Ecotoxicity and pollutant concentrations of wastewater from mineral processing

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ABSTRACT

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Ecotoxicity and pollutant concentrations of wastewater from mineral processing

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In this project, the ecotoxicity and pollutant concentration of wastewater from mineral processingwas studied. The wastewater from the process contains high amounts of heavy metals and sulphates. Wastewater treatment was tested using three different Biological systems. The efficiency of the three systems and the conditions to improve the efficiencywas tested. Theecotoxicity and pollutant concentration were analysed in order to show how the pollutants can be removed by the systems. This study contains the preliminaryresults of the treatment as well the wastewater was management options in order to obtain the best results in pollutant removal.

Two test runs were done. In the first run, wastewater was used to check how the microorganisms can be sustained in the presense of wastewater. In the second test run, the systems were first enriched with nutrient water to sustain and grow the microorganism population in order to reduce the water toxicity. After that, the wastewater was added periodically into the systems. This did not, however, improve the efficiency of the systems. The biggest problem in the wastewater was the low pH due to high sulphate concentration that makes this water also difficult to be treated by biological systems. The results of the study show that pretreatment is needed before biological system can be used to treat this wastewater.

Key words: mining wastewater, toxicity, heavy metals, sulphates, biological treatment.

CONTENTS

ABBREVIATIONS AND TERMS

TAMK	Tampere University of Applied Sciences
cr	credit
WST	Willow Stack tower
ATS	Algae Turf Scrubber
Ni	Nickel
Zn	Zinc
Na	Sodium
K	Potassium
TN	Total nitrogen
TP	Total phosphorous
BOD	Biochemical oxygen demand
AAS	Atomic Absorption Spectrophotometry

1 INTRODUCTION

In this thesis the ecotoxicological and effects and heavy metal and sulphate concentrations of wastewater from mineral processing are studied. Three different biological treatment systems were tested in the treatment of the wastewater and the ecotoxicological effects and pollutant concentrations analysed accordingly.

1.1. Characteristics of wastewater from mining processes

Different waste residues are characterized depending on the mining process. The biggest amount of waste is produced in floating and leaching operations. The main environmental problems appear because of the slowly settling fine particles, dissolved metal ions and chemicals retained in the effluent. The effects of disolved metal ions, pH and sediments should be reduced in order to not to contaminate the environment. Legal and environmental aspects of metal mining wastes have to be considered.

The extraction and the concentration of the metals which are in the Earth crust do not produce important quantity of waste. The main reason of it is because in this part of the mining process purely mechanical operations are involved. However the chemical treatment of the ores produced large quantities of waste.

In the most part of mining processes, the ores treated are slurried in water. Due to the big quantities of water required for the process, underground and surface water must be added, that it has to be pumped up and discharged. All this water has to be treated before discharging in the environment.In mining process, extensive amounts of organic and inorganic chemicals are used to alter the surface characteristics of the minerals (floating processes) and to obtain solutions and precipitates in leaching processes.Chemicals are added in order to precipitate, to neutralize and to adsorbe on the produced mineral surfaces, but some times dissolved substances of a pollutant nature remain in the effluent. This is particularly the case in leaching plants, where precipitation and neutralisation of effluent impurities are important economic objectives.

Different kinds of extraction are done in order to obtain metals from the ores with the most possible efficient way and all of them have some negative environmental effects. (Alianza mundial de Derecho Ambiental, 2010)

IN SITU LEACH MINING

In situ leach mining has more environmental and security advantages than conventional mining. In this kind of mining, the ore is left in the ground, and the minerals are recovered from it by dissolving them. This process uses a solution called `lixiviant´ to extract the metal from underground ore bodies in place.Lixiviant, which typically contains an oxidant such as oxygen and/or hydrogen peroxide mixed with strong acids that depend on the kind of metal that is extracted, is injected through wells into the ore body in a confined aquifer to dissolve the metal. The `pregnant´ solution is pumped to the surface, where the minerals can be recovered. Consequently there is little surface disturbance and no tailings or waste rock generated. However, the orebody needs to be permeable to the liquids used, and located so that they do not contaminate groundwater away from the orebody. (World nuclear association)However, ore body is disolved with strong acids than normally dissolve metals in the rock as well. The waste fluids that are obtained in the process contain high concentra-tions of metals, being an important risk to nearby ground and surface water sources. The low pH also produces acidification of the aquatic environment. (Massachusetts Institute of Technology, 2012)

HEAPLEACHING

Heap leaching is an industrial mining process to extract metals from ore via a series of chemical reactions that absorbs specific minerals.

In the heap leaching, the ore is stacked onto an impermeable base and is irrigated with a process solution that liberates the product from the ore and mobilises it into solution.
The pregnant solution is then intercepted at the base of the heap leach pad via a series of underdrains and transferred via solution channels to process solution ponds. The pregnant solution is treated according to the specific processing requirements of the commodity, and the barren solution is circulated back for reuse in the circuit: thus, the process reagents are maintained within a closed process water circuit. (Kappes, 2002) Normally, it is a step in the process. The main problems in this kind of mining extraction are the failure to keep process solu-tions within the heap leaching circuit. Release of toxic heap leaching fluids into the environment can affect the health of both the surrounding ecosystem and human population. Water is crucial in the heap leaching project design because of the possibility of the overflow of solutions containing toxic concen-

trations of heavy metals after a heavy rainfall or rapid snowmelt. (Massachusetts Institute of Technology, 2012)

In some mines, cyanide is used to extract metals from ores and the resulting leach ponds are an important cause of wildlife mortality.

BRINE MINING

The brine mining is used to in salt mines from subterranean caverns or deposits. In them, water is used as a solution to dissolve the salt or halite deposits.In this mining process, brine solutions are extracted by pipe and evaporated to remove harmful compounds which can be released into the environment.The drilling and transport of brine solutions can disrupt existing ecosystems and well casings, pipelines, and storage tanks are subject to corrosion due to the high salinity content of the solutions that they are exposed to, which can lead to leaks and contamination of adjacent bodies of water. It can produce the reduced groth of the aquatic plants and the death of the fish. (Massachusetts Institute of Technology, 2012)

FLOATING

The floating is a phisic-chemical process in which the separation of mineral species is done by selective adhesion of mineral particles to air bubbles.In this process, the particles that were crushed before the floating are pulped with water, and the surface of the mineral of interest selectively made hydrophobic through the addition of an organic species, which is termed a collector. The mineral hydrophobicity allows the adhesion of solid particles to the air bubbles that pass through the pulp. The bubbles attach to the hydrophobic particles, forming stable foam on the water surface which keeps the particles on the surface.

The reagents used in the process are flotation collectors, depressants, activators and modifiers, whose primary actions are to induce and inhibit particle hydrophobicity and provide stability to the foam. Some examples are Alky Aryl Oxime, Petroleum Distillate, Sulfosuccinate surfactant, Alkyl xanthate salt, Nalco 7873 and Alcohol/hydrocarbon blend. Many of the reagents and chemical additives used in the flota-

tion constitute a danger of contamination of the waters because of that; it is important controlling the use of this kind of reagents. (Freeman, 2009)

BIOHEAPLEACHING

This kind of extraction is a cost effective and environmentally friendly exploitation system of metal extraction using bacteria in presence of water and air. It is a simple operation system and it produces high value products. In this mine, the main product that is obtained is nickel but there are other important metals that are extracted as zinc, cobalt and copper. (Biotech, 2008)

All kinds of mines have in common two aspects: the extraction and the concentration of the metals which are in the crust earth. But depending on the mineral that is wanted to obtain the process of each mine is different. In spite of the high concentration of metals that can be extracted in a mine, the amount of waste is too high. Because of this in the mines the first step is the trituration and the milling of the rocks in order to obtain small quantities of the metal and to be able to eliminate the no metal part of the rock. The milling is one of the most expensive parts in the mining process that makes easier the metal extraction but releases more contaminants from the sample. (Biotech, 2008)

The extraction starts with large open pit-mining. The mining has four stage of crushing in which the 80% of ore sample is triturated to 8 mm. In the extraction of the mineral several physical and chemical separation tecniques can be used as concentration by gravity, magnetic separation, electrostatic separation, flotation, extraction by solvents, leaching, precipitation and amalgation.

After that, the leached efluent is recovered and it goes to the second leach platform. After the second leaching, the ore remains in the second leach platform permanently. In the metal recuperation process, the dilution in which the metals are present is precipitated.

After the crushing of the rocks, the mineral are stacked in bioleaching stacks. There the metal extraction starts. The biological process name is bioheapleaching. It consists in the mineral leaching in the main leach platform during 14 months. The leaching platforms were inoculated with endemic bacterias that are naturally growing in the ore be-

cause of that they are well adjusted to the prevailing environmental conditions. The amount of bacteria that is inoculated is in the range between 106 to 108 cells / ml. These bacterias are either mesophilic or thermophilic. (Biotech, 2008)

The biological oxidation of the pyrite and cryolite that are present in the ore is an exothermic reaction which releases substantial amounts of energy. Temperature can increase until 75ºC during the leaching. Several physical, chemical and microbiological process parameters can be modified in order to enhance and speed up the metal recovery process. To reach and maintain the temperatures required for enhanced sulphide mineral leaching, different microbial populations are required to be present over time and the microbial growth rates need to be optimum. (Biotech, 2008)

When the leaching finishes, the metals recuperation is done by precipitation. Nickel, zinc, copper and cobalt from the pregnant leach solution are precipitated and filtered. After the metals are removed, the solution is further purified and returned to irrigate the heaps.The solution is collected at the bottom of the heaps and either recirculated through the heap or fed to metals recovery. (Biotech, 2008)

1.2. Elements presents in wastewater from mining processes

Nickel

Nickel is a silvery white metal with a hint of gold. The main characteristics of this metal are that it does not conduct well the electricity and heat. It is ductile and maleable because of that it can be laminated, polished and forged. It presents ferromagnetism with room temperature. It is a very dense metal. (Lenntech, 1998)

Most part of thecomercial nickel is used as stainless steel and as other corrosion resistant alloys. Nickel when it is finely divided is used as hydrogenation catalyst. It can be found in a lot of different igneous minerals (0.01%), in the meteorites and in the earth principally in the center of it where is the second most abundant metal. In the earth crust there is about 0.008% of nickel. Two important ores are nickel sulphide, iron sulphide, pyrrhotite, pentlandite and garnierite ore. (Lenntech, 1998)

The nickel is present in some plant and animals, sea water, fuel and in most parts of the carbonmineral. Metallic nickel is strong and hard. When it is finely divided, it is black. It is moderately reactive. It is alkaline corrosion resistant and it will not burn into chunks, but very thin wires can ignite. In metallic form is a strong reducing agent. (Lenntech, 1998)

Nickel is an element that is in the environment in small levels. Its main use is in metal products but we can find it in food, plants, cigarettes and soaps.
In small quantities the nickel is essential but when it is present in high levels it is dangerous for humans. (Lenntech, 1998)

Taking high amounts of nickel has the following consecuences:
- High probability of lung, nose, larynx and prostate cancer.
- Diseases and dizziness after being exposed to nickel gas.
- Lung embolism.
- Respiratory failure.
- Birth defects.
- Asthma and chronic bronchitis.
- Allergic reactions.
- Disorders of the heart.

(Lenntech, 1998)

Environmental Effects

Nickel is released to air in energy plants and in waste incinerators. It is deposited on the ground or it fall down after reacting with the raindrops. It takes a long period of time to eliminate the nickel in the air. (Lenntech, 1998)

Nickel can be found in water when it is leaked with wastewater. Most part of the nickel compounds that are released to the environment are absorbed by sediment or soil particles. In acid soils, nickel binds to become more mobile and it often reaches the groundwater. (Lenntech, 1998)

At high concentrations in sandy grounds, nickel can damage plants and algaes can be damaged, when it is present in high concentrations in water surface. The microorganism growth can be decreased when the nickel is present although they normally develop their resistance to nickel. In ecotoxicologicanalysis, it has been observed that life expectancy of Daphnia is disturbed when the nickel concentration in water is higher than 40 ppm. (Lenntech, 1998)

For the animals, nickel is essential in low concentrations but when it is present in high concentration it can cause cancer in animals. It is not usually that animals and plants absorb nickel. It is not present in the alimentary chain. (Lenntech, 1998)

Zinc

Zinc is a grey metal, which is malleable and ductile.The main use of zinc is to be a protective coating of other metals. When zinc is used for it, it is called zinc galvanized and they are the coat of iron and steel. It can be done by dipping the article in molten zinc (hot-dip process), by electrolytically depositing zinc on the article as a dip plating (electroplating), by exposing the article to zinc powder near its melting point (Sherardizing) or by spraying with molten zinc (metallic). (Lenntech, 1998)

Zinc is one of the least common metals. It is presented in the crust earth on a 0.0005 to 0.02%. The main mineral is sphalerite, zinc marmatite or sphalerite. It is an essential element for the development of many kinds of plants and animals. Zinc deficiency in the human diet produces damage in the growth and maturity and also produces anemia.

Insulin is a protein that contains zinc. Zinc is present in most food, especially those that are rich in protein. (Lenntech, 1998)

Pure zinc and just polished is bluisk-white, lustrous and moderately hard. Humidity in the air causes its surface fogging, became it into gray. Pure zinc is ductile and malleable and can be rolled up and tighten, but small amounts of other metals as contaminants can become it into brittle. (Lenntech, 1998)

Zinc is good conductor of heat and electricity. Pure zinc is not ferromagnetic.It is a chemical active metal. It can be ignited with some difficulty producing a greenish blue flame in the air and release zinc oxide in the form of smoke. (Lenntech, 1998)

Health Impact

Zinc is common substance in the nature. Many foodstuff and also potable water contain zinc. The concentrations in foodstuffs that can increase if it is stored in metal tanks. Industrial sources and toxic waste places can be one reason of why potable water contains zinc. (Lenntech, 1998)

Zinc is a trace element fundamental for the human health. If people do not take enough zinc they can suffer loss of appetite, sensitivity decrease, taste decrease and odor decrease as well as small sores and rashes. The accumulation of zinc may even cause birth defects. (Lenntech, 1998)

Although humans can manage large amounts of zinc, too much zinc exposures may also cause health problems as stomach ulcer, skin irritation, vomiting, nausea and anemia. High levels of zinc can damage the pancreas and disturb the metabolism of proteins and cause arteriosclerosis. Exposures intensive zinc chlorate can cause respiratory disorders. People who work n contact with zinc can suffer the flu that is known as metal fever. Zinc can harm unborn children and newborns if their mothers have absorbed large concentrations of zinc. Children can be exposed to it through blood or milk from their mothers. (Lenntech, 1998)

Environmental Effects

Zinc is in the nature in the water, air and ground, but the concentration of it is inceasing because of unnatural causes. The main cause is the human activity. Zinc is added during industrial activities like mining, coal combustion and waste and steel processing. World production of zinc is increasing which means that more zinc is in the environment.Water is polluted with zinc because industrial wastewater is not purified satisfactory. One of the consequences of this is that the rivers are depositing polluted sludge on their banks. Zinc can also increase acidity of the waters. (Lenntech, 1998)

Some fishes can acumulate zinc because of the presence of high zinc concentration in river's water. When zinc enters in the bodies of these fish it can form part of the food chain. (Lenntech, 1998)

In the aquatic toxicity test, it is observed that, when the zinc concentration in water is 6 mM, more than 93% of daphnia are death.

