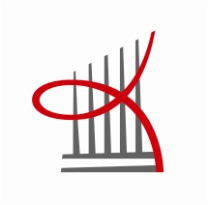

Freshwater Algae: Essential Nutrients and Factors

Zarrin Fatima

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Environmental Engineering

TAMPEREEN AMMATTIKORKEAKOULU
Tampere University of Applied Sciences

ABSTRACT

Tampereen ammattikorkeakoulu
Tampere University of Applied Sciences
Environmental Engineering
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Zarrin Fatima
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Algal growth in an acidic environment was analyzed using Algae Turf Scrubbers (ATS). The main objective was to assess and understand the role of nutrients and other environmental factors including temperature, pH and turbidity necessary for algal growth. The acidic environment was achieved by the addition of heavy metal contaminated processed wastewater to the ATS and monitoring the growth pattern over a course of 6 weeks. A total of 10 freshwater species were first cultivated separately and later on added to the ATS systems. The results demonstrated that only a few freshwater species survived namely *Anabaena, Synechococcus, Fragalaria* and *Scenesdesmus.* It was observed that a low Redfield Ratio, suitable temperature and low turbidity played a crucial role in the significant increase in cyanobacteria population. The ATS simulated the natural freshwater environment and helped get valuable insight regarding algae and heavy metals.

Key words: algae turf scrubber (ATS); redfield ratio; pH; temperature; turbidity; freshwater algae species; heavy metal; wastewater

CONTENTS

1 INTRODUCTION

Algae are found in a range of habitats on Earth, including ’freshwater, oceans, moist soils, rice paddies, natural hot springs, stone or concrete surfaces, snow, and deserts’ [Harrison, Griffiths, Langley, Vengadajellum & Hille, 2004:57]. The fact that they are found living in all climatic zones, that is, areas stretching from the tropics to the arctic and the antarctic demonstrates their diversity in form and irrelavance of the need of a perfect habitat, and at the same time makes them difficult to define in a simple manner [Harrison et al, 2004:57].

The importance of algae in water arises not only because of their presence, but also due to the various effects that can they can have in the aquatic ecosystem, such as odors and production of toxins. However, algae also have a postive influence in the aquatic environment because they produce oxygen as a result of their metabolism, and are also the primary producers in the food chain, supplying a source of food for the zooplankton and the small fish [Harrison et al, 2004:58]. Additionally, algae also serve as indicators of the aquatic environment. Algae react rapidly to a wide range of pollutants, and hence are able to provide signals of a changing environment [Mosleh, Manssor, Malek, Milow & Salleh, 2012:1].

Algae have the capability to remove a number of minerals from the water, including ‘phosphorous, ammonium, calcium, magnesium, and certain heavy metals’. Their removal ensures water quality in the lakes and rivers. Nevertheless, certain blue-green algae are also able to take up atmospheric nitrogen and supply it to other organisms [Harrison et al, 2004:59].

Increasing population and urban expansion will result in increasing demands and eventually an increase in the amount of waste generated. Heavy metal pollution of aquatic environments occurs due to the ‘disposal of industrial and domestic wastes’ and causes a threat life to the aquatic organisms. Therefore, it is extremely important that wastewaters are treated well before they are discharged into the waterbodies. Previous methods, such as chemical precipitation or adsorption have proven ineffective due to a number of reasons, and biological treatment of metal enriched waters is inexpensive and efficient. Bioaccumalation of metals by microorganisms, in this case algae, has been known for decades, but began to be explored further only in the past few years. Algae

are an excellent alternative to physiochemical methods to remove heavy metals [Mehta & Gaur, 2005:2].

2 Overview on algae

The word ‘algae’ is said to have Latin origins, and means ‘seaweed’ [Bellinger & Sigee, 2010:1]. The term describes a plethora of prokaryotic and eukaryotic organisms with varying morphologies and phylogenies. Harrison characterizes microalgae as ‘small, photosynthetic, heterotrophic, or occasionally, phagotrophic, and unicellular or colonial aquatic plants’. Macroalgae, on the other hand, are larger in size, multicellular, and eukaryotic [Harrison et al, 2004: 577]

In *Freshwater Algae – Identification and Use as Bioindicators*, algae are described as autotrophic organisms that allows them to obtain nutrients from inorganic sources, and are also photosynthetic allowing them to utilize light energy and carbon dioxide to produce complex compounds. Some algae are hetrotrophic which enables them to obtain essential complex molecules [Bellinger & Sigee, 2010:1] either by ingesting particles through a process called phagotrophy or the organisms are able to take up organic molecules via a process called osmotrophy [Graham & Wilcox, 2000:11].

Nevertheless, several algae demonstrate a mixed mode of obtaining nutrition, that is, they are able to photosynthesize and are also able to utilize osmotrophy and phagotrophy to obtain nutrients, hence are able to utilize both inorganic and organic carbon. Such a process is known as mixotrophy [Graham & Sigee, 2012:12].

An article by Michael D. Guiry suggests that algae are present in not just a few numbers, but it has been estimated that they may include anything beginning from 30,000 to 1 million species. Research indicates uncertainties regarding what organisms can be defined as algae and what exactly a species is with regard to algal phyla and classes. Despite the existing uncertainties, results give a value of 72,500 algal species, 44,000 of which have been published with names, and 33,248 names have been included in the Algaebase. [Guiry, 2012: 1057]

Lakes, ponds and streams comprise of similar planktonic and benthic microalgae, however it is not surprising to find specific freshwater algae to form colonies. Freshwater phytoplankton are the base of the aquatic food chains without the existence of which the freshwater fisheries will not be able to survive. [Graham & Wilcox, 2000:6]

Freshwater algae species are excellent indicators of the aquatic habitats because they are sensitive to the environmental conditions, and hence provide an early indication of changing environmental conditions such as the trophic status or nutrients level [Mosleh et al, 2012]. Algal populations belonging to Heterokontophyta and Chlorophyta divisions (see page 10-11) especially desmids - for instance, *Scenesdesmus* - are extremely sensitive to changes occurring in the aquatic environment, thus they are considered good bio-indicators. Nevertheless, certain species of algae produce harmful toxins that have an unpleasant taste and odor. In eutrophic lakes, green algae are the most abundant. Cyanobacteria pose a global problem because of toxicity and it usually occurs in eutrophic lakes. It has been found that 75% of lake water samples contain toxin producing cyanobacteria, which is why monitoring the cyanobacteria concentration has been included as 'a factor of risk assessment plans and safety level' for example by World Health Organization (WHO). [Mosleh et al, 2012:2]

2.1 Size and Shape

From cells that measure one micro-meter in diameter to giant kelps that stretch as long as fifty meters, algal species go unnoticed most of the time unless certain environmental conditions lead to a proliferation of their population, mainly because of the human activities (Graham & Wilcox, 2000:1).

If algae is present in a planktonic environment, the size range can be small prokaryotic unicells having a diameter of less than 1 micrometer to a large colony of blue-green algae where a cell can have a diameter of 2000 micrometer and is seen with the naked eye. In a benthic environment, the size range is bigger and algae exist as small unicells occupying freshly exposed exteriors or the algae is filamentous, for example, filaments of *Cladaphora* extend several centimeters. Moreover, these macroscopic algae often have small epiphytic colonial algae and unicells. [Bellinger & Sigee, 2010:4]

Algal cells range from unsophisticated single non-motile circular structures to complex multi- celled structures. FIG 1 shows An example of the simplest structure *Chlorella*. The simple sphere can change its shape by developing flagella (c), change the body shape (a) or develop elongate spines (d). The figure is an extract from *Freshwater Algae – Identification and Use as Bioindicators* by Bellinger & Sigee.

Motility is usually achieved with the aid of flagella, but some algae are able to move by secretion of surface mucilage which is a slimy organic material. Mucilage also influences the size and shape of the colony. While size and shape are significant factors for identification and classifying of algal species, they are equally vital for processes and features such as exchange of gases, adsorption of light, rate of growth and cell division, motility, and grazing by zooplankton. [Bellinger & Sigee, 2010:5]

An interesting question arises when thinking why algae need the ability to move or swim. As suggested, algae need to exchange molecules such as oxygen, carbon dioxide and ammonia with the environment.

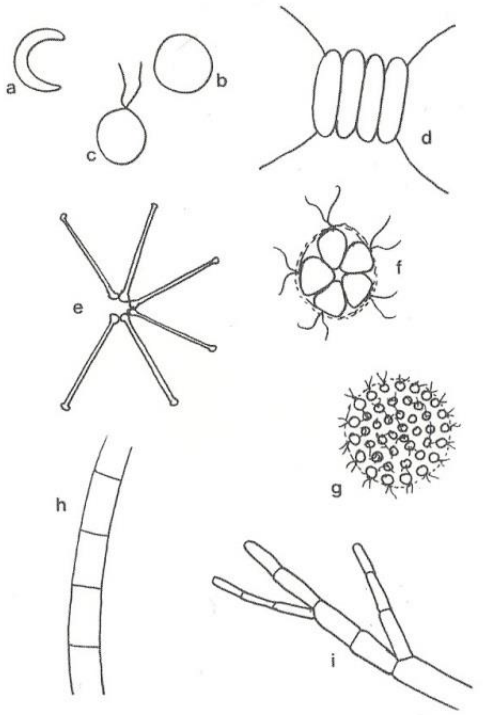

Figure 1.2 General shapes of algae. Non-motile unicells: (a) *Selenastrum*; (b) *Chlorella*. Motile unicell: (c) *Chlamydomonas*. Non-motile colony: (d) *Scenedesmus* (e) *Asterionella*. Motile colony: (f) *Pandorina*; (g) *Volvox*. Unbranched filament (h) *Spirogyra*. Branched filament (i) *Cladophora*. Reproduced, with permission, from Bellinger (1992).

FIGURE 1: General shapes of *Chlorella*

Source: [Bellinger & Sigee, 2010]

However, Bellinger explains the concept of no-slip boundary condition between a solid and liquid medium that creates a boundary where the velocity of the water is reduced and hence the nutrient availability. Therefore, algae use their structural features to generate movement relative to the water and overcome the deficiency in nutrient supply. [Barsanti, 2006:88-89]

2.2 Algal Divisions

Algae is grouped into 10 phyla (divisions) according to microscopic appearance, biochemistry (study of processes) and cytology (study of cells) [Graham & Wilcox, 2010:5, Barsanti & Sigee, 2006:4]

- Cyanophyta
- Prochlorophyta
- Glaucophyta
- Rhodophyta
- Heterokontophyta
- Haptophyta
- Cryptophyta
- Chloroarachniophyta
- Euglenophyta
- Chlorophyta

FIG 2 on the following page is an extract from 'Microalgal Culture as a Feedstock for Bioenergy, Chemicals, and Nutrition' published in *Manual of Industrial Microbiology and Biotechnology*. It describes some microalgal types including their morphology, dominant storage product, examples of known species and applications [Harrison et al, 2004: 578-579]. TABLE 1 on page 11 highlights the names of the freshwater species cultivated for use in the project.

Group (common name)	Description	Morphology	Dominant storage product	Well-known organisms	Applications[a]
Chlorophyta (green algae)	Diverse group. Origin of higher plants. Usually photoautotrophic but can be heterotrophic.	Large range of cellular structures—unicellular flagellates to complex multicellular arrangements. Cell walls contain cellulose.	Starch (α-1,4-glucan)	*Chlamydomonas*, *Chlorella*, *Scenedesmus*, *Spirogyra*, *Volvox*	*Chlorella* is commercially grown as nutraceutical and aquaculture feed. *Haematococcus pluvialis* is used to produce astaxanthin. *Dunaliella salina* is grown for β-carotene.
Cyanobacteria (blue-green algae)	Nonmotile, gram-negative, prokaryotic eubacteria. Most widely distributed algal group. Dominate particularly in oceans. Important component of picoplankton.	Can be unicellular, filamentous, or colonial.	Starch and cyanophycin (arginine and asparagine polymer)	*Spirulina* (*Arthrospira*), *Anabaena*, *Oscillatoria*	*Spirulina* biomass sold commercially to health food and nutraceutical market.
Dinophyta (dinoflagellates)	Important components of microplankton. Nutritionally diverse. Half the known species are obligate heterotrophs.	Typically unicellular flagellates. Have armor-like cell covering beneath cell membrane.	Starch (α-1,4-glucan)	*Gymnodinium*, *Crypthecodinium cohnii*	Known for blooms and toxin production; many exhibit bioluminescence. Responsible for red tides. Some species produce DHA.
Euglenophyta (euglenas)	Occur in freshwater, brackish water, and marine environments, mostly in soils and mud, especially in highly heterotrophic environments. Obligate mixotrophic, as require B vitamins. Colorless. Species are phagotrophic. More closely related to trypanosomes than any other algal group.	Unicellular or colonial flagellates.	Paramylon (β-1,3-glucan)	*Euglena gracilis*	Unique cellular and biochemical features; may have pharmaceutical applications.
Haptophyta (also known as Prymnesiophyta)	Generally marine. Mostly photosynthetic but can be heterotrophic or phagotrophic.	Largely motile unicells. The best-known haptophytes are coccolithophores, which have an exoskeleton of calcareous plates called coccoliths.	Chrysolaminaran (β-1,3-glucan)	*Pavlova lutheri*, *Isochrysis galbana*, *Prymnesium parvum*	Several species grown as food for fish and other aquaculture organisms such as bivalves and abalone.

