Efficiency of Algae Combinations in heavy metal removal from wastewaters using photo-bioreactor

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ABSTRACT

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The aim of this thesis was to compare the efficiency of different algal combinations in heavy metals removal from wastewater using algae-based photo-bioreactors. Twelve different strains of algae were divided into four groups and were introduced into twenty-four photo-bioreactor bottles: twelve contained wastewaters only while the other twelve contained wastewaters contaminated with 90 mg of heavy metal. Parameters such as temperature, pH, light and conductivity, which are believed to affect the rate of metal uptake by algae were monitored.

At the end of the experiment, it was discovered that an average of 88 percent of the metal content in the wastewater had been removed. For nickel removal, the best results were obtained with an algae combination of *Anabaena cylindrical* and *planktothrix rubescene* with an average removal rate of 100%. The algae group containing *Anabaena cylindrical* and *Scenedesmus Specie*, on the other hand was more efficient in removing with an average removal rate of 93.3%. The algae biomass was harvested and analyzed, which revealed that more than 50% of the metal removed from wastewater was retained in the algae biomass.

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1 INTRODUCTION

The environmental conditions in many part of the world are deteriorating at an alarming rate. In most large cities the environmental situation is approaching a saturation point where many enviromental pundits have predicted that sooner infrastrutures and technologies available might not be able to cope with the level of environmental pollution. The world's main source of pollutants are industrial effllluent, sewages and farm waste (Lakherwal.D. 2014).

The most serious environment pollution disaster Finland had faced in almost a century was the leakage of wastewater containing heavy metals from a gypsum pond at the Talvivaara industrial mining site in November 2012. It was estimated that more than one million cubic meter of contaminated liquid which mainly contained high concentration of heavy metals such as cadmium, uranium, nickel, copper and zinc overflew the gyspum pond into the mining safety areas and might have found a way into nearby lakes (Nuclear Heritage, 2014).

During the early studies of heavy metals, the initial concerns of environmental engineers and scientist was to understand the reactions and impacts of heavy metals in the environment (Marson.P. 2013). However, since various studies have shown that heavy metals are non-biodegrade in nature and have also been proven to be bio-accumulative and carcinogenic (Srivastava et al., 2006). The focus of concerns has shifted from its impacts and effects to remediation and recovery especially from waste and contaminated waters.

There are quite a number of methods that can be used to remove heavy metals from wastewaters; these methods include chemical precipitation, ion exchange, reverse osmosis, electro-dialysis, ultra-filtration, nano-filtration, coagulation, flocculation etc. However these methods have several disadvantages; such as high reagent requirement, unpredictable metal ion removal and generation of toxic sludge (Srivastava et al., 2006). Biosorption and bioaccumulation mechanisms used by algae to remove heavy metals have proven to be more economical, effective, versatile and above all more environmentally friendly when compared with other conventional methods (Abass et al 2014).

1.1 Theoretical background

1.1.1 Algae

Algae is a word used to classify a large group of organisms, algae consist of different taxonomic division of organisms but can be generally referred to as aquatic plants that are mostly photosynthetic and oxygenic autotrophs (John and Maggs, 1997). Algae exist in various structural forms with a very large size disparities, as they can exist in minute forms as small 0.2μm in Pico-planktons to as tall as fifty meters in kelps (Norton et al., 1996).

Just like plants, most algae are autotrophs i.e. they convert energy from sunlight, carbon dioxide and a few nutrient such as nitrogen and phosphorus to into biomass, while some can also survive as heterotrophs i.e. photosynthesize in the absence of sunlight by using starch or sugar instead of sunlight or a mixture of both process known as mixtrophics (Andrea L.A, 1994). This rare characteristic exhibited by algae has made it possible for them to survive in most extreme and sterile environments around the world.

The study of algae is called phycology. During the early studies of algae, it was discovered that algae are important producers of organic matter in the lower base of the aquatic ecosystem food chain (Bold and Wynne 1985) and because of their vast number present in the ocean, it is estimated that more than 60 percent of global oxygen is produced by algae during photosynthesis (Jack Hall, 2011), but recent studies have shown that the importance of algae in nature are far beyond being just a producer in the aquatic food chain or a mere supplier of global oxygen.

Studies have now shown that algae posse high oil content that is rich in valuable nutrient such as vitamins, protein, fatty acid, antioxidants, pigment and sterols (Hu et.al 2013). Some marine microalgae are being used as an essential food source for protein in many part of the world; Countries like China and Niger have a long history of including some algal specie as an essential part of their daily menu (Kim Se Won, 2011) while some species of algae have proven to be useful in the medical field in producing drugs to treat different diseases, a good example of this are some strain of cynobacteria that produces some compounds that can act as anti-tumour (Graham and Wilcox, 2000).

In-depth studies into the Physiochemical composition of algae had helped to reveal the usefulness of microalgae in the field of environmental pollution control, especially in the area of heavy metal removal from domestic and industrial wastewaters. Some algae have been observed to have shown extra tolerance and survived in water polluted with relatively high concentration of heavy metals. Analysis of the physiochemical composition of algal cell shows that algae have ability of binding and accumulating heavy metals through various mechanisms such as cell wall binding, chelation with phytochetalins (PCS), vacuolar compartmentalisation and cell accumulation(Hagan and Kristina, 2009) .

1.1.2 Heavy Metals

Heavy metals are toxic non-biodegradable metallic chemical element with atomic mass greater than 22 and a specific gravity index of more than 5.0 g/ml. Heavy metals exists naturally in the earth crust but become problematic when exposed and it come in contact with the soil, air or water (Perpetuo et. al. August 2011).

Although there are few cases where heavy metals are released into the environment by natural agents such as wind and flood, most recorded cases of heavy metal pollution are by various anthropogenic activities especially mining. Metals can exist in soil and water solution in free forms (e.g., Ag^{2+}, Zn^{2+}, Al^{3+}) or in complex organic and inorganic ligands ($CdHCO_3^+$, $ZnCl^+$, $CdCl_3^-$). Ligands is a term used to describe the association of atoms or molecules while a complex is a geometrical arrangement of atoms or molecules bonded by a centrally located metal ion to form a chemical unit (McLean and Bledose, 1992).

In most cases heavy metals present in the air and soil ends up in water bodies, due to precipitation and water run-offs. Heavy metals in water sources pose a great threat to the health of all living creature especially humans as it has been found to be bioaccumulative and carcinogenic (Srivastava et. al. 2006) and acute metal intoxication can also lead to the damage of the central nervous function. Cardiovascular and gestroinstestinal system disorder, kidney, lungs, endocrine glands malfunction are all diseases associated with heavy metal intoxication (Lakherwal, 2014).

Copper and nickel are of interest and would be investigated during this project, because they were among the metals released into the environment during the Talvivaara mining site pollution disaster and also there have been previous studies on these metals at Tampere University of Applied Science with useful background information.

Nickel and copper are essential trace minerals needed daily by the human body. Trace amount present in water solution do not pose any threat to humans as the human body can regulate their level homeostatically. However, ingesting a large or acute dose can be harmful. Nickel and Copper are phytotoxic (Poisonous to plant) and have been studied to be bio-accumulative in the aquatic food chain (Wase and Foster, 1997).Nickel is an unreactive element while copper is moderately reactive, nickel is not soluble in most acidic solution at room temperature and does not combine with oxygen and water but becomes more reactive at higher temperature. Copper combines readily with oxygen and water and dissolves in most acidic and alkaline solution at room temperature (Chemistry Explained,Undated).

1.1.3 Biosorption and bioaccumulation

The main mechanism algae use for metal removal is called biosorption. Biosorption is a term used to describe the physiochemical properties of a certain biological material to bind and remove none easily degradable pollutants (mostly metals and metalloids) from a solution (Glad, 1990).

Biosorption is generally regarded as a quick metabolic independent binding of metals to its cell which can either be ionic and covalent. Precipitation or crystallization of metals around the cell wall of algae could also be considered as biosorption but in most cases it is highly dependent on the cell metabolism, therefore can be best described as bioaccumulation because metal uptake that requires energy before it can be transported should not be considered biosorption (Graham et. al.,1991).

There are two phases involved during the metal accumulation by algae; Biosorption and bioaccumulation. During the first phase, metals are rapidly bound to the cell surface by a metabolism independent mechanism and usually preceded by a much slower metal binding phase which is caused by simultaneous increase in growth and surface and surface adsorption, active or intercellular uptake by active diffusion (Graham et. al., 1991).

