THE ROLE OF ALGAE IN HEAVY METALS REMOVAL FROM MINING WASTEWATER

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ABSTRACT

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Nature is the key solution for human need and problems emerging from man-made structures.

Inefficiently purified industrial and municipal wastewater discharges cause so far many prominent pollution cases.

In this work experimental scale algae turf scrubbers (ATS) have been used to remove excess amounts of nutrients and heavy metals from mineral wastewaters. ATS are mechanical systems designed for growing algae under controlled conditions to absorb the excess nutrients or/and pollutants from wastewaters.

The wastewater used in this study was mining process wastewater from Talvivaara mining site. The study started with a range finding experiment of wastewater and ten algae species cultivated together. Then ATS testing were done mainly for Zinc and Nickel removal, by it setting-up two replicates and monitoring pH, illumance and measuring metal concentrations in the beginning and at the end of experiment. Lastly a range finding experiment on the Copper tolerance of the ten algae species tested separately was conducted

The conclusion was that the ten algae species cultivated together in the ATS stabilized the Zinc within 16 days with a removal rate of 99%. The ratio used was the one found optimal in the first range finding experiment, a concentration of 2% pH adjusted mining wastewater respectively. . The expected Nickel removal didn't happen probably because to the air stones used for aeration that most probably leached Ni. The 10 algae species cultivated separately presented variable Cu removal potential.

CONTENTS

1 INTRODUCTION

The scientific field that investigates the algae is called *Phycology*. *Phykos* stands for alga in Greek. While Romans referred to it as *Fucus* and the Chinese by Tsao. The history of phycology is as old as man's interest in botany. The information and data produced in this field is a fruit of many years of investigation, which began with the invention of the microscope. *Antoni Leeuvenhock* gave the spark for the field of microbiology by reporting a number of unicellular algae and flagellates on September 7, 1674 (Sarabhai and Arora,1995).

Hence, until nowadays more research and discoveries are made with the development of analytical tools that helps understand better the physiological, biochemical and even the molecular mechanisms of these species.

With a better understanding of algae anatomy and physiochemical composition and processes, it came to the point of applying it for the man's need in varied fields.

Human health foods are produced by the cultivation of microalgae such as food additives, protein extracts and few vitamins.

Some species of algae are also know for their ability to produce unsaturated poly-hydrocarbons trough the photosynthesis of lipids in their surface cells. These then can be used later as lubricants and surfactants in industry and in the medicinal field (Graham and Wilcox, 2000 p70).

Extracts of algae in cosmetics is another field of application particularly in face, hand and body creams and lotions. However the use of algae themselves in cosmetics rather than the extracts is still limited (Barsanti and Gualtieri, 2006 p 280).

Different types of algae have proven to be the source of many antibiotics and other pharmacological useful product. These are subject of in-depth medicinal studies, which of them revealed the ability of anti-tumor compounds production, for instance *Cyanobacteria.* Thereafter once algae species are of a medical use, the algae then are cultivated in large scales in bioreactors, harvested, and the active material extracted, purified and marketed (Graham and Wilcox, 2000 p 77).

Algal Turf Systems are known for the mineral nutrient removal in wastewater effluents, which is another application field of algae. Municipal wastewater effluents and industrial wastes were mainly with a regular basis discharged to naturals waters for purifications. Since the populations grew in densities, then wastewaters treatment plants were constructed primarily for removing particulates and pathogenic microorganisms. Some municipal wastewater treatment plants till nowadays discharges the water to natural water bodies with a relatively high concentrations of phosphates and nitrogen, due to the high cost of removing them with conventional processes. As a result, discharging waters with high concentration of the previously mentioned compounds would provide enough nutrition for algae to grow. Excess of algal growth will cause then some species to produce toxins and destroy the aquatic ecosystem where this water would end-up; algal blooming and eutrophication for instance.

Other effluent systems incorporate micro algal ponds or raceways, where treated water is channeled just prior to discharge. During this process algae remove inorganic nutrients and add oxygen. One drawback of such systems is that water treatment needs a continual mixing to keep the algae suspended and to maximize the nutrients uptake and oxygen enrichment. Moreover the micro algal growth harvesting is difficult. Alternatively for the mixing and oxygenation, artificial streams are installed with a slope with the use of filamentous algae.

Accumulation of the biomass is easily harvested later with the Algae Turf scrubber. The lower portions of the algal filaments attached to substrates allow regrowth to occur.

Mineral nutrients and heavy metals are removed by Periphyton[1] algae in several ways: they trap particles, absorb ions, assimilate and store nutrients, and, at high pH, precipitate layers of calcium carbonates, which may entrap pollutants. Regular harvesting within the system stimulates the production by removing shade canopies and keeping graze population low so that pollutants are not transmitted to higher trophic levels and oxygen levels are not depleted.

Naturally formed algae mats are of three ecological types. First, basal mat-forming cyanobacteria such as Oscillatoria. Second one is filamentous green algae such as Cladophora (in warm water) or Oedogonium (in colder waters) which grow trough the cyanobacterial surface to form a canopy. Third type is an epiphytic diatom, which grow both on the canopy filaments and within the mats.

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[1] Attached algae

In Patterson California within a large-scale wastewater treatment facility, an algae turf system has proven a useful reduction of total phosphorus, soluble reactive phosphorus and an increase in dissolved oxygen level. A similar case was in Florida where periphytic algae successfully reduced phosphorus levels from effluent waters from fertilized sugarcane fields. Algae turf systems are also designed for temperate cold climates for nutrients removal (Graham and Wilcox, 2000 p 76).

The ability of algae to sorb high concentrations of heavy metals makes them a suitable candidate for removing these ions from the wastewaters. This would be possible by building an efficient and commercially viable algal technology based upon filling the gap of knowledge concerning metal sorption by algae (Critical Reviews in Biotechnology, Use of algae for removing heavy metal ions from wastewaters 2005, p 145-146).

And since heavy metal pollution in aqueous systems is certainly a big problem in today's world, technologies for bioremediation have been developed. These technologies target the bioremediation of heavy metal contaminated water by living alga cells or nonliving alga cells that became of an important concern. High Rate Algal Ponds and Algal Turf Scrubber have been deployed practically in some industrial countries (Lijun Plant Resource Institute, Bioremediation of heavy metal contamination by algae, 2014).

The scope of this work is to test Algae Turf Scrubbers as a bioremediation technology to remove heavy metals from mineral mining wastewaters.

2 TEORETHICAL BACKGROUND

Algae have proven tolerance towards heavy metals in scientific researches. The mechanisms possible for heavy metal tolerance, storage or up-take are varied depending on different factors ranging from the anatomy of the algae species to the environmental conditions or the growing mediums. Different mechanisms of metal tolerance in algae are possible.

2.1 Binding of metals on the cell surface

The metal ions binding onto the extracellular surface of the algae due to polymeric material nature of the cell walls is another mechanism of metal tolerance in algae and microbes. Many cyanobacteria and algae secrete copious amounts of mucilaginous[2] materials of different types; primarily composed of polysaccharides. This material has a substantial ability of various metal ions binding, which is one of the possible mechanisms used by algae and cyanobacteria to protect their selves from extreme and harmful environmental conditions.

These cell walls have a negative net charge due to the carboxyl (-COO⁻), phosphatic and other groups that involve metals binding through ion exchange and other mechanisms. It has been reported that thicker cell walls of some algae species has a better-modulated toxicity compared to thinner walls species. Thicker cell walls are more abundant of carboxyl, phosphatic or other group that acts as a metal binding agent.

Whether the cell wall and the membrane material act to increase or decrease intracellular metal uptake may vary depending on the organism and the adsorbing material. The question here then is whether the adsorption acts to increase or decrease the bioavailability of the metal. This could be answered in such a way that strong adsorbers; with strong ligands in their polymeric material; reduces the bioavailability of free ionic metals. While other ligands would rather contribute to the transport of the heavy metals inside the cell such as the polyphosphate case of metal storage that would be mentioned in section 2.5. (Rai and Gaur, 2001 p 366).

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[2] a viscous secretion or bodily fluid.

2.2 Extra cellular ligands for metal-complexing

Natural waters usually contain a range of ligands to which metal ions get adsorbed and become less available for living organisms. Some species of algae for instance secrete themselves such substances. These are named *siderophores*, which is a strategy for microorganisms used to overcome Iron deficiency; or to escape Cu toxicity.

Some of the *siderophores* produced are known to have strong metal complexing ligands (e.g. hydroxamates) while others are weaker metal-complexing ligands (e.g.organic acids). Each particular organism usually biosynthesies a specific kind of ligand, in some cases more than one can be produced. This usually serves the algae as a defense mechanism from metals toxicity.

However the researches on the metal-complexing ligands of algae have focused primarily on Cu^{2+}, while a pressing need to assess the binding by algae exudates on other metals is to be done (Rai and Gaur, 2001 p 367-368).

2.3 Efflux of metal ions

Efflux of metals from algae and cyanobacteria is another phenomena that relate algae and metals. This happens basically by accumulating fewer metals inside the cells. Hence the metal influx to the algae is decreased which could happen for different reason. An important one is the alteration of the cell membrane permeability for metal ions leading to an efflux.