Heterokontophyta (includes brown algae, golden algae, and diatoms)	Large group containing the *Chrysophyceae*, *Xanthophyceae*, *Eustigmatophyceae*, and *Bacillariophyceae* (diatoms). Largely marine, but some freshwater varieties.	Cells with two different flagella (as opposed to isokont—two the same). *Bacillariophyceae* are unicellular, brown cells with a silica cell wall (unique type of casing made of two frustules that fit together like a lid on a box).	Chrysolaminaran (β-1,3-glucan)	*Amphora*, *Nitzschia*, *Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, *Nannochloropsis*	Several species grown as aquaculture feed; others known for EPA production.
Rhodophyta (red algae)	One of oldest and largest groups of algae. The accessory pigments phycobiliproteins give them their red color.	Free-living, unicellular.	Starch (α-1,4-glucan)	*Porphyridium purpureum*	Some species secrete calcium carbonate—could be used in CO_2 sequestration. Most economically important macroalgae are from this family, e.g., dulse (*Palmaria palmata*), nori (*Porphyra*), and species used to make agar, carrageenans, and other food additives.

FIGURE 2: Characteristics of some algal groups relevant to microalgal biotechnology
Source: [Harrison et al, 2004: 578-579]

2.3 Species provided by SYKE for this project

TABLE 1: Names of freshwater algae species

Species Name	**Group Name**
Selenastrum	Chlorophyta
Pediastrum simplex	Chlorophyta
Anabaena cylinderical	Cyanobacteria
Fragalaria crotoneis	Heterokontophyta
Scenedesmus sp.	Chlorophyta
Navicula pelliculosa	Heterokontophyta
Haematococcus pluvialis	Chlorophyta
Synechococcus sp.	Cyanobacteria
Chlorophyta sp. (Pekari strain)	No information
Purpuraemus	No information

3 Aim of the work

The gradual increase in population and production rates has increased water use, creating a corresponding escalation in the quantity of wastewater generated. The existing technologically advanced methods demand a large capital investment, continuous maintenance and have proven to be in-efficient. This requires for treatment methods that actively remove the by-product and pollutants from the water so that the effluent can be discharged safely while meeting the environmental regulation policies. [Kiepper, 2013]

The project work utilized the concept of Algae Turf Scrubbers (ATS). It is based on the idea of a low-energy input system with microalgae and the natural process of photosynthesis [Kiepper, 2013]. The purpose was to observe how freshwater algae species grow in a controlled and heavy-metal polluted environment/system and how nutrients are consumed. The processed mining water samples were provided by Talvivaara described in the following pages.

At the beginning of the project, the following questions were of interest:

1. The types of algae species – which ones survive better in mine water?
2. What is the maximum concentration of heavy metals that the algae can survive in?
3. How does each metal affect the algae?
4. How much can the algae adsorb?
5. What factors are important for algal growth?

4 Algae Turf Scrubbers

An article written by Walter H. Adey claims that the surface waters in the United States have become eutrophic, and certain regions have very low oxygen levels. The Algae Turf Scrubber (ATS) is a system engineered to allow wastewater to flow over a sloping surface and where filamentous algal species are allowed to grow simultaneously. Walter H. Adey also mentions that the system has been tested for treatment of farm waste, streams, and aquaculture systems, and in certain situations the system has been able to treat 40 -80 million liters per day. The algal biomass produced is a lot higher than the mass obtained from other algae producing sources. The biomass can be harvested and fermented to produce ethanol, butanol and methane. ATS also provides a cost effective solution for production of biofuels. [Adey, 2011:1]

The original ATS was designed to mimic the wild ecosystems of coral reefs that are low in nutrients and have limited exposure to light, but demonstrate high levels of primary production. The design was later modified during the 1980s to control water quality with high nutrient content and greater light intensity, and the system successfully removed nitrogen, phosphorous and biological oxygen demand. The article written by Walter H.Adey discusses an interesting idea that due to the fact that the freshwater benthic algae behave in a similar manner as to the marine algal system, freshwater Algae Turf Scrubbers were also designed. Over the years, the turf scrubber design has come far from being implemented in small units to areas as large as 3 hectares handling 150 million liters of waste water per day. [Adey, 2011:2]

FIG 3 on the following page shows setup of the Algae Turf Scrubber system in the laboratory. It consists of the algal community growing in shallow troughs or basins (also referred to as raceways) and through which water is pumped. For our project work, the algal community comprised of 10 various species mentioned on page 11. The algae take up the inorganic compounds and produces oxygen through photosynthesis. The water flows down the raceways and the algae is able to remove the nutrients in the water. In this project, the system had four raceways and the water circulated through them all, and at the end it was collected in the storage tank and pumped back to the raceways. The water entering the storage tank has a low nutrient concentration because they have been ‘scrubbed’ from the water and stored within the algae cells. Important parameters for good results include maintaining the flow rate of the wastewater/water, the inclination

of the raceways, and the frequency of adding of nutrient rich water to the systems [Adey, 2011: 2]. Maintaining the correct pH, temperature and ample amount of sunlight also affects the productivity of the systems.

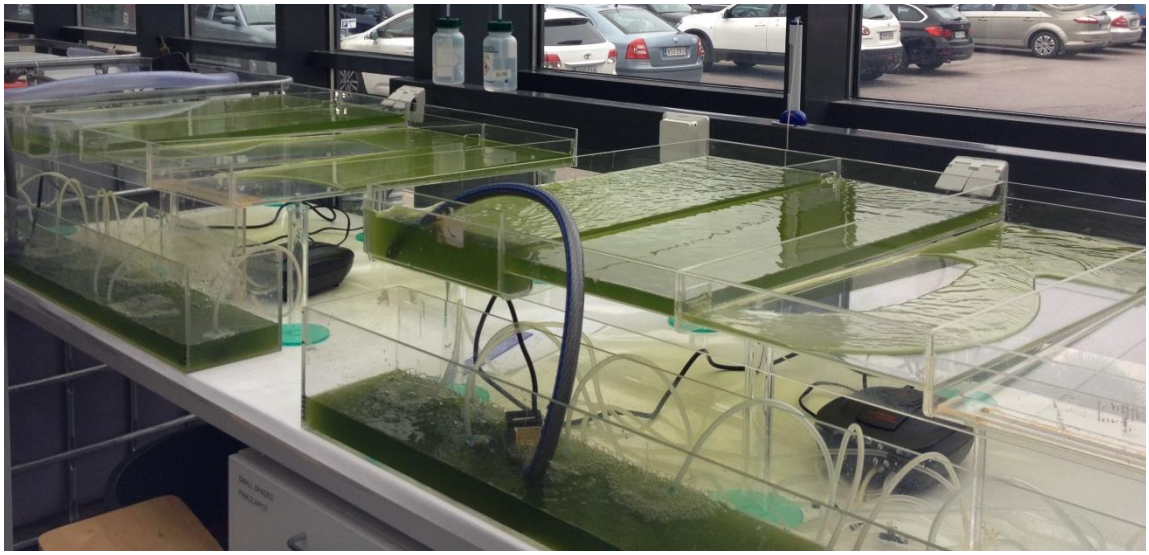

FIGURE 3: Setting up of ATS 1 (left) and ATS 2 (right). Taken on 08.08.2013

The water flows through the turf scrubber in a wave like motion which enhances the rate of uptake of nutrients [Grobler, 2013: 11]. In this case, the first two turf scrubbers contained mining water and the wave motion affected the rate of removal of pollutants from the water. The system used during the project was designed by G. Grobler and consisted of four raceways. One raceway was 700mm x 1000mm x 50mm high wall made of acrylic that was 5mm thick. The raceways were connected to each other at an angle of one degree. The flow rate was 4,7L/min. The water starts from the raceway on the left and gradually made its way to the fourth raceway, eventually collected in the storage tank and ready to be circulated again. The storage tank had a capacity of 30L and is covered with aluminum foil or mesh to prevent too much evaporation and splashing by the air pumps [Grobler, 2013: 16].

5 Talvivaara Mining Company Plc

Talvivaara is an internationally recognized metal mining company with a focus on nickel and zinc. Other mineral resources include copper, cobalt and uranium. The largest site is located in Sotkamo in eastern Finland. Talvivaara's polymetallic deposits are claimed to contain the largest amount of sulphide nickel resources in Europe. Talvivaara utilizes the process of bioheapleaching to extract the metals where metals are recovered or leached from the ore as a result of bacterial activity. The first metal production was during the year 2008. [Talvivaara, 2009]

Talvivaara's production process consists of 4 stages: large scale open pit mining, materials handling where crushing takes places, bioheapleaching, and metals recovery respectively. After the ore is recovered, it is transferred to the primary crushing where particles are crushed and screened to make them suitable for the bioheapleaching. The next stage involves agglomeration of the particles in order to combine the fine and coarser particles after which the ore is stacked eight meters high on a primary heap pad for 13-14 months. The heap is continuously aerated to stimulate bacterial activity. Ore from the primary heap is transferred to the second heap for further leaching which continues for three years. The heaps are constantly irrigated from the top with water containing microbes, leaching solution, and sulphuric acid. Once there is a definite concentration of metals in the pregnant leaching solution (PLS), metal sulphides are precipitated using chemicals and each metal is recovered one at a time. The final solution is treated for purification and neutralization, and then fed back to the leaching cycle. [Talvivaara, 2009]

The wastewater that was used for this project came from the last stages in the production processes and during November 2012, the polluted water entered into the gypsum ponds and safety areas [Sprock, 2013:8]. The occurring of the event demanded an efficient and cost effective solution to be found. TABLE 2 on the following page shows the heavy metal concentration of the processed mining wastewater used in the Algae Turf Scrubbers.

TABLE 2: Composition of wastewater used for the project work

	Tank	
	TAMK 3	**TAMK 1**
pH	3	3.9
Al (mg/L)	175	207
As (mg/L)	0.54	0.6
Ca (mg/L)	318	378
Cd (mg/L)	0.26	0.3
Co (mg/L)	1.38	1.63
Cr (mg/L)	<0.0121	<0.0121
Cu (mg/L)	<0.0121	<0.0121
Fe (mg/L)	1360	1595
Mg (mg/L)	1736	2045
Mn (mg/L)	1318	1559
Na (mg/L)	824	952
Ni (mg/L)	64.6	77.7
Si (mg/L)	13	15.4
Zn (mg/L)	121	143
U (mg/L)	0.77	0.82

6 Algae and metal binding

The use of microorganisms for heavy metal removal is a familiar concept and the idea has existed for quite many years, however, it is being implemented on large scales only nowadays because of its potential application regarding environmental protection, and also because it provides a cost effective way to recover metals. The term used to describe the process of metal uptake is called 'biosorption' and although it is used together with the term 'bioaccumulation' at times, the former is used for metal adsorption on dead biomass and includes the metals binding to extracellular and intracellular ligands of the microorganism. Nevertheless, Mehta & Gaur emphasize in the article that the term 'biosorption' can also be referred to when describing metal uptake by live biomass. [Mehta & Gaur, 2005:113]

Metal binding in algal cells takes places through two processes; a fast passive uptake followed by a slower active uptake of ions. In the passive phase, the metal ions adsorb to the cell surface rapidly and this is metabolism-independent. During the active phase, the ions move into the cytoplasm and bind to intracellular molecules, such as 'cytoplasmic ligands, phytochelatins and metallothioneinds' [Mehta & Gaur, 2006:117]. Phytochelatins (PCs) and metallothioneins (MTs) are the best known heavy-metal binding ligands found in plant cells and research indicates that algae synthesize 'a series of cysteine-rich peptides' called phytochelatins after an exposure to a range of heavy metals, and metallothioneins are already existing 'evolutionarily strongly conserved proteins of low molecular weight and high cysteine and metal contents' [Gekeler, Grill, Winnacker & Zenk, 1988:1]. Mehta & Gaur also claim that a variety of algal species and chemical sorbents are used to demonstrate metal binding. The two species *Scenedesmus* and *Synechococcus* are described to have a metal sorption capacity of 30,18mg/g and 3,17 mg/g for Nickel respectively and *Scenedesmus quadricauda* is able to bind approximately 0,04 mg/g of Zinc [Mehta et al, 2005: 116]. Sample analysis during the project work also showed both *Scenedesmus* and *Synechococcus* species and demonstrated that they are capable of surviving in an extreme environment and also well capable of metal binding – in our case, it was a simulated freshwater polluted environment in the ATS.