The principle of metal absorption and accumulation can also be distinguished by understanding the method algae uses to transport the metal across its cell wall. According to Robert Mason (2013), metals are transported across biological membranes of aquatic organisms through three transportations mechanisms; Passive diffusion, facilitated transportation and active uptake. Passive diffusion and facilitated transportation are mechanisms employed by algae in moving metals across its cell wall during the biosorption phase. These transportation mechanisms make it possible for dead algal cell to be able to uptake heavy metals because their occurrence depends highly on the structure of the algal cell and does not require any form of energy while the active process used during bioaccumulation phase depends solely on the availability of energy source to facilitate the transportation process (Robert Mason, 2013).

1.1.4 Reason for algae use in removing heavy metals from wastewaters

Algae use during wastewater treatment process has mainly been to help eliminate or reduce the concentration of heavy metals from wastewaters. Through extensive research, it has been discovered that many algae species posses' features which they can use to effectively extract heavy metals from contaminated wastewaters. One of these features is the ability to bind metals on their cell surface. Studies have shown that algal cell walls carry a negative net charge due to the presence of Carboxyl (-COO-) and phosphate and other groups used for bonding metals through ion exchange (Rai and Gaur, 2001).

Some species of algae secrets a special kind of substance called ligands. Ligands are ion or neutrally charged molecules that bond to a central of metal or metalloids ion. The bonding of these ligands to metal makes them less available around the cell's environment. They are also able to revitalize themselves during a metal induced damage. When metals bond to the algal cell, it damages its protein structure and breakdown the oxidative balance in the cell, thereby producing antioxidants such as ascrobate peroxide (APX). The extent of damage caused by metal bonding can be estimated by the amount of antioxidants and protein produced in the cell while the ability to defend itself against the damage defines the algae's tolerance capacity. Excretion and exclusion of metal from cell, protein such as proline and other binding factor production such as metallothioneins (MTs), glutathione (GSH) are some mechanisms employed by algae to help counter-act the metal induced damage (Zhang et al., 2008).

It is quite interesting to note that both living cells and dead biomass can be used to remove heavy metals from contaminated waters, as both have the ability to absorb metals available in their surroundings. However, Living micro-algae cell are more efficient during wastewater treatment because of its ability to uptake more metals using both bio- absorption and bio-accumulation mechanism and also its ability to retain the metals absorbed for a longer period of time (Hu et. al., 2006).

Micro-algae species found to have survived in sites contaminated with high level of heavy metal concentration are known to posses the ability to accumulate more metals than those found on non contaminated sites. As living but immobilized microalgae have also been observed to be more efficient in heavy metal absorption than the free living microalgae cells (Hu et. al., 2006)

1.2 Factors effecting the rate of metal uptake

There are quite a number of factors and parameters that are capable of influencing the efficacy during biosorption. Some are associated to the metals and biomass while others are external factors from the environment. In order to achieve optimum metal removal, these parameters needs to kept at conditions that supports algal growth (Ajena et. al., 2007).

1.2.1 Temperature

Temperature range of 20 - 35º C does not influence the rate of biosorption but an increase in temperature such as 50 º C may damage the algal living cell and this might lead to a decrease in the rate of metal uptake. Since adsorption reaction is exothermic, the rate of adsorption should increase as the temperature decreases. Although some studies have shown that there could be a relative increase in metal uptake as temperature increases but this has been recorded in very isolated cases (Abass et. al., 2014).

1.2.2 Characteristics of biomass

The nature of biomass is an important factor that determines the volume of metal that can be absorbed in a solution by a certain biomass. There have been strong evidences from examining different microbial biosorption systems that living cells biomass are more effective that dead cells biomass while the immobilized but living biomass are also more efficient for metal uptake than their free moving counterparts. Other biomass characteristics that could influence the rate of biosorption are biomass growth, nutrition and age which is due to changes in cell size, wall structure, etc (Abass et. al., 2014).

1.2.3 pH

The pH is unarguable the most important parameter influencing rate of biosorption. Since the process undergone by microbes during biosorption is quiet similar to ion-exchange but in this case the biomass acts as the material of exchange; microbial biomass contains material which are generally weak acidic and basic in nature (Abass et. al., 2014). The study of biomass surface charge showed that the availability of free binding site is highly influenced by the pH of the solution (Dwidvedi .S. 2012), the solubility of metal is also highly dependent on the pH of the solution. According to Abass et al. (2014) the rate of biosorption by any type of biomass is expected to decrease, if the pH of the solution decreases from 6.0 to 2.5 and there will be no noticeable metal removal once the pH of the solution is less than 2.

1.2.4 Biomass concentration

Biomass concentration in solution can influence the specific uptake (Perpetuo et. al., 2011). The increase in biomass concentration increases the electrostatic between the cells, hereby decreasing the amount of metal uptake (Gadd et al., 1988) but Fourest and Roux (1992) refuted this hypothesis and insinuated that the decrease in the specific metal uptake was due to low concentration of metals in the solution.

1.2.5 Availability of other metals

The ultimate goal behind many of the numerous biosorption researches is to develop an efficient biosorption process that can be applied in an industrial scale to remove heavy metals from wastewaters; which might contain more than one heavy metal. Sakaguchi and Nakajima (1991), claimed the presence of manganese, cobalt, copper, cadmium, mercury and lead did not affect the uptake of uranium from the same metal solution by bacterium, Fungus and yeast but noticed that the uptake of cobalt by these set of microbes was completely inhibited by the presence of other metal ion. Tsezos and Volesky (1982) also observed that the presence of Zinc (Zn^{2+}) and Iron (Fe^{2+}) inhibited the uptake of uranium by *Rhizopus arhizus.*

2 AIMS OF THE PROJECT

During this project, algae photo-bioreactor will be used for heavy metal and nutrient sequestration from wastewater. The Following questions were of interest during the planning of the project.

1. Are algae able to remove heavy metals from wastewaters and what is the removal efficiency of algae Photo-bioreactors?
2. What combination of available algal strain can be use for optimal removal for each metal tested?
3. Is there a correlation between pH and metal removed?
4. How much of the metal removed can be found in the algae biomass?
5. What is the effect of algae’s biomass concentration during metal uptake?

3 MATERIALS AND METHODS

3.1 Materials

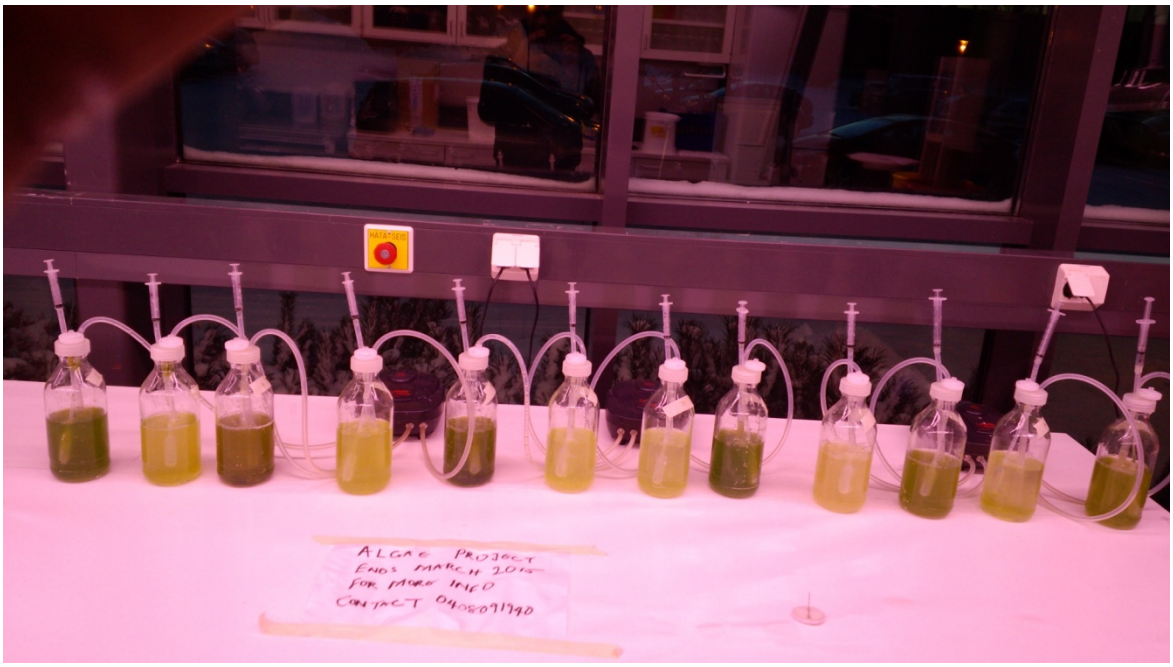
Wastewater samples used during this project were taken from the Viinikanlahti wastewater plant in Tampere, Finland. It should be noted that the Viinikanlahti wastewater treats only domestic wastewaters, so therefore the wastewater was not expected to possess a high concentration of heavy metals.

