BIOREMOVAL OF ZINC AND COPPER IN WASTEWATER BY LIVING MICROALGAE

SIEW CHUN GHEE

CHEMICAL ENGINEERING

UNIVERSITI TEKNOLOGI PETRONAS

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by

SIEW CHUN GHEE

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Dissertation submitted in partial fulfillment of
the requirements for the
Bachelor of Engineering (Hons)
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32610, Bandar Seri Iskandar,
Perak

CERTIFICATION OF APPROVAL

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Approved by,

_____

(Dr. Nurlidia binti Mansor)

UNIVERSITI TEKNOLOGI PETRONAS

BANDAR SERI ISKANDAR, PERAK

September 2015

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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SIEW CHUN GHEE

ABSTRACT

One of the major causes of pollution is heavy metal contamination due to anthropogenic activities. Heavy metals are toxic to living organisms and are not chemically and biologically degradable. Various methods have been developed over the years to remove heavy metals such as chemical precipitation, membrane technology and electrochemical treatment. However, these methods are not practical as they produce toxic sludge, consume large amount of energy and are inefficient at low concentration of heavy metal. Removal of heavy metals by using microalgae is an alternative method designed to replace the conventional technologies. Generally, wastewater discharge carries more than one type of heavy metal. Hence, the study on the effects of a combination of heavy metals can represent the actual environment. This research work focuses on the bioremoval of zinc and copper by using a marine algae *Nannochloropsis oculata*. The main objectives are to investigate the bioremoval of combined heavy metals by using living microalgae, effect of pH value as well as ionic strength on the bioremoval process. Samples are collected every 24 hours. The metal content is quantified by using Microwave Plasma Atomic Emission Spectroscopy (MP-AES). It was found that the bioremoval by living *N. oculata* is more affected by its growth which is dependent on the pH value and ionic strength of the medium and types of heavy metal it is exposed to. *N. oculata* also shows that it is not tolerant towards copper and the bioremoval in medium of zinc and copper mixture shows non-interactive effect when compared to experiment medium with zinc and copper individually. Bioremoval by living *N. oculata* also exhibits fluctuations.

ACKNOWLEDGEMENT

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CHAPTER 1
INTRODUCTION

1.1 Background of Study

In recent years, heavy metal pollution of waters has become an issue of great environmental concern. Heavy metals are commonly defined as metals which have a specific density more than 5g/cm^3 [1]. In general, the term heavy metal refers to any metallic chemical element that has high density and is poisonous or toxic at low concentrations [2]. Traditional technologies used to sequester heavy metals from solutions such as lime precipitation, ion exchange, electrochemical treatment or evaporation are not very practical and appropriate as they are expensive. This conventional methods become not economically effective when the undesired heavy metal ions are present in large solution volumes or at very low concentration.

Also, it has been known for years that many living or dead biomass are able to remove unwanted heavy metal ions from solutions [3]. Land and aquatic plants and algae have all caught considerable attention for their capacity to remove heavy metal. Therefore, a thorough study in this uptake phenomenon is necessary as this technology can be utilized in cleaning up wastewater and possible recovering of metals.

In this project, the bioremoval of zinc and copper by using living *Nannochloropsis oculata* will be studied. Zinc pollution is usually caused by mining and metallurgic operations. Primary anthropogenic sources of zinc in soil and water are discharges from smelter slags, mine tailings, and the use of commercial products such as fertilizers that contain zinc [4]. Besides this, copper is released into the environment via mining

operations, municipal and industrial solid waste and agriculture. Milling and mining contribute the most waste [4].

In short, unlike many other pollutants, heavy metals removal from the environment is essential and challenging as they cannot be biologically or chemically degraded and are ultimately indestructible [5]. Microalgae can be a promising tool for heavy metal remediation.

1.2 Problem Statement

Due to urbanization and industrialization, heavy metal pollution has become one of the most serious environmental problems. Heavy metals pollute the water, soil and air. Heavy metals cannot be destroyed or degraded. Thus, heavy metal pollution must be cleaned up and remediated.

Conventional techniques in sequestering and removing heavy metals is not economical because the separation cost tends to become very high when the amount of solution to be treated is tremendous, not efficient when the metal concentration is low and might have toxic leftover. One of the alternative methods is using microalgae. It has been found that various species of microalgae have the abilities to uptake heavy metals. This method is ecofriendly and has shown to have high removal efficiency.

Generally, wastewater discharge would carry more than one toxic substance. There is a possibility of joint actions which can be categorized as synergism, antagonism and non-interactive or additive. Therefore, the study of the effects of heavy metal ions in multi-metals solution can represent more of the actual environment compared to single metal studies. However, the studies regarding effect of metal mixtures on microalgae is very limited.

Bioremoval of zinc and copper by living *N. oculata* has not reported yet. Therefore, the study of bioremoval of zinc and copper and their mixture on living *N. oculata* will be studied.

1.3 Objective and Scope of Study

The objectives of this project are:

i. To study the bioremoval of heavy metals by using living microalgae
ii. To study the effect of ionic strength as well as pH on the bioremoval of zinc and copper
iii. To investigate the bioremoval of zinc and copper mixture using living microalgae

The scope of study of this project is to investigate the bioremoval of combination of different heavy metals by marine algae *N. oculata*. The effects of ionic strength on bioremoval of heavy metals will also be studied. Being one of the most significant affecting parameter, pH value will be varied to investigate the uptake of heavy metals.

The mass of microalgae, concentration of heavy metal and temperature will be held as fixed variables in this project. Variation of pH values and ionic strength will be studied to investigate the behaviour of uptake of heavy metals in two different experiments.

CHAPTER 2
LITERATURE REVIEW

2.1 Conventional Metal Removal Methods

There are several conventional methods for heavy metal removal such as chemical precipitation (sulfide, hydroxide and carbonate precipitation), chemical redox, ion exchange, lime coagulation, solvent extraction, reverse osmosis, evaporation recovery, cementation, adsorption by activated carbon, electro-dialysis and electrodepostion [5]. However, most of these conventional heavy metal removal techniques require large amount of energy and reagents [6], provide incomplete metal removal [7], have limited tolerance to change of pH value [8] and have low or no selectivity[9], leave over toxic sludge [6] and require high investment and cost of regeneration [10]. Therefore, bioremediation technologies serve as a promising tool to contribute in removal of heavy metals from contaminated medium. By using microalgae in metal bioremoval, it comprises of the benefits such as eco-friendly, rapid metal uptake capability, year round occurrence, time and energy saving, ease of handling, reusable, faster growth rate compared to higher plants, low cost, high efficiency, high selectivity, large surface to volume ratio, no toxic waste produced, can be applied in both batch and continuous system, no synthesis required, and can be applied to solution containing high or relatively low contaminant levels [11].