Another reason suggested is the possible modifications in the cell wall physiology or biochemistry leading again to the same phenomena. As an illustration, in a study comparing Cu-tolerant and Cu-sensitive strains the transport of Cu^{2+} was reduced due to the greater lipid content and improved membrane integrity for the Cu-tolerant strain. Another study of Al tolerance suggest that strong surface negativity; which is primarily due to carboxyl and phosphatic groups at the end of the polysaccharides cell membrane; could result in greater binding of the positively charged Al ions to the membrane rendering membrane structure and functions more sensitive to it (Rai and Gaur, 2001 p368-369).

2.4 Metal sequestration by Phytochelatins and Metallothioneins

2.4.1 Definitions

Metallothioneins is a family of metal-binding protein, which has shown cytosolic concentration regulation of free metal ions in algae. Same function is observed by Phytochelatins that are enzymatically-synthesized peptides.

Algae produce a class of metal-binding peptides, which differ from the animal occurring Metallothioneins. These metal-binding peptides contain a thiol group (-SH) and are named Phytochelatins.

To clarify the ambiguity between characteristics between the above-mentioned protein and the polypeptide, the Metallothioneins (MT) could be classified as follows:

- MT 1: Polypeptides mostly found in vertebrates but not reported in algae, their function resembles those found in horse kidney.
- MT 2: Polypeptides distantly related to those of horse kidney Metallothioneins, but found in some cyanobacteria species.
- MT 3: or Phytochelatins are non-translationally synthesized peptides found in higher plants, eukaryotic algae and certain Fungi. Phytochelatins (PC) are chemically relate to glutathione (γ-Glu-Cys-Gly)

The MT 3 or Phytochelatins, which are the focal point, are not encoded by any structural genes. And in contrast with other MTs Phytochelatins are non-protein polypeptides, and made of the three amino acids: Glutamine (Glu), Cyseine (Cys) and Glycine (Gly). The previous amino acids form the building block for the polypeptides of metal-binding character; and which occur in algae; namely glutathione and of the following structure (γ-Glu-Cys-Gly). To form a polypeptide from glutathione, the peptide bond then come between γ or side chain carboxylate (R-COO⁻) group of the glutamic acid. The general structure of the polypeptide is given by the general formula (γ-Glu-Cys)$_n$-Gly where n ranges from 2 to 11. The discovery of γ-carboxyamide linkage in polypeptides made it as a third class Metallothioneins. Hence that's what makes it different from mammalian MT since the linkage between the peptide does not require any ribosomal activity for its synthesis. Moreover that is the main reason why PC are considered non-translationally synthesized peptides.

Several researches worked on the isolation of metal-binding proteins from different groups, which has proven mostly a high affinity for Zn^{2+} and Cu^{2+} (Rai and Gaur, 2001 p370-372).

2.4.2 Metal Sequestration of Phytochelatins

Binding to different metals is the fundamental property of Phytochelatins. Indirect evidences suggest binding with Hg, Ag and weak association with Zn. In these analyses pH influence on peak absorption of metals has been observed. Moreover, polypeptides with higher polymerization values bound to Cd for instance more intensively compared with lower values of n. Other researches demonstrate that the number of polymerization is not of the primordial reason, but the abundance of S^{2-} in the polymer.

Before the metal-complexation by Phytochelatins, its biosynthesis has to be induced. This induction could be triggered due to different heavy metals such as Cd^{2+}, Ag^{2+}, Bi^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+} and Au^{2+}.

In return Glutathione biosynthesis has to be induced as well, since it is considered to be the building block for Phytochelatins polypeptides. Scientists believe at this point that biosynthesis of both glutathione (GSH) and Phytochelatins might involve common enzymes. Glutathione biosynthesis involves two steps:

1. Glu $+$ Cys $\rightarrow \gamma$-glu-Cys

2. γ-glu-Cys $+$ Gly $\rightarrow \gamma$-glu-Cys-Gly

An enzyme called γ-glutamylcysteine synthetase catalyzes the first step. While the second one by glutathione synthetase. The γ-glutamylcysteine synthetase itself is inhibited by buthionine sulfoximine (BSO). The inhibition of Phytochelatin biosynthesis by BSO suggests the involvement of this enzyme in Phytochelatin biosynthesis in higher plants.

Environmental factors were also demonstrated to affect the PC production, such as light abundance and temperature.

The whole process could be summarized into three steps:

- Induced synthesis of the enzyme phytochelatin synthase, due to intracellular heavy metals accumulation resulting in phytochelatins production.

- Decreased concentration of unbound metal ions in the cytosol by sequestration/chelation of by phytochelatins.

- Reduced metal toxicity because of the lower concentration of unbound metals (Rai and Gaur, 2001 p373-376).

2.4.3 Metallothioneins in Cyanobacteria

As mentioned earlier Metallothioneins were subdivided into three classes. And the one that is present in Cyanobacteria takes the second place and presented here since it is the focal point while the first class of Metallothionein is not the focal point as long as it concerns animal MT.

Cyanobacterium of *Synechococcus* has been identified capable of MT synthesis on exposure to heavy metals. Moreover the *Synechococcus* MT has been reported to be peculiar in its biosynthesis, meaning that it can be enhanced by Cd and Zn but not Cu.

The genes identified to be responsible for MT2 biosynthesis are smtA and smtB, while the second is considered to be its divergent. The divergent gene has the function of trans-acting smtA and reported to exert ion-inducible negative control over smtA transcription. As it has been observed that smtB binds directly with Zn and inhibit repressor-DNA complex formation. More importantly, another report stated that smtA transcripts increased significantly with the exposure of *Synechococcus* PCC6301 to Cd^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, Co^{2+} or Ni^{2+}.

Last but not least many evidences suggest that cyanobacterial smt is involved in the homeostasis of Zn^{2+}, while lack of functional Metallothionein locus (smt) has proven hypersensitivity to Zn^{2+} (Rai and Gaur, 2001 p377-378).

2.5 Polyphosphates Sequestration of Heavy Metals

Phophorus is a building block for all living cells, which constitutes of nuleic acid, phospholipids and complex carbohydrates. It plays a primordial role for the anabolic and catabolic pathways of energy conversion of cells via phosphoanhydride bonds, a reaction that involves also the posttranslational regulation of enzymes activities.

Phosphorus is the growth-limiting nutrient for the phytoplankton productivity.

Inorganic phosphates can be immediately utilized by phytoplankton, while organic phosphates usually need to be hydrolyzed first. Then the released phosphates can be incorporated in the respective inorganic phosphate transport system. Part of the organic phosphate compounds are mediated by the phosphatases[3], which are excreted to the cells into the extracellular space.

A number of functions, besides that of a phosphates store have recently been attributed to the polyphosphates: energy store and ATP[4] substitute, as buffer, chelation for metal ions (Rai and Gaur, 2001 p66-68).

In an excess phosphorus environment, many cyanobacteria and eukaryotic microalgae consumes more phosphorus that is needed for their metabolism. The surplus of the phosphorus then is sequestered in the polyphosphates bodies.

The polyphosphates are in form of orthophosphate polymers that can be mobilized by the organism for its metabolic needs in case of a phosphorus deficiency. Abundance of phosphorus has often shown the ability of metal ion toxicity mitigation to algae.

Such phenomena has been related to the polyanionic character of polyphosphates that allows attraction of electrophilic cations to the electron-rich oxygen atoms of the polyphosphates.

Polyphosphates also work as an osmotically[5] inert sink for metallic cations, such as K^+, Na^+, Ca^{2+} and Mg^{2+} that are required for the metabolism. Many of these required cations that are complexed to polyphosphate bodies, can be lost upon high concentrations of

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[3] Is an enzyme that removes the phosphate group from its substrate by hydrolyzing phosphoric acid monoester into phosphate ion with a free hydroxyl group.

[4] Adenosine triphospate, co-enzyme that trasports chemical energy within the cells for metabolism.

[5] a process by which molecules of a solvent tend to pass through a semipermeable membrane from a less concentrated solution into a more concentrated one, thus equalizing the concentrations on each side of the membrane.

heavy metal exposure to the algae. On the other hand X-ray energy dispersive analysis has shown that polyphosphates have the ability to accumulate several heavy metals:

- Pb in *Diatoma tenue* and *Navicola inerta*
- Cd, Cu, Co, Hg, Ni, Pb and Zn in *Plectonema boryanum*
- Al in *Anabaena cylindrica*
- Al, Fe, Cu and Zn in *Chlorella* (in elevated concentrations)

Yet other strains are not successful in the heavy metal sequestration through polyphosphate bodies, for instance Cu in *Anabaena variabilis* even tough the strain has proven Cu-tolerance properties.

Sequestration of metals in polyphosphates bodies is not a final solution for metal toxicity problems. Since any deficiency of phosphorus will bring back the sequestered metals in the polyphosphates due to the demand of orthophosphates for metabolism. As result the organism will be in the situation of stress again after storing them (Rai and Gaur, 2001 p379-380).

2.6 Algae mechanisms to counter-act metal induced damage

When metal damage is caused then algae is exerting different mechanisms for counteracting it. Yet after the damage has been caused then not the whole of it is recovered. For instance some studies showed that when the thiol (-SH) pools are depleted after the metals got bond to them, the antioxidant level inside the cell makes it vulnerable to an oxidative stress condition.

Another situation is that elevated concentrations of metal-induced oxygen disruption, enhances the lipid peroxidation followed by membrane disruption making the algal cells lifetime shorter.