3.1.1 Algae strains

Algal strains used in this project were supplied by SYKE. Below is the list of algae used during this project, more about these algae has been written in a previous thesis by Chuoaib Benchraka " The role of algae in heavy metals removal from mining wastewaters".

1) *Selenastrum capricornutum*
2) *Pediastrum simplex*
3) *Anabaena cylindrical*
4) *Scenedesmus sp.*
5) *Chlorphyta sp (Pekari strain) Blue green*
6) *Purpuraemus sp*
7) *Haematococcus*
8) *Planktothrix rubescence*
9) *Chlorella pyrenoidosa – green algae*
10) *Desmodesmus subspicatus*
11) *Golekinia brevispicula*
12) *Crucigenia tetrapedia*

As it had been stated in the theoretical background that age of biomass also affect the rate of biosorption. It is therefore, important to state that prior to the start of the experiment, the algae supplied by SYKE had been cultivated for two months, in order to generate enough biomass required for experiment. The algae strains were cultured separately in 600ml bottles which were filled up to 300ml. The 300ml level was maintained throughout the cultivation phase as water loss due to evaporation was replaced to the above said level. 1ml of fertilizer substral was added to the stock once a week to help supply nutrients to the algae.



Picture 1: Algae cultivation set-up; showing the 300ml fill up level of the mother stock

3.1.2 Algae Photo bioreactor

Algae Photo bioreactors are basically closed or open equipment that enables phototrophic microorganism such as algae to be cultivated outside their natural environment (algen.si). A closed photo-bioreactor system was used during this project because it provides an environment where all parameters could be controlled and effectively monitored.

Picture 2: Experimental set-up of algae photo-bioreactor bottles

It is essential to have a uniform condition in the photo-bioreactors, this makes it imperative to have mixing mechanism that enables nutrients and biomass to be mixed evenly, as it also helps to avoid flocculation and sedimentation of the algae. In order to achieve this mechanism, three holes were drilled on the cap of the bioreactor bottles. One served as an inlet for the aeration tube which facilitates the mixing while the other two holes were used as water sample extraction outlet and air circulation outlet respectively.

3.2 Methods and procedures

3.2.1 Initial Readings

A two experiment implementation phases of three weeks each was planned for both nickel and copper testing. It was intended that testing of parameters such as light intensity, nitrate, phosphate, total nitrogen, pH, temperature, conductivity and nickel concentration would be carried out, twice a week within a three and four day intervals. Prior to the introduction of the algae strain into the wastewater, the initial values of parameters that were intended to be tested during the course of the project were taken and it readings are presented in table 1 and table 2 below.

Table 1: Initial values wastewater parameters during nickel test

pH	Conductivity	Temperature	Nitrate (NO_3^--N)	Phosphate (PO_4^3)	Total Nitrogen	Nickel
7.2	1073 µs/cm	22.1º C	210 mg/L	32 mg/L	717 mg/L	0mg/L

It was observed after subsequent testing during the nickel implementation that the introduction of different algal combinations into the wastewater group samples induced some changes in the concentration of their mineral contents. Therefore a more comprehensive initial wastewater parameters testing was done during copper implementation.

Table 2: Average values of initial wastewater parameters during copper test

Sample Code	Phosphate Av. (mg/L)	Nitrate Av. (mg/L)	Total Nitrogen (mg/L)	pH	Conductivity (µs/cm)	Temp. ˚C	Cu (mg/L)
A group	19.4	36.5	90	7.2	1100	21.8	0
B group	10.5	22	54.8	7.4	822	21.2	0
C group	36.5	18.5	48.7	7.2	802	22.2	0
D group	8.75	17	45.5	7.2	903	21.8	0

The HACH system was used to measure nitrate, phosphate and total nitrogen concentrations. The HACH Lange Kit providing a measuring range 1-16mg/L was used to measure the total nitrogen after the wastewater sample had been digested in a digester at 100ºC for an hour to help convert inorganic nitrogen to its organic form. Phosphate was measured with HACH PhosVer 3 (Ascrobic Acid) method with a measuring range of 0.02 to 2.50mg/L PO_4^{3-} while the nitrate was measured with HACH Cadium reduction method with a measuring range of and 0.3 to 30 mg/L NO_3^--N.

The nickel and copper concentrations in initial wastewater samples during were measured with an AAS (AAnalyst 400) manufactured by Perkin Elmer. 232 frequency wavelength was used to measure Nickel while 216.5 was used for Copper.

3.2.2 Photo-bioreactors Coding and arrangements

Twenty four photo-bioreactor bottles were used for this project. The bottles were divided into group A to D. Group A to D had 6 bottles each, which were further divided into two subgroups of 3 bottles using alphanumeric codes 1a's to 1c's and 2a's to 2c's for easy individual identification. The cultivated algal strains were also divided into a four group combination. Group one contained all twelve algal strains, group two contained four algal strains, while group three and four both contained two combinations of algal strains as it is described in Table 4.

It was intended that every bottle in each group would contain wastewater sample and the same algal combination i.e. photo-bioreactor bottles in group A would contain wastewater and group 1 algae combinations but metals will only be added to the bottles in the subgroup of A2a to A2c. The same combination pattern was repeated for group B C and D with algal group combination of four, two and two respectively. Below is a table that further explains the combination arrangement.

Table 3: Photo-bioreactor bottle coding arrangement with metal and algal combination

Bottle Code			Algae Combination	Metal Concentration
A1a	A1b	A1c	All Algae Strain	Blank
A2a	A2b	A2c	All Algae Strain	30mg/L
B1a	B1b	B1c	*Selenastrum capricornutum* *Pediastrum simplex* *Scenedesmus sp.* *Haematococcus*	Blank
B2a	B2b	B2c	*Selenastrum capricornutum* *Pediastrum simplex* *Scenedesmus sp.* *Haematococcus*	30mg/L
C1a	C1b	C1c	*Anabaena cylindrical* *Scenedesmus sp.*	Blank
C2a	C2b	C2c	*Anabaena cylindrical* *Scenedesmus sp.*	30mg/L
D1a	D1b	D1c	*Anabaena cylindrical* *Planktothrix rubescence*	Blank
D2a	D2b	D2c	*Anabaena cylindrical* *Planktothrix rubescence*	30mg/L

All bottles were filled with 3 litres of wastewater samples. 50ml of algae was transferred from each mother stock to the algae group which was later divided proportionally among each bottle. Group A received a total of 600 ml of combined algae from the 12 algal mother stocks; group B received 200ml of algae stocks while group C and D received 100ml algae combination each. According to the metal concentrations in the initial wastewater sample which were recorded in table 1 and 2, it indicated that there was no measurable metal present in the wastewater; this prompted the addition of 30mg/L of nickel and copper to all bottles in the metal subgroup. Hence, since the volume of wastewater was 3 litres, a total volume 90ml of nickel and copper were added to each bottle in the metal subgroup.

Due to the number parameters needed to be tested on each test day. It was impossible to perform all the required testing in a day, so samples for AAS testing for Ni and Cu concentration for each test day were preserved to be tested at the end of the experiment. Hence, 100ml of samples were preserved with 1ml of nitric acid and were stored at temperature below -6ºC. Nitric acid reduces the pH of the solution to a pH value of 2 and most metal do not precipitate at low pH and so will it concentration would remain constant would remain normal in the solution.

3.3 Biomass Harvesting

The nutrient and metal sequestration phase lasted for three weeks. At the end of the three weeks, it was assumed that the algae should have absorbed some nutrient and metals from the wastewaters. In order to ascertain the amount of metal concentration which should have been absorbed or adsorbed during these test periods, the algae were harvested and their biomasses analyzed for metal content. The biomass harvested was also used to calculate the amount of biomass generated during the experimental time frame.