2.2 Bioremediation

Bioremediation involves bioremoval and bioaccumulation [5]. Bioremoval is a metabolically passive process (the amount of contaminants removed is dependent on the composition of the cellular surface of a sorbent and on kinetic equilibrium). The pollutant will be adsorbed onto the cellular structure. Bioaccumulation refers to an active metabolic process which is driven by the energy from a living organism. The contaminants will be transferred onto and within the cellular surface. Compared to the traditional methods, these biological technologies would be able to make removal of heavy metal from dilute solutions more efficient and at lower cost. The term "bioremoval" is as the accumulation of pollutants from aqueous solutions by using biological materials, facilitating the recovery and environmentally acceptable disposal of the pollutant [5]. Also, the mechanisms of removal of metals by microorganisms from solutions can be classified into extracellular accumulation, cell surface sorption and intracellular accumulation [12].

In living microalgae, the accumulation of heavy metals is a two-stage process [11]. The first stage is a passive removal of metals by the cell which occurs rapidly at the cell surface, may it be living or non-living. The second stage is a much slower process which occurs inside the cell. In the first process, heavy metal ions are adsorbed to the functional groups present on cell surface by electrostatic interaction. The functional groups differ in their affinity for specificity binding and metal. In this stage, the process comprises of physical adsorption, chemisorption, ion exchange, chelation, complexation, entrapment in the structural polysaccharide network and diffusion through cell membrane and cell wall [11]. Whereas, in the second stage which occurs within the cell, the process is a metabolism-dependent process. This process involves transportation of metal ions across cell membrane barrier which leads to subsequent accumulation inside the cell. This latter process does not involve non-living cell, is irreversible and slow. In this second stage process, the metal uptake is due to mechanisms such as covalent bonding, redox reactions, surface precipitation, diffusion into the cell interior and crystallization on the cell surface [13]. When the extracellular concentration of heavy metal ions is higher that the

intracellular concentration, binding groups on the surface will transport those metal cations into the cytoplasm via the cell membrane to be compartmentalize [11].

2.3 Affecting Parameters

In bioremoval process, the removal capacity is depending upon factors such as the characteristics of the metal ion (valency and atomic weight), environmental conditions (temperature, pH, ionic strength, contact time, and biomass or metal concentration) and nature of the biosorbent which can affect selectivity and affinity to those metal ions. Species, growth condition, cell age, biomass concentration (living or dead) and physiological state of the organism may all affect the binding mechanism of the heavy metals [14]. Extrinsic factor such as presence of other metals will also affect the sorption of metals by microalgae [11].

2.3.1 pH Value

Abiotic factor pH is one of the most important parameter in controlling the uptake of metal from aqueous solutions by microalgae (Indhumathi, PS, Shoba, & Saraswathy, 2014) [15]. pH can influence the algae tolerance and speciation of metals in solution [16]. At pH value lower than 3, the uptake capacity decreases because metal ions will be competing with hydrogen ions for binding sites [16]. Whereas, at pH value above 6.5, heavy metals tend to form hydroxide precipitate and only a small amount of heavy metal would remain in the solution to form complexes with ligands. pH can also affect the solubility and toxicity of heavy metals in water.

The pH dependence of uptake of metal is closely related to the metal chemistry in the solution as well as the acid-base properties of different functional groups on the cell surface of the microalgae. At low pH, the hydronium ions H_3O^+ will be associated with the cell wall ligands. This phenomenon restricts the approach of heavy metal cations as a result of repulsive force. This causes the functional groups to be associated with H^+ ions

subsequently hampering the metal cations from binding due to repulsive forces [11]. Whereas, when the pH value increases, the negative charge carriers such as carboxyl, amino, phosphate and imidazole groups will be exposed which would then ensue an attraction of positively charged metallic cations via process of bioremoval onto the cell surface. In other words, when the pH value increases, those functional group sites will be deprotonated (negative charges increase) and this leads to greater extend of binding of metal cations. In brief, higher pH will encourage more metal uptake because the cell surface will be more negatively charged.

Precipitation of metals tends to occur at high pH levels. The optimal pH has to be determined as precipitation decreases the extent of uptake of metal. Also, at different pH ranges, different functional groups will be available for binding the cations. For instance, at pH 2-5, bindings will be dominated by carboxyl groups, whereas at pH 5-9, phosphate groups will dominate. Whereas, at pH 9-12, phosphate, carboxyl and hydroxyl (or amine) groups are also suitable [17]. As the carboxyl groups have a wide range of pH susceptibility, it possesses the ability to chelate various types of metals. Whereas, the hydroxyl and amino groups play an important role at high pH [18].

2.3.2 Ionic Strength

Ionic strength reflects the effects of interionic interaction and charges of electrolyte activities [19]. Ionic strength plays an important role in metal ion removal. When ionic strength decreases, the removal efficiency increases [20]. Provided with a fixed pH value, the number of functional groups will remain the same but the sites available for uptake of metal ions decrease with increasing ionic strength. Hence, with stronger ionic strength, the ion removal would be less. If monovalent cations such as Na^+ and K^+ presence in high concentration, ionic strength will increase which will subsequently decrease the metal bioremoval capacity of biomass [21]. With increasing ionic strength and pH, proton binding decreases [22]. And, with increasing pH and decreasing ionic strength, Cu and Ni binding were observed to increase.

It has been reported that sodium perchlorate can be used to adjust the ionic strength. It is found that by decreasing the ionic strength from 0.5 mol/L to 0.0005 mol/L the uptake efficiency increases from 80% to 90% [20].

2.3.3 Salinity and Hardness

Salinity affects the removal of heavy metals. The optimal salinity values differs for different metals [23]. Metal toxicity (Ni, Zn, Cu, Sn and Cd) decreases as salinity increases [24].

