# ECOLOGICAL CONDITION AND MICROFLORA OF SHYMKENT LEAD-ZINC WASTE

**Akmaral Issayeva, Assel Tleukeeva**

*M.Auezov South Kazakhstan State University, 160012, Kazakhstan, Shymkent city, Tauke khan avenue, 5, +7 701 3245044, akissayeva@mail.ru*

**To determine the feasibility of bioleaching lead-zinc waste is necessary to know the structure of the data microbiocenosis of waste. As a result of microbiological studies found that the microflora lead-zinc waste Shymkent presented Thiobacillus ferrooxidans, Leptospirillum ferrooxidans, Nitrosomonas sp., Aspergillus niger, Penicillium sp., which are involved in natural microbial bioleaching process. The presence of species Amoeba limax and Tokophria sp., algae Oicomonas sp., Navicula sp., Shlorococcus sp. indicates a strong mineralization aqueous solutions around the lead-zinc waste. Modelling of bioleaching processes shown that the use of microbiological method for the extraction of valuable components of lead and zinc waste.**

*Keywords: microflora, lead and zinc waste, bioleaching, thiobacteria*

## 1. Introduction

Kazakhstan is one of the largest mining and metallurgical regions of the world and takes in terms of total production of solid minerals 13th place out of 70 mining and metallurgical countries. Production growth, of course, leads to an increase in raw material processing, in particular primary, and as a result increase the output of waste and the need to address environmental problems in mining regions.

Recycling of mining and metallurgical complex is one of the major components of the total of the management and control of natural resources in the region. Do not dispose of waste and stockpiled in the region, often very dangerous and toxic, ultimately cause significant harm to the environment. According to experts, the volume of waste mining and metallurgical complex in the dumps, tailings and slime is about 12 billion tons, of which 9.2 billion cubic meters of overburden and rock, 2.5 billion tons of tailings, 327 million tons of metallurgical slag.

One of the promising areas of extraction of metals is bacterial and chemical leaching, environmentally friendly and does not require much material and energy costs (Bollag, Dec & Bollag, 2000, Ciminelli & Garcia, 2001, Gerike, Pinches & Van Rooyen, 2001, Brandl, 2001, Ilyas, Anwar, Niazi & Ghauri, 2007, Pant, Joshi, Upreti & Kotnala, 2012). It is known that the methods of bioleaching to recover valuable components are used mainly in the processing of mineral raw materials - various types of ores for heap, and underground agitation leaching (Marca, Massacci & Piga, 2002, Olson, Brierley & Brierley, 2003, Choi, Cho, Kim & Kim, 2004, Ilyas, Ruan, Bhatti, Ghauri & Anwar, 2010, Akcil, Deveci, Jain & Khan, 2010, Lee & Pandey, 2012). There are different cultures of microorganisms and modified the conditions of cultivation (Brierley & Brierley, 2001, Rawlings, 2002, Faramarzi, Stagars, Pensini, Krebs & Brandl, 2004, Yang, Xu, Wen & Yang, 2009, Xiang, Wua, Zhua, Zhang, Liu, Wu & Li, 2010, Ilyas, Lee & Chi, 2013). For more efficient leaching of precious metals from various fields required bank cultures of microorganisms that must be isolated from the natural environment - from ores and mine waters, conducting pilot tests will provide additional data for the development of technological regulations and issue recommendations for the implementation of proven technology in production.

## 2. Material and methods of investigation

The object of the study were lead-zinc waste lead plant joint stock company “SouthPolyMetal” (JSC "SPM"), located within the boundaries of the city of Shymkent. The company JSC "SPM" is located on the banks of the river Badam, in the south-western part of the city of Shymkent **(Figure 1).**

Territory and sanitary protection zone enterprises fit snugly to the uptown. Lead-zinc waste production are stored in three kilometers from the plant site on the other side of the river. Total volume dumps containing in its composition, and Pb2 + ions Cd2 +, is 0.5 million tonnes. Areas of greatest risk of pollution is the territory of Abai district and neighborhood "Kazgurt" of Shymkent city, located in the direction of prevailing on the ground south-west and north-west winds. Chemical analysis of soil on the content of heavy metal ions was carried out at 500, 1000, 1500 and 2000 meters from the territory of "SPM", and 100, 500, 1000, 2000 and 5000 meters from the waste heaps. The dynamics of migration of heavy metals in the horizons of the soil profile was analyzed based on the results of chemical analyzes of soil samples collected at depths of 10, 20, 30 and 40 cm. As a control, were selected areas located at 8,000 meters from the analyzed sites.