To get the biomass out, water samples left in the bottles were centrifuged. Centrifugation, forced the biomass to settle at the bottom of the centrifugal tube and water in the tube was easily extracted with the aid of a pipette. For optimum biomass recovery from the tubes; ethanol was used to further recover biomass that got stuck while transferring from centrifugal tubes to the evaporating dish. Ethanol was used in preference to water because it will not dilute the metal concentration in the biomass and it also has high volatility.

In order to obtain only the algae biomass and also to determine the actual mass of biomass generated in each bottle. The biomasses were transferred into evaporating dishes and were placed in a drying oven for 24 hours at a temperature of 55ºC for continual water and liquid content removal. Before the biomasses were transferred the weight of each evaporating dish was taken and was retaken after the biomass transfer. The final weight of the dishes containing the dried algae biomass was taken to ascertain actual mass of the biomass. Biomass harvested was not 100% algae as it also contained some organic matter from the wastewater.

3.4 Digestion of biomass

One of the aim of the project was to ascertain what concentration percentage of the removed metal was retained in the biomass. In order to achieve this, wet digestion method was used to extract metal present in the biomass and it concentration measured with the AAS. About 100mg of dry biomass was taken from each metal sample and were transferred into 50ml volumetric flask containing 10ml of nitric acid (HNO_3) and 3ml of hydrochloric acid (HCL) and were allowed at a 100˚C for one hour.

During the boiling period, approximately 10ml of extra HNO_3 was added to each sample. This was done in order to maintain a 5ml solution range in each volumetric flask as solution in all samples evaporated below the 5ml required. The solution left after the boiling period were filtered and diluted appropriately before being measured with the AAS.

4 RESULTS AND DISCUSSIONS

Below are tabular and graphical representations of nickel concentrations and pH of the metal groups as recorded during various testing days.

4.1 Results and discussion

4.2 Nickel test

Samples for nickel analysis were preserved from all bottles used during this experiment phase but it was discovered during the AAS analysis of preserved blank samples still had 0mg metal concentrations, so therefore their results are insignificant and would not be displayed or discussed in this section.

Table 5: Nickel concentrations in water samples during test days and final % removed

Sample Code	0 day (mg/L)	4 day (mg/L)	7 day (mg/L)	10 day (mg/L)	13 day (mg/L)	17 day (mg/L)	% Removed
A2a	30	12.9	9.5	7.8	11.8	11.6	61.3
A2b	30	10.5	10.6	11.8	17.2	19.4	35.3
A2C	30	8.7	8.2	6.3	10.7	11.9	60.3
B2a	30	10.7	4.0	4.8	2.2	1.5	95.1
B2b	30	8.3	6.1	2. 7	1.4	1.8	93.6
B2c	30	0	2.2	0	0	0	100
C2a	30	4.2	2.4	1.4	0.1	3.7	87.6
C2b	30	17.2	10.0	4.0	1.4	0.2	99.3
C2c	30	4.1	2.1	0.4	0.1	0	100
D2a	30	9.3	3.9	1.2	0.2	0	100
D2b	30	10.1	4.6	1.4	0.3	0	100
D2c	30	9.9	4.0	0.6	0	0	100

Table 4 above shows the nickel concentration in wastewater samples during different test days and also the removal percentage of nickel concentration in each individual sample bottle. Although all samples recorded above 65 percent of nickel content removed except A2b but it could be observed that there is a huge variation in nickel content removed among the groups at the end of the experiment. Percentage range of nickel removed was between 35.3% and 100 % in individual samples, while the mean percentage of nickel removal was 76.2%.

Figure 1: Graphical trend of nickel concentration in samples during different test days

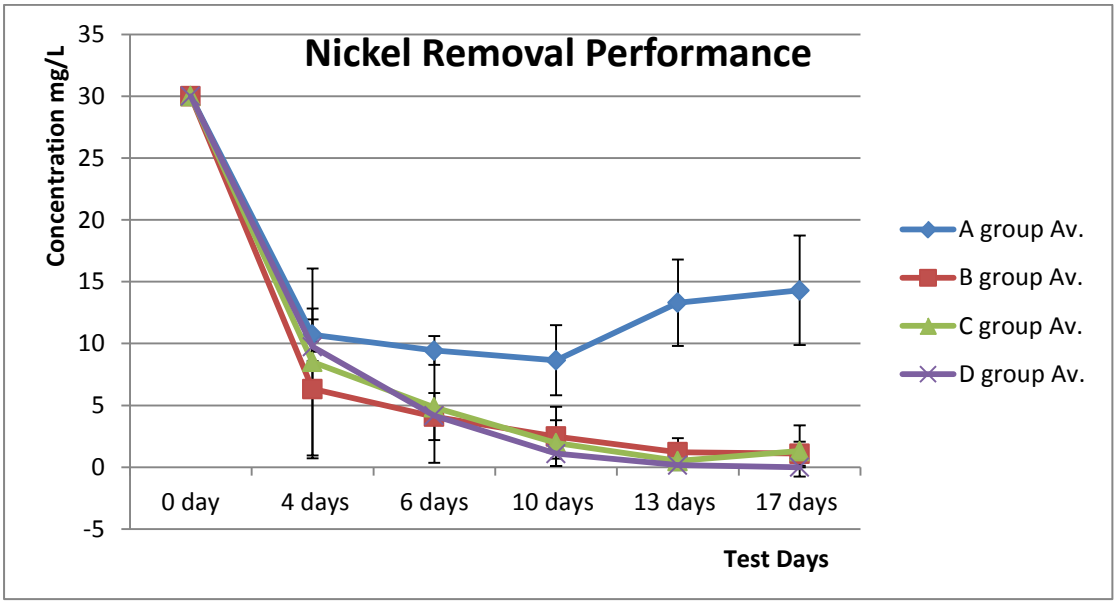
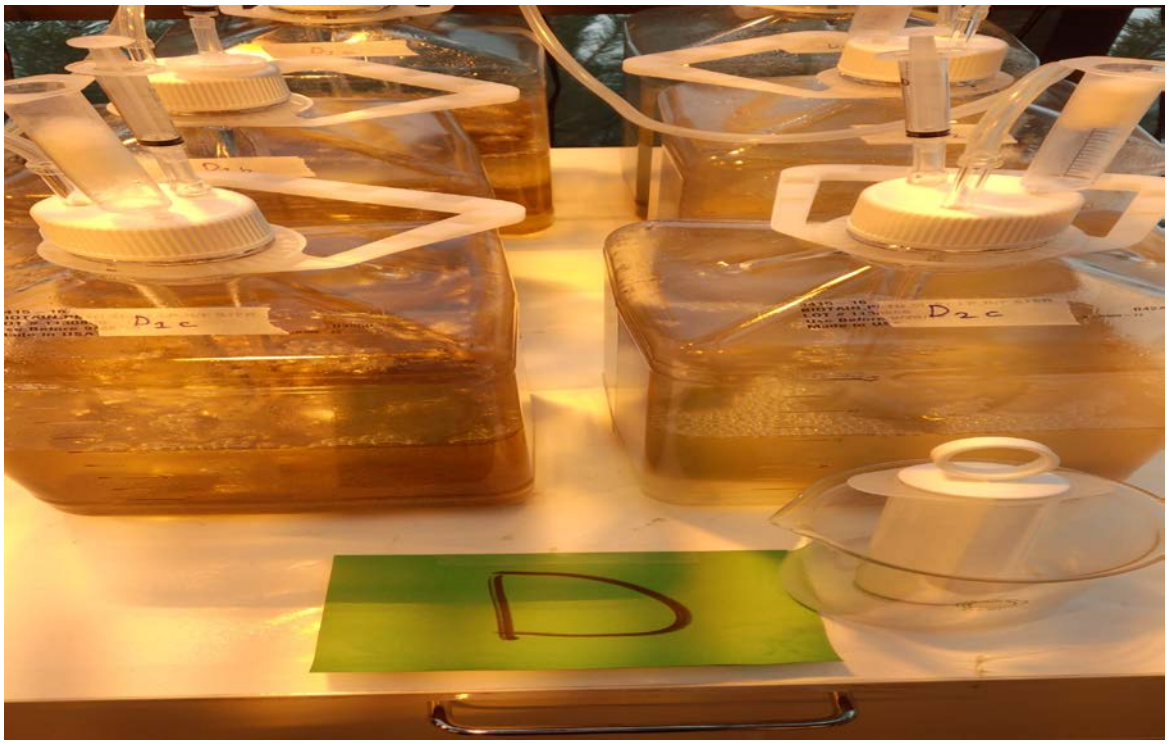


Figure 1 above is a graphical representation of the average nickel concentration present in each sample group during different test days with deviation marks showing the variance between the concentration values. It could be seen from the graph that there was a rapid uptake of nickel by algae between 0 and 4th day, the nickel concentrations by more than 65% of initial concentration in all sample groups which was then preceded by a more slower uptake which lasted through the experimental periods. According to Graham et. al., (1991) there are two phases involved during metal uptake by algae, biosorption; when metals are rapidly bound to the cell surface by a metabolism independent mechanism which is usually preceded by bioaccumulation; a much slower binding phase which is caused by a simultaneous increase in growth and surface adsorption, active or intercellular uptake by active diffusion.