2.3.4 Temperature

Temperature affects various parameters which are important for metal ion bioremoval such as stability of metal ion species, ligands and its complexes and solubility of the metal ions [5]. Temperature has significant effect on metal speciation as most chemical reaction rates are very sensitive to changes of temperature [25]. Metal ions are more soluble if the temperature is higher. In such case, the bioremoval of metal ions will be weaker [26]. Bioremoval favours high temperature if the binding is endothermic. If it is exothermic, the bioremoval would be weakened. Metal-amine interactions are exothermic while metal-carboxylate interactions are endothermic. However, the effect of temperature might vary depending on types of metal [5]. The influence of temperature is lower compared to that of pH [26].

2.3.5 Metal Speciation

The toxic effect of trace metals on microalgae depends on the species of metallic ion which is dependent upon the pH [27]. Metal speciation and charge in the solution would also affect the binding of metal cations onto microalgae [11]. Metal can appear in various chemical forms such as free ions, adsorbates on particulate, complexes with

organic/inorganic ligands. The free metal ions in are the most toxic form and can bind the furthest into microalgae [5].

2.3.6 Effect of Combined Metals

Wastewater discharged usually contains more than one toxic substance and the toxic effect of the constituents are often not the summation of all individual effects. There could be a possibility of synergism, antagonism and non-interactive or additive effect. Synergism refers to the combined toxic effect which is greater than the summation of all individual toxicities. Antagonism refers to the combined toxic effect which is less than the summation of all individual toxicities. Whereas when the combined effect is same as the summation of all individual toxicities, this phenomenon is called as additive.

The interaction between heavy metal ions appear to be complicated and without a set pattern [28]. 10 metals were individually on algae and found that it was not toxic to the algae but when the metals were mixed at same concentration, the mixtures were strongly inhibitive [29]. It is found that in the presence of Cd^{2+}, the uptake of Zn^{2+} decreases [30]. The presence of one metal can prevent the adsorption of other metals because of electrostatic and steric effects [26]. The preferential binding is related to the relative strength of interaction between types of biomass and metal ions.

2.3.7 Effect of Initial Concentration of Metal and Microalgae

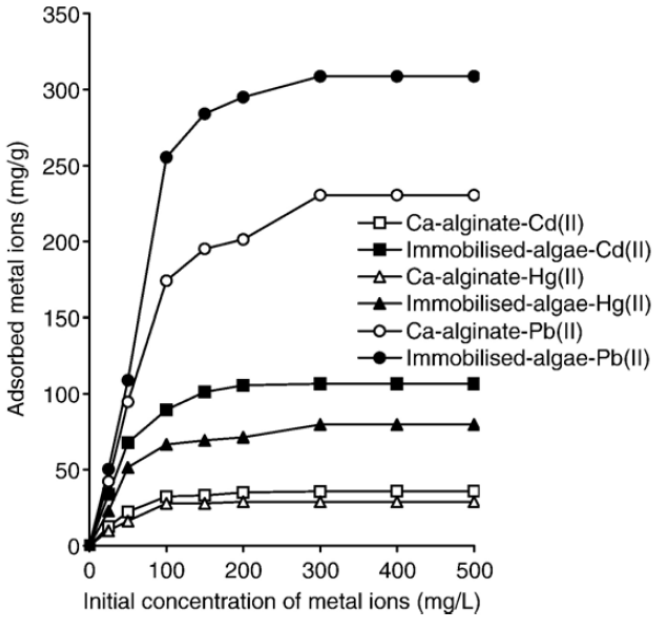

FIGURE 2.1 Bioremoval capacities of the Ca-alginate and immobilized-algal preparation for Hg(II), Cd(II) and Pb(II) ions at pH 6 and 25^{o}C [31]

As shown in Figure 2.1 above, when the initial concentration of metal ions is higher, more metal ions will be absorbed.

With increasing metal concentration, the uptake rate of metal ion will increase if the concentration of microalgae is kept constant. However, the bioremoval capacity of metal ions is inversely proportional to the initial concentration of the microalgae provided that the concentration of metal ions is kept unchanged. Increasing the concentration of microalgae will increase the number of sorption sites available.

When the microalgae concentration is low, heavy metal ions will not only be absorbed onto the cell surface but also diffuse into the intracellular part through facilitating the metal ion concentration gradient [32].

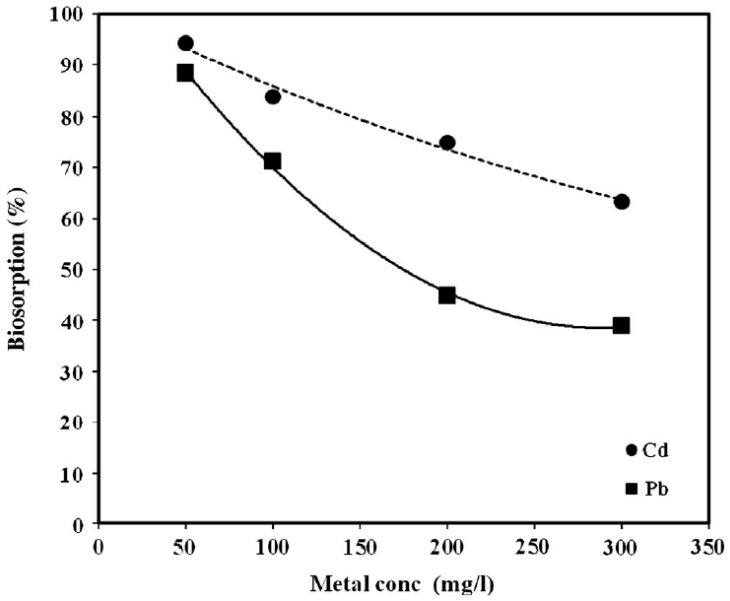

FIGURE 2.2 Bioremoval of cadmium and lead by *Anabaena spaerica* as a function of initial concentration at the optimum removal conditions [33]

Figure 2.2 above shows how the uptake efficiency is affected by the initial metal concentration.