Various mechanisms of protection are thought about by researchers and scientists. As an illustration some studies showed that heavy metal stressed-algae showed a high accumulation of free proline[6], which grows in concentration with growing heavy metal concentration. The suggestion given afterward is that proline perhaps protected the

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[6] An amino acid constituent of most proteins

plasma membrane from metal toxicity; blocking in other words the efflux of metals inside the cell.

However researchers were not sure of the manner with which intracellular proline showed a protective effect: complexation of metals, reduction in production or scavenging of harmful oxyradicals (Rai and Gaur, 2001 p380-381).

3   MATERIALS AND METHODS

3.1 Algae species used

Ten species of algae are presented below with definitions, illustrations and pictures. The Finnish Environment Institute (SYKE) provided these species, which were used in the experiment on the mining wastewater.

Anabaena Cylindrica

Sometimes spelled *Anabaina,* in freshwaters and marine waters (such as the Baltic sea) are considered primarily planktonic. It consists of filaments of spherical cells closely resembling to strung beads embedded in a mucilaginous matrix (Graham and Wilcox, 2000 p 129). *Anabaena Cylindrinca* has akinetes or *Siderophores* of an extremely resistant property to drying and freezing, as they function as a long-term anaerobic storage of the genetic material species. Its *Siderophores* can remain in sediments for many years enduring very harsh conditions as well as they remain viable for the continuance of the species (Barsanti and Gualtieri 2006, p 11).

Chlorphyta sp

This species contains chlorophylls a and b, β- and γ-carotene, and several xanthophylls as accessory pigments. The chloroplasts are surrounded by two-membrane envelope without any endoplasmic reticulum membrane (Barsanti and Gualtieri 2006, p 28).

Fragalaria crotoneis

Is a freshwater diatom green algae (Graham and Wilcox, 2000 p.55). Cells are joined to form a ribbon like colonies, displaying girdle views (Graham and Wilcox, 2000 p. 268).

Phytochelatins are the primary peptides present in this species (Rai and Gaur, 2001 p.372).

Haematococcus Pluvialis

Haematococcus occurs in brackish seashore rock pools; not of high salt concentrations; and appears as a biflagellate[7] unicells whose protoplast is connected to the wall by multiple thin strands of cytoplasm (Graham and Wilcox, 2000 p482-483).

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[7] a slender threadlike structure, esp. a microscopic whip-like appendage that enables many protozoa, bacteria, spermatozoa, etc., to swim.

Navicula pelliculosa

This species is present in freshwaters or soil (Graham and Wilcox, 2000 p36) and exists as single cells or ribbons of cells. The valves are boat-shaped as each bears a raphe (Graham and Wilcox, 2000 p263).

Yet some of the *Navicula* are reported to exist in sea ice (Graham and Wilcox, 2000 p263).

Pediastrum Simplex

Occurs commonly in freshwaters, and is a very distinctive coenobial colony having a flattened, often star like shape (Graham and Wilcox, 2000 p.49,465).

Purpuraemus

This specie was not found in a textbook nor an electronical database. However the specie was provided by SYKE.

Scenedesmus sp

Scenedesmus is a freshwater green alga, which can produce spiny cell extensions and long bristle like structures that make the organism appear large to herbivores and help reduce the sedimentation (Graham and Wilcox, 2000 p48)

Selenastrum Capriconutum

Appears in planktonic lakes and swamps, which is commonly used as a laboratory bioassay test organism. *Selenastrum* is a colony of 4-16 sickle-like or curved cells, each having a single parietal chloroplast with pyrenoid (Graham and Wilcox, 2000 p486).

Synechococcus sp.

Is a tiny cylindrical unicell that is an important primary producer in the plankton of fresh and marine waters, and has also been collected from the surfaces of algae and plants. This specie has been associated to the "whiting" events, the production of suspended fine-grained carbonates that eventually contribute to sedimentary carbonate deposition (Graham and Wilcox, 2000 p121).

Last but not least the variety of these algae species gives more chances for the metal detoxification. The reason behind this is that some of the species will actually be able to act intracellular metal uptake, while others extra-cellular by the mean of *Siderophores*

as well as some of the species will rather adsorb the metals to their cell surfaces. Hence this would be theoretically helpful reduce metal content of the process mining wastewater that is subject of this experiment.

3.2 Procedure

Algae Turf Scrubber is a medium equipped with a 4 lines system, which lets a fluid to flow from one basin to another with a short retention time (picture 2). This retention time is enough in case of microorganisms to grow and enable biological process to happen. Hence it was a good medium to test the algal tolerance towards heavy metals.
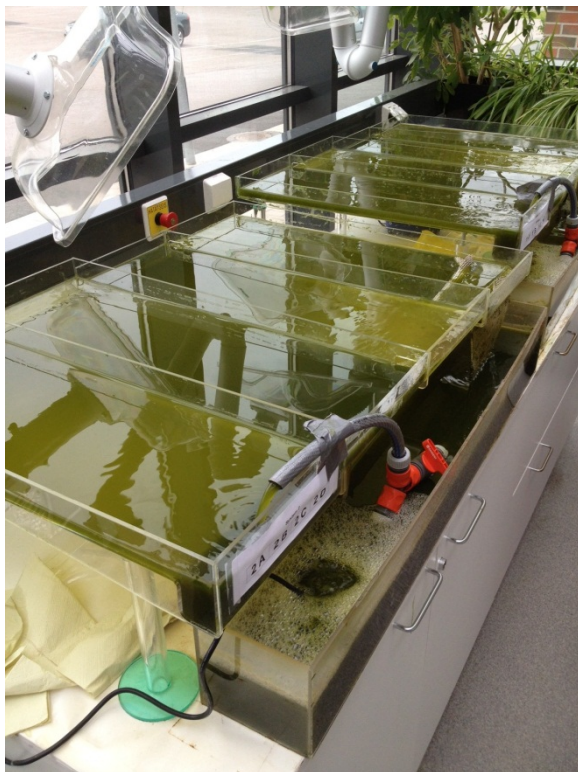
Picture 2 Algae Turf Scrubber (Grobler 2013)

The mining water provided by Talvivaara was the process mining wastewater, meaning that this water was used in the chemical and physical processing conducted within Talvivaara's process plant (See Appendix 3). This water was of initial pH of 2,4. Hence before moving to the Algae Turf Scrubber experiment, the best range of metal removal by the algae had to be tested. For this reason two tests were conducted during the whole experiment; best range finding then Algae Turf Scrubber testing.

The ten algae species previously mentioned were cultured together and used in both tests.

Additionally we were interested in single metal removal capability of the algae species involved by range finding experiment on Cu^{2+} standard solutions.

### 3.2.1 Range Finding for Mining Water

In this test two batches of the mining wastewater were used. One with original pH of 2.4, and the second one with raised pH using an aqueous solution of $Ca(OH)_2$ to the pH point of 4.5. The reason behind this was to see possible effect of the pH range on the metal removal by the algae. At the same time algae was cultured, by growing the ten algae species. A cylindrical glass tank was hosting the ten species mixed together. The culture received diluted solutions of nutrients every three days. The nutrient solution was prepared using concentrated solution of nutrients "Substral" into tap water; with a ratio of 1,5ml in 2l of tap water. Meanwhile temperature, pH and turbidity were measured. After three weeks of culture the algae was transferred to Erlenmeyer flasks. Soon after that amendments of the mining wastewater were added in concentrations presented in Table 1.

Table 1 Amendments used

No pH adjustments		pH adjusted	
Volume of Algae (ml)	Volume of amendment (ml)	Volume of Algae (ml)	Volume of amendment (ml)
300	0	300	0
299	1	299	1
298	2	298	2
295	5	295	5
290	10	290	10
285	15	285	15
270	30	270	30
240	60	240	60

When the amendments were transferred to the Erlenmeyer soon after that, air stones were added connected to hoses connected to air pumps. As the Erlenmeyer heads were blocked by cottons and covered by an aluminum foil to reduce the evaporation rate.

The test was conducted for three weeks. During this time a daily verification of the set-up was checked. And on a regular basis nutrients were added and measurements were done as well. The measurements taken were pH, temperature and turbidity. However Atomic Absorption Spectroscopy (AAS) was conducted only at the end of the experimental time.
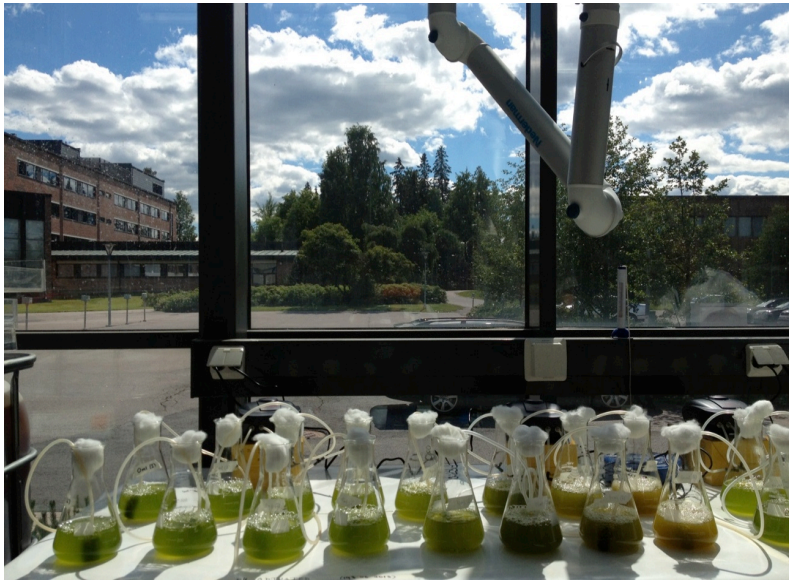
Picture 3 Range finding test set-up (by Zarrin Jafri)