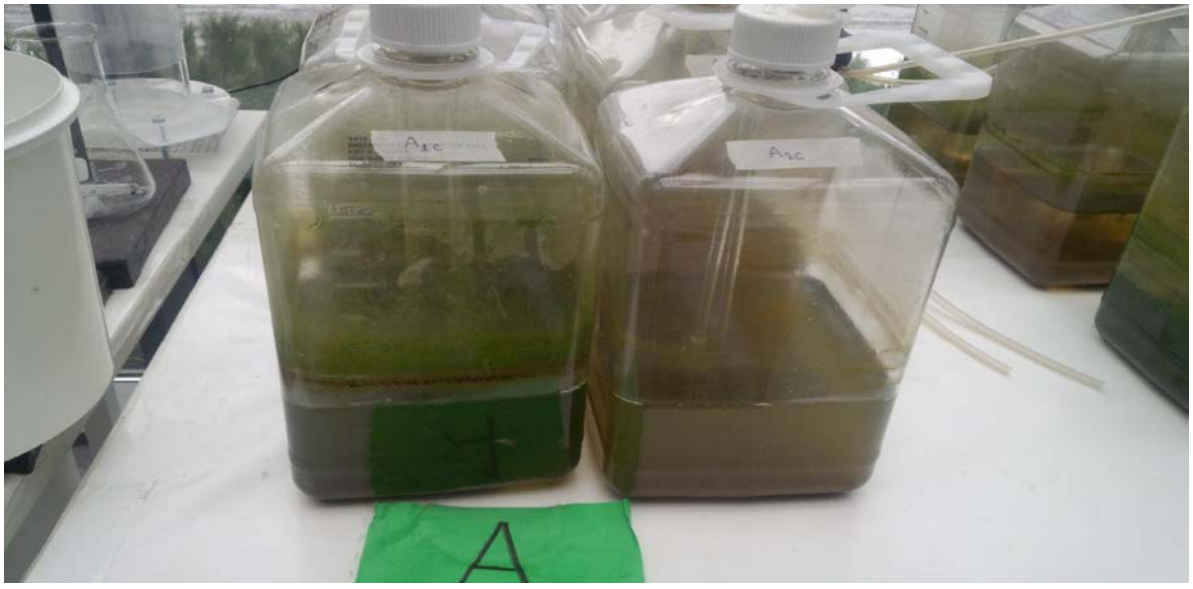
4.3 Observations during nickel testing

The wastewater collected from the wastewater treatment plant was murky and had a thick brown colour. Few hours after the experiment was set-up, some solid matter was noticed to have settled at bottoms of the PBR bottles but all samples still maintained their brown colouration. Twenty-four hours after the experiment was set up, wastewaters in Blank samples were relatively clear with no intense colour while those with nickel still had high suspended colloidal particles and were still murky as seen in picture 3.



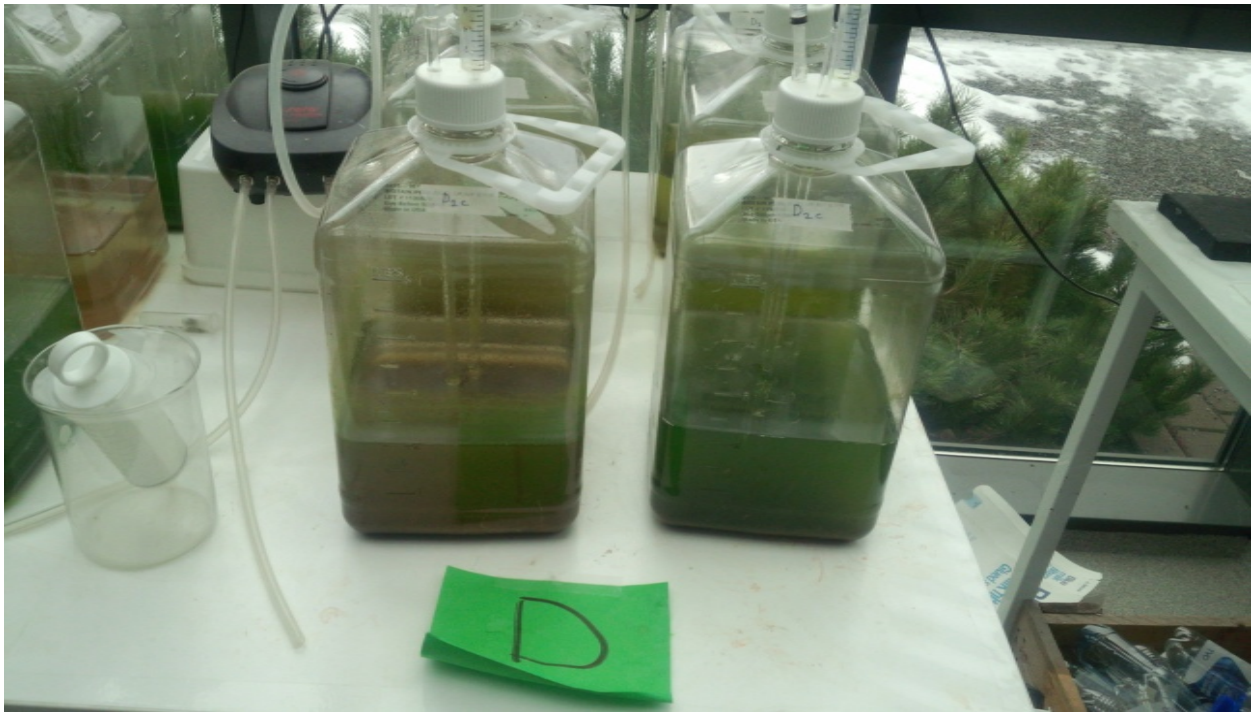
Picture 3: Physical difference between Blank and metal samples after twenty-four hours

Colloids are negatively charged particles which will continue to be suspended in a liquid if its zeta potential (electro-kinetic potentials of a suspended particle) is below or above ±30 mV (Al. Qasim et. al. 2012). Although zeta potential was not measured during this experiment but it could be presumed that the presence of nickel in the samples had affected its zeta potentials, therefore keeping the colloids in a more stable state than the blank sample.