The bioremoval efficiency drops with increasing initial metal concentration. This phenomena is mainly due to the fact that, in the beginning, all binding sites on the microalgae cell surface are vacant indicating high metal bioremoval efficiency. However, with increasing initial metal concentration, the bioremoval efficiency of metal decreases because the number of binding sites available on the cell surface has decreased [33].

2.3.8 Effect of Contact Time

Bioremoval of heavy metal on living microalgae can be divided into two steps. The first step is fast and occurs on the cell surface. The second step is a slow intracellular diffusion process.

Figure 2.3 below shows the effect of contact time on bioremoval of A*nabaena spaerica* on cadmium and lead.

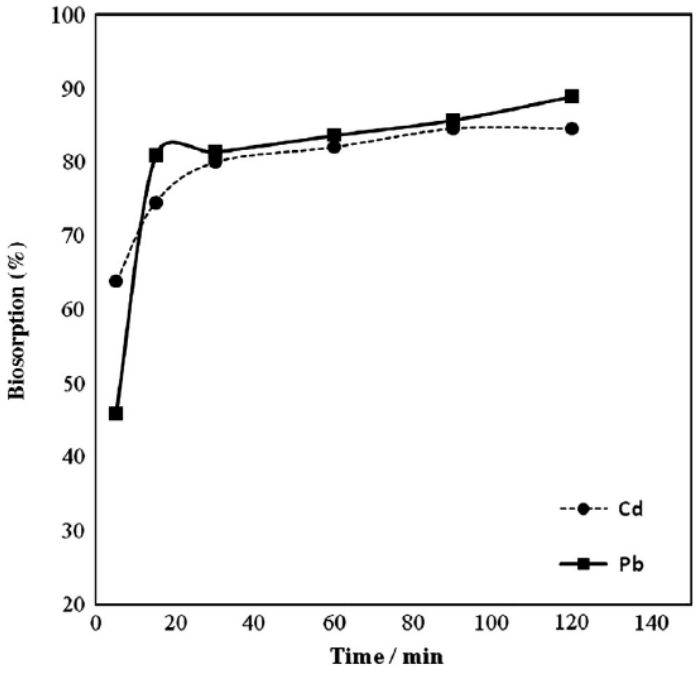

FIGURE 2.3 Graph of bioremoval efficiency against time [33]

2.4 Heavy Metal Tolerance of N.

The following table shows the comparison of EC_{50} values of effluent from the wastewater in Tucson, AZ.

TABLE 2.1 Continuous flow metal toxicity in effluent from the Ina Road Wastewater Reclamation Facility in Tucson [34]

Metal	Average concentration in secondary effluent (μg/L)	Average concentration in centrate wastewater (μg/L)	EC_{50} reported for algae (μg/L)	Ratio metal EC_{50}/ secondary effluent conc.	Ratio metal EC_{50}/ centrate conc.
Copper	7.22	23.6	50.	6.95	2.12
Zinc	47.36	23.4	24	5.07	10.26
Cobalt	< 0.20	2.23	52	> 2600	233.2
Lead	< 1.0	0.60	68	> 680.0	1133
Nickel	1.56	7.40	41	262.8	55.4
Cadmium	< 0.22	0.29	95.	> 434.1	329.3
Mercury	< 0.20	< 0.20	27.	> 135.0	> 135.0
Silver	< 0.02	< 0.02	2.8	> 140.0	> 140.0

From the information above shown in Table 2.1, experiments were conducted to find out the effect of concentration of heavy metals in f/2 medium on the growth inhibition of living microalgae, *N. Salina*. Metals concentrations are multiples of the EC_{50} values found.

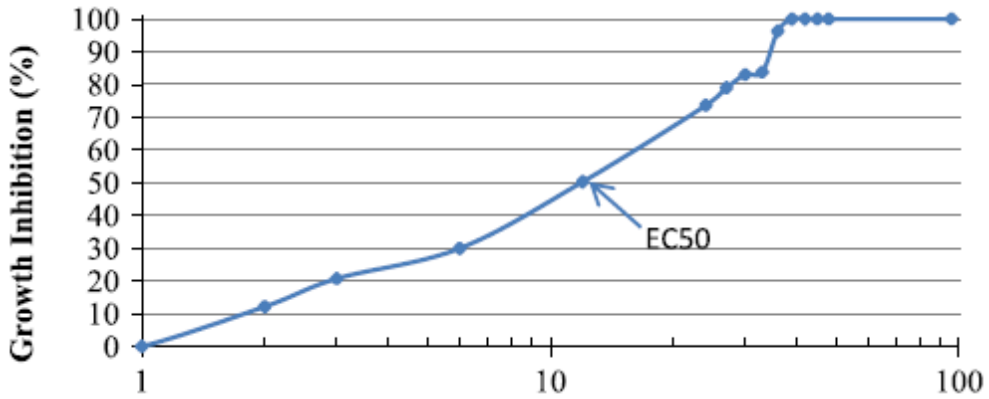

FIGURE 2.4 Growth of *N. Salina* in standard medium supplemented with mixtures of metals in Table 2.1 [34]

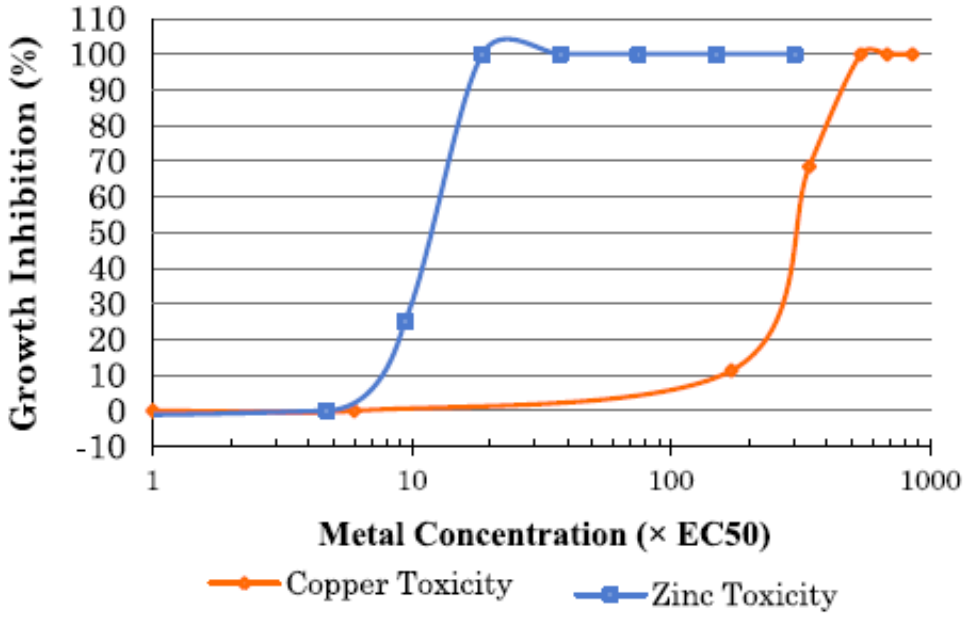

FIGURE 2.5 Individual metal (zinc and copper) experiment [34]

It is found that at about 10 times and 250 times the EC50 values of zinc and copper respectively, the growth of the microalgae is inhibited by 30%.