The isolation of bacteria: For the isolation of *Leptospirillum spp* and *Acidithiobacillus ferrooxidans* bacteria 9K media with ferrous iron as a source of energy was infected with samples from lead-zink slag and incubated at 37oC for 7-10 days (Markosyan, 1972, Biogeotechnology of Metals, 1989). The growth of isolated *Leptospirillum* sp. was observed in the range of temperature 30-40$^0$C with the optimal temperature of 37oC. The influence of pH on the growth of bacteria and its iron oxidation activity has been studied during the first days of cultivation until no significant change in pH value occurs. Optimal pH value was 2.0-2.2. Mixed cultures were routinely sub-cultured in an incubator shaker at 32-35 0C and a pH of 2.0 in 9K medium consisting of 3.0 g (NH4)2SO4, 0.5 g K2HPO4, 0.5 g MgSO4.7H2O, 0.1 g KCl, 0.01 Ca(NO3)2, and 44.2 g FeSO4.7H2O used as energy source dissolved in 1000 ml distilled water. Micromycetes were isolated with using of Zhapek medium consisting of sucrose -30,0 or glucose - 20,0; $NaN0_3$-2,0; $K_2HPO_4$- 1,0; $MgS0_4x7H_20$ - 0,5; KC1 - 0,5; $FeS0_4x7H_20$ - 0,1; (Biogeotechnology of Metals, 1989). For the isolation of Nitrosomonas sp. was used Vinogradskii medium consisting of glucose - 20,0; $K_2HP0_4$ - 1,0; $MgS0_4x7H_20$ - 0,5; $CaCO_3$ - 20,0; yeast extract-10,0; trace element solution - 1.

In modeling the bioleaching process waste glass used percolators where to put the waste in the different fractions of solid to liquid ratio (S: L) 1: 3. The exposure time of 72 hours at a temperature of +22+24$^0$C. In the study were used microscopes «Tauda» and «Mikmed-5", by light microscopy. Before microscopic solid samples averaged by quartering. The abundance of aquatic organisms was determined visually according to the following scale: 1 individually, 2 rarely, 3-lot 4 profusely.

The elemental composition of the samples before and after bioleaching was conducted on inductive plasma spectrometer «Varian 820" spectrometer AAnalyst 800 spectrophotometer SPECORD 75 IR.

## 3. Results of investigation and discussion

### 3.1 Characteristics of lead-zinc slag

The analysis of the waste showed that it consists of two types of slag: rich-after primary production, and poor-after processing **(Table 1).**

However, due to the fact that the waste was stored for decades in the open air, because of the influence of climatic conditions are active processes of wind and water erosion. In addition, the expansion of the boundaries of the city has led to the fact that, at the moment, toxic wastes are located in the city of Shymkent. 250 chemical analyzes carried out in various parts of Shymkent found that in the surrounding of "SPM" areas of the city the concentration of lead in the soil and the air is 3564.9 mg / kg and 5.0 mg / m3, respectively, and its maximum value at certain points, respectively, to reach 24900.0 mg / kg and 31.4 mg / m3 [20]. Among the studied children attending kindergartens in the area of sustainable impact of lead plant, found in 66% of the excess of limit values of lead in the blood for a child's body. At this concentration established at a rate of 10 g / dl, were 3-4 times higher than in the other cities in Kazakhstan investigated. Research has shown that the most polluted residential areas within a radius of 1500 meters in the north-east of JSC "SPM" **(Table 2).** In established five areas of concentration of ions of lead and cadmium in the soil are in direct proportion to the distance from the source of contamination and vary accordingly within 2345.6 ± 24,4 mg / kg (or 73.5 maximum - allowable concentration (MAC)) and 20.3 ± 2.0 mg / kg (or 40.6 MAC).