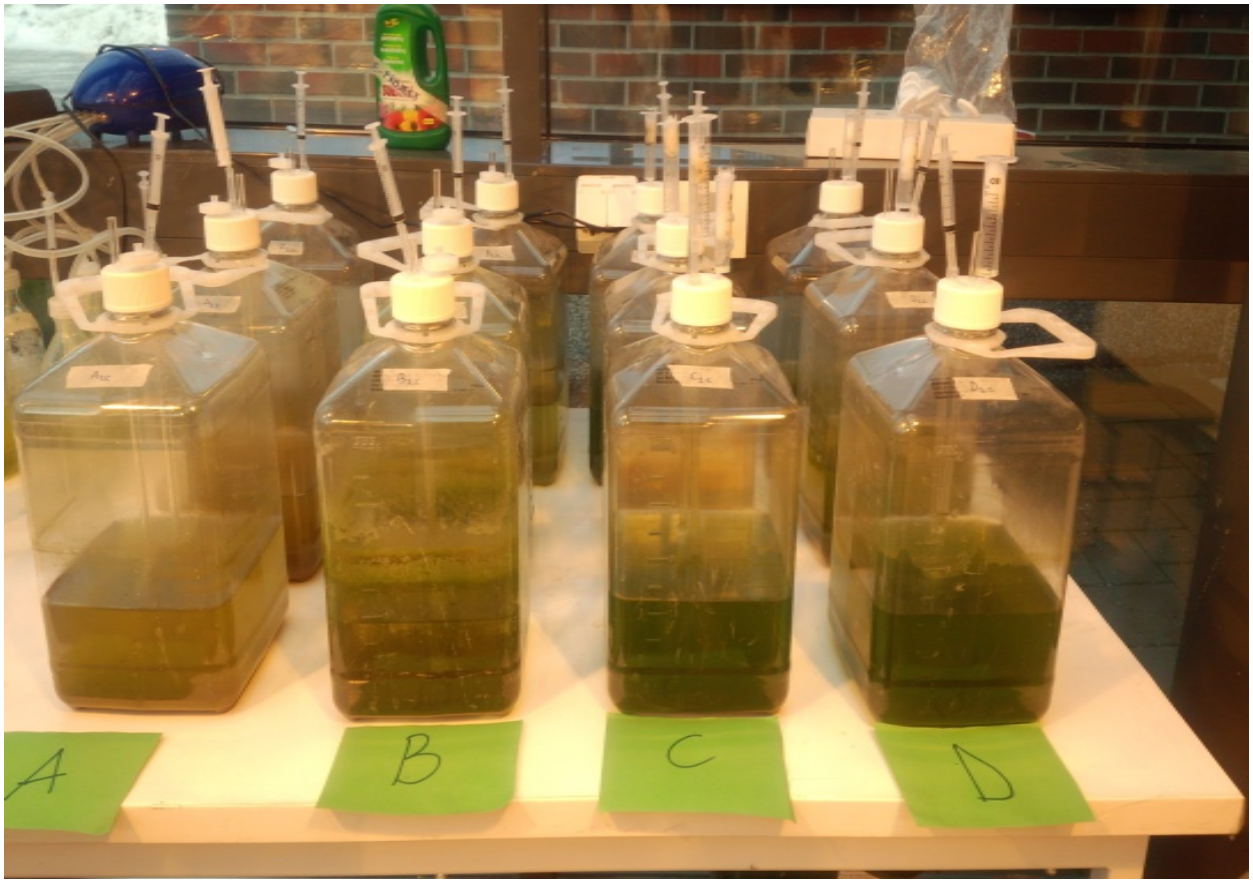


Picture 4: Physical condition of samples showing the effect of presence of nickel in biomass growth after 10 days of the experimental set up; left PBR (blank sample) and right PBR (metal sample)

Picture 4 shows the condition of sample groups on the 10th day after the experimental start-up, green colouration which indicates algae growth could be noticed in the blank samples while most of the metal samples were still characterized with high turbidity.



Picture 5; State of the most blank and metal samples on the 17th day before harvesting



Picture 6; State of metal samples on 17th day of nickel implementation

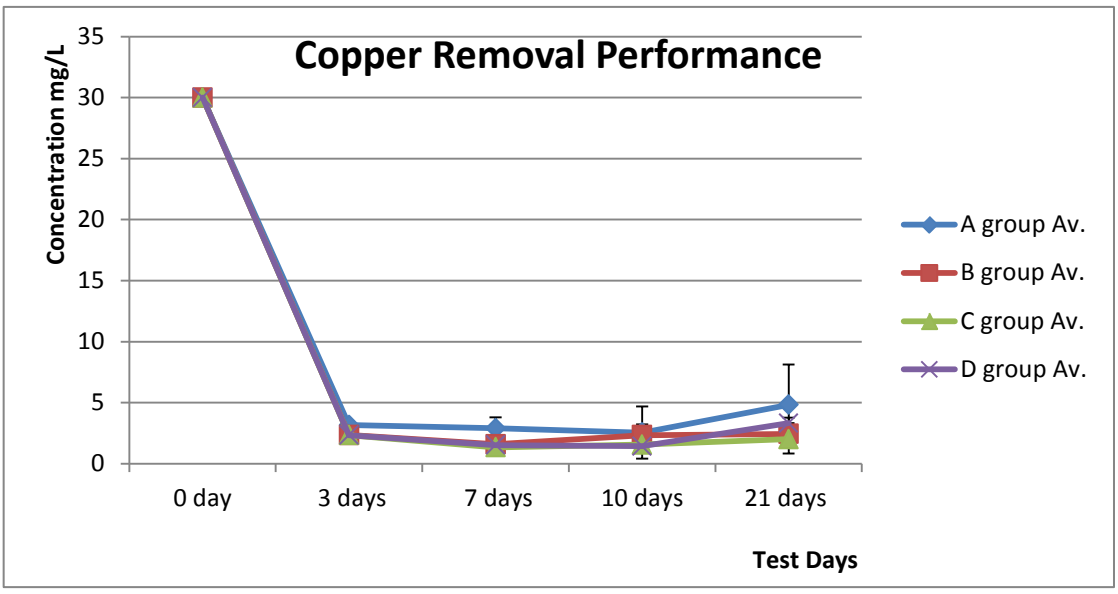
Between the 12th and 17th day, there was a rapid growth in the algae biomass. It could be seen from picture 5 (left side) that there was no visible difference between blank and the nickel sample in group D except group A which according to readings still had a considerable high amount of nickel. It could then be concluded that once heavy metal are successfully accumulated in the algae biomass, it does not affect the biomass growth because the algae in nickel samples looked as healthy as those in the blank sample.

4.4 Copper results

Table 6: Copper concentrations in water samples during test days and final % removed

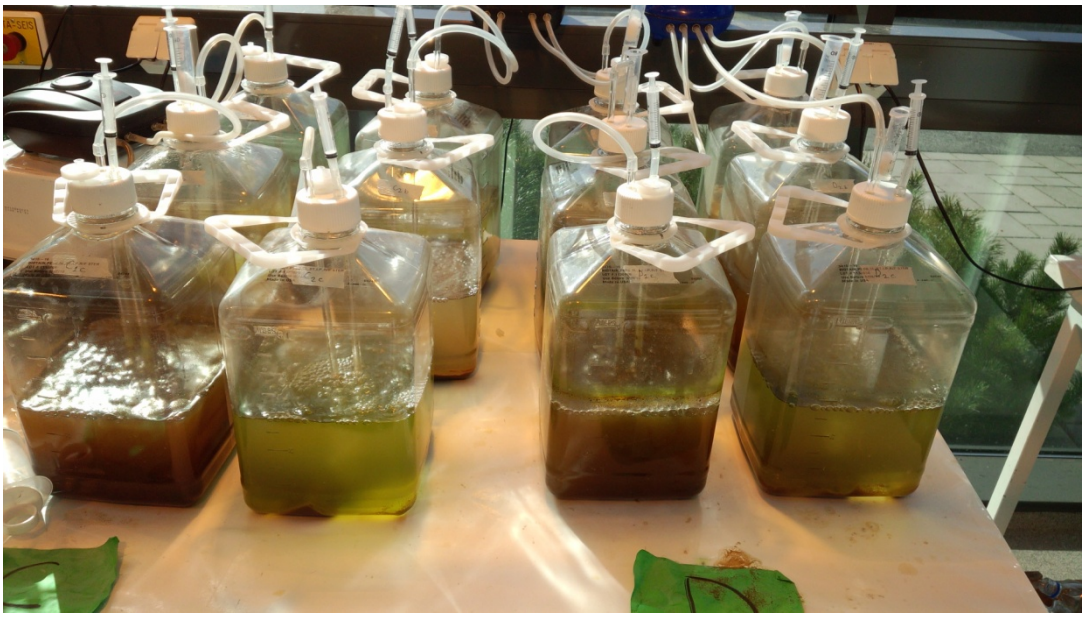
Sample Code	0 day (mg/L)	3 days (mg/L)	7 days (mg/L)	10 day (mg/L)	21 day (mg/L)	% Removed
A2a	30	3.4	3.3	1.6	4.0	86.6
A2b	30	2.9	1.9	1.1	2.0	93.3
A2C	30	3.2	3.6	5.0	8.4	71.8
B2a	30	1.8	1.7	1.9	2.3	92.1
B2b	30	2.2	0.9	1.7	3.3	88.8
B2c	30	3.1	2.1	3.4	1.6	94.6
C2a	30	2.6	1.4	1.2	1.2	96.0
C2b	30	2.8	1.9	2.2	3.4	88.8
C2c	30	1.7	0.8	1.3	1.5	95.0
D2a	30	2.7	2.0	1.3	3.9	87.2
D2b	30	2.2	1.4	1.8	3.0	90.1
D2c	30	2.1	1.3	1.2	3.2	89.4

Figure 2: Graphical trend of nickel concentration in samples during different test days



Unlike the nickel test which had a wide removal percentage range, the removal percentage during the copper test was quite narrow, ranging from 83.9% to 93.3%. However, no algae combination recorded 100% removal but the mean percentage removal of the experiment was 89.5%.