Large quantities of zinc can be found in the ground. If animals absorbe this concentratios, they will damage their health. Zinc can damage the plants too; they can not survive on zinc rich grounds. Despite manure that contains zinc is still applied.Zinc can disturb the activity in soil; it negatively influences the activity of microorganisms and earthworms. The decomposition of organic matter may be slower because of this. (Lenntech, 1998)

Copper

Copper is one of the transition metals and it is an important non ferrousmetal.It is used extensively because of its chemical, physical and mechanical characteristics, as well as its electric characteristics and its abundance. (Lenntech, 1998)

It has not high chemical activity. It is very heavy. It is not a magnetic metal, it is a bit paramagnetic. Its thermic and electric conductivity is very high. It is one of the metals that can be obtained in the most pure state. It is moderately hard; it is tenacious in the extreme and fatigue resistant. Its mechanical, physical and chemical characteristics depend on the size of the metal grain. (Lenntech, 1998)

Copper was one of the first metals used by humans.The main applications of copper compounds are found in agriculture, especially as fungicides and insecticides, as pigments, electroplating solutions, in primary cells, as mordants in dyeing, and as catalysts. (Lenntech, 1998)

The most part of copper in the world is obtained from the sulphure ores like chalcocite, covellite, chalcopyrite, bornite and enargite. The oxidized ores are cuprite, tenorite, malachite, azurite, chrysocolla and brochantite. (Lenntech, 1998)

There are huge quantities of copper in the earth for the future use if the minerals with lower concentration of copper are used and there is not probably that cooper runs out in a short period of time. (Lenntech, 1998)

Health impact

Copper is a substance present in the nature that is distributed by a lot of natural phenomena. Humans use copper in several parts of their lives. It is used the industria and in agriculture. Copper production has increased in the last decades and because of this the amount of copper in the environment has increased too. (Lenntech, 1998)

Copper can be found in a lot of kinds of food, in freshwater and in the air. Copper ingestion is necesary because copper is an essential trace element in human health. Although humans can handle big quantities of copper, manage too many copper can cause health problems. (Lenntech, 1998)

Copper soluble compounds are the greatest threat to human health. Copper compounds are usually water soluble and they appear in the environment because of the agricultural activities. (Lenntech, 1998)

Copper concentrations in air air are too low so this is not an important way of pollution but people who lives near copper foundry industries can suffer breath problems.People, who live in houses with copper pipes, are exposed to high levels of copper because copper is released by pipe corrosion. (Lenntech, 1998)

The copper exposition in work can cause a flu named "metal fever".If someone is exposed to copper in a long period of time; it can produce nose, mouth and eyes irritation and cause headaches, stomachaches, dizziness, vomiting and diarrhea. A large intake of copper can cause liver and kidneys damage and even death. If the copper is carcinogenic has not been determined. (Lenntech, 1998)

Environmental effects

World production of copper is still growing what means that more copper is present in the environment. The rivers are depositing mud on its banks that are contaminated with copper, due to the discharge of wastewater contaminated with copper from industries. Copper is in the air by releasing it during the combustion of fuel. Copper is present in the air because of the fuel combustion. (Lenntech, 1998)

Copper can be released in the environment by both human activities and natural activities. Examples of natural activities that release copper are dust storms, decaying vegetation, forest fires and marine aerosols. Examples of human activities are mining, metal production, wood production and phosphate fertilizers production. (Lenntech, 1998)

When the copper is in the ground, it is strongly connected to the organic materia and minerals because of that it is difficult that copper is present in subterranean water. Copper can be acumulated in plants and animals when it is present in the ground. In grounds with high copper concentration, plants can not survive. Copper can seriously affect agricultural land, depending on the soil acidity and the presence of organic matter. Despite this manure containing copper is still used. (Lenntech, 1998)

Copper can disturb the activity in the soil because of its negative influence on the activity of microorganisms and earthworms. The decomposition of organic matter may decrease because of this. (Lenntech, 1998)

Animals can absorb copper concentrations that damage their health.

Cobalt

Cobalt is ferromagnetic hard metal. It is tension resistant and it has thermal properties and electrochemical behaviour. Water and air do not affect cobalt in normal conditions and it is attacked by sulphuric acid, clorhidric acid and nitric acid. Hydrofluoric acid, ammonium hirdróxido and sodium hydroxide attack it slowly. Cobalt presents variable valent and form complex ions and colored compounds, as all compounds of transition. Cobalt and its alloys are fatigue resistant and corrosion resistant at high temperatures. (Lenntech, 1998)

One of its most important commercial aplications is to form part of alloys that are resistant to the high temperatures, of magnetic alloys, alloys for machines and tools, for metal sealing for glass and for the medical and dental alloy named vitallium.Plants and animals need small quantities of cobalt. (Lenntech, 1998)

Its isotope cobalt-60, that is produced artificialy, is widely used in the industry, medicine and investigation. (Lenntech, 1998)

Cobalt compounds have a variety of industrial applications, including for use as catalysts, and in agriculture to remedy cobalt deficiency in soil and natural vegetation. (Lenntech, 1998)

It is extensively distributed in the nature and it constitutes approximately the 0.001% of the total igneous rocks present in the crust earth. It is present in meteorites, stars, the sea, freshwater, soil, plantsand animals and in the manganese nodules found on the ocean floor. It can be obserbed cobalt traces in a lot of iron, copper, nickel, silver, mangenese and zinc ores but the most important minerals that are commercial are arsenides, oxides and sulfides. (Lenntech, 1998)

Health Impact

Cobalt is distributed in human environment so they are exposed to it when they breathe, drink waterand eat food with cobalt. (Lenntech, 1998)

Cobalt usually is not freely available in the environment but when it does not join to soil particles and it does not sediment, plants and animals can take it and it can be acumulated in plants and animals. (Lenntech, 1998)

Cobalt is useful for humas because it is part of B-12 vitamin. It is used to treat anemia in pregnant women, because it helps to create red corpuscles.When cobalt is present is high concentrations, it can cause health problems. If high cobalt concentration air is breathed unhealthy effect in lungs can be appear as asthma and pneumonia. Normally these problems appear in people who work with cobalt.

When plants grow in grounds in which cobalt is present, they can have small cobalt particles, principally in the parts that are eaten as fruits and seeds. It is important to control the plants that grow near mines. (Lenntech, 1998)

The health effects that result of taking high concentrations of cobalt are:

- •Vomiting and nausea
- • Vision Problems
- • Heart Problems
- • Thyroid damage

Health effects may also be caused by radiation from radioactive isotopes of cobalt. This causes sterility, hair loss, vomiting, bleeding, diarrhea, coma and even death. This radiation is sometimes used in cancer patients to destroy tumors. These patients also suffer hair loss, diarrhea and vomiting. (Lenntech, 1998)

Environmental Effects

Cobalt is an element that is present in the environment in a natural way. Humans release cobalt in small quantities to the air when carbon is burnt and in mining process. (Lenntech, 1998)

Radiactive cobalt isotopes are not present in the environment in a natural way; they are released in nuclear energy plants. But they do not need a long period of time to be desintegrated so they are not too dangerous.It is too difficult eliminate the cobalt when it is release to the atmosphere and more when it is absorbed by either soil particles or water. (Lenntech, 1998)

Sulphates

Sulphates are presented in a natural way in the environment and it can be found in natural water in different concentration. They are also used in the chemical industry, to control the algaes in the water and as additive in foodstuff.Wastewater from mining processes has high concentration of sulphates due to the oxidation of sulphate ores.

Mining water has large quantities of sulphates due to the pyrite oxidation and the use of sulphuric acid.

Sulphate salts are moderately soluble in water. Calcium and magnesium sulphates contribute to the water hardness and they make up the water permanent hardness.High levels of sulphates in water can give taste to the water and cause laxative effects. When high concentratios of sulphates are presented in acid water, it can have corrosive properties. (Clark, 2002)

1.3. Environmental effects of wastewater

The effluents of the mining are different due to the fact that they depend on their origin and their productive activities of the company, even each company can have more than one effluent with different composition. Because of that the wastewater can have different caracteristics and there is no unique way to remove the contaminants. (Dharmappaet al, 2000)

The volume and the chemical composition of the wastewater depend on:
- The process of the mining
- The hydrology of the mining
- The nature of the mineral
- The benefit of the company

It is very important to define the water quality model in order to know what would happen if the wastewater was leaked in the environment. The quality water impacts come from both puntual discharges and diffuse sources. (Environmental protection agency, 1997)

Spilled effluents in ecosistems produce local impacts and extended impacts. Local impacts are related with the zone were the wastewater is flowed into the ecosistem. The extended impacts are the most permanents and they affect the water quality in a long time.Diffuse sources can be various and they are more difficult to locate. One example of them is when the contaminant is present in the land due to the action of the precipitation. (Environmental protection agency, 1997)

There are five important impacts with which the quality of water is perturbed.

MINING ACID RAIN

When big quantities of ores in which sulphates are present are digged out in open pit way, this ores react with the air and water and they forme sulphuric acid. When the water has a particular acid level, a kind of bacteria called "TiobacilusFerroxidante" can appear and they increase the speed of the oxidation process and the acidification, leaching more the waste metals present in the water. This process continues until all the sulphates present in the ore are extracted completely. The acid is transported by mining water, raining or superficial water and it is deposited in water tanks, rivers, lakes and

other acuaticecosystems. The mining acid drain degrades severally the water quality and it can produce the death of the acuatic life. (Environmental protection agency, 1997)

HEAVY METALS AND LEACHING

Heavy metal pollutants are produced when some metals which form part of the ores are in contact with the water. Metals are extracted and they are taken to the rivers while water washes the ores surface. Although metals can be removed in neutral pH conditions, leaching is accelerated in low pH conditions. (Environmental protection agency, 1997)

CHEMICAL CONTAMINATION

This kind of contamination happens when some some chemical reagents which are used for the separation of the metals from the ore are leaked. These chemicals can be really toxic for humans and animals. (Environmental protection agency, 1997)

EROSION AND SEDIMENTATION

Mining development produces disturbence in the ground and in the rocks because of the construction of roads, landfills and open pit excavations.When there is not neither preventions nor control estrategies, ground erosion that is exposed can transport high amounts of sedimentation to the rivers and lakes.Excessive sedimentation can cause the obstruction of the riverbank, it can destroy the vegetation of it and the animals and the acuatic organism habitat. (Environmental protection agency, 1997)

MINING WASTE DUMPS

Once minerals have been processed and recovered, the useless part of the ore is taken to the dumps as mining waste. The dumps have the same toxic heavy metals and acid ores that the waste of the ore produces. They can contain chemical waste used to process the ore too. Dumps are usually located on the surface, in concentration areas or in oxidation lakes. (Environmental protection agency, 1997)

If they are not controlled, minerals can leach to the surface or to the underground water producing the pollution of this water.

As it was said, the contaminants present in the wastewater can be differents depend on the characteristics of the mine and the kind of extraction that is done in it.

The principal contaminants present in mining waste water can be classified in five different groups.

Main categories	Sub categories
Physical	Suspended solids (SS) Turbidity Color Temperature Taste and odor
Chemical (Organic)	Coal Oils and Grease Soaps and Detergents Rubber Dyes and Phenolic compounds

Chemical (Inorganic)	Heavy Metals (Ni, Zn, Cr, Cu, Hg, Pb, etc.)
	Acids
	Alkalis
	Cyanide
	Dissolved salts
	   - Cations: Mg, Ca, K, Na, Fe, Mn, etc.
	   - Anions: Cl-, S042-, NO3-, HCO3-, PO4-, etc.

Biological	Bacteria, viruses and small organisms
Radiological	Uranium Tritium and other radioactive substances from mine tailings

TABLE 1. Typical contaminants in mine wastewater (Sivakumar et al, 1992)

- Biological contaminants: In this kind of contamination, different kind of pathogenic microorganisms can cause diseases. Other kind of non pathogenic microorganisms are present too and they can produce the diseases in people with low defenses.The microorganisms that get used to this kind of contamination can be cause toxic substances and infections. (Dr Raghu N. Singh, 2006)

- Chemical contaminants (organic and inorganic): Depend on the microorganism's metabolisms; there are two kinds of contaminant. (Dr Raghu N. Singh, 2006)

    • Biodegradable: when they are descompounded in the intestine in presence of amine of some foods, they can produce nitroamines which can produce cancer. (Dr Raghu N. Singh, 2006)

    • Non biodegradable: They are compounds that are produce by chemical synthesis. There are not organisms that can transform them. They can change the taste, smell and colour, produce foam and can be toxic due to the bioacumulation in acuatic organisms. (Dr Raghu N. Singh, 2006)

- Physical contamination: Thermic contamination comes from the use of water to hot or cold the different products in the process. It affects the oxigen concentration in water. (Dr Raghu N. Singh, 2006)

- Radioactive contamination: It comes from natural sources or human activities. It is mutagenic and carcinogenic. (Dr Raghu N. Singh, 2006)

Physical and biological pollutants are the most critical in most of the mines. The temperature is an important physical pollutant but it is not really important in the wastewater due to the fact that mines use the necessary energy quantity. (Dr Raghu N. Singh, 2006)

In this thesis sulphates and heavy metals are measured. PH is considered too because it plays an important role in the bacterial and algae growing that is necessary in our systems. For the nutrient and organic material analysis of this wastewater, Lorena Lorilla Thesis, 2013 has to be showed. (Dr Raghu N. Singh, 2006)

- pH: It is the measurement of the molar concentration of hydrogen ions in the solution. The metal solubility depends on the pH. Moreover the high sulfur content coal is the reaction of sulphur, oxygen and water and in the presence of bacteria forms sulphuric acid and lowers pH. (Dr Raghu N. Singh, 2006)
  It is, with the sulphate concentration, one of the biggest problems of our wastewater. (Dr Raghu N. Singh, 2006)

- Sulphates: In bioheapleaching process, the oxidation from sulphur to sulphates is done by the bacterias in the process. Moreover sulphuric acid is also added to regulate the pH in the irrigation water solution. The sulphates present in water have a laxative effect when the water is consumed and an unpleasant taste. The sulphates also change the pH level as it is previously said. (Dr Raghu N. Singh, 2006)

The high sulphates concentration makes our wastewater too acid to work with live organisms. One of the main objectives in the project is reduce the sulphates concentation in order to increase the pH of the water. (Dr Raghu N. Singh, 2006)

- Metals: Metals can be divided into two groups, the metals which are toxic and the metals which are non toxic. (Dr Raghu N. Singh, 2006)

The elements such as K, Na, Fe, Mn and Ca are the most important non toxic metals in mining water. The Ca and Mn produce the hardness of the water while Fe and Mn cause discoloration in water. The deposition in pipes should be avoided. (Dr Raghu N. Singh, 2006)

The toxic metals such Zn, Al, Ni, Co, Cr and Ba are present in the mining water. The presence of each metal depends on the process and the kind of mine. The characterization, assesment and control of these discharges are an important part of the environmental control program of the mine. As it has been said, the metal solubility in water depends on pH. With low pH, the compound metals are formed. (Dr Raghu N. Singh, 2006)

In the following some case studies about environmental effects of wastewater from mineral processing are presented:

The Summitville Mine in Colorado has become a case study of environmental damage as a result of mining. Gold was mined there from 1870 until 1992. Some of the following events affected the environment at the mine: Geologic characteristics at the mine site contributed to the generation of both natural and mining-related acid drainage; the height of the containing dike for cyanide leach solutions (used to chemically extract gold) was below the level required for snowstorms and spring runoff; broken pump lines and a French drain beneath the leach pad caused cyanide-contaminated solutions to be released into the local watershed ; several waste rock piles at the mine reacted with rain and snowmelt to form acidic waters that flowed into area streams; an underground tunnel released large volumes of contaminated waters; and mining deforested much of the land. (Pollution Issues, 2008)

Another case study is the Iron Mountain Mine in California. Mining for copper, gold, silver, and zinc began in 1879 and continued until 1963 using underground and open-pit methods. The site contains inactive mines and numerous waste piles from which harmful quantities of untreated acidic; metal-rich waters were discharged. Mining operations fractured the mountain, changing the hydrology and exposing the mineral deposit to oxygen and water, which resulted in intense acid mine drainage into nearby creeks and waterways. These caused numerous fish kills and posed a health risk to the area drinking water. (Pollution Issues, 2008)

An important case of environmental effects of wastewater without controlling are China´s mines. Current estimates state that around 20,000 tons of heavy metals are illegally mined and exported from "off-grid" mines in China every year. It is unlikely that any of these illegal mines have environmental safeguards in place, which means that contamination, dust, and other wastes are not being addressed. This affects the health of the workers as well as destroys the surrounding environment. (Schuler et al, 2011)

2 OBJECTIVES

This thesis concentrates on the ecotoxicological effects of wastewater from mineral processing and possibilities to reduce them using innovative biological wastewater treatment methods. The tests are done in a laboratory scale pilot plants. The first aim of this project is to testthetoxicity ofwastewater to terrestrial (*Eiseniafetida*) and aquatic organism (*Daphniamagna*) The other aim is to check the efficiency of three different biological wastewater treatment methods: two biofilter systems (Willow Stack Tower and EBB-flow system) and algae truf scrubber used in theses of Alberto Freire Lopez (2011) and Gerbrand Grobler (2013). The hypothesis is that the pilot plants would be able to reduce the toxicity of mining water. In addition to the toxicity studies the heavy metal concentrations of the tested water were monitored in this study.

Wastewater from mineral processing was treated in order to decrease the concentration of pollutants and the toxicity. The treatment was done with three biological systems during two periods of time. The effectivity of these systems and with which parameters it is higher will be analyzed.