7 Factors affecting algal growth

7.1 Light

Photosynthesis allows light energy to be converted into chemical energy to stimulate growth. Organisms that acquire most or all of their energy in this manner are referred to as phototrophic or photosynthetic [White, 2007:147]. Although it depends upon the photosynthetic system, light energy is known to have the following characteristics:

1) Light energy can stimulate the phosphorylation of ADP (Adenosine Diphosphate) to make ATP (Adenosine Triphosphate) in all photosynthetic systems (also referred to as the process of photophosphorylation)
2) In certain photosynthetic systems, light energy can also force the transfer of electrons from H_2O to $NADP^+$ (Nicotinamide adenine dinucleotide phosphate). This process is called photoreduction of $NADP^+$. [White, 2007:149]

During the process of photoreduction of NADP+, the water is oxidized to oxygen gas and NADP+ molecule becomes reduced to NADPH. The synthesis of ATP and NADPH allows electromagnetic energy to be absorbed by the photo-pigments present in photosynthetic membranes and subsequently converted to chemical energy. The author also mentions that the light stimulated oxidation of chlorophyll as the most important aspect that results in the electron transport and eventually the generation of Δp (the potential energy to allow protons to move across the membrane) thereby leading to the synthesis of ATP and NADPH [White, 2007]. Both molecules play a significant role in the Calvin cycle to 'fuel incorporation of CO_2 (Carbon dioxide) to sugar' [Graham & Wilcox, 2000: 112]

Anabeana cylindrical and *Synechococcus sp.* were the two species of cyanobacteria used for the project work (see TABLE 1). Cyanobacteria are oxygenic prokaryotic phototrophs that make use of H_2O as an electron donor for the photo-reduction of $NADH^+$ to NADPH. They are found growing in a variety of aquatic habitats. They only have one type of chlorophyll – chlorophyll *a* - and light harvesting pigments called phycobilins, and also carotenoids that help prevent photo-oxidation under high light intensities [White, 2007:150].

The efficiency of photosynthesis depends on the operation of Photosystems I and Photosystems II (also commonly referred to as Reaction Center I and Reaction Center II)

and that must always be energized to maintain an electron flow and reduce $NADP^+$. Light energy required for photosynthesis is not absorbed directly by the reaction centers, but is absorbed first by the light harvesting pigments present in the photosynthetic membranes or within granules called phycobilisomes and transferred to the reaction centers [White, 2007:169]

White describes that photosynthesis efficiency decreases immediately at 685nm despite the fact that chlorophyll is still absorbing light. Hence, it became known that there are two different systems – one that operates at 700nm only and the other only operating at lower wavelengths of light. The solution lies in the provision of light of lesser wavelengths, for example, using fluorescent lamps to keep both reaction centers energized so that photosynthesis is not inhibited. [White, 2007]

7.2 Nutrients required for growth

In addition to sufficient light, algae also require a variety of inorganic nutrients for growth, such as carbon dioxide nitrogen, phosphate, iron and silica that are limiting nutrients for algal populations. TABLE 3 summarizes the important nutrients required for proper algal growth.

TABLE 3: Some essential nutrients and functions [Graham & Wilcox, 2000: 24]

Element	**Examples of Functions/ location in algal cells**
Nitrogen	Amino acids, nucleotides, chlorophyll, phycobilins
Phosphorous	ATP, Deoxyribonucleic acid (DNA), phospholipids
Chlorine	Oxygen production in photosynthesis, trichloroethylene
Sulphur	Some amino acids, nitrogenase, dimethylsulfoniopropionate (DMSP)
Potassium	Osmotic regulation, cofactor for many enzymes
Silica	Diatom frustules, silicoflagellate skeletons
Calcium	Alginates, calcium carbonate
Magnesium	chlorophyll
Iron	Nitrogenase, nitrate and nitrite reductase
Bromine and Iodine	Halogenated compounds with anti-herbivore or allelopathic functions

In *Algae: Anatomy, Biochemistry and Biotechnology*, Barsanti & Gaultieri write that 6 major elements occupy approximately 99,9% of the entire biomass of algae, and they are carbon, oxygen, hydrogen, nitrogen, sulphur, and phosphorous. In addition, there are also several trace metals such as calcium, potassium, sodium, chlorine, magnesium, iron and silicon. [Barsanti & Gaultieri, 2006:160]

The incorporation of elements inside the algae also emphasizes upon the role the algae play in recycling of elements in the biogeochemical cycle, allowing equilibrium to be reached between various elements. While it is of great importance for all elements to be recycled well, the recycling of growth-limiting nutrients and the rate at which they are recycled is the main objective to observe, and these are known to be nitrate (NO_3^-), iron (Fe), phosphate (PO_4^{3-}), and dissolved silicon $Si(OH)_4$ [Barsanti & Gaultieri, 2006:159-160]

7.3 Liebig's Law of Minimum

The concept of the limiting nutrient is known as Liebig's Law of Minimum which states that the growth is not dependent on the total nutrients available, but the nutrient that is present in the smallest quantity. Barsanti & Gaultieri explain the point further by stating that if the supply rate of the essential nutrient is less than the rate at which the algae takes up the nutrient, then at the end the algae is only 'slightly' nutrient deficient. However, not all algal species will be limited by the same nutrient. For instance, silicate is vital for diatoms and their growth rate is affected if silicon concentration is low. The climatic conditions and influent entering the lakes and rivers also determines the concentration of limiting nutrients. [Barsanti & Gaultieri, 2006:160]

7.4 The Redfield Ratio

Globally, the two most important limiting nutrients are nitrogen and phosphorous. In *Algae: Anatomy, Biochemistry, and Biotechnology,* it has been suggested that all nutrients are present inside algal cells in a 'species-specific structural ratio' commonly referred to as the Redfield Ratio. It enables the species nutrient requirement to be deter-

mined, but the value depends on the environmental conditions. Alterations in nutrient ratios will cause changes in phytoplankton populations and trophic linkages.

The *American Scientist* claims that 'synthesis of organic matter is a highly selective process' that results in products existing in a specific composition [Redfield, 1958: 206]. More importantly, the quantity in which the organic matter is produced depends on the requirement of the biochemical cycle. The validity of the argument can be described by comparing the elemental composition of carbon, nitrogen and phosphorous (C:N:P) found in plankton in sea water. Analysis showed that the average ratio is 106:16:1. The ratio describes the 'quantitative relations of the cycles of the separate elements involved in the biochemical cycle as a whole…It provides a quantitative criterion…' [Redfield, 1958: 207]. In accordance with Liebig's law defined above, the nutrient present in the smallest quantity will become the limiting factor and the Redfield ratio defines the requirements. While nitrogen-phosphorous exist in that ratio, carbon - existing as carbonate - is always found in great excess with the phosphorous-carbon ratio as 1:1000 while the requirement is 1:105. Therefore, carbon is never a limiting nutrient. [Redfield, 1958: 207]

The 'Redfield Ratio is an exception rather than a rule in freshwater' [Hecky, Campbell & Hendzel, 1993:709]. The ratios are variable and substantially higher for lakes than the Redfield Ratio of 106:16:1. In freshwater lakes, a variety of conditions exist, for example, nitrogen-phosphorous deficiency or nitrogen-phosphorous sufficiency. But despite the variation, data suggests that C:N:P ratio of freshwaters should be similar to the marine system [Hecky et al, 1993: 709].

7.5 Carbon dioxide

The Calvin cycle describes the pathway of carbon fixation by algae, the reduction to carbohydrate, and regeneration of the CO_2 acceptor. Carboxylation is a natural process where one molecule of CO_2 is added to an acceptor molecule called ribulose biphosphate (RuBP). The enzyme catalyzing the reaction is Ribulose biphosphate carboxylase (RuBisCo). It allows the conversion of inorganic CO_2 to reduced (organic) carbon in all photosynthetic oxygen evolving organisms (1). Reduction occurs here and

the result is phosphoglyceric acid (PGA). It should also be mentioned that this enzyme is also called ‘oxygenase’ because it can use oxygen to produce phosphoglycolate (2).

$$RuBP + CO2 + H2O \rightarrow 2PGA \quad (1)$$

$$RuBP + O2 \rightarrow PGA + phosphoglycolate \quad (2)$$

Reaction (2) is the pathway for photorespiration and is unfavourable because it leads to loss of organic carbon. Reaction (1) or carboxylation is stimulated by high levels CO_2 [Graham & Wilcox, 2000:32-33]. The energy required to reduce inorganic carbon to organic form is supplied by ATP and NADPH – the high energy chemical intermediates formed during light reactions. The final stage of the Calvin cycle regenerates the CO_2 acceptor molecule RuBP, and this final step is also dependent on energy supplied by ATP [Barsanti & Gaultieri, 2006:153-154]

Carbon sources for algae are HCO_3^- and CO_2. At pH 8-9, the bicarbonate ion concentration is a lot higher than CO_2, and vice versa if pH value is lower. Modern algae are known to produce carbonates and these include the cyanobacteria, green and brown seaweeds, the freshwater green algae, some red algae, and the haptophyte algae also known as coccolithophorids. The process of manufacturing calcium carbonate is called calcification, and scientists claim that in certain species of algae calcification may be an important process in the acquisition of CO_2 neccessary for photosynthesis. Despite active transport and passive diffusion mechanisms to take up CO_2, algae have also adapted carbon-concentrating mechanisms (CCMs) to continuously supply CO_2 to the enzyme Rubisco and carry out photosynthesis, and these include:

- cell membrane inorganic carbon transporters that help convert bicarbonate to CO_2
- enzymes including carbonic anhydrase to interconvert CO_2 and bicarbonate
- calcification-linked processes
- specialized cellular structures called carboxysomes and pyrenoids [Graham & Wilcox, 2000:32-33]

7.6 Nitrogen

The importance of the element for algae is explained by nitrogen being an essential component of ‘amino acids, proteins, enzymes, nucleic acids, chlorophylls, energy mol-

ecules (ATP and ADP) and other nitrogen containing cellular constituents [Graham & Wilcox, 2000:25, Barsanti & Gualtieri, 2006: 164]. Certain algae are able to use organic forms of nitrogen, such as urea and amino acids, whereas some are able to use extracellular enzymes to take up ammonium from dissolved amino acids [Graham & Wilcox, 2000:25]. Concentration of nitrogen in the ATS was found by measuring Total Nitrogen which is the sum of 'nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N) and organically bonded nitrogen' [Asa Analytics, 2013].