4.5 Observation during the Copper test

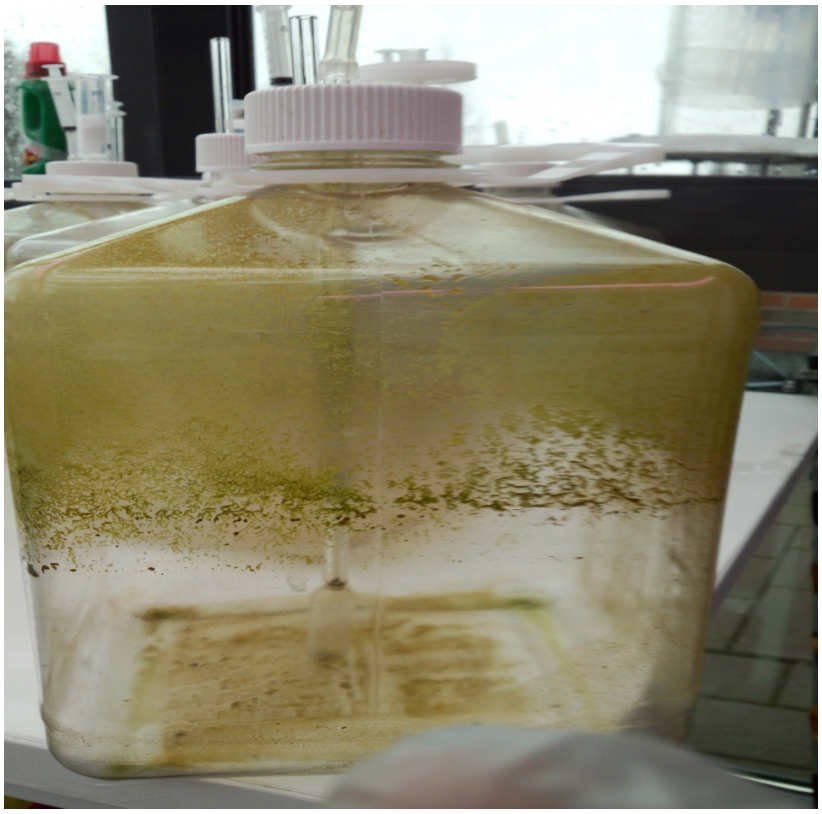


Picture 7: Six days after experimental start up for copper test

Six days after the experimental set up, there was high visibility of algae growth in both the blank and metal samples but algae growth was more visible in the metal samples. There was faster growth than in the case of nickel, when it took 8 and 12 days to have visible algae growth in blank and metal samples. Since there was no background information indicating that any of the algae strain used in this project is chemotrophic, it could then be assumed that the availability of more hours of sunlight during the copper testing might have contributed to the algae's early rapid growth. Microalgae need sunlight/light for optimal photosynthesis. They need light to produce Adenosine triphosphate (ATP) which serves as energy carrier in all organisms and Nicotinade adenine dinucleotide phosphateoxidase (NADPH) a membrane bound enzyme complex (Al-Qasim et. al., 2012).

Picture 8: Cu metal groups (Left) and blank groups (Right) on the 20th day

At the end of the experiment it was observed that algae growth was more pronounced in the samples containing copper than in blank samples. There was no clear explanation or theory to support this trend but a considerable number of literatures exist, which suggests that the presence of Ni and Cu should inhibit algae growth. According to Spencer and Nichol (1983), the growth of algae tested were inversely related to the concentration of nickel in the solution, thus nickel inhibition of algae growth appears to be similar to Copper, Zinc and Cadmium.



Picture 9: Photo-bioreactor after biomass harvesting

After harvesting the algae from the bottles, a considerable amount of algae was noticed to be stuck to the bottle wall and were not harvestable. This was observed to have occurred during both test periods.

4.6 The efficiency of different algae combination in Ni and Cu removal

In this section, analysis of metal removed achieved by various algae group was used as the criteria to determine the efficiency rate of the four algae combination group as described in table 4.

Table 7: Algae combination efficiency for nickel removal

Algae group	Metal group	Percentage Removal in individual sample			
		a	b	c	Av. % removal
1	A2	61.3	35.3	60.3	52.3
2	B2	95.1	93.7	100	96.3
3	C2	87.6	99.3	100	95.6
4	D2	100	100	100	100

Group 4 which consisted of *Anabaena cylindrical* and *Planktothrix rubescence* algal strains was the most efficent algae combination for nickel remediation during this project while algal combination in group 1 which consisted of 12 algae recorded the lowest efficency percentage.

Perpetuo et al (2011) and Gadd et al (1988) stated that biomass concentration in a solution can influence metal uptake, as increase in biomass concentration increase electrostatic force between algal cells hereby decreasing the amount of metal uptake. Considering that all samples were subjected to the same condition throughout the experiment and the biomass concentration was the only factor, not constant among the groups.

Table 8: Algae combination efficiency for copper removal

Algae group	Metal group	Percentage Removal in individual sample			
		a	b	c	Av. % removal
1	A2	86.6	93.3	71.8	83.9
2	B2	92.1	88.8	94.6	91.8
3	C2	96.0	88.8	95.0	93.3
4	D2	87.2	90.1	89.4	88.9

Algae Group 3 was the most efficient combination during the copper implementation. Group 1 also recorded the lowest percentage removal during this test implementation. However, a critically analysis of the result did not indicate that this was due to high biomass concentration. Fourest and Roux (1992) in one of his publication had refuted the earlier assertion by Gadd et al (1998) by insinuating that a decrease in specific metal uptake was due to low concentration of metal in solution and not because of biomass concentration.

4.7 Correlation between pH and metal removal

According to many literature and academic journals reviewed during the course of researching on this project. All writers agreed that the pH is the most important parameter that influences the rate of biosorption. Dwidvedi .S. (2012) stated that the study of biomass surface charge showed that the availability of free metal binding site is highly influenced by the pH of the solution while Abass et al. (2014) noted that the solubility of metal is highly dependent on the pH.

Table 9: correlation between pH value and metal removed

Sample Code	Nickel	Copper
A2a	-0.269014726	0.53320051
A2b	0.542996682	0.973687017
A2c	0.885546562	-0.688178736
B2a	0.908322568	0.39590425
B2b	0.504213791	0.288257679
B2c	-0.125966469	0.084561399
C2a	0.439204759	0.996126023
C2b	0.892324795	0.892569604
C2c	0.744764711	-0.98914215
D2a	0.683958666	-0.56991522
D2b	0.596159006	0.232820679
D2c	0.598064656	0.999176312

Table 10: Average correlation of each group

Sample Code	Nickel	Copper
A2	0.386509509	0.272902811
B2	0.42885663	0.25624111
C2	0.692098088	0.299851159
D2	0.626080841	0.220693924

Correlation values in table 8 represent the relationship between the pH value and metal removed from each sample. The value displayed in table 8 does not follow a definite correlation pattern as some samples in the same group displayed extreme opposite correlation pattern while others showed little correlation relationship.

However, the average correlation values displayed in table 9 gives a clearer view of the correlation pattern as it can be seen that group A and B during the nickel implementation showed relatively low linear correlation but correlation values for group C and D indicates that as pH value increases, the amount of metal content removed also increased while correlation values during the copper test, indicates that there is no strong correlation pattern between the pH value and metal removed.