3.2.2 Algae Turf Scrubber

In this test, that was the second part of the experiment, algae culture of the ten species was taking place again. Then transferred to the Algae Turf Scrubber (ATS) 1 and 2 as a replicate.
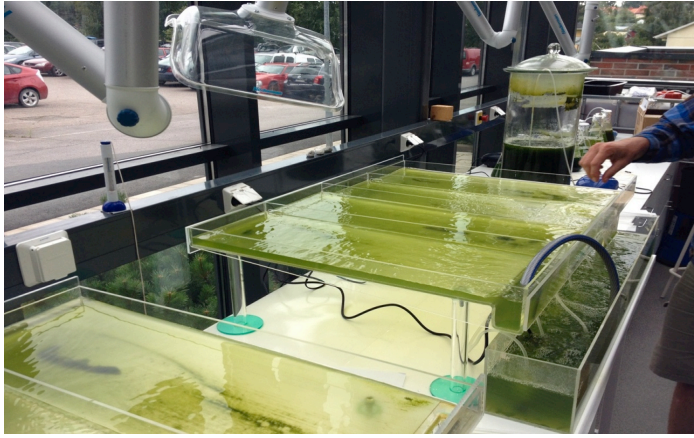
Picture 4 Both ATSs at the begining of the experiment (photo by Zarrin Jafri)

On a regular basis the two ATSs were fed nutrients till the point a thin film layer was formed. The nutrient solution here was prepared using phosphate, urea, glucose plus an organic nutrient solution. After mixing them in warm water it was added to the system.

After that when the volume of the fluid in both replicates reached 25 liters, 500 ml of adjusted mining wastewater was added; which was 4.8 of pH. This ratio and adjusted pH only were chosen because of the results generated on the first part of the experiment; which is presented in the results part.

The test run for three weeks or exactly 20 days while nutrient solutions were added three times a week and measurements were taken. Sensors monitored the pH and illuminance measurements each 10min and data was transferred to a computer registering it. However turbidity, phosphates and AAS were taken on a regular basis.

3.2.3 Range finding experiment for Copper

The copper is also amongst the heavy metal produced during the processing at Talvivaara. And the idea was to see if the most efficient species to absorb copper were combined would be an efficient way to treat the wastewater onsite right after the heavy metal release. In the prospect of being able to remove the heavy metal and recover it rather than discharging the whole metals in the same wastewater and then treated later.

Range finding experiment has been done on the ten algae species separately, however the contaminants used was rather standard solutions of Cu^{2+} with the following matrix:

Table 2 Amendments and algae species

	Algae Species (in each growing replicates was a total of 300ml)									
Cu^{2+} Amendments (ml)	1	2	3	4	5	6	7	8	9	10[8]
0										
5										
10										
15										
30										
60										

Each amendment had two replicates, and the procedure followed is the same one used in the range finding part of the experiment. This was in the purpose of testing the efficiency of the ten algae species singly to sequester copper.

3.3 Calculations

Range finding calculations were based upon the following equations:

$$C_m = \frac{m}{V} \qquad (1)$$

where C_m : mass concentration, m : mass of the contaminant, V: is volume of the sample.

Hence equation (1) was used alternatively to calculate the mass of the contaminant:

$$m = C_m . V \qquad (2)$$

For the percentage removal the following relative equation was used:

$$\%R = \frac{m_i - m_f}{m_i} . 100 \qquad (3)$$

where $\%R$: percentage removal, m_i initial mass of contaminant, m_f final mass of contaminant.

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[8] See Appendix 4 for the corresponding names

3.4 Correlation of pH with other variables

The pH during the ATS experiment was controlled by different factors. Heat and light which was measured in terms of lumens and represents the algal photosynthesis. Then the biochemistry of the site since adding the heavy metals altered the pH, and the fact of heavy metal removal during the experiment by algae affects the pH of the water since the chemistry of it changes and reduces in amounts. Turbidity here is encountered in the correlation since it reflects visually also the status of the water; meaning that clear water at the end of the experiment should be of a stable pH variation since chemical processes should be stable by then.

Moreover the data produced for each variable is of different patterns. Some of them are stable over period of time while others are not. Hence the time where most of the variables data is stable correlation between other variable could be observed then. Especially the correlation between the pH and lumens because then it reveals a chemical stability over the water, which means end of the detoxification process.

For this reason correlation calculation were done to produce each of the figures 24 and 30 were based upon the matrix below (Table 3):

Table 3 Correlation table

	Turbidity FAU	pH	Lumens µmol s-1 m-2	Zn (mg/L)	Ni (mg/L)
ATS 1					
ATS 2					

3.5 Methods of analysis

The pH and lumens were monitored by pH Lab Electrodes and a LiCor Quantum sensor that were connected to a controller then to a notebook were the data was stored in every 10 min time lapse.

Turbidity was measured diluting samples 10 times then reading by the Filter-photometer P-600 that gave turbidity values in FAU.

For measuring the Amount of Zn and Ni in the ATS, an atomic absorption spectroscopy analysis was done. First standard concentration solution for the heavy metal were prepared then the Atomic Absorption Spectrometer AAnalyst 400 was used for the analysis. Calibration of the instrument was done by the mean of the software and calibration curves were produced before the samples reading started.

Phosphates measurements were done using the HACH Lange LCK. The samples here were diluted by distilled water then digested after adding reagents provided by the manufacturer. After the values were obtained when the vials were introduced in the instruments, where a barcode help it define the chemical under investigation and the range of measuring it.

4   RESULSTS AND DISCUSSION

Heavy metal analysis in both parts focused on Zinc and Nickel which were of the main importance, since they are the metals of specialization for the company and hence the main problem for their wastewaters.

4.1 Range finding of mining water

This section includes the pH-adjusted results and the pH non-adjusted both for Zinc (Table 5) and Nickel (Table 4).

Table 4 Nickel results

V (mL)	Nickel					
	pH adjusted			pH non adjusted		
	Amount Ni added to treatment (mg)	Amount Ni remained after treatment (mg)	Removal %	Amount Ni added to treatment (mg)	Amount Ni remained After treatment (mg)	Removal %
1	1.01	1.70	-	1.07	2.42	-
2	2.02	1.77	13	2.13	2.50	-
5	5.06	2.01	60	5.33	2.79	48
10	10.1	18.24	-	10.65	25.99	-
15	15.17	19.77	-	15.98	26.61	-
30	30.33	22.25	27	31.95	28.74	10
60	60.66	24.50	60	63.9	31.02	51

The values presented in the 30 and the 60 ml range are not considered relevant because algae died at these high mining water concentrations and the metals precipitated as inorganic precipitates. The optimum removal is observed at 5 ml. We also got negative removal rates (increase in Ni concentration during experiment). These are not included in the Table 4, since we suspect air stones are the source of the extra Ni in our system (still needs to be confirmed).

Table 5 Zinc Results

V (mL)	Zinc(Zn)					
	pH-adjusted			pH non-adjusted		
	Amount Zn added to treatment (mg)	Amount Zn remained after treatment (mg)	Removal %	Amount Zn added to treatment(mg)	Amount Zn remained after treatment (mg)	Removal%
1	0.25	0.26	-	0.3	0.28	6.5
2	0.49	0.33	32	0.6	0.50	16
5	1.23	1.18	4	1.5	1.16	22
10	2.45	1.40	43	3	0.87	71
15	3.68	2.69	27	4.5	1.83	60
30	7.35	5.84	21	9	7.17	20
60	14.7	12.17	17	18	12.06	33

As from Table 5 it can be seen that Zn shows a maximum removal at the 10 ml range. However for Zn removal more biased results were obtained.

The highest concentration values are not representing one of the highest removal values since the samples looked dead few days after the transferring of the amendments, making it just a precipitation but not a stabilization of the algae to the metals. While the other high values are signs of best range since the samples didn't precipitated right after the addition of the amendment.

These values belonged to the Nickel part of the results that as well showed negative values, which is contradictory, yet this is suggested to be mainly due to the air stones used. Believing that they contain high amount of Nickel.

Considering both results for Nickel and Zinc the best amendment that knew the highest amount of removal are the 5, 10 and 15 ml.

Hence the best amendment that was chosen afterward was the 5ml since it shows best results for Zn and Ni. Making it a ratio of 1,666% ≈ 2% of pH adjusted mining wastewater amongst the cultivated algae.