Inorganic nitrogen sources are ammonium (NH4+) and nitrate (NO3-) and algae are able to import them directly through the cell membrane. Ammonium is preferred mostly because it can be used efficiently for the synthesis of amino acids. In order to convert nitrate to ammonium, energy is required and also the presence of the enzyme Nitrate Reductase (NR) which is present in a variety of algae. Nitrate Reductase consists of iron and molybdenum as cofactors, and at times iron concentrations can be low in neutral to alkaline natural waters because iron is bound to less soluble compounds, such as, iron hydroxide. However, the rate of uptake of ammonium is independent of iron availability. It is also known that aquatic nitrifying bacteria easily convert ammonium to nitrate which can limit the availability of ammonium for algae. Research indicates that cyanobacteria have a distinct form of Nitrate Reductase, and that the enzyme exists in two other forms in eukaryotic algae. [Graham & Wilcox, 2000:26]

Nevertheless, the activity of the enzyme is 'inducible by low ammonium levels' hence, the enzyme concentration increases as nitrate becomes available again [Graham & Wilcox, 2000:26]. But activity is inhibited if concentration of ammonium is too high. The concept of enzyme activity provides an effective way to measure and observe how nitrate is incorporated in algae.

Cyanobacteria are oxygenic phototrophic organisms that are able to convert gaseous nitrogen to ammonium, and the metabolism is known as diazotrophy. Since the atmosphere is made up of at least 70% gaseous nitrogen, the cyanobacteria readily supply nitrogen to the aquatic life. Nevertheless, nuisance cyanobacterial blooms can also easily develop in freshwater and marine waters because of the abundance of nitrogen in the air. Graham & Wilcox claim that freshwater lakes rich in phosphate, cyanobacterial blooms created by *Anabaena* and *Aphanizomenon* are common. An interesting aspect regarding cyanobacteria is that they are able to excrete approximately 40-60% of fixed

nitrogen into the water, thus providing algal cells and aquatic plants with the essential nutrient. [Graham & Wilcox, 2000:26]

Cyanobacteria are considered to have dominated the earth for a long time before the eukaryotic life began to develop. Nevertheless, their ability to convert nitrogen is still intact and is performed with the help of the enzyme Nitrogenase.

7.7 Phosphorus

The element helps build up the lipid portion of the cell membranes, several coenzymes, DNA, RNA, and most importantly, the ATP. Barsanti & Gaultieri claim that phosphates exist in three basic forms: orthophosphate, metaphosphate, or organically bound phosphate. All three forms of phosphate are found in living, decaying or dead organic matter as ‘free ions or weakly chemically bounded in aqueous systems, chemically bounded to sediments and soils, or as mineralized compounds in soil, rock and sediments’ [Barsanti & Gaultieri, 2006:163]. Concentration of phosphorus in the ATS was found by measuring Total Phosphorous which is the sum of ‘reactive, condensed and organic phosphorous’ [Asa Analytics, 2013].

Under aerobic conditions, when algal growth is at the maximum, there is also an excess of algae dying off, at which point the bacteria begin to decompose the organic matter and release the phosphates through a process known as ‘ recycling or internal cycling’. The phosphates are precipitated as iron phosphate and stored in sediment, and are released if anoxic conditions develop. If phosphorous is limited, the first impact is on nucleic acid synthesis which means that transcription, decoding, and translation of genetic code by RNA to produce proteins will be effected. Since the rate of protein synthesis is reduced, photosynthesis is affected which in turn affects cell metabolism. More importantly, the rate of manufacture and regeneration of substrates essential to the Calvin cycle is affected which affects the process of carbon fixation. Consequently, there is a decrease in phospholipids and increase in cell volume since no nucleic acids are produced and so there is no cell division. [Barsanti & Gaultieri, 2006:162]

7.8 Iron

Scientists claim that in the past, iron was a more common ion in the anoxic oceans than in today's oxygen-rich waters. Therefore, the cells had an ample supply of Fe^{2+}, and iron became a co-factor for many enzymes, for instance glutamate synthetase, nitrate and nitrite reductase, nitrogenase, and ferredoxin – an electron carrier for the process of nitrogen fixation. Graham & Wilcox point out that each nitrogenase complex is made up of 32-36 iron atoms, and that decomposition of dead organic matter also releases small amount of iron. If iron is present in a plentiful quantity, algae are able to store it in the form of protein aggregations called phytoferritin. [Graham & Wilcox, 2000:38]

7.9 Sulphur

Algae require sulphur for biosynthesis of two amino acids: cysteine – where sulphur plays an important role in the formation of covalent disulfide bond and protein structure, and methionine – that influences biosynthesis of dimethylsulfoniopropionate (DMSP) and consequently the global sulphur cycle. Moreover, sulphur is also required for sulphur containing thylakoid lipids. Energy is required for the uptake of sulphur, and in freshwater ecosystems, the concentration of the available sulphur can be significantly decreased by anaerobic bacteria that convert sulphur to hydrogen sulfide (H_2S). Hence, sulphur may become a limiting nutrient at times, nonetheless, certain species of algae have high-affinity sulphur transport mechanisms. [Graham & Wilcox, 2000:38-39]

7.10 pH, redox potential and temperature

It is difficult to define temperature limits reliably, and it maybe assumed that microorganisms only grow according to the temperature of their location, for example, certain microorganisms may only grow at +10℃ and not at higher temperature. However, temperatures between 16℃ and 35℃ are usually found to be suitable for algal growth [Harrison et al, 2004:583]

Guidelines regarding experimental work recommend pH and redox potential values to be always quoted, and also explains that redox potential helps understand the activity of microorganisms. However, the presence of sulphate and hydrogen carbonate ions can

modify the redox behaviour of metal species. Algae prefer a pH value that is between 6-8, and it has been found that in some natural and artificial locations microorganisms can demonstrate optimum growth at very acidic and alkaline pH values. [EFC, 1992:12]

8 Materials and Methods

8.1 pH, Light sensor and Turbidity

The equipment to used to measure pH and illumination was Phidget pH Lab Electrodes and LiCor Quantum sensor. As mentioned by S.Sprock in his thesis, pH-meters and light sensor were connected to a computer where the data was recorded and saved every ten minutes. Turbidty was monitored with a turbidity meter. A more detailed description can be found in G.Grobler's thesis work – *Algae Cultivation for Wastewater Reclamation.*

8.2 HACH

The HACH system was used to measure Total Nitrogen and Phosphate Phosphorus on a frequent basis. To measure Total Nitrogen, HACH LANGE LCK 138 kits were used and the measuring range was 1-16mg/L. For Phosphate Phosphorus, LCK 139 kits were used and the measuring range was 0,05-150mg/L PO_4-P and 0,15-4,50mg/L PO_4. Detailed instructions can be found in G.Grobler's thesis.

8.3 Test Run

The algal species provided by SYKE were already being cultivated separately in 800ml Erlenmeyer flasks. To obtain a mixture of algal species, 400ml of each species was transferred to a big glass cylinder and 15L of distilled was added so that at the end the total volume of algae mixture in the cylinder was 19L.

At first, algae was allowed to grow in two 1L Erlenmeyer flasks with distilled water, however, the pH values were observed to be very low (below 5.0), and addition of tap water significantly improved the pH. Therefore, for entire length of the project tap water was used.

A trial run using various mining water concentrations was performed to understand how algal growth is affected. The initial pH of the mining water was 2,4 and it was adjusted to 4,5 using Calcium hydroxide ($Ca(OH)_2$). The trial was perfomed during June & July 2013 with 8 mining water amendments as follows:

1) 16 Erlenmeyers containing algae and pH adjusted mining water
2) 16 Erlenmeyers containing algae and no-pH adjusted mining water

The mining water amendments were 0ml, 1ml, 2ml, 5ml,10ml,15ml,30ml, and 60ml (for both pH and no pH amendments). It had also been decided that the total volume of algae and mining water will be not more than 300ml in each Erlemeyer flask and all treatments to be done in two replicates (see Appendix 2 for description & calculation of mining water amendments).

Values for pH and temperature for algae were monitored for several weeks, and Atomic Absorption Spectroscopy (AAS) was also performed to observe the metal removal by algae in each flask, however only Zinc and Nickel concentrations were measured. The end result demonstrated that algal growth was best with a mining water amendment of 5ml. There was no significant difference in algal growth whether it was pH-adjusted or no-pH-adjusted mining water and microscope obervations also revealed that there was no significant growth in mining water amendments higher than 10ml. Hence, the final decision was to use pH-adjusted mining for the final project analysis

8.4 Algae Turf Scrubbers used in the project

At the beginning of August 2013, five liters of the mixed algae was culture was transferred from the big cylinder to each Algae Turf Scrubber and 15L of tap water was added to each system. In addition, the algae were provided with an ample supply of nutrients to stimulate growth. The nutrients were supplied in the form of Biobact fertilizer, di-sodium-hydrogen phosphate (Na_2HPO_4), glucose and urea. The nutrients were added in a specific amount depending on the amount of evaporation from the turf scrubbers (see Appendix 11).

Both systems were allowed to run without any addition of mining water until 19th August 2013 in order to obtain a sufficient amount of algae biomass. The total amount of algae mixture in each turf scrubber at that time was 25L (25000ml). Based on the Erlenmeyer data (Appendix 2), it was calculated that 5ml of mining water in a 300ml Erlenmeyer flask is approximately 2%. Accordingly, 2% of 25L gives 500ml, and this was the volume of pH-adjusted mining water added to each Algae Turf Scrubber.

Due to an error in adding the correct amount of nutrients to the Algae Turf Scrubbers and the fact that the tests for Total Nitrogen for the original samples were not done correctly, a third Algae Turf Scrubber was setup on August 29th 2013. It contained only the algae mixture and no mining water.

9 Results and Discussion

Graphical representation of pH and Temperature data for ATS 1, 2 and 3.

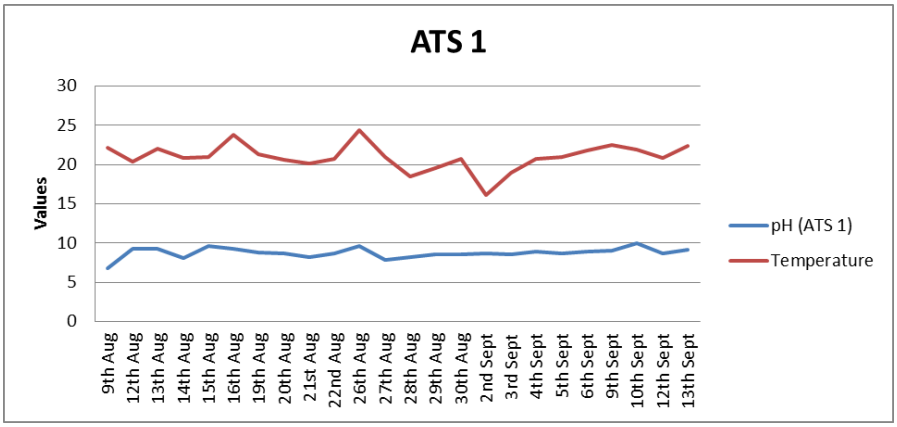

FIGURE 4: pH vs Temperature for Algae Turf Scrubber 1 (ATS 1)

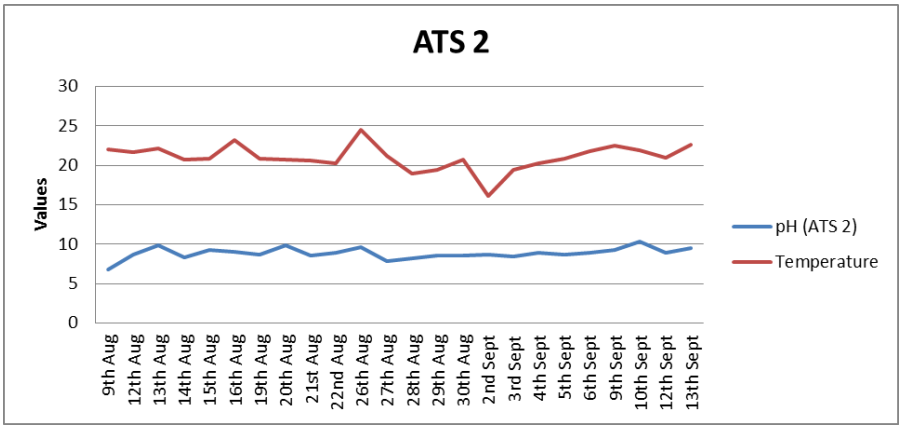

FIGURE 5: pH vs Temperature for Algae Turf Scrubber 2 (ATS 2)