4.8 Biomass Analysis

Table 11: Comprehensive analysis of nickel implementation results

Sample Code	Harvested Biomass (g)	Left in solution (mg)	Amount in Biomass (mg)	Missing (mg)
A2a	1.1	34.8	25.8	29.3
A2b	1.2	52.2	18.9	12.8
A2c	1.3	35.3	19.3	35
B2a	0.8	4.4	36.8	48.8
B2b	1.3	5.8	56.1	28.2
B2c	1.2	0	0.3	89.7
C2a	1.1	11.2	25.4	53.4
C2b	0.5	0.6	19.9	69.5
C2c	2.7	0	34.9	55.1
D2a	1.4	0	54.8	35.2
D2b	1.8	0	57.3	32.7
D2c	1.6	0	60.5	29.5

Table 12: Comprehensive analysis of Copper implementation results

Sample Code	Harvested Biomass (g)	Left in solution (mg)	Amount in Biomass (mg)	Missing (mg)
A2a	2.4	12.1	58.8	19.9
A2b	2.8	6	60.6	23.4
A2c	2.2	25.4	46	18.6
B2a	3.1	7.1	65.3	17.6
B2b	2.6	10.1	59.8	20.1
B2c	2.2	4.9	45.6	39.6
C2a	2.6	3.6	56.5	29.9
C2b	1.9	10.1	47.4	32.6
C2c	2.4	4.5	54.2	31.3
D2a	2.1	2.6	49.2	40.8
D2b	1.7	3	50.6	39.4
D2c	2	2.7	48.5	41.5

16g and 29g of biomass were harvested to be analyzed from nickel and copper implementations respectively. The amount of biomass harvested was very important during the biomass analysis because they were used to estimate the total concentration of metal in biomass after the metal concentration removed and digested is known. Results from the biomass analysis showed that 16% of the total metal content during the nickel implementation was still in the remaining wastewater samples, 43% was retained in the biomass and 43% could not be accounted for, while result from the copper implementation revealed that 8.8% was not removed from the samples, 58.5% was found in the biomass while 32.7% could not be accounted for.

5 CONCLUSIONS

The metal content removal percentages of 76.2% and 89.5% recorded during this project have shown that algae photo-bioreactor use to remove or remediate nickel and copper from polluted wastewater is efficient. The graphical analysis of the trend of removal has also shown that there are two uptake phases during metal removal; biosorption and bioaccumulation phases, as all samples (except A2a during nickel implementation) recorded more than 60% metal content removal before the next test days. Although it was hard to ascertain if major uptake occurred within hours or days but graphical results indicated that it occurred within a short period after the experimental start-up.

Biomass concentration apparently affected the uptake of nickel but had no effect on copper. There was positive linear correlation between the pH value and metal removed during the nickel implementation. However, there was weak or no correlation between pH value and metal removal during the copper implementation.

For nickel removal, the best results were obtained with an algae combination of *Anabaena cylindrical* and *planktothrix rubescene* with an average removal rate of 100%.The algae group containing *Anabaena cylindrical* and *Scenedesmus Specie*, on the other hand was more efficient in removing with an average removal rate of 93.3%. By wet digesting the harvested biomass it was discovered that 43% of total metal content during the nickel testing was retained in the harvested biomass and 58.5 % was retained during the copper test while 41% and 32.7% could not be technically accounted for, at the end of the project.

The physical state of photo-bioreactor bottles after biomass harvesting indicates that there might be a considerable amount of the unaccounted metal content in the algae stuck on the bottle walls. Some of the unaccounted metal concentration might also be missing due to human errors and factors which could be from the introduction of the initial 90mg metal content, suboptimal harvesting of biomass or improper biomass digestion.

In future research, wastewater samples should be filtered to get rid of organic matter before being used for the experiment because it’s influences on the experiment was not known. Better biomass harvesting and digestion methods should be developed for optimal results.

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Figures

Tables

Pictures

APPENDICES

Appendix 1. pH readings for nickel implementation

Sample Code	0 day	4th day	6th day	10th day	13th day	17th day
A1a	7.2	7.86	6.47	6.28	5.95	6. 4
1A1b	7.2	6.76	6.39	6.81	6.91	7.16
A1c	7.2	7.19	6.33	5.92	6.01	6.25
A2a	7.2	7.59	8.02	7.25	7.74	7.55
A2b	7.2	7.87	7.94	6.99	6.83	6.34
A2C	7.2	7.4	8.01	7.73	6.94	6.5
B1a	7.2	7.51	7.74	7.47	7.93	7.68
B1b	7.2	7.59	7.69	7.61	7.97	7.64
B1c	7.2	7.29	6.92	7.57	7.86	8.16
B2a	7.2	7.55	8.17	8.12	8.04	8.18
B2b	7.2	7.68	8.23	7.96	8	8.26
B2c	7.2	7.63	7.99	7.53	8.01	8.4
C1a	7.2	8.09	7.57	7.67	8.04	8.08
C1b	7.2	8.04	7.59	7.7	8.1	8.19
C1c	7.2	7.97	7.44	7.56	7.9	8.05
C2a	7.2	7.93	7.83	7.9	8.27	8.1
C2b	7.2	8	8.02	8.12	8.1	8.09
C2c	7.2	7.56	7.98	7.99	7.83	8.29
D1a	7.2	7.7	7.76	7.87	8.08	8.4
D1b	7.2	7.82	7.78	7.98	8.06	8.38
D1c	7.2	7.83	7.65	7.83	8.07	8.42
D2a	7.2	7.76	8.22	8.1	7.97	8.27
D2b	7.2	7.79	8.22	8.13	7.93	8.24
D2c	7.2	7.81	8.12	8.06	7.99	8.64

Appendix 2. pH values of Copper implementation

Sample Code	0 day	3 days	7 days	10 days
A1a	7.2	6.7	6	5.9
A1b	7.2	6.5	6	5.9
A1c	7.2	6.4	6.2	6.6
A2a	7.2	6.5	7.8	7.8
A2b	7.2	7.3	8.2	8.5
A2C	7.2	6.9	6	5.4
B1a	7.4	6.8	5.9	5.5
B1b	7.4	6.8	5.6	5.7
B1c	7.4	6.4	6.8	6.9
B2a	7.4	6.4	5.8	5.7
B2b	7.4	6.6	7.4	8.4
B2c	7.4	6.4	6.1	5.9
C1a	7.2	6.5	5.6	6
C1b	7.2	6.3	5.5	5.6
C1c	7.2	7.1	7.5	7.4
C2a	7.2	7.1	7.6	7.8
C2b	7.2	7.9	8.3	8.4
C2c	7.2	7.5	7.7	8.2
D1a	7.2	7.5	7.6	7.6
D1b	7.2	7.9	7.5	7.6
D1c	7.2	6.7	5.5	5.5
D2a	7.2	7.4	7	7.2
D2b	7.2	6.9	7	6.6
D2c	7.2	6.8	7.2	7.3

Appendix Biomass weight of Algae during Nickel Implementation.

Sample Code	Evaporating Dish (g)	Wet Biomass + Dish (g)	Dried Biomass + Dish (g)	Final Biomass (g)
A2a	37.8	46.6	38.9	1.1
A2b	33.5	44.5	34.6	1.2
A2c	38.7	51.8	40	1.3
B2a	31.5	39.4	32.3	0.8
B2b	40	51.9	41.2	1.3
B2c	37.9	53.4	39	1.2
C2a	78.6	90.9	79.7	1.1
C2b	76.6	83.4	77.1	0.5
C2c	61.8	90.3	64.5	2.7
D2a	40.2	62.5	41.6	1.4
D2b	40.2	61.9	42	1.8
D2c	69.9	88.2	71.4	1.6

Appendix 4. Biomass weight of Algae during Copper Implementation

Sample Code	Evaporating Dish (g)	Wet Biomass + Dish (g)	Dried Biomass + Dish (g)	Final Biomass (g)
A2a	37.7	61.9	40.1	2.4
A2b	36.4	63.9	39.2	2.8
A2c	31.4	52.9	33.5	2.2
B2a	39.8	73.5	42.9	3.1
B2b	35.5	61.3	38.1	2.6
B2c	32.4	61.1	35	2.5
C2a	35.9	64.8	38.5	2.6
C2b	32.8	58.2	34.7	1.9
C2c	33.5	61.4	35.9	2.4
D2a	38.5	61.9	40.6	2.1
D2b	38.3	57.5	40	1.7
D2c	40.2	64.5	42.2	2

Appendix 5. pH value of cultivated algae stock

Sample bottle	**Algae Name**	**PH Value**	**Temperature °C**
1	*Selenastrum capricornutum*	5.41	18.5
2	*Pediastrum simplex*	5.88	19.0
3	*Anabaena cylindrical*	5.86	18.9
4	*Scenedesmus sp.*	5.46	17.9
5	*Chlorphyta sp (Pekari strain) Blue green*	4.96	18.9
6	*Purpuraemus sp.*	5.38	19.1
7	*Haematococcus*	5.67	18.7
8	*Planktothrix rubescence*	5.61	19.2
9	*Chlorella pyrenoidosa – green algae*	5.68	19.3
10	*Desmodesmus subspicatus*	6.09	20.0
11	*Golekinia brevispicula*	5.65	19.9
12	*Crucigenia tetrapedia*	5.53	19.9