These results were obtained from the analysis of soil samples from native horizon, which survived only in certain residential areas. The concentration of heavy metals in a lower 10- 20 and 20-40 cm soil depths, respectively, was 45.0 ± 3.7% and 20.0 ± 2.1% by volume of the total set of heavy metal ions. The content of heavy metals in soil, within the limit values, it was found at a depth of 50.0-60.0sm.

### 3.2 Microflora of lead-zinc waste

The studies found that in aqueous solutions, selected from a pool near the waste JSC SPM meets 2 types amoeba- Amoeba limax and Tokophrya sp., algae Oicomonas sp., Navicula sp., Chlorococcus sp., commonly found in highly mineralized standing water bodies **(Figure 2).**

In liquid and solid samples marked micromycetes in the amount of $2.5 \pm 0.2 \times 10^4$ cells / ml and $3.1 \pm 0.2 \times 10^3$ cells / g. Taxonomic characteristics micromycetes were assigned to species Aspergillus niger and Penicillium sp. Nitrifying 1 phase were allocated in the amount of $10^2$-$10^4$ cells / ml. In the samples taken at the border: lead-zinc waste-soil were found nitrifying 2 phase in an amount of 10-$10^2$ cells / g. Denitrifiers weren`t found. Thiobacteria Acidithiobacillus ferrooxidans, marked in the amount of $5.0 \pm 0.4 \times 10^2$ cells / ml, mobility and activity. These bacterias are single rods of 0.5 x 1 μm size, motile (single polar flagellum), obligate chemolitotroph and autotroph, strictly aerobic. Optimum temperature for the growth is 30-35 ° C. Optimum pH is 2.2- 2.5. Leptospirillum ferrooxidans number ranges of $10^4$-$10^5$ cells / ml. Small curved-rod shaped Gram-negative cells of 0.3-0.6x1.0-3.3 μm, motile by a single polar flagellum. They grow in acidic environment on mineral medium containing ferrous iron at an optimum pH 2.5-3. Only Fe2 + is used as an energy source, unable to use sulfur or organic compounds for growth. The cells are aerobic and mesophilic. Mixed culture and Acidithiobacillus ferrooxidans Leptospirillum ferrooxidans is capable of oxidation 8.6 ± 0.6 g / l of divalent iron per day.

### 3.3 Modelling natural processes bioleaching lead-zinc waste

In percolation tests were used poor lead-zinc slag, having in its composition, µg / kg: Ti-409.616; V-29.768; As-138.875; Ag-18.697; Sb-43.223; W-8.381. During the experiments was used a mixed culture micromycetes Aspergillus niger and Penicillium sp. with a titer of $10^7$-$10^8$ cells / ml, the ratio S: L = 1: 3, 0.5-1.0 mm particle size fraction, the exposure time of 72 hours at a temperature of + 20 + $25^0$C. After time in the leaching solution is removed, g / l: Ti-258.468; V-16.730; As-114.017; Ag-10.190; Sb-26.842; W-3.671, that is: on the Ti-63.1, by V -56.2, by As-82.1, by Ag-54.5, on Sb-62.1, W-43.8% extraction. It has been found that by reducing the size 0.25-0.5mm fractions to the extraction of metals in the solution increases. Thus, the Ti content in the solution is increased to 292. 555 (71.4% recovery rate); To V-26.416 (88.7%); 13.17 Ag-up (70.4%); Sb- to 31.748 (73.4%); W- to 5.183 (61.8%) g / l. Further reduction in grain size fractions leads to reduction of dissolved metals recovery.
In the next experiment were used batch culture thiobacteria, micromycetes, nitrifying 1 phase. The results were compared with the results of bioleaching and sulfuric acid leaching **(Table 3).**

As can be seen from Table 3, the use of different groups of microorganisms in the solution will extract the valuable components. Modelling of bioleaching processes shown that the use of microbiological method for the extraction of valuable components of lead and zinc waste. Using composition Acidithiobacillus ferrooxidans +Leptospirillum ferrooxidans extracts in solution Ge and Au. It is interesting to note the use of nitrifying and micromycetes for bioleaching, because their life is optimum in the neutral pH range, and will not lead to further pollution acids. Using them as a leaching agent in the solution will extract Ti, As, Sb, W.