2.5 Effect of Nitrogen Sources on Cell Growth

Sodium nitrate is chosen to represent nitrate because it is the source of nitrogen in growth medium. Besides this, it is also less costly compared to potassium nitrate. This makes it an advantage in industrial processes.

There is an optimal concentration of sodium nitrate on cell growth. If the concentration is higher than a critical limit, it will be inhibitive to the cell growth. Chlorophyll is a nitrogen-rich compound. When the nitrogen in the growth medium is depleted, the cell starts to utilize intracellular nitrogen pool for its growth and chlorophyll is one of the most easily accessible intracellular nitrogen pool [35].

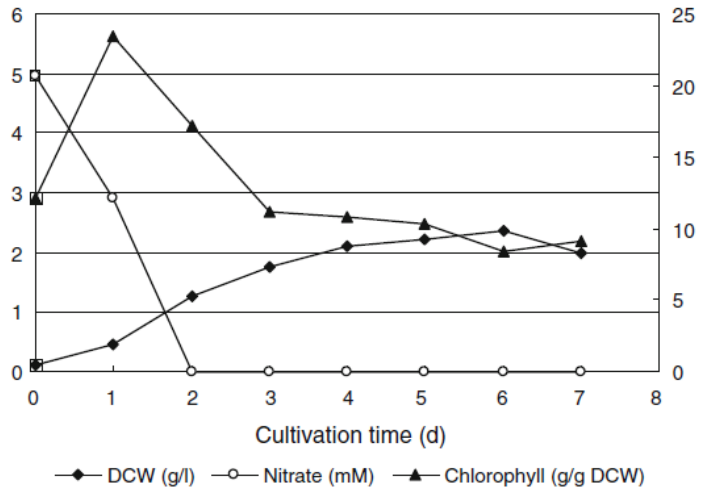

FIGURE 2.6 Nitrogen consumption of *N. oleoabundans* in the media of different sodium nitrate [35]

When the external source of nitrogen is exhausted, the intracellular nitrogen pool will be consumed and hence causes the chlorophyll content to drop drastically [35]. Figure 2.6 shows how depletion of nitrate affects the dry cell weight and chlorophyll content of *N. oleoabundans*.

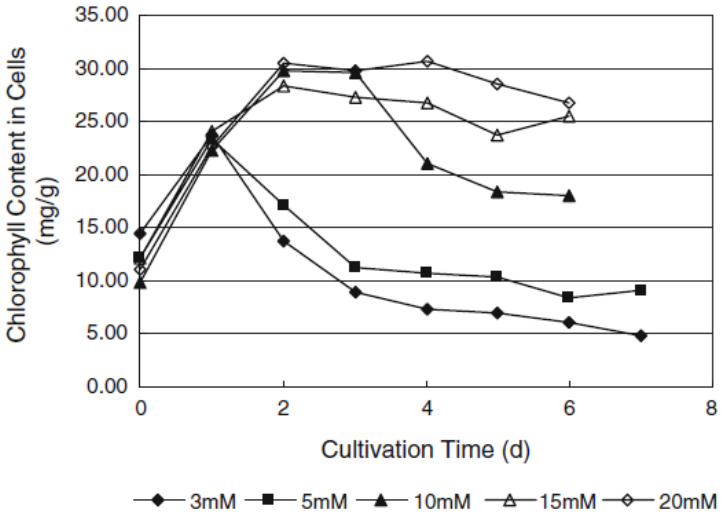

FIGURE 2.7 Cell growth of *N. oleoabundans* in medium with different sodium nitrate concentration [35]

Figure 2.7 shows how concentration of sodium nitrate affects the cell growth. It is reasonable to hypothesize that the critical value is in between 10 and 15 mM because cell chlorophyll content decrease occurred at 10 mM but not at 15 mM nitrate.

CHAPTER 3
METHODOLOGY

3.1 Experimental Design

3.1.1 Preparation of f/2 Medium

The f/2 medium is a general enriched seawater medium which is commonly designed for the use of growing coastal marine algae. Table 3.1 below shows the chemicals needed and final concentration of each components. It also shows the amount of stock solutions to be prepared and amount of quantity to be added to the final product.

TABLE 3.1 Chemicals and their concentrations needed for preparation of f/2 medium

Components	**Stock solution (g/L)**	**Quantity**	**Final concentration (M)**	**Final concentration (g/L)**
Macronutrients				
$NaNO_3$	15	5mL	8.82×10^{-4}	0.075
$NaH_2PO_4\,H_2O$	5	1mL	3.62×10^{-5}	5×10^{-3}
$Na_2SiO_3\,9H_2O$	30	1mL	1.06×10^{-4}	0.03
Micronutrients				
$FeCl_3\,6H_2O$	---	3.15g	1.17×10^{-5}	3.16×10^{-3}
$Na_2EDTA\,2H_2O$	---	4.36g	1.17×10^{-5}	4.36×10^{-3}
$CuSO_4\,5H_2O$	9.8	1mL	3.93×10^{-8}	9.8×10^{-3}
$Na_2MoO_4\,2H_2O$	6.3	1mL	2.6×10^{-8}	6.3×10^{-3}
$ZnSO_4\,7H_2O$	22	1mL	7.65×10^{-8}	0.022
$CoCl_2\,6H_2O$	10	1mL	4.2×10^{-8}	0.01
$MnCl_2\,4H_2O$	180	1mL	9.1×10^{-7}	0.18
Vitamins				
Thiamine HCl (B1)	---	0.2g	2.96×10^{-7}	
Biotin (H)	1	1mL	2.05×10^{-9}	1×10^{-3}
Cyanocobalamin (B12)	1	1mL	3.69×10^{-10}	1×10^{-3}

1. The medium prepared will be autoclaved for 20 minutes at 121^{o}C.
2. The remaining vitamin stock solutions will be stored in refrigerator or freezer.