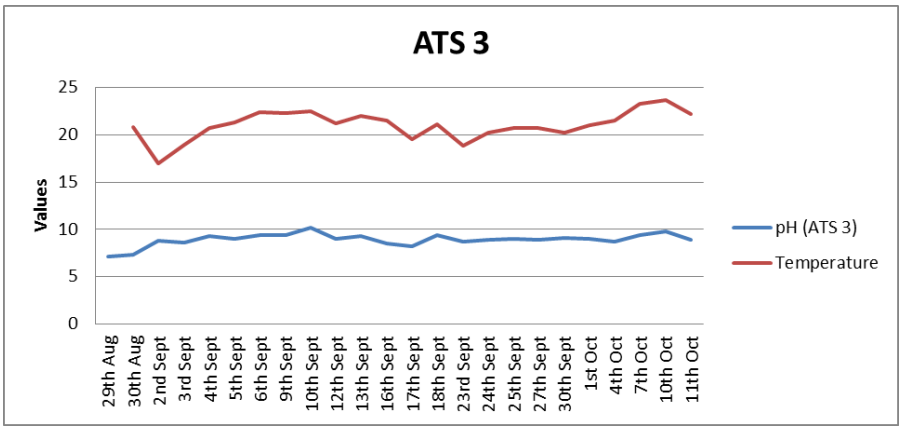

FIGURE 6: pH vs Temperature for Algae Turf Scrubber 3 (ATS 3)

TABLE 4: Average pH, Temperature (℃) and Turbidity (FAU) values for Algae Turf Scrubber 1, 2 and 3.

	pH (ATS 1)	**Temperature (ATS 1)**	**pH (ATS 2)**	**Temperature (ATS 2)**	**pH (ATS 3)**	**Temperature (ATS 3)**	**Turbidity (ATS 1)**	**Turbidity (ATS 2)**	**Turbidity (ATS 3)**
Average	8,77	20,99	8,87	21,04	8,92	21,03	123,19	255,13	189,00
Max	9,98	24,10	10,30	24,50	10,18	23,70	680,00	780,00	850,00
Min	6,78	16,10	6,82	16,10	7,15	17,0	14,00	19,00	38,00

According to TABLE 4 above, all the systems had similar average pH and temperature values during the entire period of time starting on August 9th and ending on September 13th. Algae Turf Scrubber 1 had an average pH and temperature of 8,77 and 20,99℃ whereas Algae Turf Scrubber 2 had an average pH and temperature of 8,87 and 20,04 ℃ degrees respectively. The third system was running from August 29th to October 11th and pH and temperature values were 8,92 and 20,03 ℃ respectively.

A reasonable explanation for the pH and Temperature values of the Algae Turf Scrubbers is found in a study done in Tinto, Odiel and Piedras rivers in southwestern Spain where researchers studied growth of algae populations in areas of acid mine drainage and natural alkaline waters. The article describes the acid mine-drainage areas as extreme habitats for algae because of their low pH values (2,5) and a very high concentration of heavy metals such as, iron, zinc, and sulphates. The article also claims that only 'extremophilous taxa' will be able to survive these harsh conditions. The team monitored several physiochemical and biological parameters of several chosen areas, such as pH and temperature. For acid mine-drainage areas, the team recorded average pH and temperature as 3,44 and $8,25^0$C. For alkaline waters, the pH and temperature values were 8,19 and 9.35^0C respectively. [Urrea-Clos & Sabater, 2009:261]

TABLE 4 shows that all three systems were alkaline and the average values for pH are more or less the same as the values observed in Spain.

Interestingly, the team discovered some algae species including *Fragalaria*, *Navicula*, and *Anabaena*, which were also the algae species we observed during the project (see Appendix 6).

Studies show that pH plays a very same significant role in the development of biological communities. The areas affected by mining drainage put physical and chemical stress on the biota. An excellent point was highlighted towards the end of the article that because few species are able to adapt to such extreme environments, tolerant species are able to compensate for their absence by increasing their own biomass, which provides an explanation why we saw a large number of *Scenedesmus, Synechoccocus, Fragalaria,* and *Navicula* species and occasionally some *Anabaena* and *Haemotococcus* because they developed tolerance when mining water was added to the both ATS 1 and 2. The remaining species including *Pediastrum simplex, Selenastrum, Chlorophyta sp* and *Purpuraemus* were probably not able to survive the extreme conditions.

The values obtained from the light sensors shows a great variation in the availability of light. The requirement for light by algae varies greatly with the depth of culture, and that approximately 1000 lux is sufficient for Erlenmeyer flasks and 5000-10,000 lux for larger volumes [Lavens, 1996]. However, even with the presence of chlorophyll *a* and the accessory pigments, approximately 40% of the light energy is utilized for the manufacturing of ATP and NADPH [White, 2007:161]. Looking at FIG 7, the available light for algae decreased over the course of 6 weeks, but it was compensated by the sodium lamps that were switched on for a few hours every day starting at the end of August.

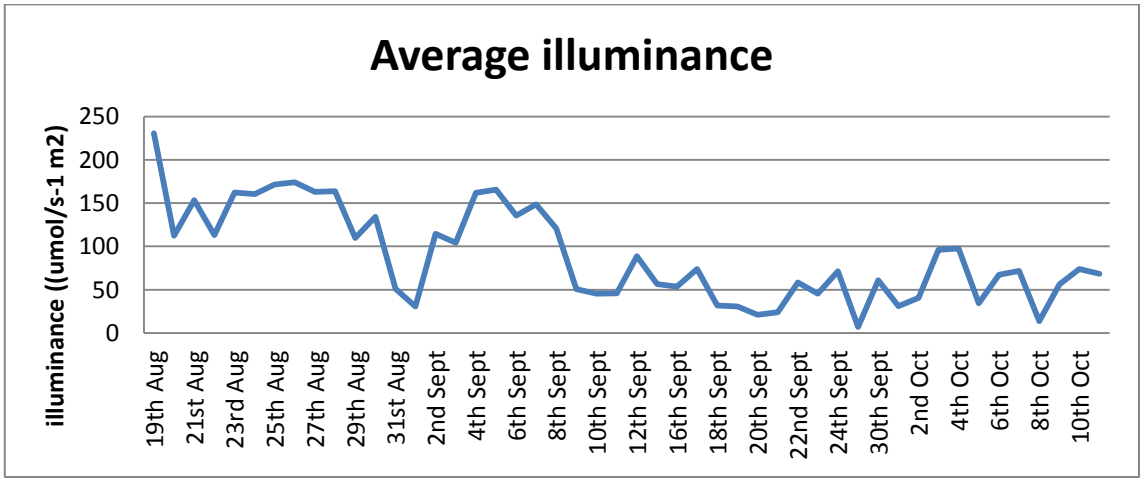

FIGURE 7: Average illuminance for all three Algae Turf Scrubber systems

TABLE 5: Average, maximum and minimum values of TN for ATS 1, ATS 2 & ATS 3

	Total Nitrogen (mg/L)		
	ATS 1	**ATS 2**	**ATS 3**
Average	24,15	24,28	29,49
Max	28,25	27,83	96,80
Min	19,40	19,40	7,77

Graphical representation of data for Total Nitrogen for the Algae Turf Scrubbers

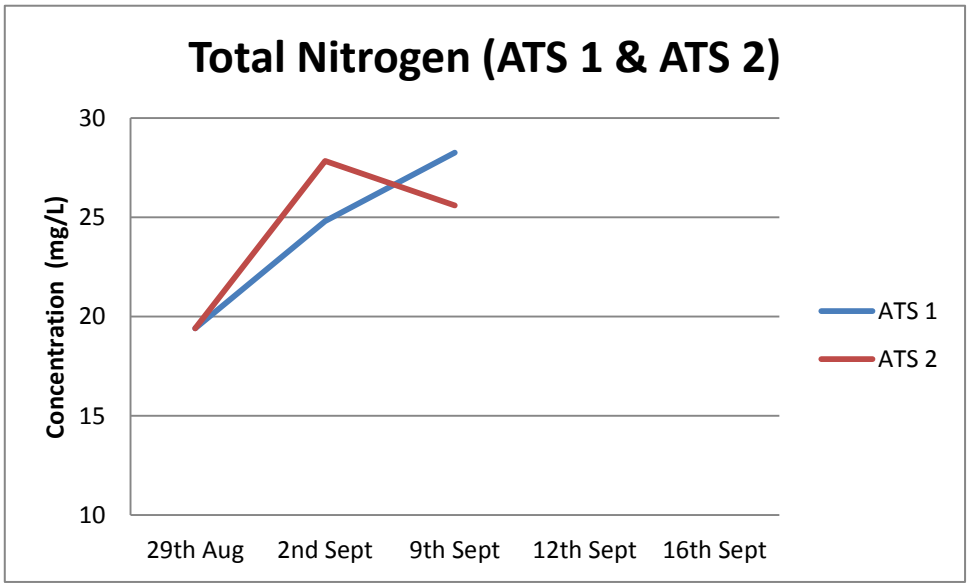

FIGURE 8: Total Nitrogen (TN) for ATS 1 & ATS 2

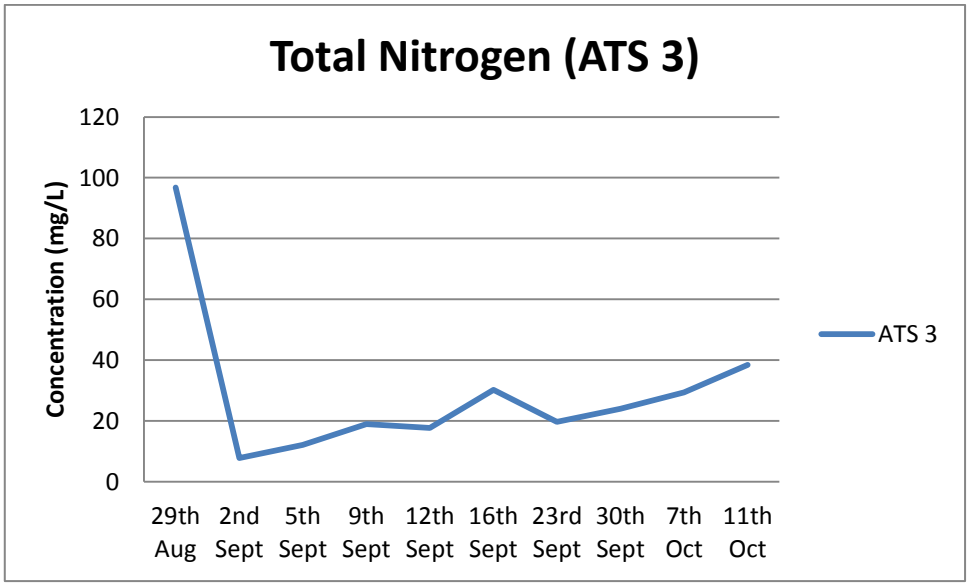

FIGURE 9: Total Nitrogen (TN) for ATS 3

Due to an error mentioned earlier, there are only a few values for TN for ATS 1 and ATS 2. However, the average total nitrogen values for both were 24,2mg/l and 24,3mg/l respectively (TABLE 5). ATS 3 served as a control system because no mining water had been added to it. Average value for TN for ATS 3 was 29,5mg/l over a period approximately one month a half. Looking at FIG 8 for ATS 1 & ATS 2, it is difficult to approach an explanation for the behavior as both the trend lines for TN follow a different path, despite the fact that both ATS 1 & ATS 2 had the same system setup. FIG 9 shows a decrease in nitrogen levels and a sudden increase again after 2nd September in ATS 3.