In this part of the study, sulphates and heavy metal will be checked as well as the wastewater toxicity. The principal metals that are presented in the water are niquel, zinc, copper and cobalt but in the test the heavy metals that are check are niquel, zinc, sodium and potassium.Sulphates will be measured too. The amount of sulphates in the water was too high so it causes problems to maintain bacteria and algae alive. The main consequence of this high sulphate concentration is that the pH is too low.Because of that one of the main objectives of this study will be to find the way to treat this wastewater trying to keep the biological organisms alive. First at all, the treatment will be done directly with the mining water to observe how the organisms are affected with it. After that some changes will be introduced. Ecotoxicity analyses are done to know the influence of the water in the environment. This ecotoxicity tests show us how this water affects to terrestial and acuatic organism.

TALVIVAARA MINE

The wastewater used in this project comes from Talvivaara mine.

Talvivaara mine is situated in Sotkamo, Finland. It belongs to the company Talvivaara Mining Company Plc..In this mine, the main product that is obtained is nickel but there are other important metals that are extracted as zinc, cobalt and copper.

The mine has two deposits, Kuusilampi and Kolmisoppi. They represent one of the biggest resources of nickel in Europe. In this deposit, the metal extraction is done by open pit extraction. Low level nickel is extracted by bioheapleaching because of the amount of sulphur present in the ore. The dissoluted metals are recovered from the pregnant leaching solution as sulphides. NiCoS, ZnS_2 and CuS are the main prroducts of the comany.

The mine production started in 2008 and the precipitation of the metallic sulphures next year, 2009.

The Talvivaara ore body is well-suited for open pit mining due to thin overburden, favourable resource geometry and a low waste to ore ratio. The ore is relatively low grade, but well-suited to bioleaching due to its high sulphide content. (Talvivaara mining company Plc., 2009)
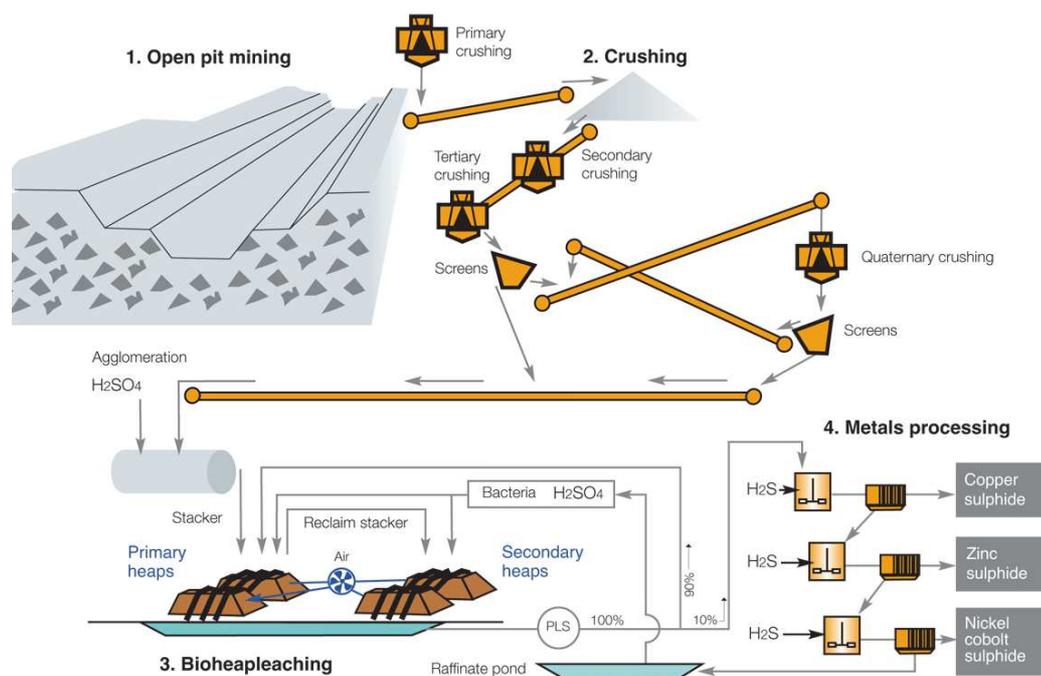
PICTURE 1. New talvivaara process (Talvivaara mining company Plc., 2009)

The water used in the tests of this project is not ordinary mine waste water but waste water from the recovery plant. It is partly purificated and stored temporarily on the plant. The valuables and some of the less valuable metals have been precipitated but the final purification is missing.

The studied waste water has the following concentrations:

Date	TANK	pH	Conductivity	Metal Concentration					
				Al	As	Ca	Cd	Co	Cr
			μS/cm	mg/l					
26.02.2013 00:00	Sample 1	3	17630	175	0,54	318	0,26	1,38	<0.0121
26.02.2013 00:00	Sample 2	3,9	18100	207	0,6	378	0,3	1,63	<0.0121

Date	TANK	MetalConcentration									SO_4^{2-}
		Cu	Fe	Mg	Mn	Na	Ni	Si	Zn	U	
		mg/l									
26.02.2013 00:00	Sample 1	<0.0121	1360	1736	1318	824	64,6	13	121	0,77	18000
26.02.2013 00:00	Sample 2	<0.0121	1595	2045	1559	952	77,7	15,4	143	0,82	18000

TABLE 2. Initial element concentrations of the wastewater used in this study; Talvivaara mining company Plc.

3   MATERIAL AND METHODS

In this project, three biological systems are used to treat the wastewater. They are two biofilters and an algae turf scrubber system. The systems are described in detail in Alberto Freire Lopez (2011) and Gerbrand Grobler (2013) theses. The pollutant concentrations and ecotoxicological effects of the treated and untreated wastewater are studied. They are done by analysing heavy metal and sulphate concentrations and doing toxicity tests with terrestrial and aquatic organisms.

The biofilter systems have been used in leachate treatment tests and they have rather good efficiency in the removing of TN, TP and BOD from landfill leachate wastewater. (Hepokorpi and Khelia, 2010) They used the same work principle but there are two different configurations for the work system. Bacterias present in the surface of the willow grow using pollutants as nutrients. In the others studies in which landfill leachate wastewater was used as influent. The results showed that if the concentration of wastewater is higher then the removal of nutrients will be higher too. (Freire López, 2012)

In the other system, algaes are used as bioremedation agents. Algaes are aquatic organism and they are photosynthetic oxygenic autotrophs. Growing conditions has to be controlled in order to mantain the algaes alive. So it is very important to maintain the sunlight conditions and the sufficient growing environment. (Grobler, 2013)

3.1. Materials

3.1.1.   Biofilters

The biofilters combines a retention mechanic action of the suspended material using filtration and a biological transformation of the contaminants that are present in the wastewater by microorganisms.The aged refused is a good alternative to leachate treatment. Because of their high porosity and specific area aged refuse has potential in removal of contaminants of wastewater. In these aged refuse, over time, bacteria become to acclimate at high concentrations. (Zhao et al.,2006; Shi et al., 2007)
In addition, it is an inexpensive alternative for cleaning nutrient rich water.

3.1.1.1. Willow Stack Tower (WST)

The willow stack tower is a system that is based on the principle of a trickling filter. This kind of systems are using in some rural zones of Finland in order to eliminate the cotaminants of leachates from landfills and compost sites. Examples of this implementation are the towers from Vaasa and Pälkäne. (Hepokorpi and Khelia, 2010) The aim of the systems is remove the contaminants of wastewater using bacteria. By this way, the microbes grow in the willows surface using the leachate contaminants as nutrients.

WST consists in a wooden frame with different floors. The water goes through the different floors in which there are willow sticks. The sticks are collocated in vertical because of the fact that the water slides along the surface of the sticks where the bacteria is located. In the bottom of the tower, a pool can be found. It is using as an open tank where the water that has been treated can be stored or recirculated.

In this project, the WST system that is used is designed with the same parameters that were used in the design of the Pälkäne's WST and with the same parameters of the waste water which was used during the experiment. (Hepokorpiand Khelia, 2010) The system was design by Alberto Freire in 2012 in a laboratoryscale. WST is a continuous open system in which a spray is used for the distribution of the water in the system.The system used in the laboratory consists in a storage tank where the wastewater is aerated with different pumps. The water is driven by a peristaltic pump to the top of the WST where a spray system distributes the water in the system. The water slides on the willow surface where the bacteria are growing using its pollutants as nutrients. The water goes down into the storage tank where the process starts again.

PICTURE 2. WST pilot plant, TAMK greenhouse

(Photo: Stefan Sprock, 2013)

3.1.1.2. EBB-Flow System

The EBB-flow is a new system that works with the same principle than the WST. The bacteriapresentin the willows surface remove the contaminant from the wastewater. But it works simulating the natural phenomena of tides movement in the sea.EBB-Flow is a semi-closed batch system. The EBB-Flow system is filled and emptied periodically. It will work as a continuous system using a recirculation tank in order to mantain the bacteria alive and keep the parameters constant the systems. It does not need a water distribution system like the WST and the airation is arranged with the ebbflow movement.

The wastewater is storaged in a 1m^3 tank. A centrifugate pump impulses the water by a dual output system. This system controls the water distribution with two ball valves. One of the outputs goes to the tank where the willows sticks are and the other one recirculates the water to the storage tank due to security reasons.

As it is said, the system works with a siphon. The siphon consists in a shape "U" invert tube with one of its endings submerged in liquid. In the siphon the potential energy is transformed into kinetic energy. The free ending should be below the ending that the ending that is inside the water because of the fact that it works by gravity. The pipe has to be filled of liquid due to the force that brings the fluid to the other branch is the weight of the liquid which is inside the pipe. (Freire López, 2012)

PICTURE 3. EBB-Flow pilot plant, TAMK greenhouse

(Photo: Stefan Sprock, 2013)

3.1.2.   Algae Turf Scrubber

Algae turf scrubber is a system that uses algae ecosystem as way of treating wastewater. The system uses principally string algae to capture the energy of sunlight and build algae biomass from carbon dioxide. The algaes capture the nutrients from the wastewater as well as many toxic organic compounds can be degraded.ATS-produced algae can be converted into energy products such as biodiesel and methane. (Adey D. W., 2012)

The most important factors to take care are sunlight and a sufficient growing environment for the algae because of the fact that algae are very sensitive organism and if they are not the correct ones the algaes will not grow and they will die.

The ATS consists of a suitably sloped surface on which the substrate can grow and the water can run off by means of gravity. Typically, it is advised in a small scale, to attach a grid-like material to your slope, upon which the algae can grow and attach themselves. This also makes it easier to harvest the algae at a later stage. Methods may vary depending on the type of algae species used. (Grobler, 2013)

This wastewater is therefore distributed in equal pulses from which the algae obtains its nutrients. It is believed that this pulse flow enhances the metabolites between the algae cells creating a better homogenisation of species on the growing surface. As a result, the algae turf consists of a dense mat of algae less than several centimetres in height. (Glober, 2013)

As water travels down the ATS, pollutants are recovered through both biological and physical processes. Organic matter (Carbon), nitrogen (N), phosphorus (P) and other elements necessary for metabolic growth are rapidly removed from the water column through biological uptake. This speed however is highly dependent on the amount of light available to the algae. The uptake rates will increase under favourable conditions including light, water temperature, nutrient concentrations pH and correct flow rate. Removal of these compounds (organic matter, N, and P) results in water quality changes within the ATS, including elevation of pH concentrations. (Grobler, 2013)

PICTURE 4. ATS pilot plant, TAMK greenhouse

(Photo: Stefan Sprock, 2013)

3.2. Laboratory analyses

3.2.1.   Toxicity test and analyses

Toxicity analysis can be used to identify criteria to evaluate the toxic effects in complex samples of the wastewater effluents in a process. They show the effects in the aquatic and terrestrial ecosystems of the wastewater.

When an organism is exposed to pollutants, a number of successive events can happen if the exposure concentration is high enough and/or the exposure duration is long enough. The microorganism responds in three phases: alterations in biochemical level, responses on a physiological level and effects on the whole organism level.

Though individual chemicals can have an impact in specific biochemical pathways, it is often difficult to extrapolate these effects to higher levels of biological organization especially in the case of complex exposure scenarios, like field exposure or whole effluent toxicity. (Ostrander, 2005)

By this way, these tests give an overview of the effect of the disturbance of processes at lower levels of biological organization.

Also indirect effects can be taken using this hypothesis. Even organism are not directly exposed to the pollutants, there may be other trophic levels that are impacted as changes in food availability and changes in food quality.

There are two kinds of biotoxicity:

- Acute test: It shows the lethal concentration of pollutants in a specific microorganism. The calculated value is called median value of letal concentration and it is the concentration which causes the death of the 50% of the experimental population in a specific period of time, normally this period of time is between 24 and 96 hours. (Metcalf & Eddy, 1995)

- Chronic test: It shows the concentration of the substance that causes an effect to the 50% of the experimental population in a specific period of time. (Metcalf & Eddy, 1995)

In this project, two different biotoxicity tests have been done. One of them is to show how the wastewater affects to an aquatic medium and the other one to show how it affects to a terrestial medium.

3.2.1.1. Aquatic toxicity test *Daphnia magna*

This test evaluates the harmful effects of wastewater in aquatic organisms. Crustaceans are used in this kind of test because of the fact that they are the principal consumers of the zooplacton in aquatic ecosystems.

The principle of this test is the determination of the inmobilization of the 50 % of *Daphnia magna* Straus after 24 hours exposed in wastewater. The results that are obtained are measured as LC_{50} whichis the concentration at which 50% of the tested group will die. It is also called median lethal concentration.The range between the higher concentration in which all the *Daphnia magna* Straus are inmobilizated and the lowest concentration in which all the *Daphnia magna* Straus are not inmobilizated can be observe with a preliminary test. Due to the fact that the water used in this project is always changing this preliminary test was omited and the range of concentrations used was always the same. The test has been done once a week during the work period.In this project the different dilutions with which the test was done are: 0, 1/1000, 1/500, 1/200, 1/100, 1/50, 1/20, 1/10. They were done from the original water. Using the table 1 from the page 27 of this project, the metal concentrations in each dilution can be calculated. It can be showed in the following table:

Dilution	Metal concentration TANK 1				
	Ni	Zn	Cu	Co	SO42-
Original water	64,6	121	0,0121	1,38	18000
1/10	6,46	12,1	0,00121	0,138	1800
1/20	3,23	6,05	0,000605	0,069	900
1/50	1,292	2,42	0,000242	0,0276	360
1/100	0,646	1,21	0,000121	0,0138	180
1/200	0,323	0,605	0,0000605	0,0069	90
1/500	0,1292	0,242	0,0000242	0,00276	36
1/1000	0,0646	0,121	0,0000121	0,00138	18

TABLE 3. Metal concentration in the different dilutions used for the *Daphnia* Test using TANK1

Dilution	Metal concentration TANK 3				
	Ni	Zn	Cu	Co	SO42-
Original water	77,7	143	0,0121	1,63	18000
1/10	7,77	14,3	0,00121	0,163	1800
1/20	3,885	7,15	0,000605	0,0815	900
1/50	1,554	2,86	0,000242	0,0326	360
1/100	0,777	1,43	0,000121	0,0163	180
1/200	0,3885	0,715	0,0000605	0,00815	90
1/500	0,1554	0,286	0,0000242	0,00326	36
1/1000	0,0777	0,143	0,0000121	0,00163	18

TABLE 4. Metal concentration in the different dilutions used for the *Daphnia* Test using TANK3

3.2.1.2. Terrestial toxicity test *EiseniaFetida*

This test is used to show the effects of the pollutants in the soil on the reproductive output of the earthworms *Eiseniafetida*. It is used to determine the water concentration which causes the mortality of the 50% of earthworms (LC_{50}). The test takes place in 7 and 14 days because of this, the test has been done at the beginning and at the ending of each running period.

In this period of time the changes in the behaviour and morphology of the worms can be observed. The adult worms can be counted and weighed after two weeks, and the number of juveniles hatched at the end of the second week can be observed.The reproductive output of the worms exposed to the test substance is compared to that of the controls in order not to determine the no observed effect concentration. The test concentrations should bracket the range between the concentration in which no changes can be observed in the worms (control) and the concentration in which all the worms die. The control normally is tap water without dilution.

In order to obtain results, the concentration of the dilutions using in this test were changed. In the first test run the wastewater concentration was too low so big changes could not be observed.

In the first test the dilutionsthat were used are: 0, 1/1000, 1/500, 1/100, 1/50 and 1/20.

Dilution	Metal concentration TANK 1				
	Ni	Zn	Cu	Co	SO42-
Original water	64,6	121	0,0121	1,38	18000
1/20	3,23	6,05	0,000605	0,069	900
1/50	1,292	2,42	0,000242	0,0276	360
1/100	0,646	1,21	0,000121	0,0138	180
1/500	0,1292	0,242	0,0000242	0,00276	36
1/1000	0,0646	0,121	0,0000121	0,00138	18
0	0	0	0	0	0

TABLE 5. Metal concentration in the different dilutions used for the *Eisenia* Test using

TANK1

In the second test the dilutionsthat were used are: 0, 1/50, 1/20, 1/10, 1/2 and 1/1.