4.2 Experiments with Algae Turf Scrubber (ATS)

The results displayed below (Figure 1 and Figure 2) are data produced during the experimentation of both replicates of the ATS.
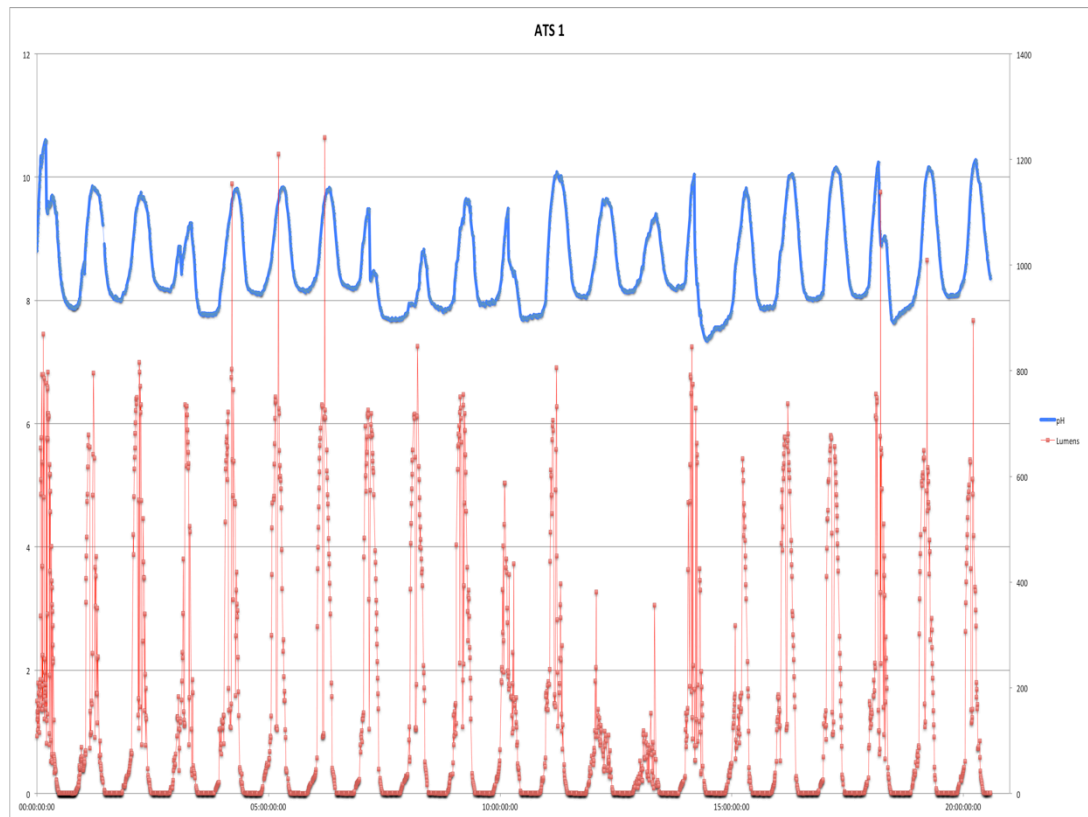
Figure 1 pH and Lumens (μmol s^{-1} m^{-2}) profile by time frame
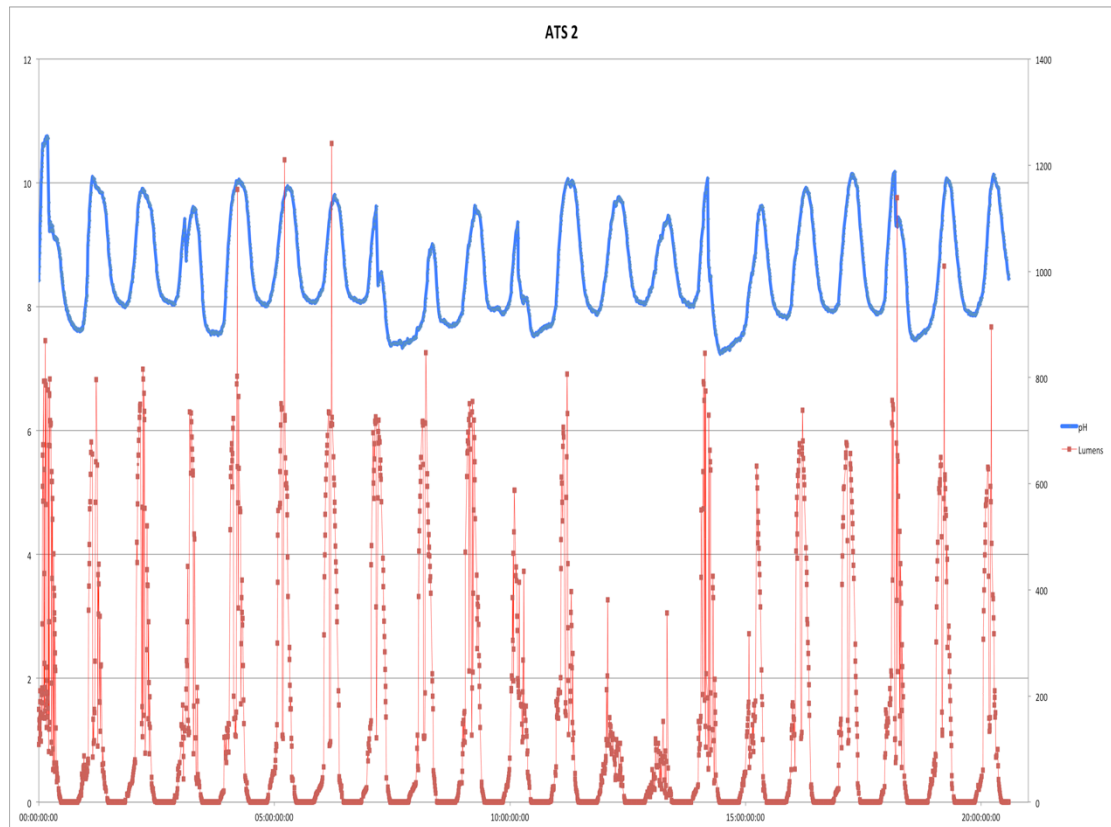
Figure 2 pH and Lumens (μmol s^{-1} m^{-2}) profile of ATS2 by time frame

The sensors connected to the computer pH and illumance produced data for 20 days in form of 10 min time frame. Both for ATS 1 and ATS 2 a dependency between illumance and pH is quite clear, for instance the difference of day/night is observed of effect on pH values.
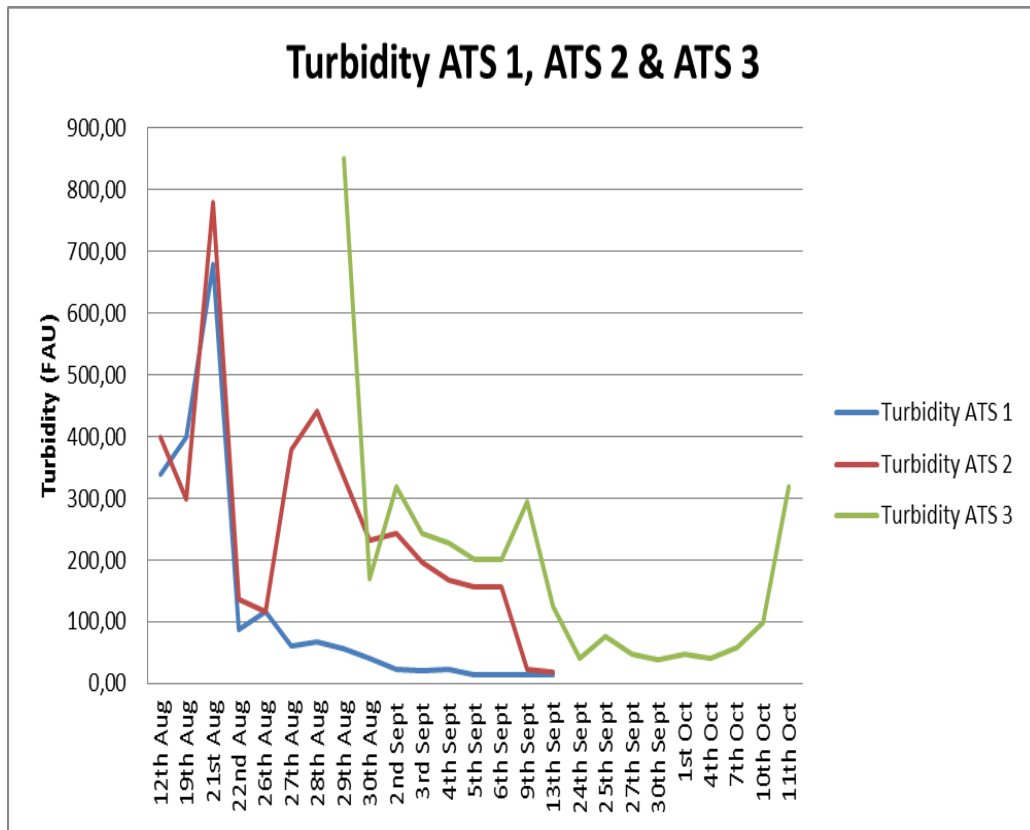
Figure 3 Turbidity profile with time frame