## 4. Conclusion

Thus, it is established that the waste of JSC "SouthPolyMetal" undergoes natural microbiological processes bioleaching, which is confirmed by the presence of different groups of microorganisms involved in the biogeochemical cycles of sulfur and iron. Modelling of bioleaching processes shown that the use of microbiological method for the extraction of valuable components of lead and zinc waste. Preliminary studies have shown that the use of mono or polycultures microorganisms facilitates the extraction of precious metals in solution. Further development of the method of bioleaching valuable components of the lead-zinc waste will provide not only economic benefits, but also reduce environmental stress in Shymkent.

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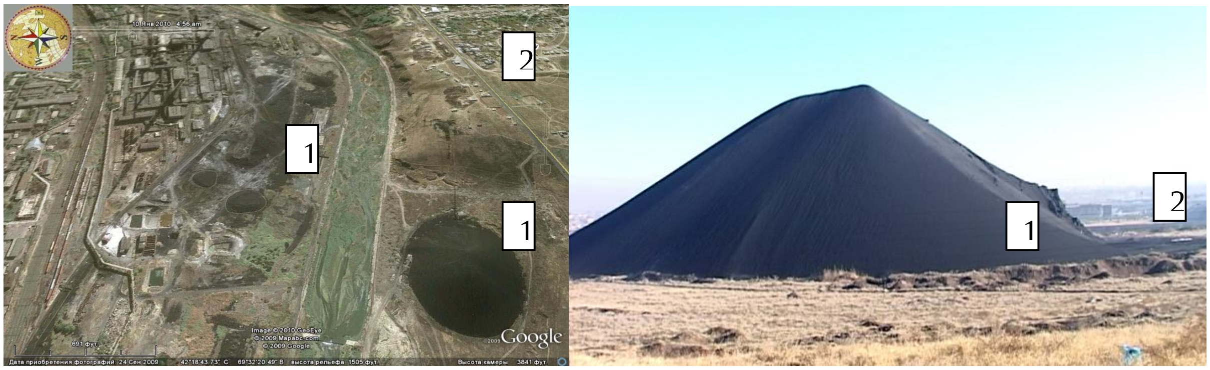


A B

1. Lead-zinc waste, 2. Human items

**Figure 1. Location of Shymkent lead-zinc waste**: A. view from space ((www.googlemap.com), B. side view

**Table 1. Composition of the lead-zinc slag of JSC "SouthPolyMetal",%**

| Type of slag | Pb | Zn | Cu | FeO | CaO | $SIO_2$ | Volume, t | Location | Year of establishment |
|---|---|---|---|---|---|---|---|---|---|
| Rich slag | 1.6±0.1 | 10.0±1.1 | 5.8±0.6 | 38.5±3.1 | 13.1±1.1 | 25.0±2.2 | 0.9±0.1 million | At the plant | C 1992 |
| Poor slag | 0.03±0,05 | 2.0±0.2 | 0.9±0.1 | 40.2±3.8 | 15.2±1.3 | 28.5±2.6 | 2.2±0.2 million | outside plant | 1975-1992 |

**Table 2 Characteristics of soil pollution heavy metal ions Shymkent**
(average for 2002-2008 according to the Research of impurity of soils of the territory Shymkent lead ions.., (2008))

| Sampling | The content of heavy metals, mg / kg | | | | |
|---|---|---|---|---|---|
| | $Cd^{+2}$ | $Pb^{+2}$ | $Cu^{+2}$ | $Cr^{+4}$ | $Zn^{+2}$ |
| 0.5 km from the JSC "SPM" | 11,8±1,1 | 350,6±10,3 | 75,7±5,7 | 12,8±1,1 | 54,3±4,7 |
| 1.0 km from the JSC "SPM" | 13,3±1,1 | 455,6±12,7 | 98,5±8,4 | 14,2±1,2 | 66,3±3,3 |
| 3.0 km from the JSC "SPM" | 5,3±0,2 | 256,3±13,9 | 65,3±3,9 | 7,4±1,1 | 37,9±3,9 |
| 5.0 km from the JSC "SPM" | 2,3±0,1 | 125,3±15,2 | 63,4±3,9 | 6,2±0,3 | 29,7±1,2 |
| MAC | 0,5 | 32 | 33 | 6 | 21 |