TABLE 6: Average, maximum and minimum values of TP for ATS 1, ATS 2 & ATS 3

	Total Phosphorous (mg/L)		
	ATS 1	**ATS 2**	**ATS 3**
Average	5,04	5,27	7,92
Max	7,77	7,77	17,9
Min	3,63	4,34	4,95

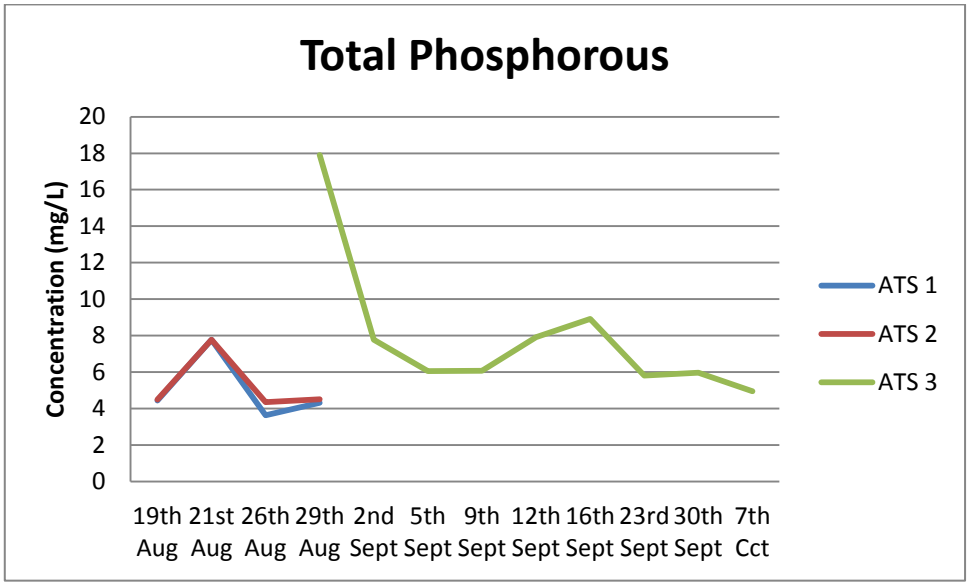

FIGURE 10: Total Phosphorous for all (ATS1, ATS 2 and ATS 3)

Average TP for ATS 1, ATS 2 & ATS 3 was 5,04mg/l, 5,30mg/l, and 7,92mg/l respectively (TABLE 6). The trend line for ATS 1 & ATS 2 shows an increase and a sudden decrease in TP concentrations (FIG 10). For ATS 3, TP concentration decreased a great deal after the first two days – from 96,8mg/L to 7,77mg/L – but the trend line shows a continuous decrease in TP concentrations after 5th September.

Comparing all the three graphs (FIG 8, 9 & 10), it is interesting to see that for ATS 1 & ATS 2, initial TN and TP concentrations were similar. For ATS 3, the initial TN and TP concentrations were also similar and there was a sudden drop in the concentration level of both TN and TP after the first two days. The point of focus here is the pattern in ATS 3; the fact that the concentration of TN began to increase at exactly the same time when the concentration level of TP began to decrease demonstrates the idea of nutrient deficiency.

Nutrient analysis not only helps determine uptake of nutrients by algae, but also explains why certain algal species are found in one type of environment and not another. Comparisons of N/P ratios can help predict the survival factor of algae species. Nevertheless, a number of factors may contribute to algae growth, for example, the ability of algae to store essential nutrients [Graham & Wilcox, 2000:24]. Although there are a variety of physiological assays in place to comprehend the nutrient status of phytoplankton, composition ratio is the simplest way to define the nutrient status in both marine and freshwaters [Hecky et al, 1993: 710]. Residence time and the likelihood of stratification also determines the cycling of carbon, nitrogen and phosphorous (C:N:P) in lakes and the composition of ratios [Hecky et al, 1993: 712].

Particulate concentration is higher and varies a lot more in lakes than in oceans. Comparing the setup of the Algae Turf Scrubbers to freshwater lakes, the mean annual concentrations of nitrogen and phosphorous have a wide range: 1.2-210µmol/L nitrogen and 0,06-7,8µmol/L phosphorous. In general, phytoplankton in lakes is classified as moderately to severely deficient in phosphorous and only moderately deficient in nitrogen. The phytoplankton must adjust to low phosphorous concentrations and maintain only those species capable of growing in a phosphorous-limited environment. [Hecky et al, 1993: 718-719]

A study performed on 51 lakes in late 1990s extending from the arctic to tropical climatic regions in the world explained that there is a broad range of C:N:P ratio, and the values can be close to the Redfield Ratio or extremely high mainly due to the fact that Redfield Ratio is a characteristic of the oceans [Hecky et al, 1993719]. A generally low variance of C:N ratio in freshwaters indicates that nitrogen deficiency is not a severe problem [Hecky et al, 1993: 721], perhaps because these lakes have an ample supply of nitrogen from surface and groundwater and nitrogen deficiency is more common in a marine environment.

Lakes contain a large number of nitrogen-fixing cyanobacteria that play a vital role in maintaining the supply of nitrogen [Graham & Wilcox, 2000:25]. A comparison of algal tests results with chemical nutrient concentrations in lakes shows that a mass N:P ratio higher than 17 indicates phosphorous limitation, a ratio below 10 indicates nitrogen limitation, and values between 10 and 17 show that either one of the nutrients maybe limiting [Ekholm, 2008]. The N:P ratio can also help estimate the risk of cyanobacterial blooms. Nitrogen fixing bacteria dominates in lakes if the total mass N:P ratio is below 22, but it is also important to realize that several other factors, for instance light conditions, may also contribute to the growth of cyanobacteria. [Ekholm, 2008]

TABLE 7: Total Nitrogen (TN), Total Phosphorous (TP) and N/P ratios for all three Algae Turf Scrubbers

	TN (mg/L)	**TP (mg/L)**	**(mass) N:P**	**TN (µmol/L)**	**TP (µmol/L)**	**(molar) N:P**
ATS 1	24,15	5,04	4.79	1725,00	162,58	10,61
ATS 2	24,27	5,27	4.61	1733,57	170,00	10,20
ATS 3	29,49	7,92	3.72	2106,43	255,48	8,24

According to TABLE 7, the total mass N:P ratio was below 22 demonstrating that the cyanobacteria species (*Anabaena cylindrical and Synechococcus sp.)* were dominating the Algae Turf Scrubbers. Nitrogen fixation occurs to balance nitrogen with phosphorous, which implies that the algae was limited by phosphorous [Hellström, 1996: 55]. Evidence demonstrates that all species of cyanobacteria are able to compete for nitrogen when it is scarce, so when there is a surplus of phosphorous in the system, nitrogen concentration decreases and as a result cyanobacteria increase in number to maintain the balance [Havens et al, 2002: 380]. This concept validates the results of TN and TP on pages 33 & 34 because the concentration of TN increased at the same time when TP began to decrease in ATS 3, which means that ATS 3 had a surplus of phosphorous which caused nitrogen to become a limiting factor. Since there were two nitrogen-fixing algal species, they helped create a surplus of nitrogen and phosphorous eventually became the limiting nutrient. Consequently, the N:P also decreased which favored the bloom of nitrogen-fixing cyanobacteria.

TABLE 9: Average values of Turbidity for all three Algae Turf Scrubbers

	Turbidity ATS 1	Turbidity ATS 2	Turbidity ATS 3
AVERAGE	123,19	255,13	189,00
MAX	680,00	780,00	850,00
MIN	14,00	19,00	38,00

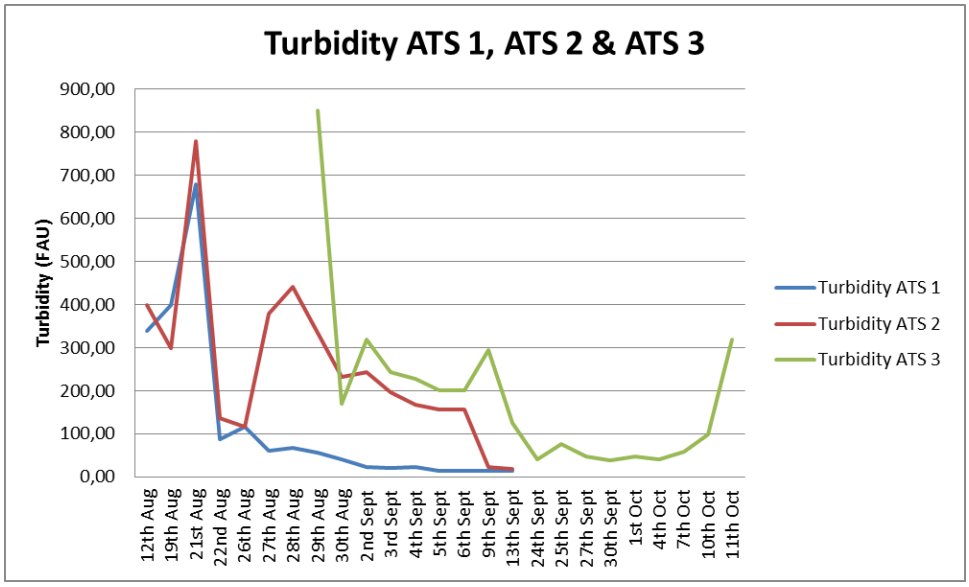

FIGURE 11: Turbidity for all three Algae Turf Scrubbers

Water turbidity greatly influences algal growth, whether it is in a river, stream or simply a tank in a laboratory. A comparative study of the Illinois River and Fox River indicated high turbidity reduces algal growth as it creates a light inhibition effect. It also showed that when turbidity was 135 Jtu gross photosynthesis was 1,6 g/m^2/day oxygen and when turbidity was 40-50 Jtu gross photosynthesis was 13,8 g/m^2/day oxygen. [Wang, 1974:3]

Light inhibition is a critical factor for algal growth, however, looking at the average values in TABLE 9 and analysing FIG 11, algal growth followed an interesting trend as the trend line increased at the beginning, dropped suddenly and intended to increase slightly again only to have a continuous decreasing trend line afterwards. High turbidity values are less of a problem than low turbidity persisting for a long time [water on the web, 2008]. FIG 11 above merely suggests that low turbidity allowed optimum algal growth – mostly an increase in the number of cyanobacteria. Flow rate is also an important factor to consider at this point, and since it was quite reasonable for all three systems (4,7 L/min, see page 14), the algae, heavy metals, and micronutrients were able

to accumulate at the bottom. At this point it is also be noted that cyanobacteria respond immediately to changes in temperature and that they have optimal growth rates at 25℃ [WSUD, 2005:349]. Since temperature values were close to 25℃, the flow rate was constant, turbidity was low and there was a sufficiency of nitrogen caused by a deficiency of phosphorous , algal growth – in this case cyanobacteria – increased greatly.

TABLE 10: Total harvested algae (wet mass)

	Total Harvested Algae (g)
ATS 1	879,0
ATS 2	782,3
ATS 3	2095,0

Algae mass production can help achieve many objectives, such as production of hydrocarbons, organic substances, and proteins [Shelef, Sukenik & Green, 1984:6]. The writers discuss the concept of High rate algal ponds (HRAP) similar to an Algae Turf Scrubber that have shallow raceways and aim to enhance photosynthetic activity. The final product of HRAP can contain upto 600mg/L microalgae, but efficient separation, dewatering and drying of algae is the most important step from an economic perspective. Microalgae are able to form stable suspensions due to their small size which also creates difficulties in their recovery [Shelef et al, 1984:7].

Algae harvesting refers to the ‘concentration of fairly diluted algae suspension (0,02-0,06% Total Suspended Solids) until a slurry of paste containing 5-25% or more is obtained’ [Shelef et al, 1984:7]. The stability of the suspension is affected by algal surface electric charge which develops intracellular forces and is influenced by ionic strength of the medium, pH and other environmental factors. It is also affected by cell dimensions and cell density that cause slow cell sinking. Planktonic algae are said to reduce their sinking rate through ‘motility, reducing cell dimensions or reducing cell density via gas vacuoles as observed in blue-green algae’ [Shelef et al, 1984:9].

Separation of algae is achieved by a number of methods including addition of chemical flocculants to cause rapid sedimentation or simply by centrifuging. The final step of algae harvesting is drying of the dewatered algae so that the moisture content is 12-

15%. Drying helps convert the biomass into a stable product, but it may not be very cost effective. Nevertheless, drying methods depend on the production scale and the final purpose of algae cultivation. Drum drying is recommended for microalgae for better digestability and a small requirement for energy and investment. Incineration is also common where the material is dried before ignition. [Shelef et al, 1984]

TABLE 10 shows the total amount of wet algae harvested from the three systems at the end of the experiment period. Algae was simply harvested by carefully scrapping the top surfaces with a small flat tool. Total amount of algae harvested from all three systems was 879,0g, 782,3g and 2095,0g for ATS 1, ATS 2 & ATS 3 respectively. TABLE 10 shows a very high total harvested biomass for ATS 3 compared to ATS 1 & ATS 2. Perhaps the reason lies in the fact that the presence of heavy metals in ATS 1 & ATS 2 inhibited sufficient algal growth hence the low biomass. Nevertheless, further studies need to be performed for a clearer understanding of the concepts.