3.1.2 Cultivation of Microalgae

1. f/2 medium prepared will be used to culture the microalgae.
2. Microalgae *N. oculata* will be cultivated in the f/2 medium.
3. The cultivation is carried out in an Erlenmeyer flask where the temperature will be maintained at room temperature and under continuous illumination at about 2600 lux.
4. Cotton wools is used to stopper the Erlenmeyer flask to prevent airborne particles contamination.
5. The growth is expected to be exponential as the microalgae is exposed to standard culture media.
6. The microalgae will be harvested on the 7th day.

3.1.3 Metal Bioremoval Experiment

1. Only batch experiments are performed. The common fixed variables in the bioremoval experiment are the concentration of metal and mass of microalgae.
2. $ZnCl_2$ and $CuSO_4 \cdot 5H_2O$ salt are used to prepare the metal solutions.
3. NaOH and HCl are used to alter the pH value of the medium while $NaNO_3$ will be used to adjust the ionic strength of the medium. Nitrate salt is used because its tendency of forming complex with most metals is low.
4. Stock solutions containing different pH values and ionic strength are prepared with 2.4 mg/L and 12.55 mg/L of Zn^{2+} and Cu^{2+} ions respectively.

pH Experiment

On the 7th day of cultivation, 20mL of *N. oculata* will be inoculated into 200mL of the stock solutions of different pH values prepared.

1. 2 same sets of samples will be prepared.
2. All Erlenmeyer flasks will be stoppered by cotton wools to prevent airborne particles contamination at the same time allowing carbon dioxide to diffuse into the flasks.
3. 12mL of samples will be withdrawn from each Erlenmeyer flask to be analyzed daily.

Ionic Strength Experiment

1. On the 7th day of cultivation, 20mL of *N. oculata* cultivated is inoculated into 200mL of stock solutions of different ionic strengths prepared.
2. 2 same sets of samples will be prepared.
3. All Erlenmeyer flasks will be stoppered by cotton wools to prevent airborne particles contamination at the same time allowing carbon dioxide to diffuse into the flasks.

Metal Analysis

1. The samples collected will be centrifuged and the supernatant will be analyzed by using MP-AES (microwave plasma atomic emission spectroscopy) for metal concentration.
2. Before running the analysis, it is necessary to prepare the standard metal solutions.
3. The percentage of uptake of metal will be calculated and recorded.

3.1.4 Monitor of Growth of Microalgae

1. The specific absorbance wavelength of chlorophyll of *N. oculata* is found to be at 688nm.
2. The absorbance value of the control and samples are recorded and monitored.
3. Samples are taken and absorbance readings are recorded every 24 hours.

3.2 Key Milestone

TABLE 3.2 Key milestones of project

Semester	Progress	Week
FYP 1	Preliminary research work	4
	Submission of extended proposal	6
	Proposal defence	8
	Submission of interim report	14
FYP 2	Commencement of experiment	3
	Submission of progress report	8
	Pre-SEDEX	10
	Viva	13
	Submission of dissertation	15

Table 3.2 shows the key milestones of project. The duration of project is divided into two sections as FYP1 and FYP2.

3.3 Gantt Chart

TABLE 3.3 Gantt chart

No.	Week / Details	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Apparatus and chemicals sourcing															
2	Cultivation of microalgae															
3	Zinc pH experiment															
4	Zinc ionic strength experiment															
5	Copper pH experiment															
6	Copper ionic strength experiment															
7	Copper zinc pH experiment															
8	Copper zinc ionic strength experiment															
9	Submission of progress report															
10	Pre-SEDEX															
11	Submission of draft final report															
12	Submission of dissertation (soft bound)															
13	Submission of technical paper															
14	Viva															
15	Submission of project dissertation (hard bound)															

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Zinc pH Experiment

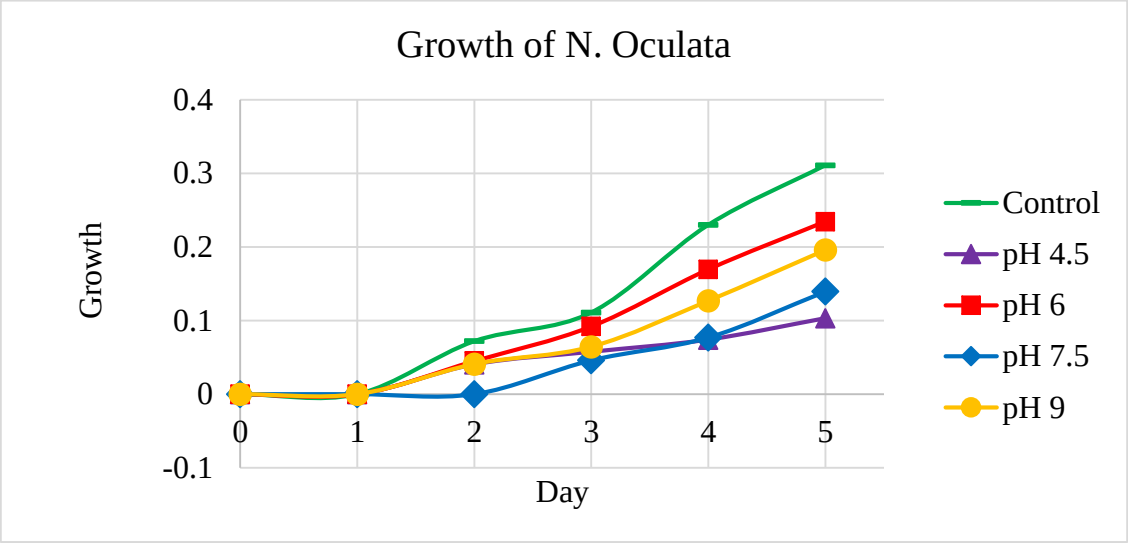

FIGURE 4.1 Growth of *N. oculata* in zinc with various pH

Figure 4.1 shows the growth of *N. oculata* recorded throughout the period of experiment. It is known that *N. oculata* can tolerate alkaline medium as growth was recorded between pH 8 and pH 13 [36].

