 32-22°38 S and 66°01 66°08 O). The mineral presents high sulphide content (main mineralogical species are pyrite, sphalerite, and galena) and it is also rich in silver. Extractive activities for lead and silver ceased in 1986 and the site was abandoned without adequate mine closure. This abandoned mine was especially chosen as the study site because it is situated 25 Km south of Laguna Pozuelos, National Natural Monument, Biosphere Reserve (UNESCO 1990) and Ramsar site (1982). The site with neutral to alkaline water hosts 44 species of birds and is under a serious risk because Cincel River, the mainly tributary of the lake, can receive direct input from acid mine drainage that drained during the summer season [3].

The aim of this work was to detect and to characterize the effect of metal concentration in the Pan de Azúcar mine site and surrounding area including the analysis of soil, water and vegetation. Microorganisms probably responsible for AMD generation were isolated and characterised by biological molecular techniques. Ecotoxicological effect of the AMD was also evaluated using standard methods.

## Materials and Methods

**Evaluation of environmental impacts in the surrounding area of Pan de Azúcar passive mine**. In October 2010, seven sampling sites were selected based on the distance from mine, topography, vegetation and slope (see Fig. 1). In order to determine the abundance of each species of plants in soils, eight 25 $m^2$ plots were analysed. Plant samples were collected from every plot. Soil samples were also collected at the same time and the same place where the plants were sampled. In the laboratory the plants were identified using Darwiniana dichotomous key and classified making a herbarium. Zinc and lead contents were analysed in roots and aerial parts. Plant roots were rinsed with abundant distilled water and separated from the aerial parts in order to determine their respective dry weights (oven dried at 50 ◦C, until constant weight). 1 g of biomass (aerial or roots plants) was exposed to acidic digestion with $HNO_3$(c)/$H_2SO_4$(c) (initial volume ratio 4:1). Additional volumes of $HNO_3$(c) were added until oxidation of the organic matter. Soil samples were sieved using sieve size 20 mesh before treatment with the same mixture of acids indicated above. The acid extracts were filtered with membrane 0.45 μm and then analysed using atomic absorption spectrophotometry (Shimazdu AA6650F Atomic Absorption Spectrophotometer).

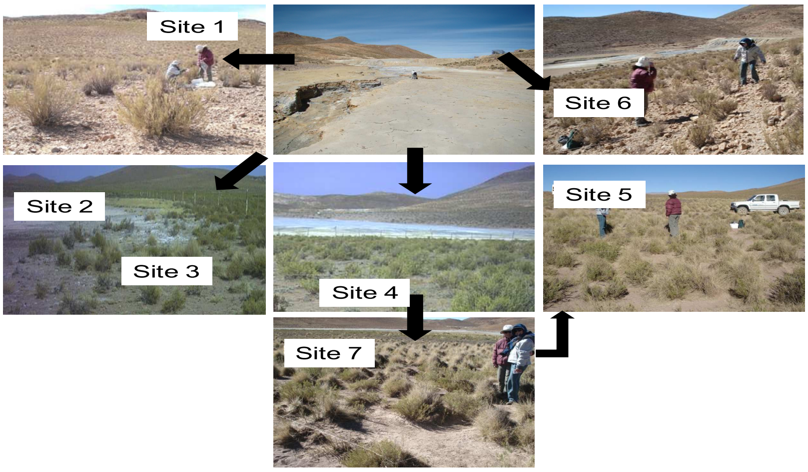


Fig. 1. Location of sampling sites in Pan de Azúcar Mine (Jujuy province).
Photo on the top and centre shows the tailings dam.

**Studies about environmental risk of acid mine drainage generation from tailings**. Five samples from the tailing (DAM N°2, located at 22°36′52′′ S and 66°03′11.7′′) were taken at different depths and identified as DC2-P2-1, 2, 3, 4 and 5 (highest number is the deepest sample). The samples were dried at 30°C for one week. Then they were homogenized and stored in dried conditions at 4°C up to their use. Samples were enriched for iron- and/or sulphur- oxidising microorganisms using 0K medium (KCl 0.1g/L; $MgSO_4.7H_2O$ 0.5g/L; $K_2HPO_4$ 0.5g/L; $Ca(NO_3)_2$ 0.01g/L; $(NH_4)_2SO_4$ 3g/L) supplemented with sulphur powder (10% w/v) and iron(II) (9 g/L) as $FeSO_4$. Enrichments were incubated at 150 rpm and 30°C. Both original samples and enrichments were analysed using Fluorescence in situ hibridation (FISH) with specific probes for *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans.* In order to evaluate the risk associated to AMD generation bioleaching of the tailing samples were carried out; for that the samples were added at two different pulp densities (2 and 5 % w/v) to 150 mL of 0K medium, inoculated with microbial communities from the enrichments, and incubated at 150 rpm and 30ºC. Sterile controls were also carried out under the same conditions.