Turbidity of both ATS replicates (Figure 3) shows similar behavior at the beginning how ever a different pattern is seen at the end of the experiment.
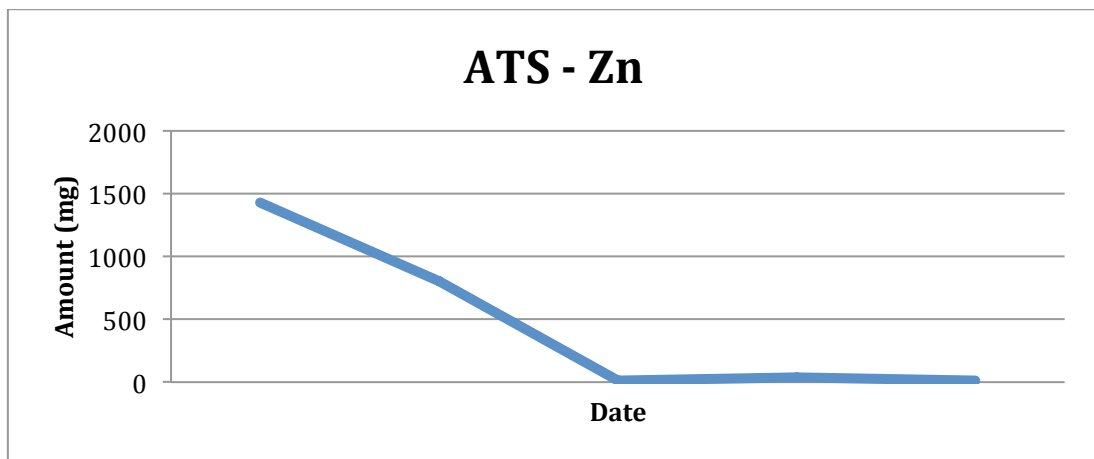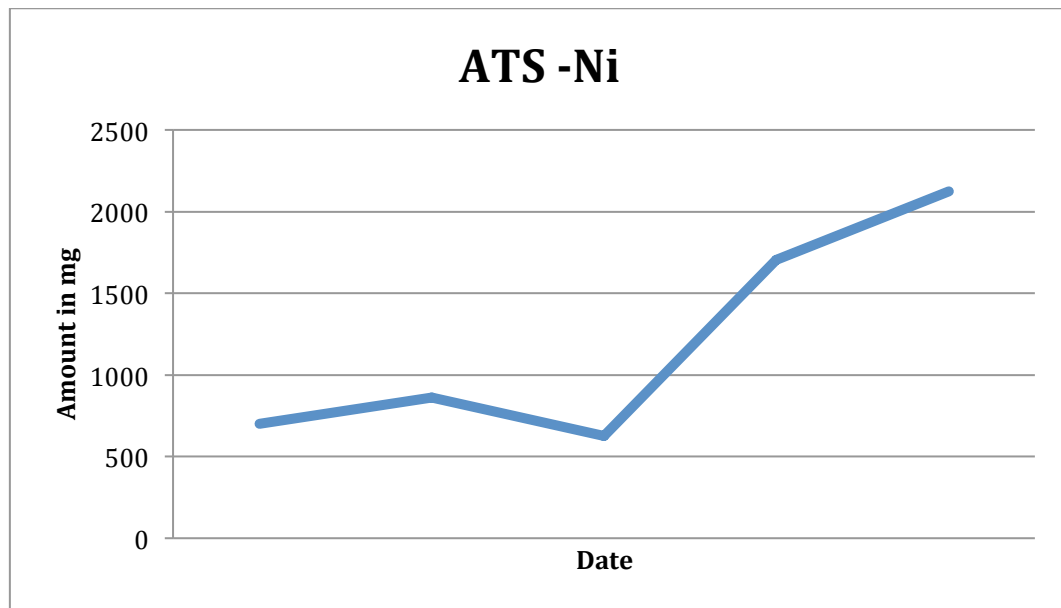

Figure 4 Amount of Zn average values in the ATS replicates during 20 days of the experiment

The graph above are presenting the concentrations measured by AAS, it includes samples from the beginning, mid and end of the experiment. For both replicates a similarity is observed.

Figure 5 Amount of Ni average values in the ATS replicates during 20 days of the experiment

Figure 5 reveals an increase in Ni concentration in ATS after 15 days of experiment this was not expected, but when it exceeded the original amount added then the air stones were suggested to be the reason of the Ni excess.

Another possible reason for Nickel overconcentration and especially not being reduced by ATS system is that the nature of the algae species. For instance as mentioned in section 2.2 some algae species have the natural tendency to store some metals to overcome future deficiencies, or make other microorganisms in their natural ecosystem to escape toxicity. Hence possibly the ten species used didn't have such natural behavior towards Ni, consequently this could in return explain the failure of Ni reduction in this experiment.
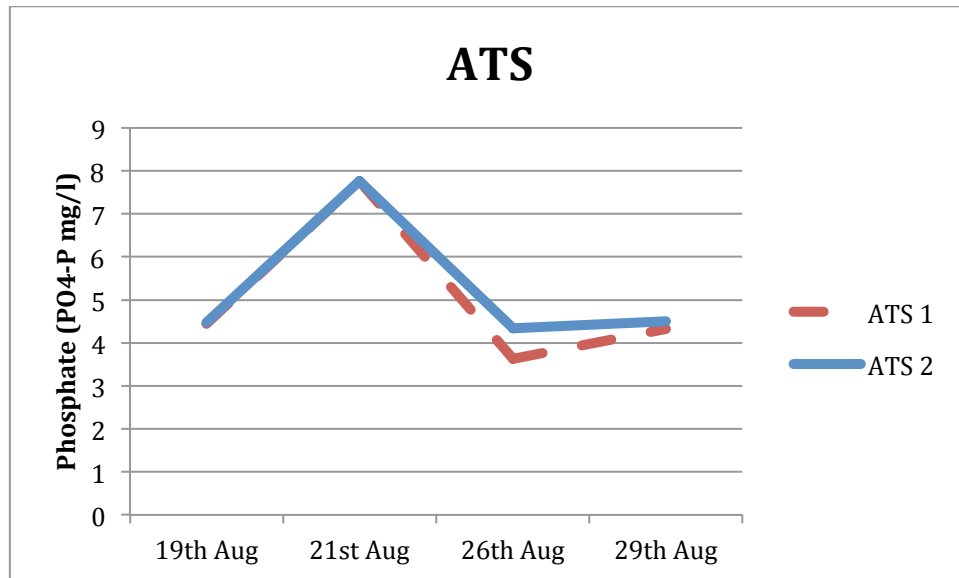
Figure 6 Phosphates levels by time frame

Phosphates level (Figure 6) during the experiment were the same for both replicates of the ATS since the replicates were kept in similar treatment and the same amount of nutrients were given on the same day to both replicates of the experiment.

The peak of the pattern might presents the fact that algae produces polyphosphates where the metals get bound, hence when the metal is trapped inside the algae with the polyphosphate then there are less polyphosphates in the water that is observed at the end of the graph. And as a matter of fact when the metal are stored inside the cell of an algae trough the Phytochelatins or Metallothionein, the Phosphates level should not be reduced so as not to induce back release of metals due to the phosphates deficiency in the environment (see section 2.5.). Phosphates level at the end of the experiment showed stable values that does not prove any phosphates deficit. Hence any metals sequestered inside algae cells were not back released.

Our results suggest that the metal then has been stored inside the cell because of the intracellular process. As the permeability of the cell membrane of the algae species used then has enabled any metal efflux out of the algae cells, which is seen on the Zn reduction of a long period towards the end of the experiment.

4.3 Correlation results

Since Nickel results for both replicates of the ATSs have shown controversies results; since the initial amount almost four folded at the end of the experiment; it was not encountered in the matrix.

While Zinc results in figures 4 shows stable variation of data from the 15th to the 20th day it was then taken into consideration in the results.

Considering turbidity as the graph in figure 3, ATS1 data is less to almost not varying from the 10th to the 20th day. However ATS2 is showing data stability from the 16th to the 20th day.

As result the range of days where the turbidity and Zn data both for ATS1 and ATS2 is stable is from the 16th to the 20th day, consequently:

Table 6 Correlation table

	Turbidity	pH	Lumens	Zn	Ni
ATS 1	16-20	*	*	16-20	-
ATS 2	16-20	*	*	16-20	-

As consequence the graphs presented in figures 1 and 2 were cropped or focused on from the 16th to the 20th day producing graphs in figure 7 and 8.