Dilution	Metal concentration TANK 3				
	Ni	Zn	Cu	Co	SO42-
Original water	77,7	143	0,0121	1,63	18000
1/2	38,85	71,5	0,00605	0,815	9000
1/10	7,77	14,3	0,00121	0,163	1800
1/20	3,885	7,15	0,000605	0,0815	900
1/50	1,554	2,86	0,000242	0,0326	360
0	0	0	0	0	0

TABLE 6. Metal concentration in the different dilutions used for the *Eisenia*Test using

TANK3

3.2.2. Sulphate Analyses

The wastewater that is used in this project has high concentration of sulphates.

The mine uses the bioheapleaching as way of obtain the metals. As it has been explained, in this process a bacterial leach is used to extract base metals from sulphide ores. Theseamount of sulphates are presented in our water because of the fact that the oxidation from sulphur to sulphates is done by the bacterias in the process. Other fact is that sulphuric acid is also used to regulate the pH in the irrigation water solution which is used to appropriate leaching conditions and maintain process water balance in the recirculation solution which returns to the heap.

The analysis of the quantity of sulphates in the wastewater is done with the Spectrophotometer.

A spectrophotometer consists in two systems, one is the spectrometer which produces the light with a determinate wavelength and the other is the photometer which measures the intensity of this light. By this way, the cell is situated between the spectrometer bean and the photometer. The photometer sends a signal to a display device. Normally a galvanometer is used in this kind of system.

To know the concentration of the sample, the amount of light absorbed by the liquid is measured. For changing the colour of the sample, a reactive is added in it and depending on the concentration, the colour will be lighter or darker. So, as the development of the color is linked to the concentration of the substance, the concentration can be measured by determining the extent of absorption of light at the appropriate wavelength.

Beer's law relates in a quantitative way the solute concentration and the intensity of the transmited light.

It is:

$$A = \log_{10} \frac{P}{P_O}$$

Where:

$A \equiv$ Absorbance

$T = \frac{P}{P_O} \equiv$ Transmittance

3.2.3.   Element Analyses

In this project four different elementsare measured with the AAS technique. In this project the non toxic metals that are going to be measured are Potassium and Sodium. The toxic metals that are going to be measured are Zinc and Nickel.In this project Ni and Zn are measured because they are the main metals that are present in the wastewater. There are other metals which are present in high concentrations too but we will focuse in both of them.

The principle of AAS method is the measurement of the amount of light which is absorbed when the light goes through a cloud of atoms. If the amount of atoms present in the dilution increase, the amount of absorved light also increases in a predictable way. The use of special light sources and careful selection of wavelength allow the specific quantitative determination of individual elements in the presence of others.

The atom cloud is produced by thermal energy that dissociates the chemical compounds in atoms. The systems have an absortion system which aspirates the sample into a flame. The flame provides this thermal energy. Under the proper flame conditios, most of the atoms will remain in the ground state form and theyabsorbe light at the analytical wavelength from a source lamp. In this analysis AAS device was used.

PROCEDURE

First, the calibration line had to be done. It is used for determining the concentration of the metal in the sample by comparing the unknown sample to a set of standard samples of known concentration. The range is different for each metal.

Nickel

The standard dilutions for the Nickel analysis were 0,0 mg/l; 5,0 mg/l; 7,5 mg/l; 10,0 mg/l; 15,0 mg/l; 20,0 mg/l.

Zinc

The standard dilutions for the Zinc were 0,0 mg/l; 10,0 mg/l; 25,0 mg/l; 50,0 mg/l; 75,0 mg/l; 100,0 mg/l.

Potassium

The standard dilutions for the Potassium were 0,0 mg/l; 0,1 mg/l; 0,25 mg/l; 0,5 mg/l; 0,75 mg/l; 1,0 mg/l.

Sodium

The standard dilutions for the Zinc were 0,0 mg/l; 5,0 mg/l; 7,5 mg/l; 10,0 mg/l; 15,0 mg/l; 20,0 mg/l.

4   RESULTS

The tests started for the willow stack tower and the EBB-Flow system with nutrient water during the fail week. It was done because of the fact that microorganisms had to be kep alive when the willow was cut. During all the test runs, the flow of the systems was the same. The work way was different in order to obtain results in the different tests. The WST work flow was:

	t (s)	V (ml)	F (l/h)
1	31,00	300	34,8
2	30,27	300	35,7
3	32,68	300	33,0
			34,5

TABLE 7. WST flow rate

There were some changes because the solids present in the wastewater block the holes of the pipe in the tests.

In the EBB-Flow system, each batch in the system took 8 minutes and 32 seconds.
In the ATS systems, the algae's were submerged in the water so the flow rate is not important to know because the wastewater is always in contact with the microorganism so the treatment operation is done during all the time of the test run.

4.1. Preliminary Test

In the preliminary testswastewater was tested without treating. In this preliminary test the different test that were going to be used were checked.For the heavy metal test the concentrations for the calibration line were determined.For the sulphate analysis, the dilution of the water was found in order to be between the ranges of the HACH spectrophotometer. It was 1/1000.

Preliminarytest for *Daphnia* toxicity test were also done. In the preliminary test, the number of *Daphnia* that there were in each cell was not the exact number that the specification papers said so there was no results of it.

In this preliminarytest week, *Eiseniafetida* test was not done because of the duration of the test.

4.2. First Test Run

The first test week started on the 11th of March for the WST and the EBB-flow system. On the 25th of March started the ATS first run. In this test run, all the system tanks were full of wastewater.

This test run lasted three weeks for the WST and the EBB-Flow system and two weeks for the algae turf scrubber. During theseperiod of time, three different analisys were done each week. These three analyses are sulphate analysis, heavy metal analysis and water toxicity analysis. The soil toxicity analysis was done in the begining and in the end of the test run.In the first test run the systems work with these parameters:

WST

The WST storage tank was filled with 32 liters of wastewater.

EBB-FLOW SYSTEM

The Ebb-flow system storage tank was filled with 1m^3 of wastewater.

ATS SYSTEM

Each ATS system storage tank was filled with 25 liters of wastewater.

4.2.1 Metal analyses

The concentrations of Nickel, Zinc, Potassium and Sodium of different treatmens can be seen in Figures 1 to 8.
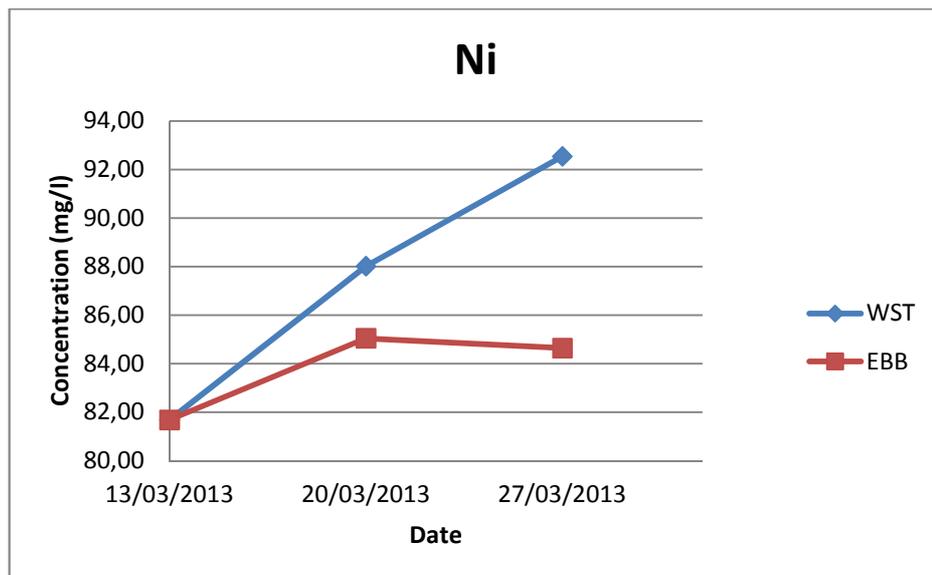
Figure 1. Nickel concentrations during the first test run in the WST and EBB flow treatments
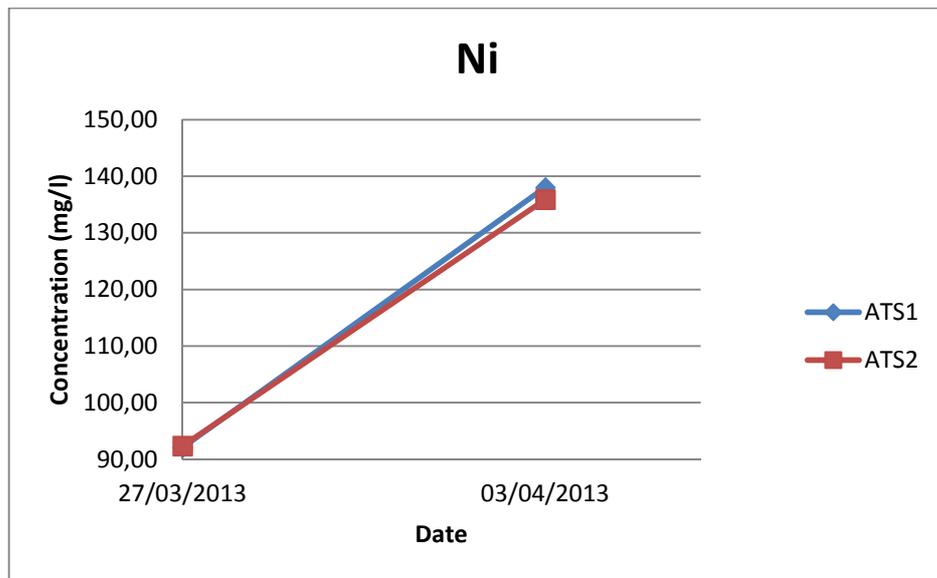
Figure 2. Nickel concentrations during the first test run in the ATS I and ATSII treatments

In the graphs, it can be observed that the nickel concentration increased in all the systems. The reason of this increase was that a lot of water evaporated due to the high temperatures in the green house and because of that the concentration of the nickel increase. The systems did have any effect in reduction of the Nickel concentrations of the wastewater.
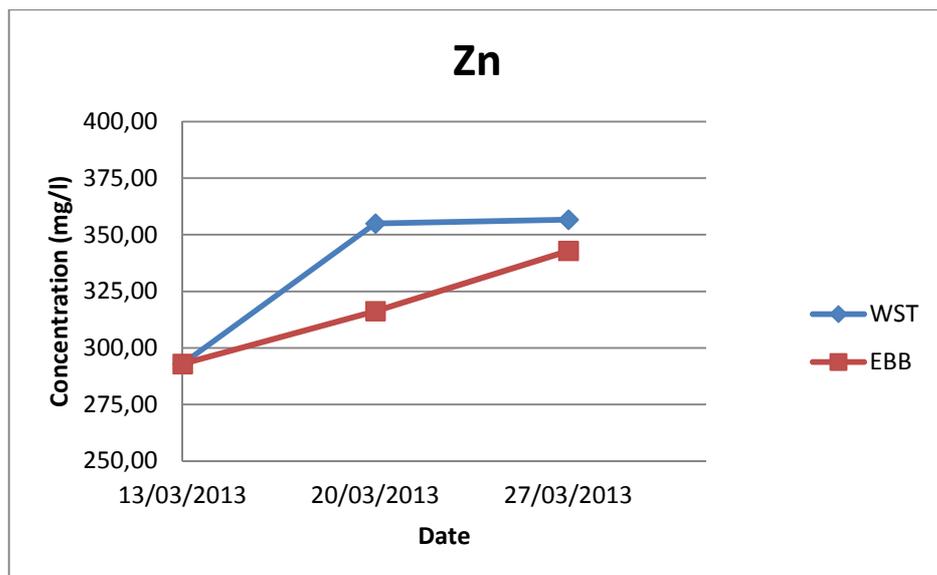

Figure 3. Zinc concentration during the first test run in the WST and EBB flow treatments
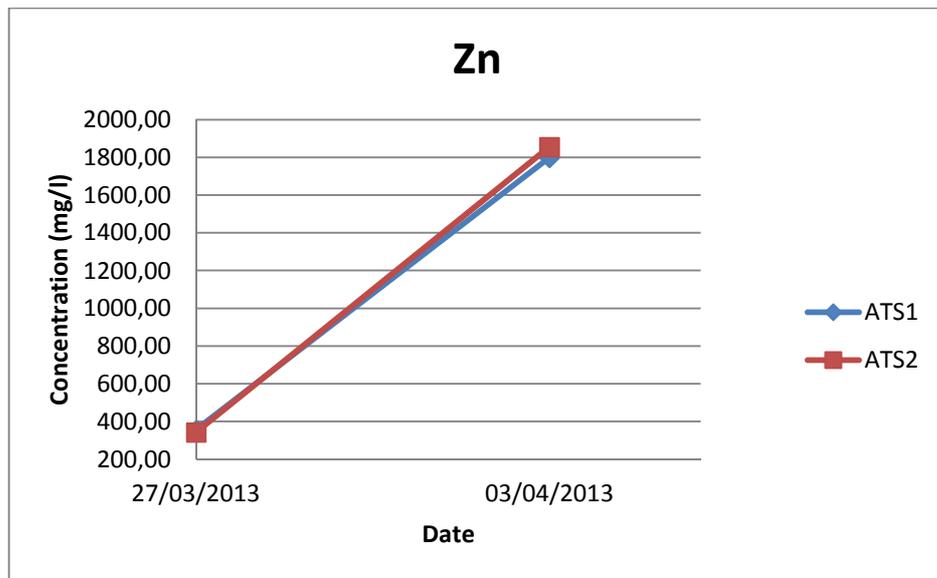
Figure 4. Zinc concentrationduring the first test run in the ATS I and ATS II treatments

In this graphs, the same increasing trendcan be observed.
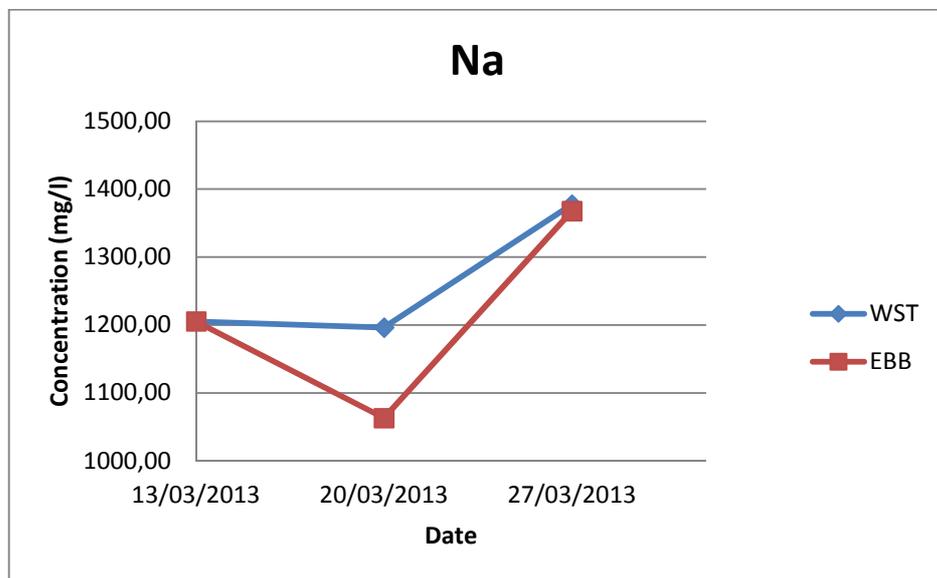
Figure 5. Sodium concentrations during the first test run in the WST and EBB flow treatments
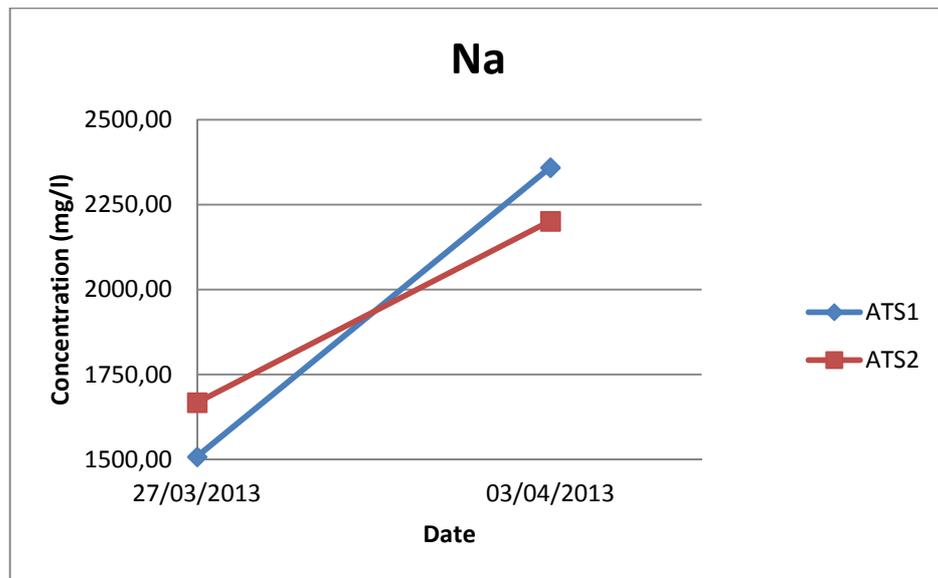
Figure 6. Sodium concentrations during the first test run in the ATS I and ATS II treatments