**Ecotoxicology bioassays.** AMD samples were collected from the tailings and their toxicity were evaluated using standardized ecotoxicology bioassays with *Lactuca sativa* seeds according to specific methods (IRAM-29114:2008 and IRAM-259117:2009). These bioassays are cheap, easy to implement and reproducible. Zinc sulphate was used as reference contaminant in toxicity control experiments. AMD toxicity experiments were done by diluting AMD using water from Cincel River; seeds were germinated at 20°C. Length of the lettuce plantlets was measured and the results were used in a dose-response analysis.

**Results and Discussion**

6 different species of plants were identified in the samples around the mine (Table 1). *Deyeuxia breviaristata* seemed to be the most tolerant plant to the metal since it was detected in sites 2 and 3 which soils showed the highest metal contents (see below). In the other sites higher number and diversity of species were found.

Table 1. Number of plants corresponding to the species found in each sampling sites

| Species *(abbreviations into brackets)* | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 |
|---|---|---|---|---|---|---|---|
| *Parastrephia lepidophylla* (Wedd) Cabrera (*Tv*) | 5 | | | 19 | 42 | 14 | 15 |
| *Parastrephia lucida* (Meyen) Cabrera (*Ta*) | 18 | | | | | 16 | 5 |
| *Festuca argentinensis* (St. Yves) (*F*) | | 8 | | 1 | 23 | | 1 |
| *Adesmia horrida* Gill. Ex Hook and Arn (*Añ*) | 10 | | | | | | 1 |
| *Distichlis scoparia* (Kunth) (*Dist*) | | | | 1 | | | |
| *Deyeuxia breviaristata* (*Db*) | | full covered | full covered | | | | |

All soils are heavily contaminated with zinc whose concentrations decreased in the following order S2 >S4 >S6 >S3 >S7 >S4 >S1. Lead was not detected in soils from sites 1, 5, and 7. Other soils contained lead whose concentration followed the following order S2 >S3 >S4 >S6 (Fig. 2a). Fig. 2b shows metal contents in the different plants sampled from different sites (it just includes some representative results). Most plants contained zinc in their biomasses while just some of them presented lead. Highest zinc concentration was found in the following species Db(S2), Añ(S1), and Ta(S6) (the site is informed into brackets). Biomass of Db from site 2 showed the highest level of lead.

Sulphur-oxidizers were detected by FISH analysis. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* strains were isolated from the enrichments. Fig. 3 shows changes in pH values, and $Fe^{2+}$ and $Fe_{total}$ concentrations during bioleaching of one of the tailing samples (DC2-P2-5) at the highest pulp density (5 % w/v). This sample was the deepest one used in our experiments. During its bioleaching, the highest iron dissolution was reached (the other data are not shown here). Bioleaching of different samples showed that unless the superficial sample (DC2-P2-1) the higher the depth the samples were taken, the more generation of acid and the higher dissolution of iron. That is why DC2-P2-2 and DC2-P2-3 do not represent serious risk for AMD formation. The deepest samples (DC2-P2-4 and DC2-P2-5) showed a continuous decrease of pH values and increase of soluble iron concentration during bioleaching. Iron concentration in inoculated systems was at least four times higher than that in sterile controls; in addition iron in inoculated systems was mainly as iron(III). In sterile controls the ratio Fe(III)/Fe(II) was very low. DC2-P2-1 showed a different behaviour and its bioleaching reached higher iron dissolution than those for the samples immediately below it; this behaviour can be explained due to this sample was taken from the layer of eroded material deposited on the surface all around the mine. At higher pulp densities, bioleaching of deepest samples showed a direct increase in iron dissolution (data not shown). Fig. 3b shows the dose-response curve of $CI50_{120}$ obtained using *Lactuca sativa* seeds in assays increasing the dilution of AMD. According to this test, even high dilutions of AMD provoke serious ecotoxicological impact.