Using the data above and correlative calculations, the following graph was produced showing the pH and illumance variation from the 16th to the 20th day (Figure 7 and Figure 8):
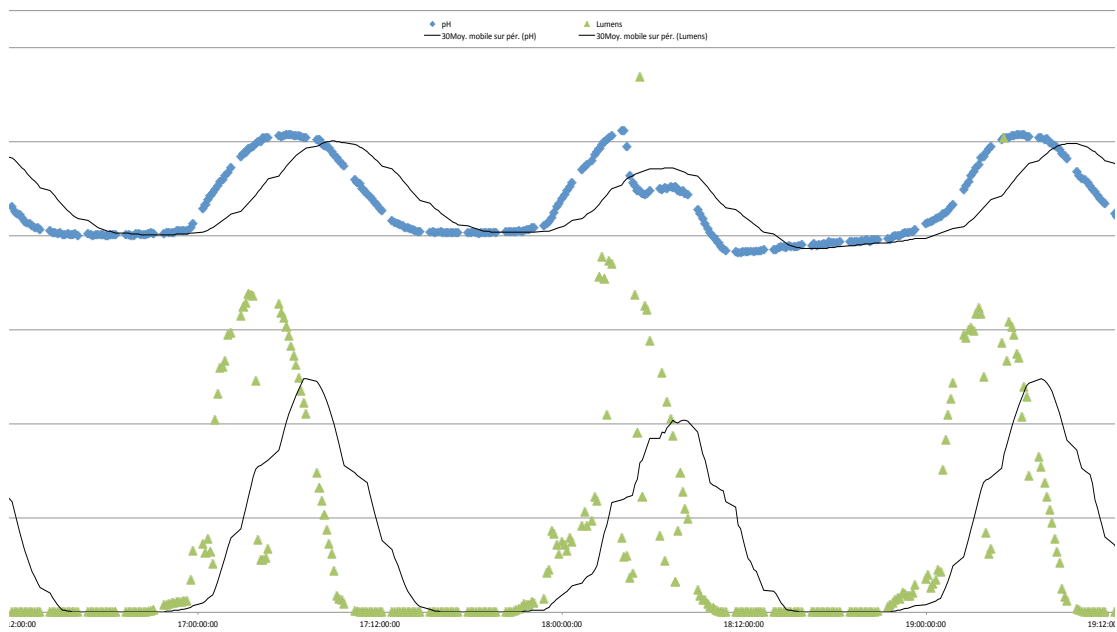
Figure 7 pH and illumance variation of ATS1 from the 16th to the 20th day
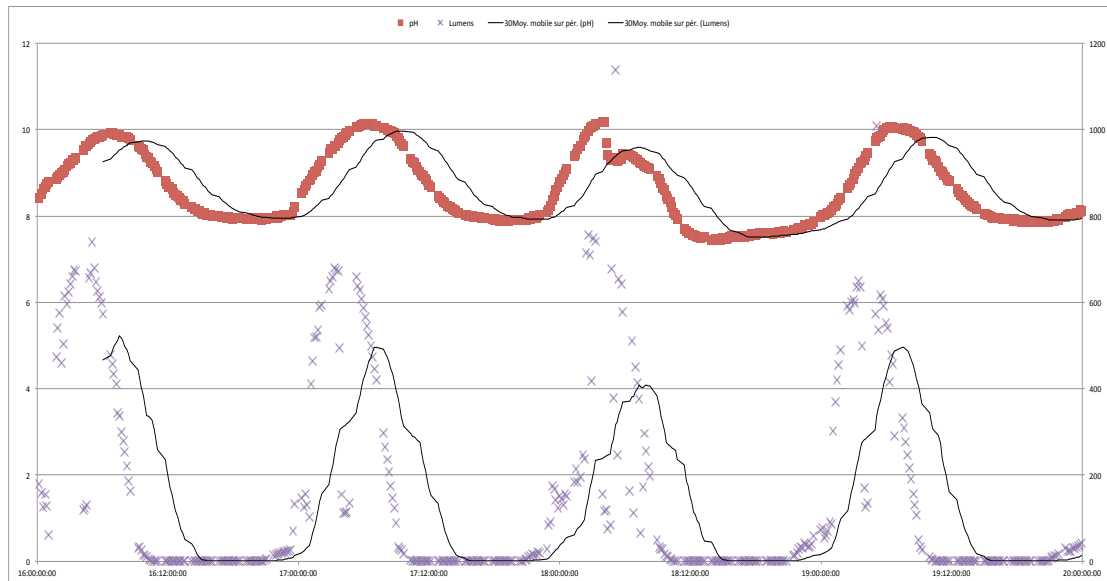
Figure 8 pH and illumance variation of ATS2 from the 16th to the 20th day

The thin black line in both graphs represents the mean average between the points, so as to get rid statistically from outliers due to sensor errors and mainly to see the behavior of the one representing the pH and its relation with the one representing the illumance. pH correlates with illuminance positively, meaning that stronger illuminance is associated with the increase of pH in the water. This is because of the photosynthesis by the algae in the presence of the light that uses the protons and CO_2 from the water causing pH to increase. The Pearson correlation coefficient obtained for ATS 1 is 0,64 and ATS 2 is 0,60 that indicates a rather strong positive correlation.

Observing graphs produced in each results part; figure 7 and 8; where each peak represents one day. The last three days then shows that when the peak of the lumens is higher the peak of the pH is higher as well. This means that when the day has been warmer or sunnier the pH has risen. And vice versa. Meaning that the pH at this point is not showing a dependency to other variables, which tells to some extent biological processes are not happening. That theoretically will alter the chemistry of the water, and in return it would be seen on the pH variation profile as well.

4.4 Results of Cu experiment

The results obtained were as follows and accordingly to the species.

Selenastrum Capriconutum

Table 6 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	0.38	-
16.7	5.93	64.50
33.4	14.36	57.01
50	**6.26**	**87.49**
100	-	-
200	-	-

Pediastrum Simplex

Table 7 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	0.80	-
16.7	3.35	79.93
33.4	**2.03**	**93.91**
50	21.85	56.31
100	-	-
200	-	-

Anabaena cylinderical

Table 8 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	0.81	-
16.7	**3.75**	**77.55**
33.4	14.55	56.43
50	20.78	58.44
100	-	-
200	-	-

Fragalaria crotoneis

Table 9 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	-	-
16.7	4.03	75.85
33.4	**4.56**	**86.35**
50	18.66	62.67
100	-	-
200	-	-

Scenedesmus sp

Table 10 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	0.45	-
16.7	8.24	50.67
33.4	**6.28**	**81.20**
50	10.90	78.20
100	-	-
200	-	-

Navicula pelliculosa

Table 11 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	0.52	-
16.7	4.23	74.66
33.4	**5.73**	**82.45**
50	10.06	79.87
100	125.13	-25.13
200	135.05	32.48

Haematococcus pluvialis

Table 12 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	1.30	-
16.7	7.04	57.82
33.4	5.76	82.75
50	**6.43**	**87.12**
100	120.45	-20.45
200	133.27	33.36

Synechoccocus sp

Table 13 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	1.07	-
16.7	8.25	50.55
33.4	**9.92**	**70.28**
50	15.40	69.18
100	116.17	-16.17
200	133.72	33.13

Chlorophyta sp

Table 14 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	1.45	-
16.7	12.53	24.93
33.4	18.56	44.41
50	**17.34**	**65.32**
100	106.22	-6.22
200	135.37	32.31

Purpuraemus

Table 15 Copper concentration and percentage removal

Initial Concentration (mg/l)	Final Average concentration (mg/l)	Percentage removal (%)
0	1.32	-
16.7	**4.85**	**70.90**
33.4	10.08	69.79
50	21.60	56.78
100	121.35	-21.35
200	129.85	35.07

The values displayed in bold are the optimum absorption, and as it shows different species has shown tolerance in different concentrations. Hence combining theses most efficient species at highest concentration in an ATS like medium scale system then the water will be decontaminated from the copper right after its release (see figure 11) and making it possible also for recovery. Moreover the idea could be applied for other heavy metal at different concentration or with the same metal including more species in the experiment.

5   CONCLUSIONS

The ten algae species stabilized the Zinc within 16 days with a removal rate of 99% using the ATS. The ratio used was of 2% adjusted mining wastewater to 4.5 pH. However the expected Nickel removal didn't happen probably because to the air stones used for aeration that suspectedly leached Ni. Algae tolerance to Copper showed high removal rates at varied concentrations tested.

More importantly the water at the end of the experiment was clear as the turbidity graphs show, and the amount of Zn was significantly reduced.

Based on our results we can conclude that only factor affecting the pH at the ending of the experiment is the lumens (or temperature), in other words the system used was successful in reducing the Zn content of the process mining wastewater.

Yet Ni results were not encountered in the analysis because of the absurd nature of its results; amount measured at the end is almost four times more than the initial amount.

The design of the algae turf scrubber used in this experiment lacked a process of scrubbing the mat of algae formed at the end, which makes it hard to make further conclusion about such system. The importance of it comes when the scrubbing has to be done manually leaving chances for metal back release due to mechanical forces. However in case when such systems involve the process of draining the water purified first before scrubbing the algae layer formed, then possible metal back-release is not presented.

On the other hand the choice of the algae species which mostly belong to cyanobacteria was perfect for such systems since these species are know of good mat-forming properties (Graham and Wilcox, 2000 p76). Consequently increasing the surface area where contaminated water can flow in reducing a theoretical retention time for the biological process.

Suggestion for Talvivaara

From another perspective studies on algae towards heavy metals should be done, as the writers Rai and Gaur states: " There is a pressing need to assess the binding by algal exudates of other metals also" (Rai and Gaur, 2001, p368). Other statements like these are still present in the same book. As a consequence more data about algae species and metal tolerance should be produced.