In this graphs, the same trendcan be observed. In the EBB-Flow system a decrease can be observed during the first week. It could be because in this system less quantity of water was evaporated. In the second week the increase of the concentration can be show in the same way than in the oders systems.
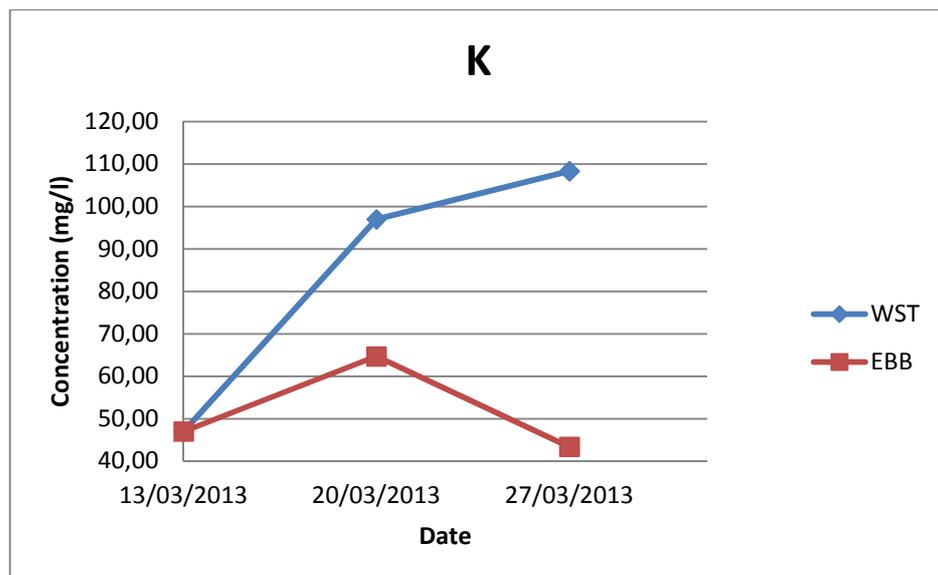

Figure 7. Potassium concentrations during the first test run in the WST and EBB-Flow treatments
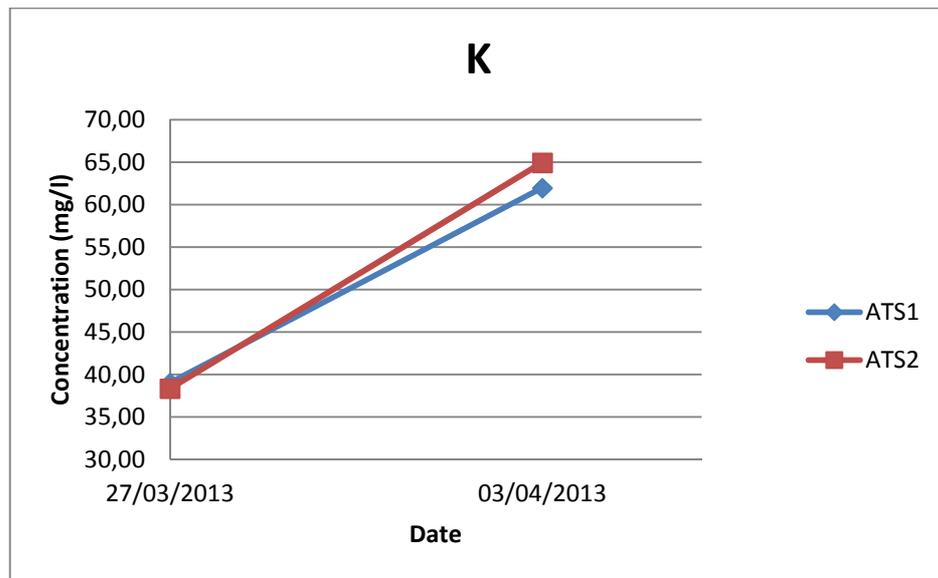
Figure 8. Potassium concentrations during the first test run in the ATS I and ATS II treatments

In these graphs, the same increase can be observed. It was for the same reason. In the EBB-Flow system a decrease can be observed during the second week.

4.2.2 Sulphate analyses

The concentration of sulphates of different treatmens can be seen in Figures 9 to 10.
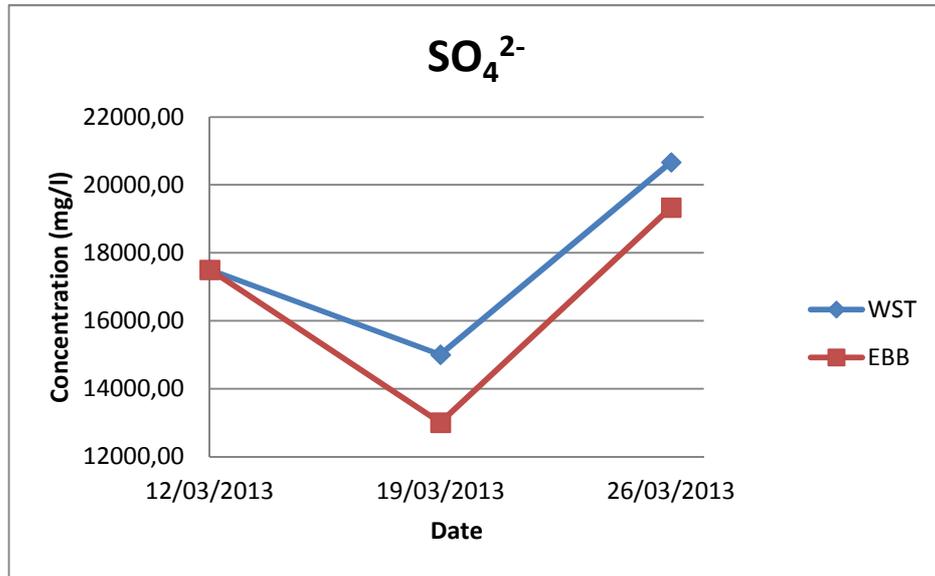
Figure 9. Sulphate concentrations during the first test run in the WST and EBB-Flow treatments
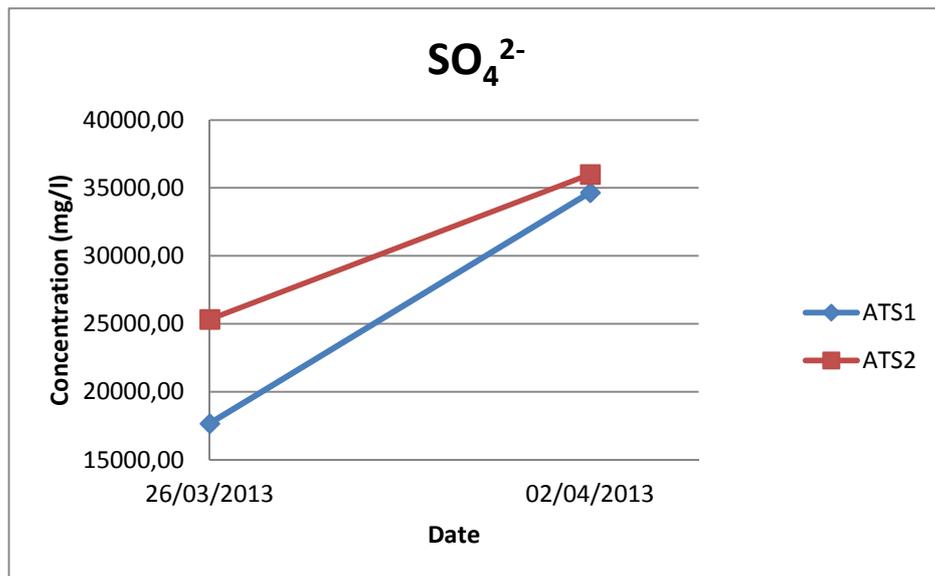

Figure 10. Sulphate concentrations during the first test run in the ATS I and ATS II treatments

Different behaviours can be observed in the sulphate removed. In the two biofilters a decrease of the sulphates in the water can be observed. It could be because some of them precipitated.In the ATS an increase can be observed because of the high evaporation of the water.Ecotoxicity tests

4.2.3. Ecotoxicity tests

4.2.3.1   Aquatic toxicity test

The results for the *Daphnia* test can be seen in Figures 11 to 14.

WST AND EBB-FLOW SYSTEMS:

This test was done for the toxicity of the wastewater before starting to treat it:
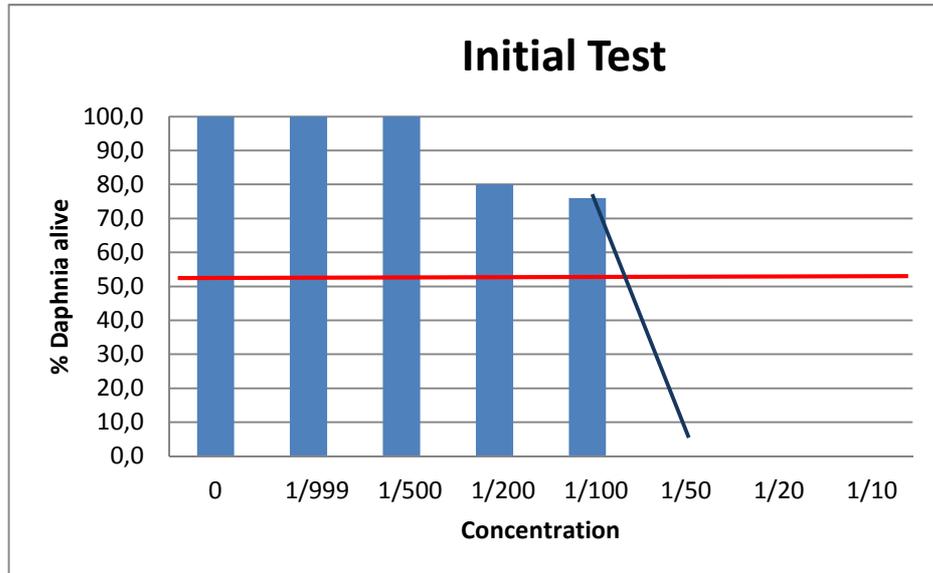
Figure 11. Percentage of living *Daphnia magna* in different mining water dilutions after 48 hours exposure

As it was explained, *Daphnia magna* test measures with which concentration the LC_{50}. Graph shows that this concentration was between 1/100 and 1/50. The exact value for this concentration is 0,0134 or 1/75.

The values for each metal concentration in this dilution are:

Dilution	Ni	Zn	Cu	Co	SO42-
Metalconcentration TANK 1 (mg/l)					
Original water	64,6	121	0,0121	1,38	18000
1/75	0,861	1,613	0,000	0,018	240,000

TABLE 8. Metal concentration for the LC_{50}

The results of the toxicity for the third week trating the wastewater were:
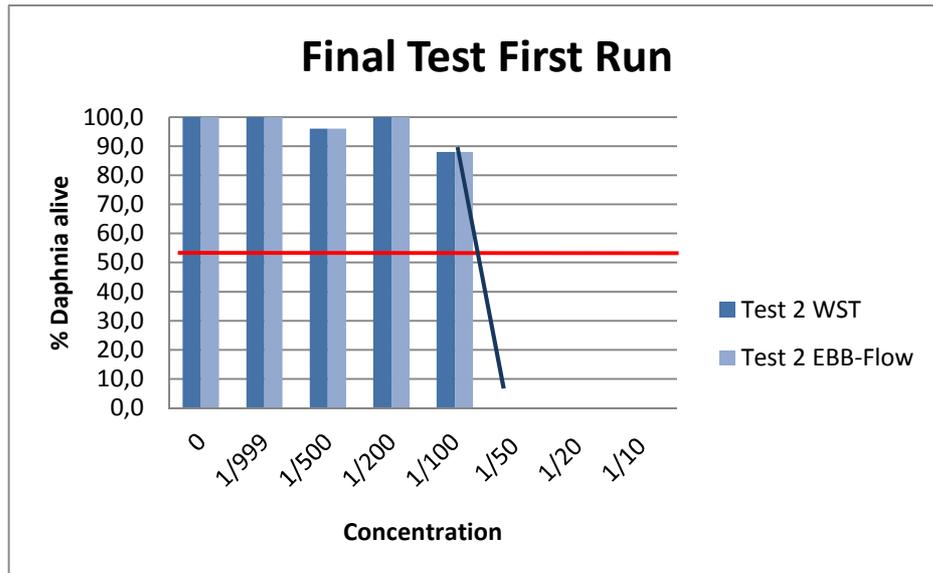
Figure 12. Percentage of living *Daphnia magna* in different mining water dilutions after 48 hours exposure Sulphate in the WST and EBB-Flow treatments

In both systems the value of the concentration is the same so we are going to calculate only one point. The LC_{50} for the WST and the Ebb-flow system after three weeks running is 0,0143 or 1/70.

The values for the metal concentrations in this dilution, if the nutrient water was not added, are:

	Metalconcentration TANK 1				
Dilution	Ni	Zn	Cu	Co	SO_4^{2-}
Original water	64,6	121	0,0121	1,38	18000
1/70	0,923	1,729	0,000	0,020	257,143

TABLE 9. Metal and ion concentration for the LC_{50}.

The LC50 changes a little bit because of the evaporation of the water what increase the concentration of the metals in the water. This is the principal reason of the value decrease and it can be showed in the metal test too.

ATS I AND ATS II

This test was done for the toxicity of the wastewater before starting to treat it:
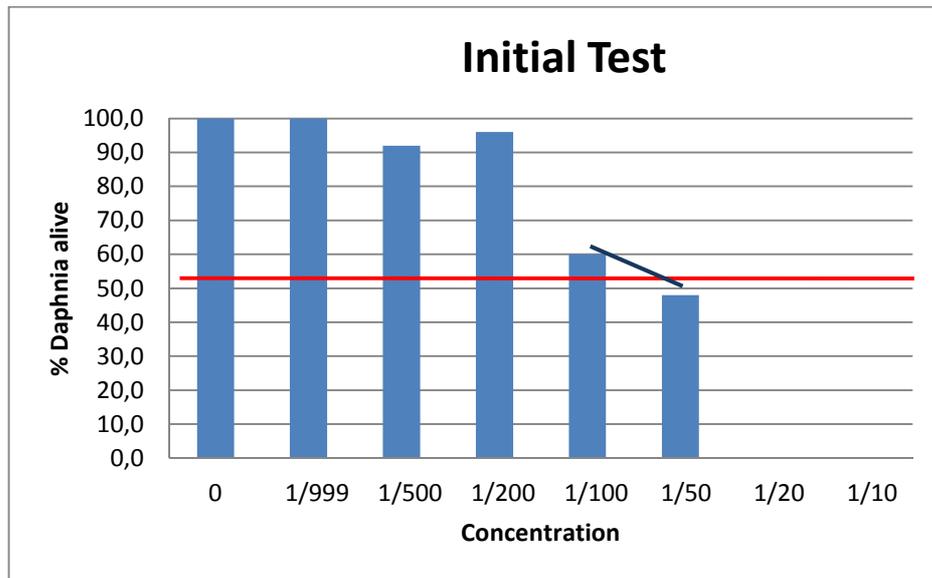
Figure 13. Percentage of living *Daphnia magna* in different mining water dilutions after 48 hours exposure Sulphate in the ATS I and ATS II treatments

The value for the LC50 is 0,018 ml wastewater/ml water or 1/55

The values for the metal concentrations in this dilution are:

Dilution	Metalconcentration TANK 1				
	Ni	Zn	Cu	Co	SO_4^{2-}
Original water	64,6	121	0,0121	1,38	18000
1/55	1,175	2,200	0,000	0,025	327,273

TABLE 10. Metal and ion concentration for the LC_{50}

The results of the toxicity for the second week trating the wastewater were:
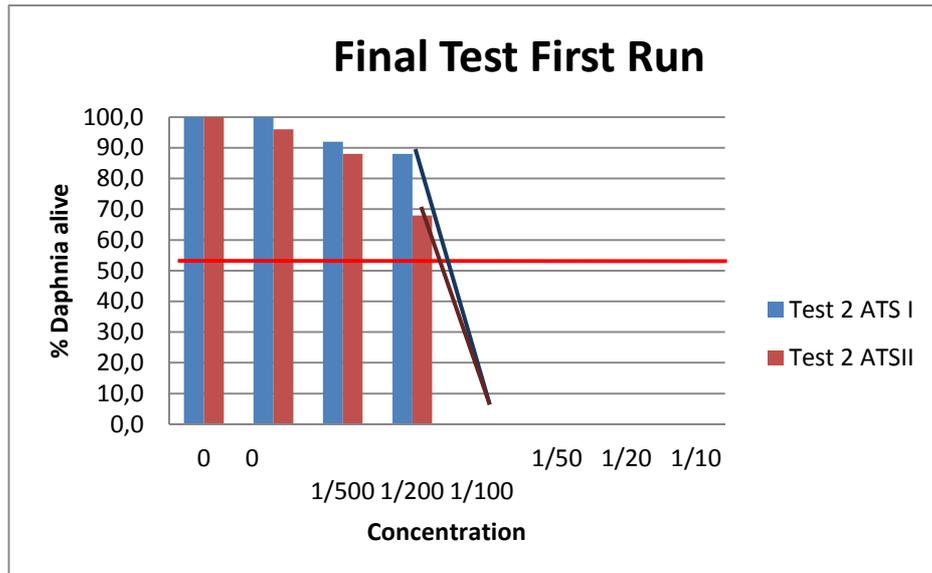
Figure 14. Percentage of living *Daphnia magna* in different mining water dilutions after 48 hours exposure Sulphate in the ATS I and ATS II treatments

The value of the LC_{50} for the ATS I is 0,007 ml wastewater/ml water or 1/140.