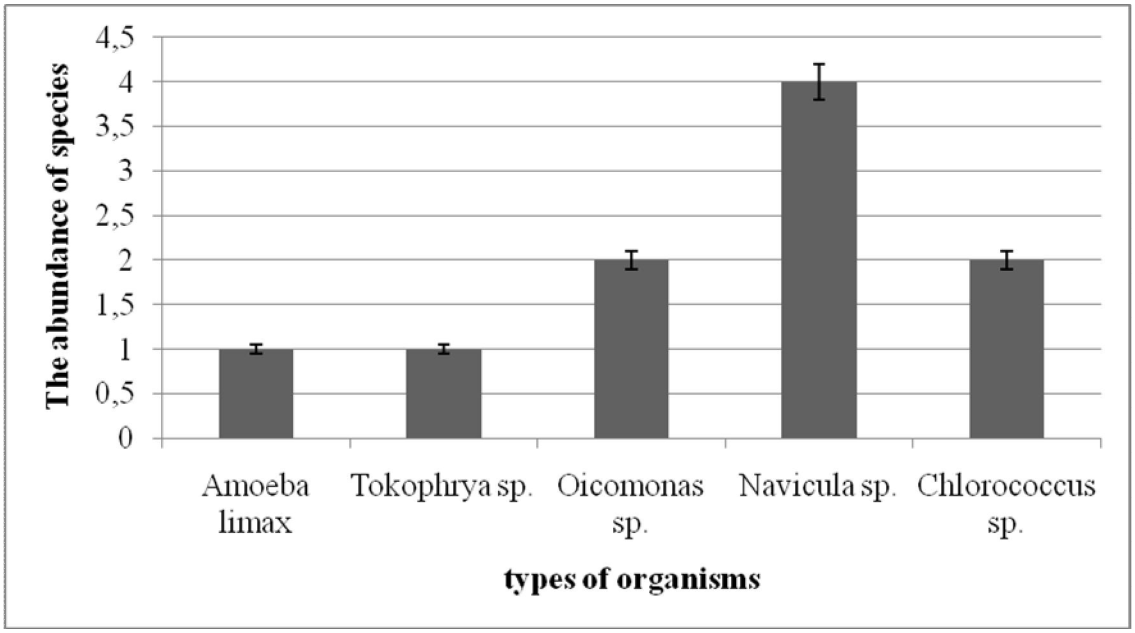


**Figure 2. The abundance of species in the lead-zinc waste**

**Table 3. The results of the leaching of lead and zinc waste Shymkent using various leaching agents**

| Elements | Nitrosomonas sp. | Aspergillus niger+Penicillium sp. | Acidithiobacillus ferrooxidans +Leptospirillum ferrooxidans | H2SO4 |
|---|---|---|---|---|
| Ti47 | 238.354 | 258.468 | 57.966 | 50.272 |
| Ge72 | 16.844 | 0.0 | 30.959 | 11.014 |
| As75 | 189.906 | 114.017 | 71.391 | 88.143 |
| Se82 | 20.064 | 0.669 | 20.154 | 0.0 |
| Mo95 | 8.525 | 6.74 | 4.592 | 3.432 |
| Ag107 | 11.09 | 10.19 | 10.33 | 2.226 |
| Sb121 | 33.495 | 26.842 | 2.249 | 1.955 |
| W182 | 3.362 | 3.671 | 0.355 | 1.047 |
| Au196 | 1.301 | 9.534 | 13.004 | 5.303 |