10 Conclusion

Critical analyses of the results demonstrates that the algae species were not the best suited for the project work because out of the ten species originally cultivated, only four seemed to have survived and these included *Scenedesmus, Synechoccocus, Fragalaria,* and *Navicula* species. *Anabaena* and *Haemotococcus* were seen occasionally. Marine algal species have been an area of focus because they have better metal binding capacity, and might provide a better option for further research work related to Algae Turf Scrubbers and heavy metal removal.

The rate of evaporation was high for all three systems, and this was compensated by the addition of nutrient water. However, subsequent addition of nutrients created an imbalance between nitrogen and phosphorous concentrations so that eventually phosphorous became the limiting nutrient. Low N:P ratio and a desirable temperature encouraged the growth of blue-green algae in the ATS. Nitrogen fixation by microalgae is a common phenomenon in aquatic systems, and so it is not difficult to assume that it will naturally occur to adjust the nitrogen phosphorus ratios in the water. Low turbidity also influenced algal growth conditions as sufficient light was available for photosynthesis, however, this phenomenon needs to be studied further for better understanding.

The original samples could not be taken into account because an incorrect amount of nutrients was added to ATS 1 & 2, and also because the tests for Total Nitrogen were not done correctly either. Hence, a third Algae Turf Scrubber (ATS 3) was setup and the data from this system was used for comparison with the two previous systems.

The Algae Turf Scrubber used in the laboratory was well adapted to suit the available area and easily maintained throughout the period. The project work provided valuable insight regarding the contamination of freshwater lakes with heavy-metal processed mining water. The Atomic Absorption Spectroscopy (AAS) was performed at several occasions for both ATS 1 and ATS 2 to see whether or not the heavy metals were removed and by how much. The process of biosorption was evident as the concentrations of both Zinc and Nickel significantly decreased in both systems. The original sample of the processed mining wastewater in the two tanks contained 64,6mg/l and 77,7mg/l of Zinc and 121mg/L and 143mg/l of Nickel. The freshwater algae growing in ATS 1 & 2 were able to bind the metals and remove them from the polluted mining wastewater.

Concentration of Zinc was lowered to 0,5mg/l and 0,4mg/l in ATS 1 and ATS 2 and concentration of Nickel was lowered to 25,0mg/l and 29,6mg/l respectively. However, AAS performed on August 26th and 28th revealed a drastic increase in the concentration of Nickel in both systems. It was believed to have been most likely caused by the deterioration of air stones used in the ATS systems, but further studies need to be performed to understand the sudden increase in Nickel and not Zinc. In general, the Algae Turf Scrubbers functioned well in mimicking the freshwater system affected by heavy metal polluted processed mining wastewater.

Improvements for future research work include more frequent nutrient analysis of nitrogen and phosphorous and also calculating the N:P ratios on a regular basis to ensure that the values are near the Redfield Ratio which will prevent blue-green algae from dominating.

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13 Appendices

Appendix 1. pH and Temperature values (℃) for Algae Turf Scrubber 1, 2 and 3 (ATS 1, ATS 2 and ATS 3).

Date	pH (ATS 1)	Temperature	Date	pH (ATS 2)	Temperature	Date	pH (ATS 3)	Temperature
9th Aug	6,78	22,2	9th Aug	6,82	22,1	29th Aug	7,15	
12th Aug	9,31	20,4	12th Aug	8,72	21,7	30th Aug	7,33	20,8
13th Aug	9,26	22,1	13th Aug	9,88	22,2	2nd Sept	8,77	17
14th Aug	8,07	20,9	14th Aug	8,36	20,7	3rd Sept	8,64	19
15th Aug	9,59	21	15th Aug	9,23	20,9	4th Sept	9,3	20,7
16th Aug	9,23	23,8	16th Aug	9	23,2	5th Sept	9,03	21,3
19th Aug	8,82	21,3	19th Aug	8,71	20,9	6th Sept	9,41	22,4
20th Aug	8,64	20,6	20th Aug	9,92	20,8	9th Sept	9,4	22,3
21st Aug	8,28	20,2	21st Aug	8,61	20,6	10th Sept	10,18	22,5
22nd Aug	8,65	20,8	22nd Aug	8,9	20,3	12th Sept	8,99	21,2
26th Aug	9,6	24,4	26th Aug	9,63	24,5	13th Sept	9,3	22
27th Aug	7,84	21	27th Aug	7,84	21,2	16th Sept	8,54	21,5
28th Aug	8,28	18,5	28th Aug	8,18	19	17th Sept	8,25	19,5
29th Aug	8,63	19,6	29th Aug	8,58	19,5	18th Sept	9,43	21,1
30th Aug	8,63	20,7	30th Aug	8,58	20,7	23rd Sept	8,71	18,9
2nd Sept	8,68	16,1	2nd Sept	8,73	16,1	24th Sept	8,86	20,2
3rd Sept	8,56	19	3rd Sept	8,47	19,5	25th Sept	9	20,7
4th Sept	8,96	20,7	4th Sept	8,96	20,3	27th Sept	8,9	20,7
5th Sept	8,75	21	5th Sept	8,73	20,9	30th Sept	9,11	20,2
6th Sept	8,98	21,8	6th Sept	8,94	21,8	1st Oct	8,98	21
9th Sept	9,05	22,5	9th Sept	9,27	22,5	4th Oct	8,67	21,5
10th Sept	9,98	21,9	10th Sept	10,3	21,9	7th Oct	9,39	23,3
12th Sept	8,71	20,9	12th Sept	8,9	21	10th Oct	9,8	23,7
13th Sept	9,18	22,4	13th Sept	9,55	22,6	11th Oct	8,88	22,2

Appendix 2. Calculation of mining water amendments

Mining water amendment (1ml)	Algae Mixture Volume (ml)	Percentage of mining water
0ml	300ml	0%
1ml	299ml	0.33%
2ml	298ml	0.66%
5ml	295ml	1.66%
10ml	290ml	3.33%
15ml	285ml	5.26%
30ml	270ml	11.10%
60ml	240ml	20%

2% of 25000ml = 500ml

Appendix 3. Total Nitrogen values for all three Algae Turf Scrubber systems

Total Nitrogen			
Date	ATS 1	ATS 2	ATS 3
29th Aug	19,4	19,4	96,8
2nd Sept	24,8	27,83	7,77
5th Sept			12,12
9th Sept	28,25	25,6	18,95
12th Sept			17,7
16th Sept			30,2
23rd Sept			19,7
30th Sept			23,95
7th Oct			29,35
11th Oct			38,4

Appendix 4. Total phosphorous values for all three Algae Turf Scrubber systems

Phosphate PO4-P mg/l			
Date	ATS 1	ATS 2	ATS 3
19th Aug	check	check	
19th Aug	4,44	4,48	
21st Aug	7,77	7,77	
26th Aug	3,63	4,34	
29th Aug	4,32	4,5	17,9
2nd Sept			7,77
5th Sept			6,05
9th Sept			6,06
12th Sept			7,92
16th Sept			8,91
23rd Sept			5,8
30th Sept			5,96
7th Sept			4,95

Appendix 5. Turbidity values for all three Algae Turf Scrubbers

Date	Turbidity ATS 1	Turbidity ATS 2	Turbidity ATS 3
12th Aug	340,00	400,00	
19th Aug	400,00	300,00	
21st Aug	680,00	780,00	
22nd Aug	87,00	136,00	
26th Aug	116,00	116,00	
27th Aug	62,00	378,00	
28th Aug	67,00	442,00	
29th Aug	56,00	334,00	850,00
30th Aug	40,00	232,00	171,00
2nd Sept	24,00	243,00	318,00
3rd Sept	20,00	197,00	244,00
4th Sept	23,00	167,00	227,00
5th Sept	14,00	157,00	202,00
6th Sept	14,00	157,00	202,00
9th Sept	14,00	24,00	294,00
13th Sept	14,00	19,00	126,00
24th Sept			42,00
25th Sept			77,00
27th Sept			48,00
30th Sept			38,00
1st Oct			47,00
4th Oct			41,00
7th Oct			58,00
10th Oct			99,00
11th Oct			318,00

Appendix 6. Calculating N:P ratios

	TN (mg/L)		**TN (mmol/L)**	**TN (umol/L**		
ATS 1	24.15		1.725	1725		
ATS 2	24.27		1.73357143	1733.5714		
ATS 3	29.49		2.10642857	2106.4286		
		TN/TP (mass)				**TN/TP RA-TIO**
		4.791666667				10.61011905
	TP (mg/L)	4.605313093	**TP(mmol/L)**	**TP (umol/L)**		10.19747899
ATS 1	5.04	3.723484848	0.16258065	162.58065		8.244859307
ATS 2	5.27		0.17	170		
ATS 3	7.92		0.25548387	255.48387		

Example Calculation:

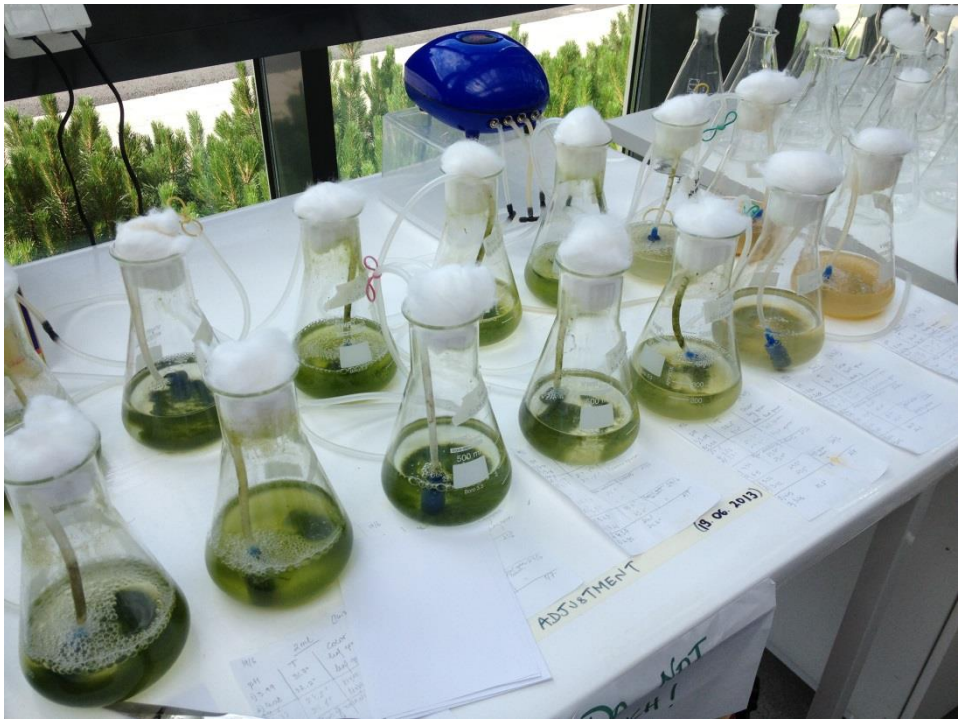
24.15 (mg/L)*1000mmol/14000mg = 1.725mmol/L