The values for the metal concentrations in this dilution are:

Dilution	Metalconcentration TANK 1				
	Ni	Zn	Cu	Co	SO_4^{2-}
Original water	64,6	121	0,0121	1,38	18000
1/140	0,461	0,864	0,000	0,010	128,571

TABLE 11. Metal concentration for the LC_{50} in ATS I

The value of the LC_{50} for the ATS II is 0,006 ml wastewater/ml water or 1/158.

The values for the metal concentrations in this dilution are:

Dilution	Metalconcentration TANK 1				
	Ni	Zn	Cu	Co	SO_4^{2-}
Original water	64,6	121	0,0121	1,38	18000
1/158	0,409	0,766	0,000	0,009	113,924

TABLE 12. Metal concentration for the LC_{50} in ATS II

In the initial test the LC_{50} is 0,018 ml wastewater/ml water or 1/55 while in the final test the LC50 in the ATS I is 0,007 ml wastewater/ml water or 1/114 and 0,006 ml wastewater/ml water or 1/147 in the ATS II. The difference between the beginning and the ending of the test is because of the evaporation like in the others systems that were used in the project. The water is more concentrated in the ending of the test. This is the main reason in the difference between the concentration in the ATS I and in the ATS II. In the first one the water evaporation is higher than in the second one.

4.2.3.2. Terrestial toxicity test

The *Eiseniafetida* test was only done for the WSTand the EBB-Flow system.

Initial values for the wastewater without treating:

solution	beginning		after 7 days		after 14 days		soil mass [g]
	number	weight (g)	number	weight (g)	number	weight (g)	
	11.3.2013		18.3.2013		25.3.2013		11.3.2013
0 "blind"	5	2,220	4	1,947	4	2,038	818
0 "blind"	5	2,458	5	3,109	5	2,821	818
1/20	5	2,400	5	2,863	5	2,335	818
1/50	5	2,347	6	3,295	6	3,077	818
1/100	5	2,359	5	2,782	5	2,515	818
1/500	5	2,667	6	3,791	6	3,006	818
1/1000	5	2,191	5	2,265	5	2,148	818

TABLE 13. The number of living worms and mass of 5 worms in *Eiseniafetida*toxicity test in the beginning of the test run

Eiseniafetida test shows the percentage mortality of *eiseniafetida* for each concentration.

This test was done with 5 worms in each vessel instead of 10 worms.
During the first test week, for some unexplained reason, some extra worms appeared in the test vessels, since. In some vessels there are six worms and not five.

Some worms were presented in the soil and they were very small when the test runstarted so they could not be found. During the first week, the conditions were not too hard to the worms for growing and they could be found one week later.

The pollutant concentrations are represented in TABLE 4, page 37.

One worm disappeared in one of the blank concentrations. It had to be because of the fact that the worm escape of the vessel. The water used for the blank is tape water, so it should not make any change into the worm morphology. There is no change in the number of worms in the rest of the concentrations. The LC_{50} could not be found.

This table shows that in the first test week, the worms grow in presence of the water. It was because the water concentration did not affect them in their morphology.

In the second test week, a worm weight decrease shows that they are affected by the wastewater. The weight decrease can be showed in the 1/20 concentration.

4.3. Second Test Run

In order to obtain results in the wastewater treatment, the second test week started working with nutrient water. The growing period for the algae and the bacteria was two weeks. During these two weeks, algae grew in good conditions and bacteria took the nutrients and started their bio-cycle. In this period of time, nutrient water was added in order to maintain the level of water in the storage tanks due to the water evaporation.

When the growing period finished, adaptation period started. Wastewater with nutrients was added in order to fill up all the storage tanks but trying to maintain algaes and bacteria alive. In this period of time, one liter of wastewater was added in each ATS system, one liter of wastewater was added in WST too and ten liters of this wastewater was added in EBB-flow system. Nutrient water was also added in ATS systems and WST in order to maintain the nutrients concentration constant in the tanks because of the water evaporation. In the Ebb-flow system nutrient water was not added due to the evaporation was not relevant.

When two weeks of the adaptation period went by, algae and bacteria died. So in this work period only one analysiswere done in order to know the concentration of the water with which all the organisms died.

WST
The WST storage tank was filled with 20 liters of wastewater.Each weekday, one liter of wastewater was added in order to replace all the nutrient water. NThe tank was filled up with nutrient water during this adaptation period in order to keep the concetration of pollutants constant due to the water evaporation.

EBB-FLOW SYSTEM

The Ebb-flow system storage tank was filled with 200 liters of wastewater. Each day 10 liters of wastewater was added.

ATS SYSTEM

Each ATS system storage tank was filled with 20 liters of wastewater. The working way was the same than in the willow stack tower.

4.3.1 Metal analyses

The concentrations of Nickel, Zinc, Potassium and Sodium of different treatmens can be seen in Figures 15 to 22.
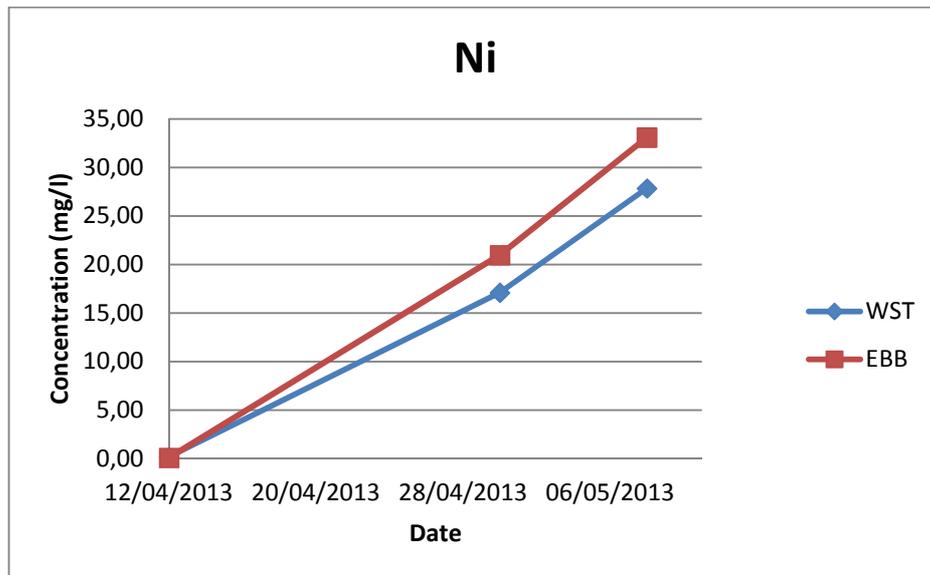

Figure 15. Nickel concentration during the second test runin the WST and EBB-flow treatments
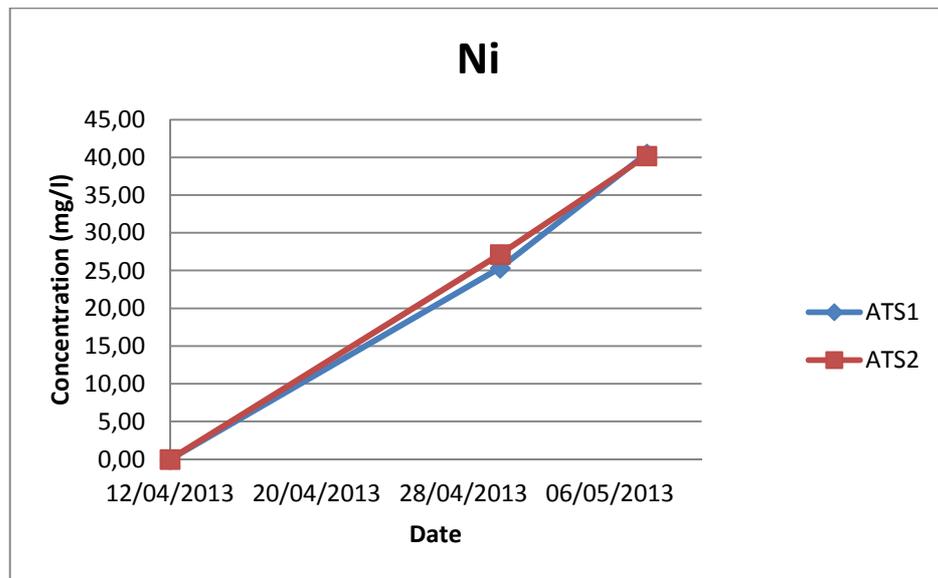
Figure 16. Nickel concentration during the second test run in the ATS I and ATS II treatments

In the second test run, the same increase of the nickel concentration than in the first one can be observed. It is because of the water evaporation.

The nickel concentration in the nutrient water added to maintain in the same level the nutrient concentration is zero so the addition of nutrient water do not increase the nickel concentration in mining water and nutrient water dilutes it.
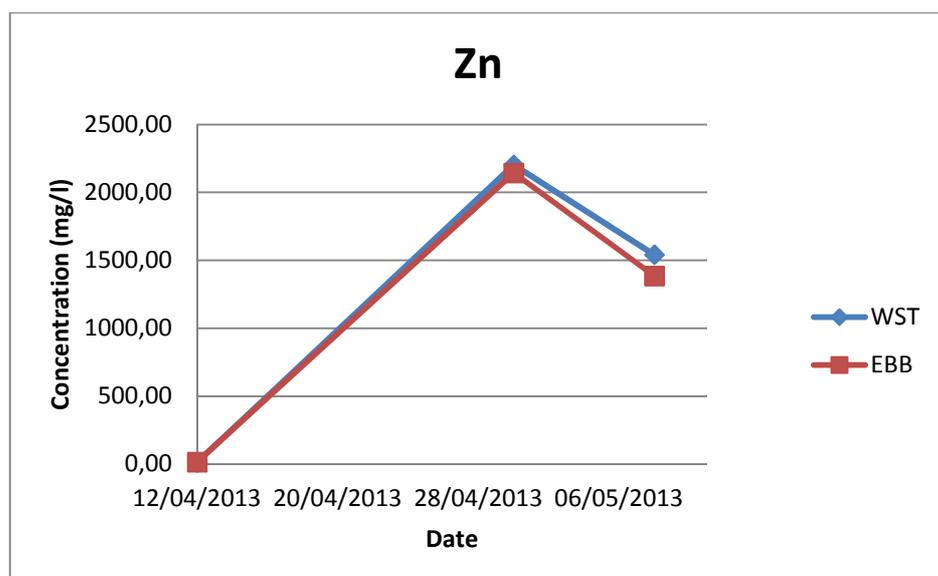

Figure 17. Zinc concentration during the second test run in the WST and EBB-Flow treatments
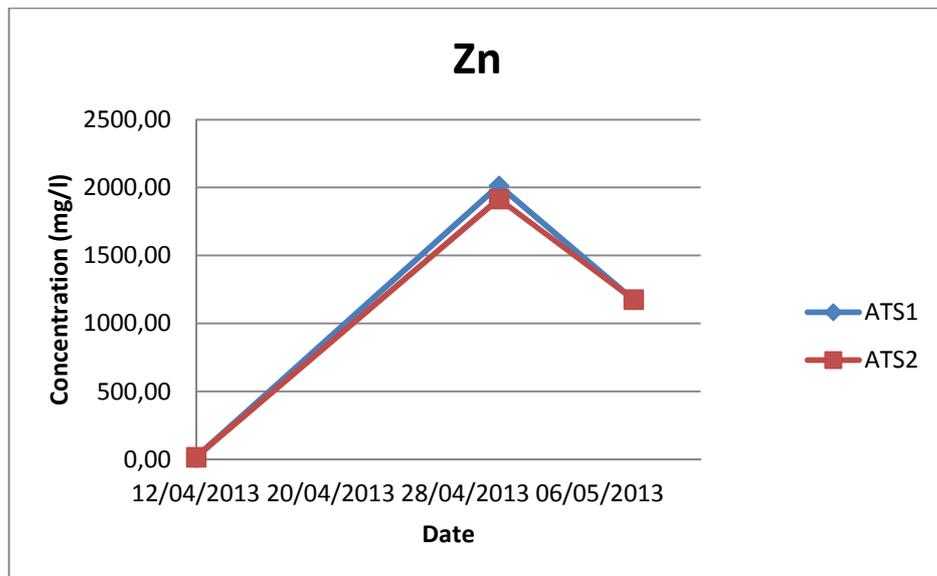
Figure 18. Zinc concentration during the second test run in the ATS I and ATS II treatments

In the second test run the zinc concentration increased in the first treatment week and it decreased in the second treatment week as it could be showed in both graphs.