In our case study, the control is maintained at about pH 8 while the other samples are at pH 4.5, pH 6, pH 7.5 and pH 9 respectively with zinc in the medium at 2.4 mg/l. We can observe from Figure 4.1 that the growth of *N. oculata* at pH 4.5 is much inhibited as compared to the others.

The growth of *N. oculata* is not only affected by the pH of the growth medium but also the amount of heavy metal in the growth medium [34]. According to Figure 2.4, the concentration of zinc when it is 10 folds that of EC_{50} value is used, the growth inhibition is expected to be 30%. In our experiment, it is recorded that the growth inhibition percentage ranges from 32.95% to 72.91%. The large difference is mainly due to the microalgae is intolerant towards acidic growth medium.

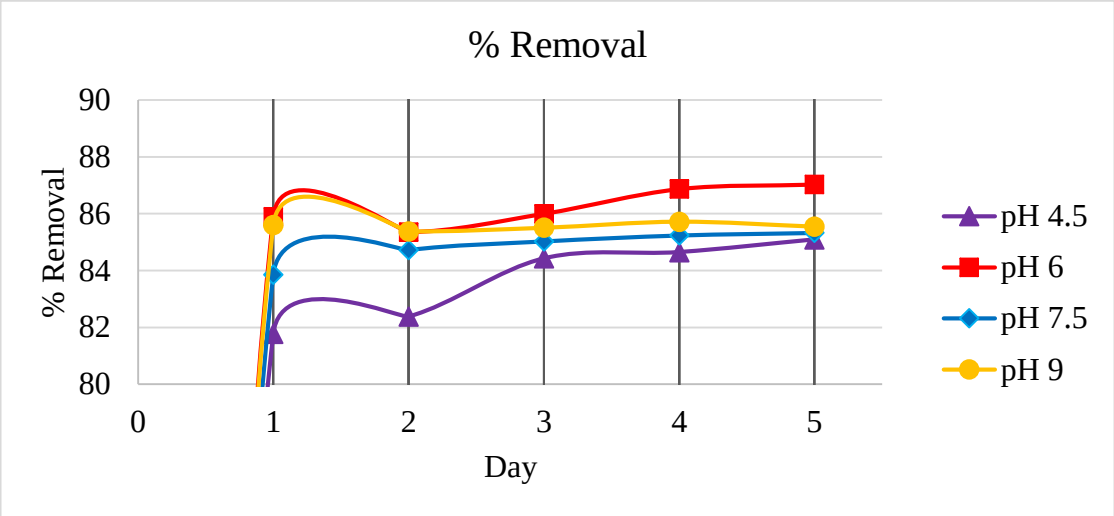

FIGURE 4.2 Percentage of removal of Zn in experiment with various pH

As shown in Figure 4.2, all experiment sets exhibit the same behavior of heavy metal absorption as time proceeds. The amount of zinc ions adsorbed increases when the duration of experiment is longer. This is because bioremoval of heavy metal on living microalgae can be divided into two processes. The first process is fast and occurs on the cell surface whereas the second process is a slow intracellular diffusion process. The longer the experiment duration, more zinc will be absorbed onto the cell surface and diffused into the cellular level. If the experiment duration is allowed to be extended, the percentage of removal might still increase because the microalgae is still within the growth phase.

As shown in Figure 4.2, the percentage of zinc removal increases with increasing pH. At low pH, hydronium ions H_3O^+ will be associated with the cell wall ligands and

thus restricting the adsorption of positively charged zinc ions. The functional groups is said to be associated with H^+ ions causing them to repulse positively charged zinc ions. When the pH value increases, the functional group sites will be deprotonated as negative charges increases due to presence of hydroxide ions OH^-. This causes greater extend of adsorption of zinc ions onto the deprotonated functional group sites [11].

However, it is also noticeable that the experiment set at pH is pH 6 appears to have highest zinc removed. This might be due to presence of higher amount of adsorbent (microalgae) in the medium due to better growth. The percentage removal of zinc increases rapidly with amount of microalgae due to greater availability of exchangeable sites for adsorption [15].

4.2 Zinc Ionic Strength Experiment

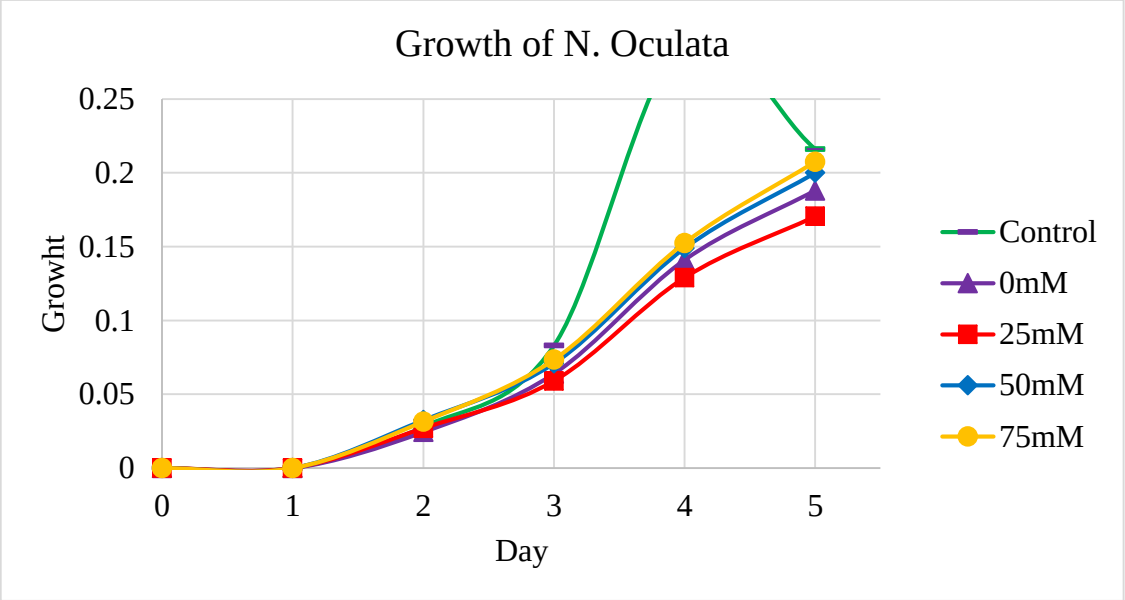

FIGURE 4.3 Growth of *N. oculata* in zinc with various ionic strength

Figure 4.3 above shows the growth of *N. oculata* when exposed to growth medium added with zinc ions with different ionic strength which is varied by using sodium nitrate. From Figure 4.3, it is not clear at this point whether the concentration of sodium nitrate has reached the critical level which can inhibit the cell growth. Preliminary experiments would have to be conducted to determine the critical point.