1.725 (mmol/L)*1000µmol = 1725µmol/L

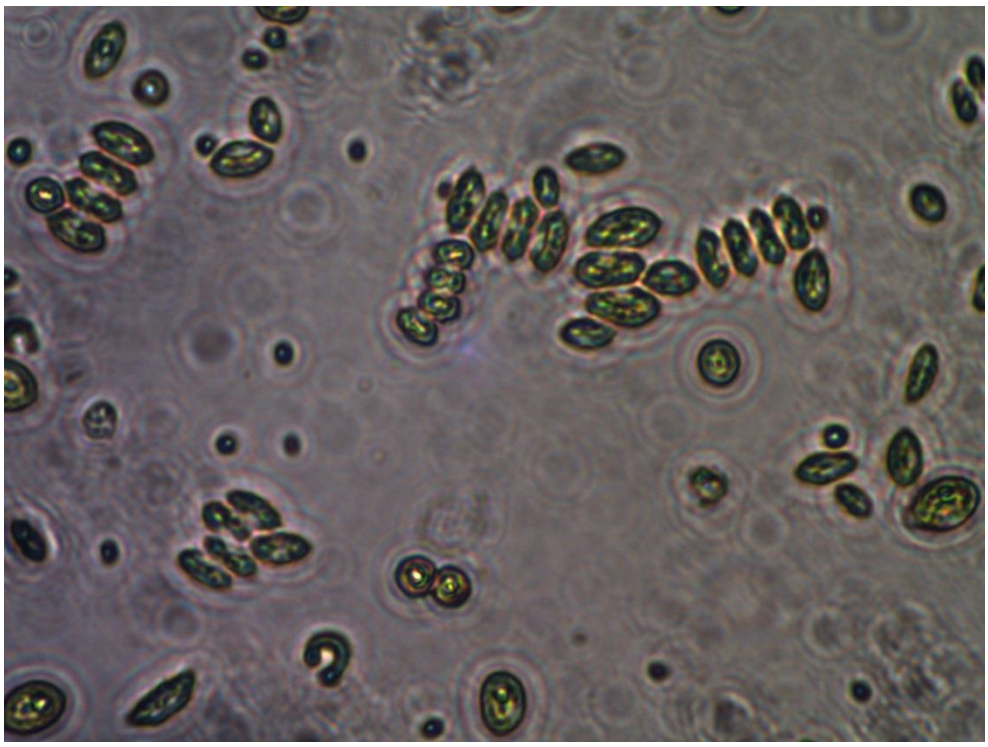
Example Calculation for N:P

1725 (µmol/L) /162.58065 (µmol/L) = 10.610119

Appendix 7 . Erlenmeyer tests (June-July 2013)



Appendix 8 . *Scenedesmus, Navicula, Haemotococcus* and *Synecococcus* species in Algae Turf Scrubber 3



Appendix 9. pH and temperature values from the Erlenmeyer tests (June-July 2013)

Algae and no pH-adjusted mining water

		No pH Adjustment	
	Date	pH	T
0ml	19-Jun	5,30	31,4
		5,34	30,7
	24-Jun	4,26	23,5
		6,27	24,3
	25-Jun	7,00	23,6
		6,63	24,2
	27-Jun	4,35	25,4
		5,75	26,1
	1-Jul	3,93	23,3
		6,23	24,1
	3-Jul	4,32	22,0
		6,00	23,4
	5-Jul	4,15	21,5
		5,63	21,8

		No pH Adjustment	
	Date	pH	T
1ml	19-Jun	4,50	32,5
		4,26	30,2
	24-Jun	4,09	23,8
		4,28	24,1
	25-Jun	5,64	23,8
		6,04	24,1
	27-Jun	4,14	25,5
		4,50	25,8
	1-Jul	4,58	23,8
		4,71	23,7
	3-Jul	4,12	23,0
		4,68	23,0
	5-Jul	4,04	21,6
		4,57	21,7

		No pH Adjustment	
	Date	pH	T
2ml	19-Jun	3,99	31,7
		4,08	32,7
	24-Jun	4,33	24,2
		4,31	21,1
	25-Jun	6,20	24,2
		5,91	24,0
	27-Jun	4,85	25,6
		4,09	25,7
	1-Jul	4,52	23,8
		4,29	23,8
	3-Jul	4,49	23,3
		3,94	22,9
	5-Jul	4,71	21,8
		4,09	21,8

		No pH Adjustment	
	Date	pH	T
5ml	19-Jun	3,46	32,0`
		3,46	30,3
	24-Jun	3,79	24,0
		3,86	24,1
	25-Jun	5,88	23,9
		6,00	23,9
	27-Jun	4,68	25,7
		4,89	25,7
	1-Jul	4,76	23,1
		5,20	24,2
	3-Jul	3,81	22,6
		4,94	23,5
	5-Jul	3,65	21,9
		4,94	21,9

		No pH Adjustment	
	Date	pH	T
10ml	19-Jun	3,19	32,8
		3,18	31,2
	24-Jun	3,32	24,1
		3,35	24,1
	25-Jun	4,34	23,9
		4,38	24,0
	27-Jun	3,63	25,6
		3,66	25,6
	1-Jul	4,37	24,1
		4,35	24,2
	3-Jul	3,92	23,2
		4,36	23,4
	5-Jul	3,88	22,0
		4,63	22,1

		No pH Adjustment	
	Date	pH	T
15ml	19-Jun	3,08	32,0
		3,06	31,0
	24-Jun	3,23	23,7
		3,21	24,0
	25-Jun	4,34	23,7
		4,15	23,9
	27-Jun	3,56	25,5
		3,42	25,5
	1-Jul	4,05	23,7
		3,76	23,7
	3-Jul	3,88	23,2
		3,89	22,7
	5-Jul	3,64	22,0
		4,06	22,1

		No pH Adjustment	
	Date	pH	T
30ml	19-Jun	2,86	32,3
		2,88	32,0
	24-Jun	2,85	23,4
		3,05	23,9
	25-Jun	3,75	23,4
		3,81	23,9
	27-Jun	3,02	24,7
		3,08	25,4
	1-Jul	3,04	22,9
		3,03	24,1
	3-Jul	2,99	22,4
		3,06	23,2
	5-Jul	2,98	21,7
		3,06	22,1

		No pH Adjustment	
	Date	pH	T
60ml	19-Jun	2,79	32,9
		2,77	30,7
	24-Jun	2,88	24,0
		2,88	24,1
	25-Jun	3,68	23,4
		3,62	23,4
	27-Jun	2,73	25,1
		2,79	25,2
	1-Jul	2,76	23,9
		2,80	23,9
	3-Jul	2,78	23,3
		2,81	23,1
	5-Jul	2,81	22,2
		2,82	22,22

Appendix 10. pH and temperature values from the Erlenmeyer tests (June-July 2013)

Algae and pH-adjusted mining water

		pH Adjustment	
	Date	pH	T
0ml	19-Jun	5,97	31,2
		6,02	29,6
	24-Jun	4,56	23,3
		4,88	23,7
	25-Jun	6,63	24,2
		6,62	24,3
	27-Jun	3,97	27,2
		6,73	27,5
	1-Jul	4,01	23,7
		8,03	23,7
	3-Jul	4,25	22,7
		7,54	23,0
	5-Jul	4,53	21,4
		7,45	21,4

		pH Adjustment	
	Date	pH	T
1ml	19-Jun	5,25	32,9
		5,47	31,4
	24-Jun	4,35	23,6
		4,38	23,5
	25-Jun	5,81	24,1
		6,25	24,2
	27-Jun	4,03	27,6
		4,01	27,1
	1-Jul	4,54	23,7
		4,17	23,1
	3-Jul	4,00	23,0
		3,91	22,4
	5-Jul	4,02	21,5
		4,04	21,2

		pH Adjustment	
	Date	pH	T
2ml	19-Jun	4,96	31,9
		5,04	30,1
	24-Jun	4,57	23,8
		4,11	23,3
	25-Jun	6,00	24,3
		6,30	24,0
	27-Jun	5,09	27,0
		5,60	27,7
	1-Jul	5,05	23,8
		6,00	23,9
	3-Jul	4,76	22,9
		5,98	23,2
	5-Jul	4,69	21,5
		6,04	21,5

		pH Adjustment	
	Date	pH	T
5ml	19-Jun	4,50	30,7
		4,62	30,5
	24-Jun	3,97	23,6
		3,94	23,6
	25-Jun	5,02	23,9
		5,39	23,9
	27-Jun	3,80	27,1
		4,22	27,2
	1-Jul	4,16	23,3
		4,61	23,3
	3-Jul	3,78	22,6
		4,03	22,4
	5-Jul	3,78	21,3
		4,05	21,4

		pH Adjustment	
	Date	pH	T
10ml	19-Jun	4,22	31,3
		4,39	30,8
	24-Jun	3,62	23,4
		3,76	23,9
	25-Jun	5,16	23,8
		4,59	24,1
	27-Jun	4,13	27,0
		3,69	27,1
	1-Jul	4,33	23,5
		4,21	23,6
	3-Jul	4,03	22,4
		4,08	22,9
	5-Jul	3,93	21,4
		4,08	21,6

		pH Adjustment	
	Date	pH	T
15ml	19-Jun	4,05	31,4
		4,13	30,2
	24-Jun	3,54	23,7
		3,38	23,4
	25-Jun	4,48	23,9
		4,51	23,8
	27-Jun	3,67	26,7
		3,63	26,5
	1-Jul	3,92	23,4
		3,93	22,8
	3-Jul	3,78	22,5
		3,62	22,6
	5-Jul	3,77	21,4
		3,50	21,4

		pH Adjustment	
	Date	pH	T
30ml	19-Jun	3,81	31,7
		3,85	30,7
	24-Jun	3,70	23,8
		3,5`	23,5
	25-Jun	4,61	24,0
		4,71	23,8
	27-Jun	3,75	25,8
		3,76	26,1
	1-Jul	3,80	23,5
		3,88	23,9
	3-Jul	3,73	22,7
		3,95	23,0
	5-Jul	3,72	21,5
		3,99	21,7

		pH Adjustment	
	Date	pH	T
60ml	19-Jun	3,69	32,1
		3,71	31,5
	24-Jun	3,75	23,4
		3,94	23,8
	25-Jun	4,78	23,9
		4,83	23,9
	27-Jun	3,77	25,5
		4,03	25,8
	1-Jul	3,62	23,4
		4,02	23,6
	3-Jul	3,51	22,5
		3,98	22,8
	5-Jul	3,50	21,4
		3,77	21,6

Appendix 11. Zinc and Nickel concentration result from Atomic Absorption Spectroscopy for ATS 1

ATS 1-Zn					
SAMPLE	REP1(mg/l)	REP2(mg/l)	REP3(mg/l)	AVERAGE	AMOUNT(mg) in the 25l in ATS
Initial Sample	58.4	56.8	56.5	57.2	1430.8
Wed-210813	31.6	32.6	32.1	32.1	802.5
Fri-230813	0.6	-	0.5	0.5	13.8
Mon-260813	2,0	1.3	1.2	1.5	37.5
Wed-280813	0.5	0.6	0.4	0.5	12.5
ATS 1-Ni					
SAMPLE	REP1(mg/l)	REP2(mg/l)	REP3(mg/l)	AVERAGE	AMOUNT(mg) in the 25l in ATS
Initial Sample	30.7	28.7	24.8	28.1	701.7
Wed-210813	34.2	35	34.3	34.5	862.5
Fri-230813	25.8	21.5	27.8	25.0	625.8
Mon-260813	66	68	70.3	68.1	1702.5
Wed-280813	81.9	84.8	88.0	84.9	2122.5

Appendix 12. Zinc and Nickel concentration result from Atomic Absorption Spectroscopy for ATS 2

ATS 2-Zn					
SAMPLE	REP1(mg/l)	REP2(mg/l)	REP3(mg/l)	AVERAGE	AMOUNT(mg) in 25l in ATS
Initial Sample	50.8	51	50.9	50.9	1272.5
Wed-210813	25.5	25.8	26.8	26.0	650.8
Fri-230813	0.1	0.1	0.3	0.2	4.2
Mon-260813	0.9	1.3	0.8	1.0	25.0
Wed-280813	0.4	0.5	0.3	0.4	10.0
ATS 2-Ni					
SAMPLE	REP1(mg/l)	REP2(mg/l)	REP3(mg/l)	AVERAGE	AMOUNT(mg) in the 25l in ATS
Initial Sample	23.6	27.3	28.6	26.5	662.5
Wed-210813	30.5	34.2	33.7	32.8	820.0
Fri-230813	27.5	29.6	31.7	29.6	740.0
Mon-260813	73.9	77.2	80.3	77.1	1928.3
Wed-280813	91.2	93.2	97.5	94.0	2349.2

Appendix 13. Nutrient Solution ratios

10L Solution	
Biobact	12,908g
Urea	0,477g
Glucose	10g
di-sodium hydrogen phosphate	0,444g

20L Solution	
Biobact	25,816g
Urea	0,954g
Glucose	20g
di-sodium hydrogen phosphate	0,888g

50L Solution	
Biobact	64,54
Urea	02,384g
Glucose	50g
di-sodium hydrogen phosphate	2,221g