The reason of this change in the concentration was because in the first week there was water evaporation and in the second week some metals precipitated.
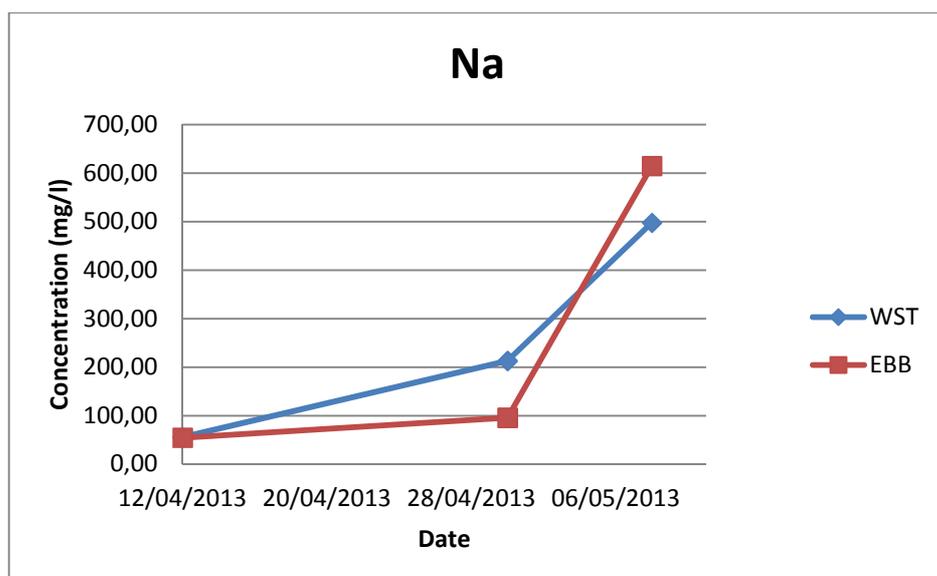
Figure 19. Sodium concentrationsduring the second test run in the WST and EBB-Flow treatments
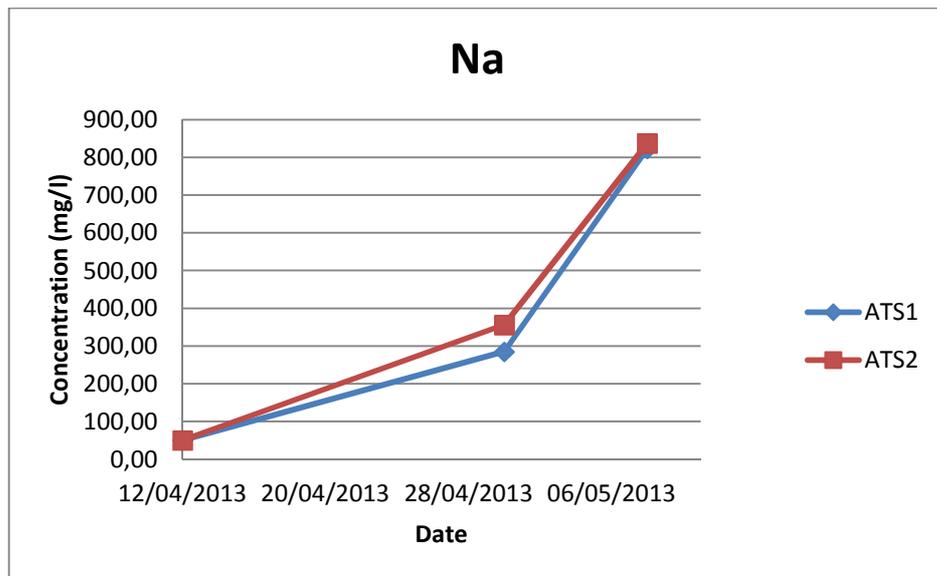
Figure 20. Sodiumconcentrations during the second test run in the ATS I and ATS II treatments

In the sodium concentration we can observed that happened the same than in the nickel concentration. It increased during the whole test. This increase was because of the water evaporation.
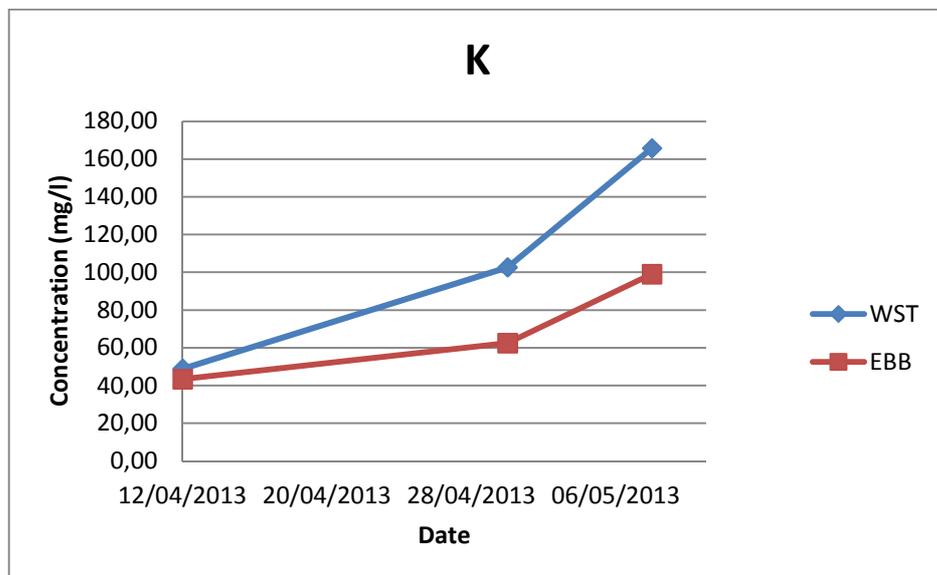

Figure 21. Potassium concentrations during the second test run in the WST and EBB-Flow treatments
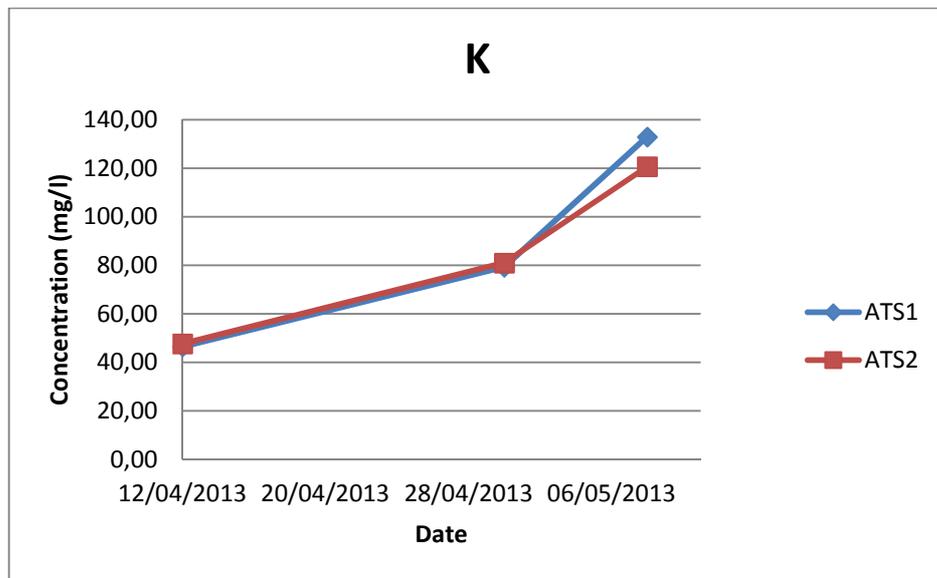
Figure 22. Potassium concentrations during the second test run in the ATS I and ATS II treatments

The same trend as with sodium took place with the potassium.

4.3.2 Sulphate analyses

The concentrations of Nickel, Zinc, Potassium and Sodium of different treatmens can be seen in Figures 23 to 24.
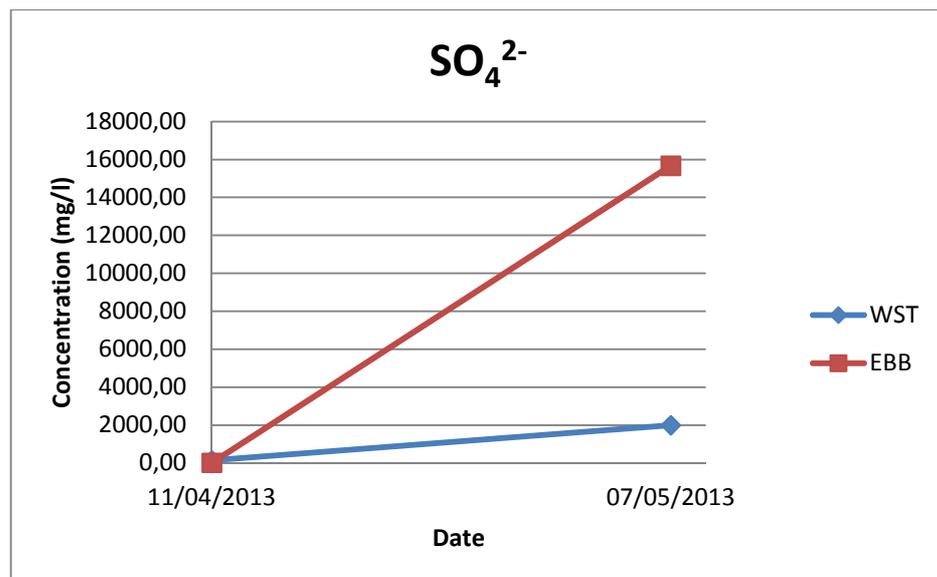
Figure 23. Sulphate concentrations during the second test run in the ATS I and ATS II treatments
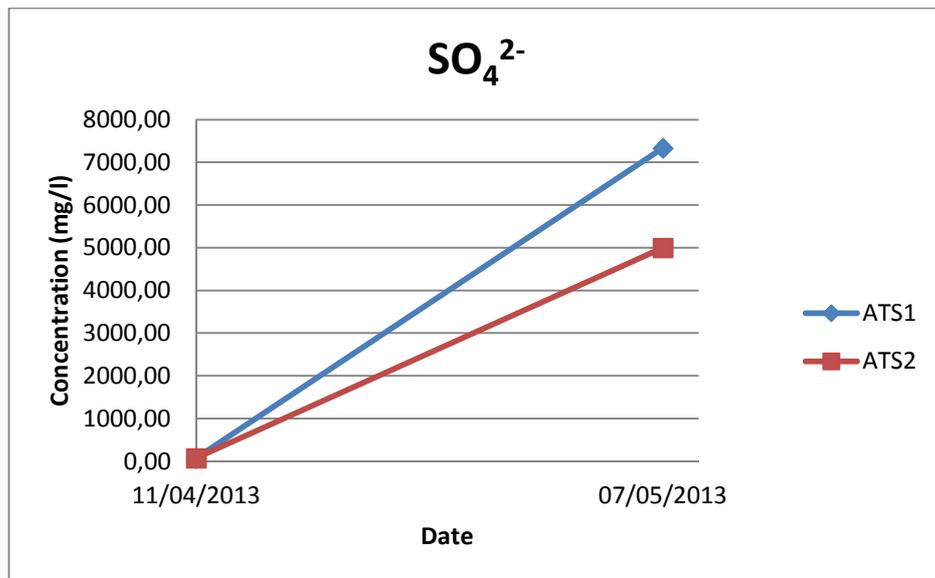
Figure 24. Sulphate concentrations during the second test run in the ATS I and ATS II treatments

The sulphate concentration increased in all the systems. The main reason was the water evaporation like in the heavy metal case. This produced a pH decrease what was one of the principal causes of the microorganism death.

4.3.3 Ecotoxicity tests

4.3.3.1. Aquatic toxicity test

The results for the *Daphnia* test can be seen in Figures 25 to 26.

This test was done for the toxicity of the wastewater after treating it:
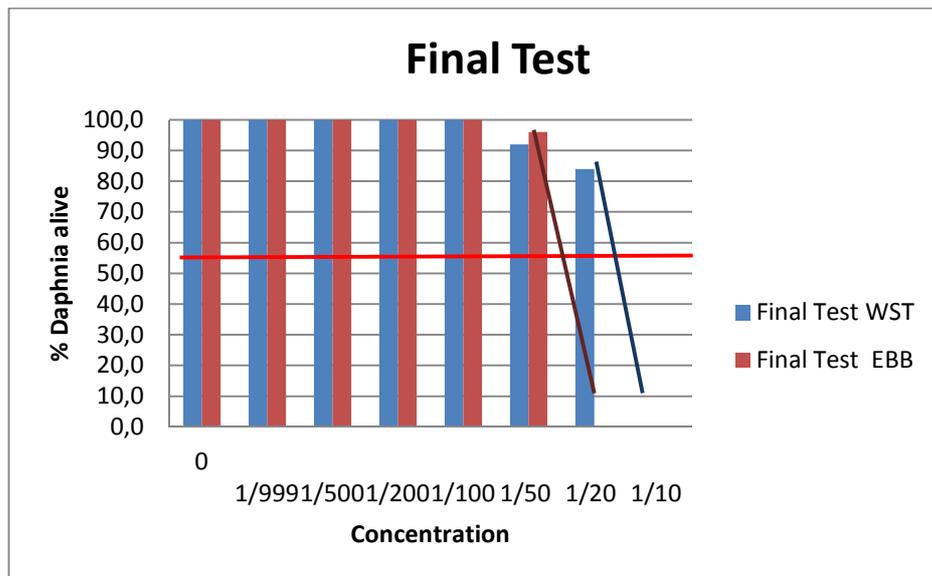
Figure 25. Percentage of living *Daphnia magna* in different mining water dilutions after 48 hours exposurein the WST and EBB-Flow treatments

The value of the LC_{50} for the WST is 0,070 ml wastewater/ml water or 1/14.

The values for the metal concentrations in this dilution are:

Metalconcentration TANK 3					
Dilution	Ni	Zn	Cu	Co	SO_4^{2-}
Original water	77,7	143	0,0121	1,63	18000
1/14	5,550	10,214	0,001	0,116	1285,714

TABLE 14. Metal concentration for the LC_{50}inWST

The value of the LC_{50} for the ATS II is 0,034 ml wastewater/ml water or 1/29.

The values for the metal concentrations in this dilution are:

Dilution	Metalconcentration TANK 3				
	Ni	Zn	Cu	Co	SO_4^{2-}
Original water	77,7	143	0,0121	1,63	18000
1/29	2,679	4,931	0,000	0,056	620,690

TABLE 15. Metal concentration for the LC_{50} in ATS II

ATS I and ATS II:
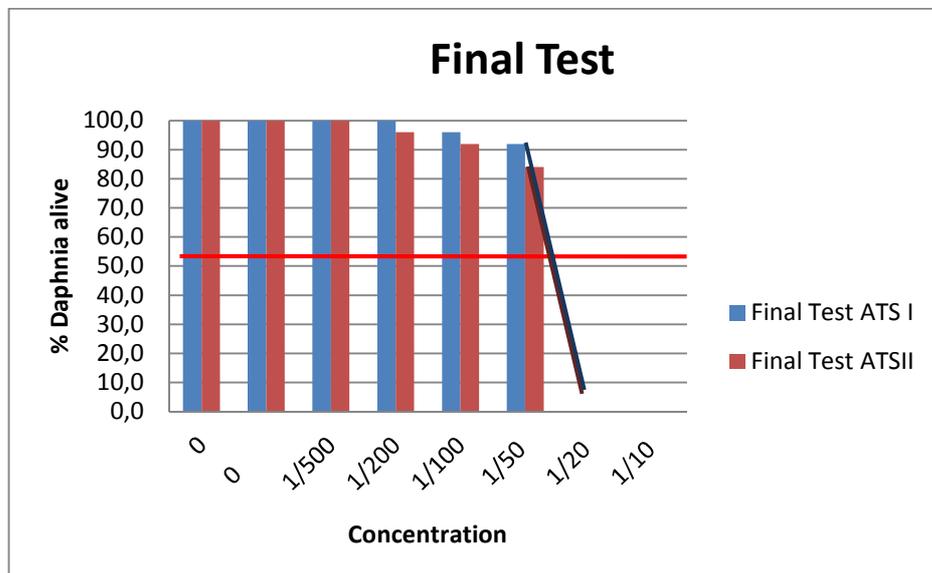
Figure 26. Percentage of living *Daphnia magna* in different mining water dilutions after 48 hours exposurein the ATS I and ATS II treatments

The value of the LC_{50} for the ATS I is 0,034 or 1/30.

The values for the metal concentrations in this dilution are:

Dilution	MetalconcentrationTANK 3				
	Ni	Zn	Cu	Co	SO_4^{2-}
Original water	77,7	143	0,0121	1,63	18000
1/30	2,590	4,767	0,000	0,054	600,000

TABLE 16. Metal concentration for the LC_{50}inWST

The value of the LC_{50} for the ATS II is 0,032 or 1/31.

The values for the metal concentrations in this dilution are:

	Metalconcentration TANK 3				
Dilution	**Ni**	**Zn**	**Cu**	**Co**	**SO_4^{2-}**
Original water	77,7	143	0,0121	1,63	18000
1/31	2,506	4,613	0,000	0,053	580,645

TABLE 17. Metal concentration for the LC_{50}inWST

In this test run, one test was only done at the end. For the willow stack tower the concentration in which the 50% of *Daphnia* died was 0,07 ml wastewater/ml water or 1/14. This concentration is higher than in the first test run because of the nutrient water that dissolved the wastewater and became less toxic.

In the EBB-Flow system, the concentration was similar than in the first test run. The reason is that at the end of the second test run most of the water was from the original wastewater.

In both algae turf scrubbers, the concentration was lower than in the first test run. It was because the amount of water that was evaporated. The result for both of them was similar.

4.3.3.2.Terrestial toxicity test

The *Eiseniafetida* test for the end of the second test run shows:

solution	WST						
	beginning		after 7 days		after 14 days		soilmass [g]
	number	weight (g)	number	weight (g)	number	weight (g)	
	11.3.2013		23.5.2013		25.3.2013		11.3.2013
0 "blind"	10	2,617	9	3,432	10	4,285	621,2
0 "blind"	10	2,967	10	3,161	10	3,735	621,8
1/100	10	2,497	10	2,727	10	3,413	621,6
1/50	10	2,018	7	1,667	8	1,243	620,4
1/10	10	2,627	10	2,741	10	3,109	621,0
1/2	10	2,152	10	2,452	10	2,985	621,8
1/1	10	2,367	7	2,069	6	1,716	621,6

TABLE 18. *Eiseniafetida* initial test WST

solution	EBB-Flow						
	beginning		after 7 days		after 14 days		soilmass [g]
	number	weight (g)	number	weight (g)	number	weight (g)	
	16.4.2013		23.5.2013		25.3.2013		11.3.2013
0 "blind"	10	2,388	9	1,991	9	2,289	621,6
0 "blind"	10	2,819	10	3,236	9	3,507	621,0
1/100	10	2,507	10	3,111	10	3,439	621,0
1/50	10	2,284	10	3,229	10	3,584	619,8
1/10	10	2,200	10	2,228	9	2,375	620,4
1/2	10	2,063	10	2,633	10	3,015	619,6
1/1	10	2,302	6	1,693	5	1,586	620,6

TABLE 19. *Eiseniafetida* initial test EBB-Flow system

In both systems, some changes in the weight and number of earthworms can be showed.