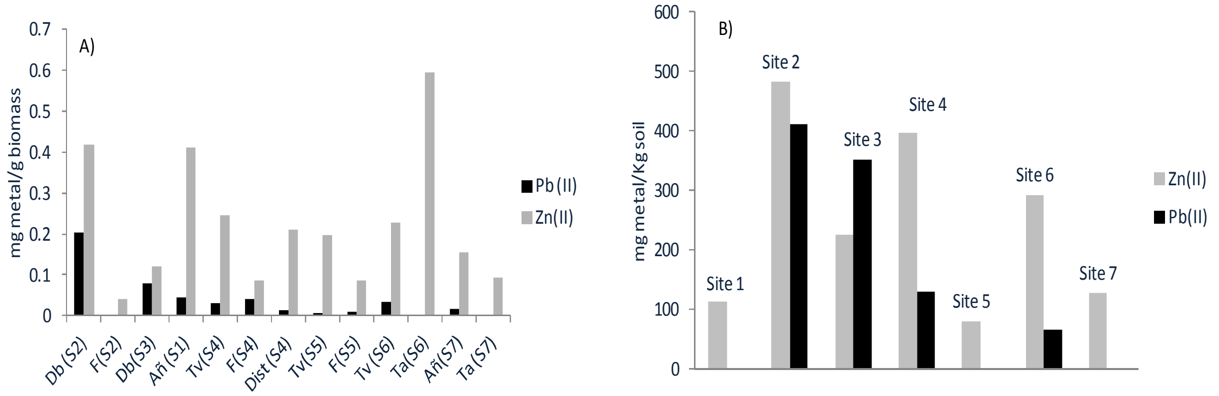


Fig. 2. Zn(II) and Pb(II) concentration: a) into the soils and b) in the biomasses of plants. In the second graph, sites are indicated into brackets. The abbreviations of plants can be found in Table 1.

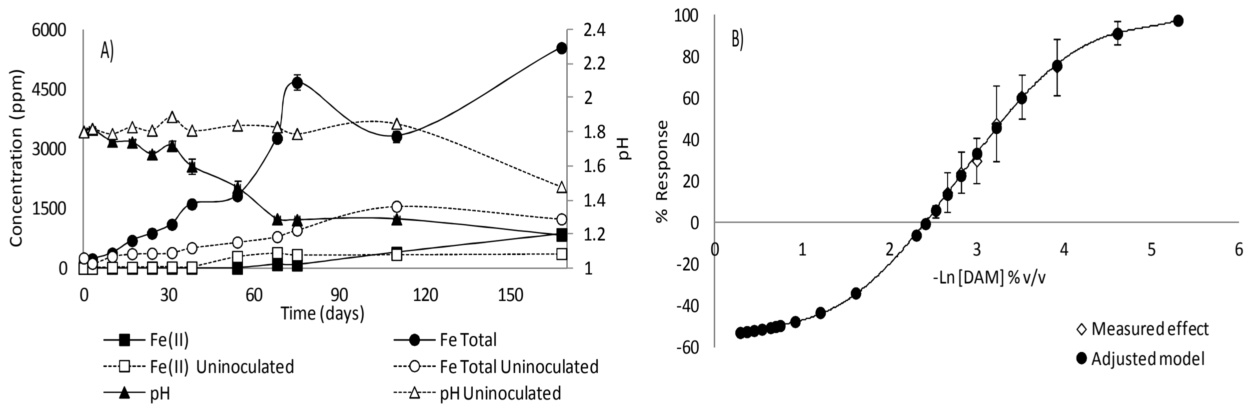


Fig. 3. a) $Fe^{+2}$ and $Fe_{total}$ concentrations and changes in pH values during bioleaching (pulp density 5% w/v) of DC2-P2-5 tailing sample. b) Dose- response curve $CI50_{120}$ employed *Lactuca sativa* seeds.

## Conclusions

Our results demonstrate that the abandoned mine Pan de Azucar is provoking a serious impact to the environment not only on the soil which presents high lead and zinc concentrations but also on the plants whose biomasses contain up to 400 and 500 ppm of lead and zinc respectively. Those plants are the main source of foods for animals in the zone which also are consumed by people living around. In addition several ADM were detected close to the tailings; these drainages produce severe ecotoxicological effects as the test used showed; even whether high dilutions of AMD reach Cincel River (and consequently Laguna de Pozuelos), the environmental situation of this Biosphere Reserve could be seriously impacted. Besides, the drinking water for people could be affected by the acidity and the presence of heavy metals constituting an additional human health risk.

## References

[1] J. Bech, P. Duran, N. Roca, W. Poma, I. Sánchez, L. Roca-Pérez, R. Boluda, J. Barceló, C. Poschenrieder: J. Geochem. Explor. Vol. 123 (2012), p.109.
[2] M.N. Rashed: J. Hazard. Mater. Vol. 178 (2010), p. 739.
[3] M. Arnosio, A. Kirshbaum, P. Piccardo, M. Vernuci, G. Roman Ross: Actas del XVII Congreso Geológico Argentino (2008), Jujuy.