For this reason and as I see it if the same system was used, yet with each species used separately then better results could be generated. However the process mining waste water used the should not be the one from the settling ponds or the one coming after the

end of the whole processes carrying different heavy metals. It should rather come from the end of each process that generates a specific heavy metal waste; the process plant shows in appendix 3. Consequently applying this idea the treatment of the wastewater would rather take place within the processing, as the following figure 9 shows:
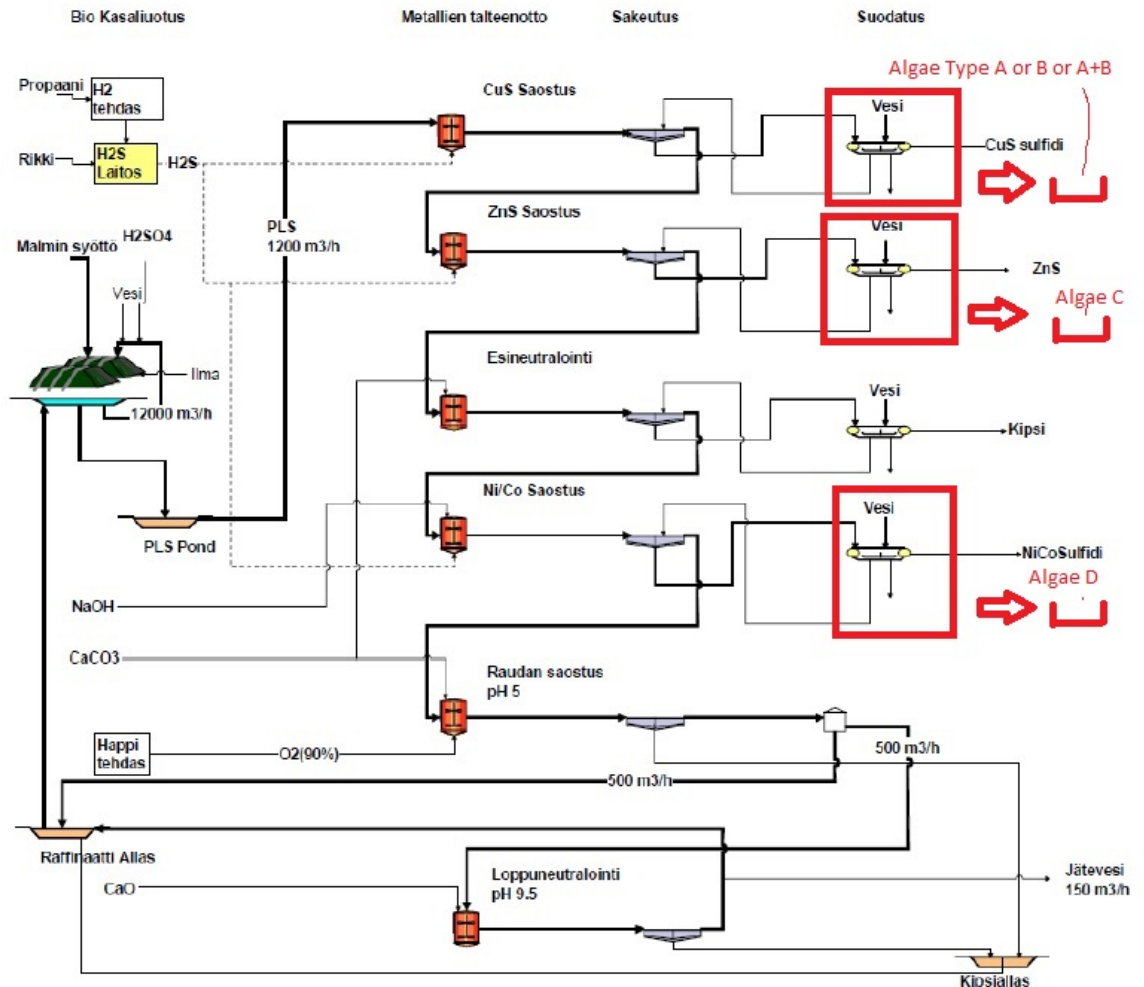
Figure 9 Theoretical modification of Talvivaara process plant

Further more with a proper knowledge of algae species tolerance toward specific heavy metals the experiment with ATS could be done again based on the above-mentioned idea. Especially that the results for the range finding experiment of Cu on the ten species prove a substantial evidence.

Scope of such idea would be then possible to recover each heavy metal separately with a proper extraction method, and possibly generating maximized heavy metal removal from mining wastewaters for instance.

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APPENDICES

Appendix 1. TAMK water samples analysis 27.02.2013

Metallianalyysit Liuos

Näytepaikka	pH	Johtokyky µS/cm	Al mg/l	As mg/l	Ca mg/l	Cd mg/l	Co mg/l	Cr mg/l	Cu mg/l	Fe mg/l	Mg mg/l	Mn mg/l	Na mg/l	Ni mg/l	Si mg/l	Zn mg/l	U mg/l	Tekijä	Raportille
TAMK3 AVOLOUHOS	3	17630	175	0.54	318	0.26		1.38	<0.0121	1380	1736	1318	824	64.6	13	121	0.77	SNI	BVH
TAMK1 AVOLOUHOS	3.9	18100	207	0.6	378	0.3		1.63	<0.0121	1595	2045	1559	952	77.7	15.4	143	0.82	SNI	BVH

Appendix 2. Limit values of Tampere's water

TAMPEREEN VEDEN VASTAANOTTAMAN JÄTEVEDEN RAJA-ARVOT

Metallit			
Arseeni	As	0,1	mg/l
Elohopea	Hg	0,01	mg/l
Hopea	Ag	0,2	mg/l
Kadmium	Cd	0,01	mg/l
Kromi, kokonais	Cr	1,0	mg/l
Kromi, kuudenarvoinen VI	Cr^{6+}	0,1	mg/l
Kupari	Cu	2,0	mg/l
Lyijy	Pb	0,5	mg/l
Nikkeli	Ni	0,5	mg/l
Sinkki	Zn	3,0	mg/l
Tina	Sn	2,0	mg/l

Appendix 3. Metals Recovery Process at Talvivaara Plant
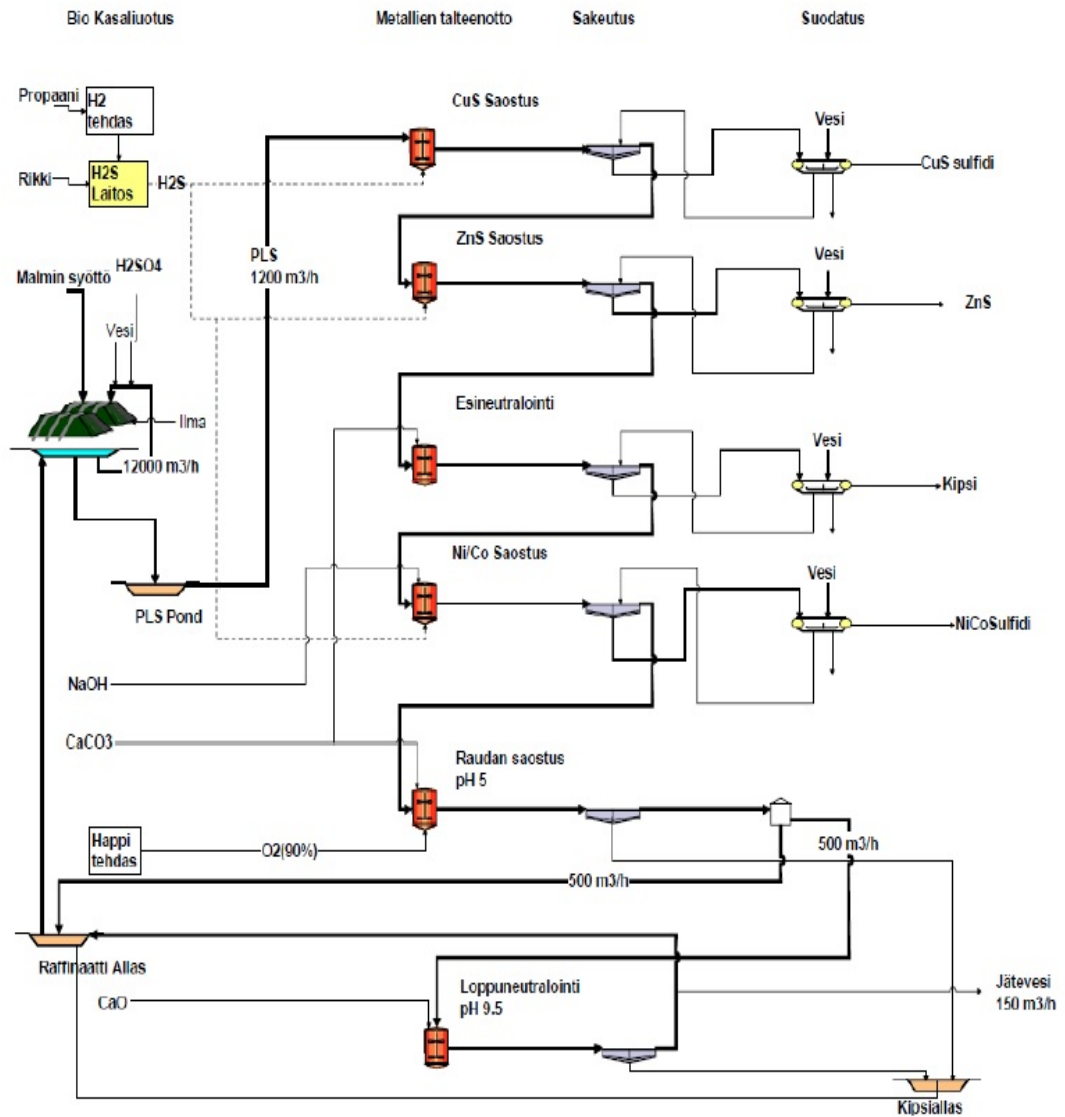
Appendix 4. Algae Species

Species Numbers	Species Name
1	Selenastrum Capriconutum
2	Pediastrum Simplex
3	Anabaena cylinderical
4	Fragalaria crotoneis
5	Scenedesmus sp
6	Navicula pelliculosa
7	Haematococcus pluvialis
8	Synechoccocus sp
9	Chlorophyta sp
10	Purpuraemus[9]

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[9] No information about it was found.