The toxicity of the undiluted wastewater caused the disapearance of 60% of the worms in the WST and 50% of the worms in the EBB-Flow system. By this way, the LC_{50} for the WST could not be calculate because the original water causes the 60% of the death worms, but for the EBB-Flow the LC_{50} is 1/1, the original water. It has to be said that this water is diluited with nutrient water but the proportion could not be calculated because of the high evaporation.We can observe that the terrestial toxicity of the wastewater did not change during the second test run. It shows us again that the pollutant concentrations of the wastewater were not reduced significantly in the three different systems. The concentrations are calculated in TABLE 5, page 37.

5 DISCUSSION

The results obtained during both test runs shows that the low pH and the concentration of pollutants present in the wastewater makes it too strong for the microorganisms to be able to grow in it.This pH appeared because of the high concentration of sulphates presents in the wastewater.

In the first test run, all used tanks were filled up with wastewater from mineral processing. In both algae turf scrubbers, the algae could not grow. The graphs show an increasing trend of all the pollutants with time. This increase is due to the fact that a lot of water was evaporated because of the high temperatures in the greenhouse.

In the other systems, the ebb-flow and the willow stack tower, the results of the first test run shows the same than in the algae turf scrubber. All the metal concentrations increase because of the evaporation. In the case of the sodium in the first period, its concentration in both systems was constant or it decrease a bit, it could be because some metals precipitated in presence of phosphates (Lorena Lorilla, 2013). The same happened with the potasium in the second period of the run.

In the aquatic toxicity test shows as that in the beginning of the test the LC_{50} is 0,0134 ml wastewater/ml water or 1/75 for the WST and the EBB-Flow system and 0,018 ml wastewater/ml water or 1/55 for both ATS. In the final test we can observe that for the WST and the EBB-Flow the LC_{50} is between the same ranges of concentrations than in the beginning test. It was 1/70 for the WST and for the EBB-Flow system. It shows that the toxicity of the water was the same after the treatment. In both ATS systems the range in the final test is between 1/200 and 1/100. It was 0,007 ml wastewater/ml water or 1/140 for the ATS I and 0,006 ml wastewater/ml water or 1/158 for the ATS II. It shows that less daphnia are alive when the sample concentration increases. It is because in these systems the evaporation of water was higher than in the other ones and the toxicity of the water increased because of this. Our results did not indicate many differences in the toxicity between the un-treated water and the treated water.

In the terrestrial toxicity test, the worms could grow in presence of the wastewater what shows us that the wastewater is not so strong for the terrestrial life although a weight decrease could be observed during the second week of the test run. There were some earthworms that dissapeared during the test but it was not because of the wastewater.

In the second test run, all the systems were initially filled up with nutrient water. With this water, both algae and bacteria could grow in the systems. The period in which the systems run with nutrient water was 10 days, by that time all the microorganisms grow succesfully.

When this growing period finished, wastewater was added gradually in order to adapt the microorganisms to the polluted wastewater. It was thought that the low concentration of nutrients in the wastewater could be the reason of the microorganisms' death but it was not. When we started to addthe wastewater, all microorganisms died in the systems. The high level of pollutants and the low pH makes the water too strong to sustain the microorganims.

When the wastewater was added into the algae turf scrubber, the colour of the algae changed. It became darker than at the begining of the test run. When the system was running during one week, the colour of the algae was brown and something white appeared in the surface of the algae turf scrubber.

In the WST and in the EBB-Flow system, the consecuences of the addition of wastewater did not appear as soon as in the algae turf scrubber. One week later, in the surface of the willow could observe that the willow was being rotten.

The result that was obtained shows that in all the systems the metal concentrations increase except in the concentration the zinc. The increase is due to the water evaporation in the systems. When a zinc decrease is observed, it could be because of the precipitation of metals with phosphates.

The results of the aquatic toxicity test shows that in the end of the second test run the range between the 50% of daphnia was alive was 0,07 ml wastewater/ml water or 1/14 for the WST and 0,034 ml wastewater/ml water or 1/29 for the EBB-Flow system. This value is higher than in the first test run but it was because the wastewater was disolved with nutrient water so the toxicity of the water decreased. It is bigger for the willow stack tower because more nutrient water was added than in the EBB-Flow system. For both algae turf scrubbers this concentration are 0,034 ml wastewater/ml water or 1/30 for the ATS I and 0,032 ml wastewater/ml water or 1/31 for the ATS II, this range is lower than in the willow stack tower because in the algae turf scrubber there was more evaporation.

The terrestrial toxicity test shows us that the wastewater continued being toxic for the earthworms, but the effect was not that dramatic as with aquatic organisms. The treatments did not reduce in the water toxicity. In the tests using undiluted wastewater the 60% of worms disappeared in the WST-treated wastewatertestand the 50% of the worms disappeared in the EBB-Flow treated wastewater test. The LC50 for the EBB-Flow system is 1/1.

It was important to remark that the zinc concentration increase a lot during both test runs. In both test runs the initial concentration of zinc was between 20 and 30 mg/l and it increase until 2000 mg/l during the first week of the test run.

For knowing the results of the nutrient concentration, TOC and BOD_5 has to be checked in Lorena Lorilla´s thesis (2013) as well as a more detail description of the algae systems can be found in Stefan Sprock´s thesis (2013).

The main result of this study was that thes three tested systems (WST, EBB-flow and ATS) alone cannot treat the wastewater from mineral processing. Two different ways of work could be done in order to get better results in the systems working. On one hand, WST could be used in order to reduce the amount of water which has to be treated which represents a big problem in the industry. The main problem of this alternative is that different working systems have to be searched to reduce the high levels of pollutants in the wastewater because the biological systems can not work in these conditions. On the other hand, different changes could be introduced in order to reduce the concentration of pollutants in the wastewater before treat it with our systems.

Finally different ways of working could be also used.

On one hand, as it was said, the WST could be used in order to reduce the amount of water that has to be treated. By this way the mine's dimension could be reduced due to the fact that the evaporation reduces a lot the quantity of water. After this biological system, the pollutant concentration is too high so, other systems have to be used to reduce this concentration. Biological systems could not be used because of the high pollutant concentration in the water so, different alternatives has to be looking for, as chemical precipitation.

Different ways of reduced the pollutant concentration before the treatment are going to be introduced:

It is obvious that some pre-treatment is needed first. This treatment could be done in different ways. The first one is a chemical treatment that decreases the level of pollutants in the water and indecrease the pH.pH test was done in order to know how the metals and sulphates concentration vary when a chemical is added to precipitate them. This test shows that at pH 9 the concentration of sulphates is too high but it could be one way to remove some pollutants and adjust the pH to keep the microorganisms alive.

The way to pretreatment could be dilute the wastewater with water. It could be a good way to reduce the concentration of pollutants in the water. This solution could be a good option when it is worked in a lab scale. The problem is that when you want to work in a real scale, high amounts of water would have to be treated and it could be not the best way on the economic point of view. In this alternative the WST would the best option to work because of the fact that in this system a lot of water was evaporated and it has high capacity of storage this amount of water.

Different studies in which the wastewater was pretreated with different systems that are been studied and they could be a good option as an innovative wastewater treatment.

There is a study that could be done as pretreatment for our wastewater. In this study, the biotreatment of acidic wastewater is done with an anaerobic baffled reactor (ABR) at constant hydraulic retention time (HRT) of 1,0day. The water used in the study contains Cr(VI), Fe(III) and sulfate (3500 mg/l) and it was studied in an ethanol supplemented.

The reactor performance was very high even at low pH (2,5–3,5) and high Cr(VI) concentration (200mg/l). Sulfate reduction and COD oxidation degrees exceeded 80% and 90%, respectively, in all cases. The alkalinity generated due to the sulfidogenic oxidation of ethanol increased wastewater pH to neutral values (7,3–8,0) and facilitated precipitation of Cr(III) compounds. The produced precipitates were retained in the ABR as a result of its compartmentalized structure and the presence of a sludge blanket acting as filter. Cr(VI) precipitates as CrO(OH) as well as chromium sulfide. Part of chromium may be also adsorbed in amorphous precipitated phases. The experimental results prove the high efficiency of the process and its potential application to tackle environmental issues related with acidic wastewaters/leachates generated in the mining and metallurgical industry as well as in chromium containing waste disposal sites. (Sahinkaya et al, 2012) This solution could be investigated due to the pH increase in the waster after the treatment and some metals precipitate. It could be a good idea to use this before our biological systems.

Other study shows how acid drainage wastewaters contaminated with Cu, Fe, Zn, Ni, Cd and Co were remediated by hydrogen sulphide precipitation reaction in laboratory-scale installation. The installation design includes anaerobic bioreactor, chemical reactor and settler, connecting in series. The recirculation of treated wastewater from settler to anaerobic bioreactor was performed by peristaltic pumps. Sulphate-reducing bacterial consortium is adhered in biofilm, which is immobilized on zeolite particles in the anaerobic bioreactor. The bacteria were cultivated on a medium containing lactate as a source of carbon and energy. Heavy metals removal was achieved in a chemical reactor by biogenic produced H_2S and bicarbonate ions. This study is more focused in decreasing the metal concentrations in the wastewater than in the decrasing of the sulphates concentration that is better for us due to the low pH. (Bratkova et al, 2012)

Other way to pretreat the wastewater is using a biological treatment with sulphate-reducing bacteria (SRB). In this study, the phylogenetic analysis of the gene sequence revealed that this consortium contains species of SRB affiliated to Desulfovibriodesulfuricans and Desulfobulbusrhabdoformis. The results show that the presence of usually lethal concentrations of Fe (400mg/l), Zn (150mg/l) and Cu (80mg/l) is not toxic for the sulphate-reducing bacteria present in this sample. As a consequence, a very good efficiency in terms of sulphate reduction and metals removal was obtained. Both ethanol and lactate can be used by this inoculum as carbon source. With the other samples tested sulphate reduction was inhibited by the presence of copper and zinc. This highly metal resistant consortium will be used to inoculate a bioreactor to carry out decontamination. (Martins et al, 2009) As the first solution, with this kind of pretreatment, the sulphates concentration could be decrease and after that, the treatment with our systems could be done in order to decrease the heavy metal concentrations.

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Appendixes

Appendix 1
Toxicity test of *Daphnia magna*

PROCEDURE

In this proyect the test has been done with the Daphtoxkitsmicrobiotest.

- Preparation of standard freshwater.
- Fill approximately half a 2000 ml volumetric flask with distilled water.
- Pour the content of vial labeled number 1 ($NaHCO_3$) in the flask.
- Pour the content of the 3 others vials in the flask: vial 2 ($CaCl_2$), vial 3 ($MgSO_4$) and vial 4 (KCl), respecting this sequence.
- Add distilled water up to the 2000ml mark.
- Shake in order to homogenize the medium.

Storage of hatching and dilution medium

- Transfer all the ephippia of the vial into a microsieve.
- In order to eliminate the traces of the storage medium, rinse the ephippia with tap water.
- Pour 15 ml pre-eareted Standard Freshwater in the hatching petri dish and transfer the ephipia into it.
- Cover the petridish.

Incubate for 72h, at 20-22ºC under continuous illumination of 6000 lux.

Preparation of toxican dilutions

A dilution series (0, 1/1000, 1/500, 1/200, 1/100, 1/50, 1/20, 1/10) of the effluent sample is prepared with Standard Freshwater.

Filling of the test plate

In order to obtain a statistically acceptable avaluation of the effects, each test concentration as well as the control has to be assayed in almost 4 replicantes. In this proyect 5 replicants has been used.

In this proyect two multiweel plates are used. Each multiwell plate is provided with 5 test wells for the controls and 6 test wells for each toxicant concentration.

- Pour 10 ml of the respective toxicant concentrations into each well corresponding rows, in the sequence of increasing toxicant concentrations.
- Transfer exactly 5 Daphnia neonates into the test wells using a micropipette.

Scoring the results

After 24 hours, put the multiwell plate on a light table. Search the number of dead and inmobilizated neonates for eac toxicant concentration and calculate the mean and the % effect.

Terrestial toxicity test.Soil Quality

PROCEDURE

Prepare the worms.

Select worms in order to obtain a homogeneous population with similar size and weight. Wash the worms and dry them with absorbent paper. After that, weigh them.

Test subtrate

- Determine the equivalent weight of substrate to 500 g (dry mass).
- Put the substrate in a glass container with a capacity of 1-2 litres to allow the exchanges between the substrate and the atmosphere.
- Determine the pH of the substrate.
    o Take a weight equivalent to 250 ml of sample volume.
    o Put it in a container and add 300 ml of distilled water.
    o Shake it for 1 h on a shaking machine.
    o Measure the pH of the sample with a pH-metre.

Water soluble substances

- Make the dilutions with the different concentrations with which will be work.

Prepare the test containers

- Add the substrate into the container.
- Take 10 warms and add them to the test container. Make a small hole and put them inside.
- Add 65 ml of each water dilution in each container and cover the worms with the substrate.

After 7 days

- Count the live worms and weight them. Remove the death ones if they are visible.
- Put the substrate inside the container again, make a hole and put the worms. Cover them.

If the substrate is too dry, some distilled water can be added.

After 14 days

- Count the live and death worms. Weight the live ones.

All the text must be done at a temperature of 20ºC ± 2ºC and a constant light intensity of 400 lux to 800 lux, controlling light/dark cycle should be to be of between 12 hours.

Sulphate Analysis. Spectrophotometer

PROCEDURE

For calculating the quantity of sulphates in the water, method 8051 is used.

- Select the program Sulphate 680 in the spectophotometer system.
- Fill the cell with 10 ml of sample.
- Add the contest of one bag of sulfaVer 4 Reagent Powder Pillow to the cell. Shake it to disolve the powder. If sulphates are present in the dilution, white turbidity has to appear.
- Press timer. A five minutes reaction period will begin. Do not disturb the cell during this period of time.
- Prepare the blank cell. Add 10 ml of sample in the second cell.
- When the timer expires, insert the blank cell into the cell holder and press ZERO.
- The display will show 0 mg/l SO_4^{2-}.
- Insert the prepare sample with the SulfaVer 4 Reagent into the cell holder and press READ. The results are in mg/l SO_4^{2-}.

The range of sulphate concentration with which results can be obtained are between 2 mg/l to 70 mg/l. Because of the amount of sulphates that were presenting in our wastewater we had to dilute the wastewater in 1/1000 ml.

Heavy Metals Analysis. AtomicAbsortion Spectrophotometry

PROCEDURE

For calculating the metal concentration in the water

Nickel

Calibration line: Prepare the dilutions with the standard dilutions of the metal.

0,0 mg/l, 5,0 mg/l; 7,5 mg/l; 10,0 mg/l; 15,0 mg/l; 20,0 mg/l.

- Choose the right light sources for the measured metal.
- Choose the right wavelenght for the measured metal. For the nikel is 341,48 nm.
- Choose the method for the measured metal. For nickel is Ni.

Zinc

Calibration line: Prepare the dilutions with the standard dilutions of the metal.

0,0 mg/l, 10,0 mg/l; 25,0 mg/l; 50,0 mg/l; 75,0 mg/l; 100,0 mg/l.

- Choose the right light sources for the measured metal.
- Choose the right wavelenght for the measured metal. For the Zinc is 307,59 nm.
- Choose the method for the measured metal. For nickel is Zn.

Potassium

Calibration line: Prepare the dilutions with the standard dilutions of the metal.

0,0 mg/l, 0,1 mg/l; 0,25 mg/l; 0,5 mg/l; 0,75 mg/l; 1,0 mg/l.

- Choose the right light sources for the measured metal.
- Choose the right wavelenght for the measured metal. For the potassium is 766,49 nm.
- Choose the method for the measured metal. For nickel is K.
-

Sodium

Calibration line: Prepare the dilutions with the standard dilutions of the metal.

0,0 mg/l; 5,0 mg/l; 7,5 mg/l; 10,0 mg/l; 15,0 mg/l; 20,0 mg/l.

- Choose the right light sources for the measured metal.
- Choose the right wavelenght for the measured metal. For the sodium is 330,34 nm.
- Choose the method for the measured metal. For nickel is Na.