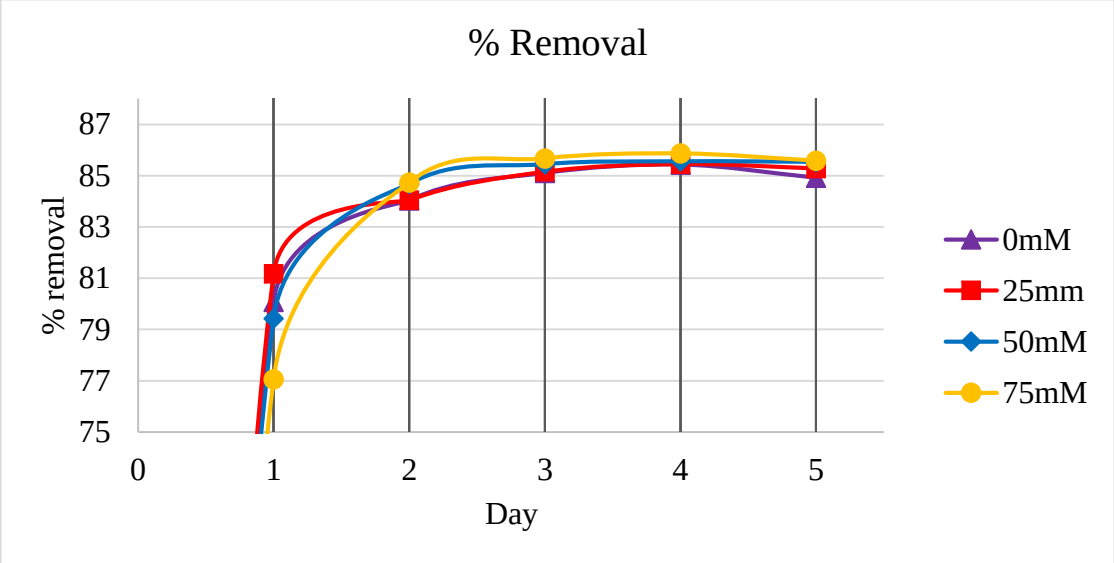

FIGURE 4.4 Percentage of removal of zinc with various ionic strength

As shown in Figure 4.4, on day 1, the percentage of zinc removal is the lowest when the sodium nitrate concentration is 75mM followed by 50mM, 0mM and 25mM. When ionic strength is high, it will cause the metal bioremoval capacity of microalgae to decrease because the number of sites available for uptake of metal ions decreases when there are more cations in the medium [20]. However, the results obtained on day 1 shows that only the experiment sets with 0mM and 25mM of sodium nitrate do not behave the way they should.

As the experiment proceeds (day 2 to 5), the results obtained do not correspond to the hypothesis which states that if concentration of monovalent cation is high, ionic strength will increase and thus decrease the percentage of bioremoval [21]. The results shows that the percentage of removal of all experiment sets are very close to each other.

The experiment might have to be repeated to minimize any possible error. From Figure 4.3, we can see that on day 5, the experiment set with 75mM of sodium nitrate has the highest chlorophyll content followed by the 50mM, 0mM and 25mM. High chlorophyll content refers to more adsorbent (microalgae). More adsorbent correlates with greater adsorption sites [15].

4.3 Copper pH Experiment

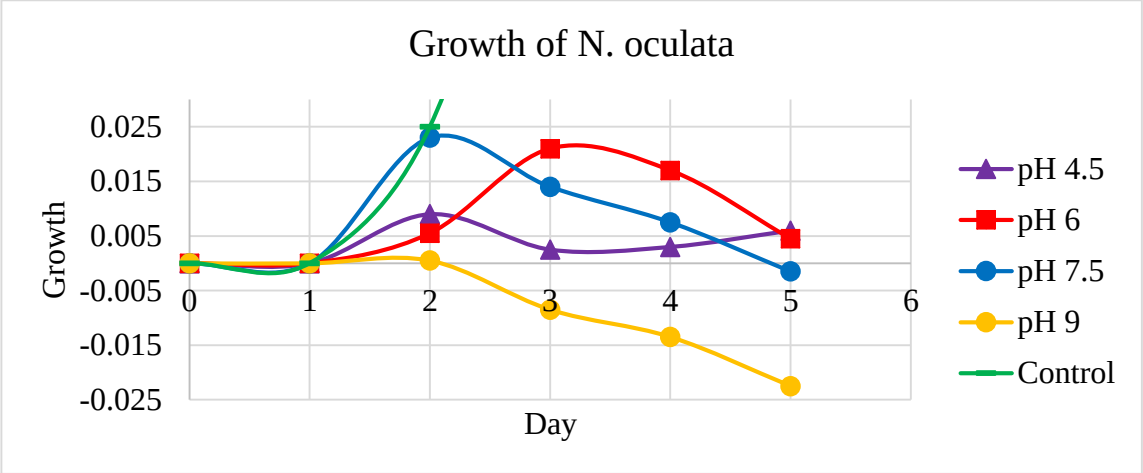

FIGURE 4.5 Growth of *N. oculata* in copper with various pH

In this experiment, the concentration of copper in the growth medium is prepared at 25 mg/l. This is 500 times the EC_{50} value of a *N. salina [34]*. As we can observe from the graph shown in Figure 4.5, all samples observed recorded slow or poor growth.

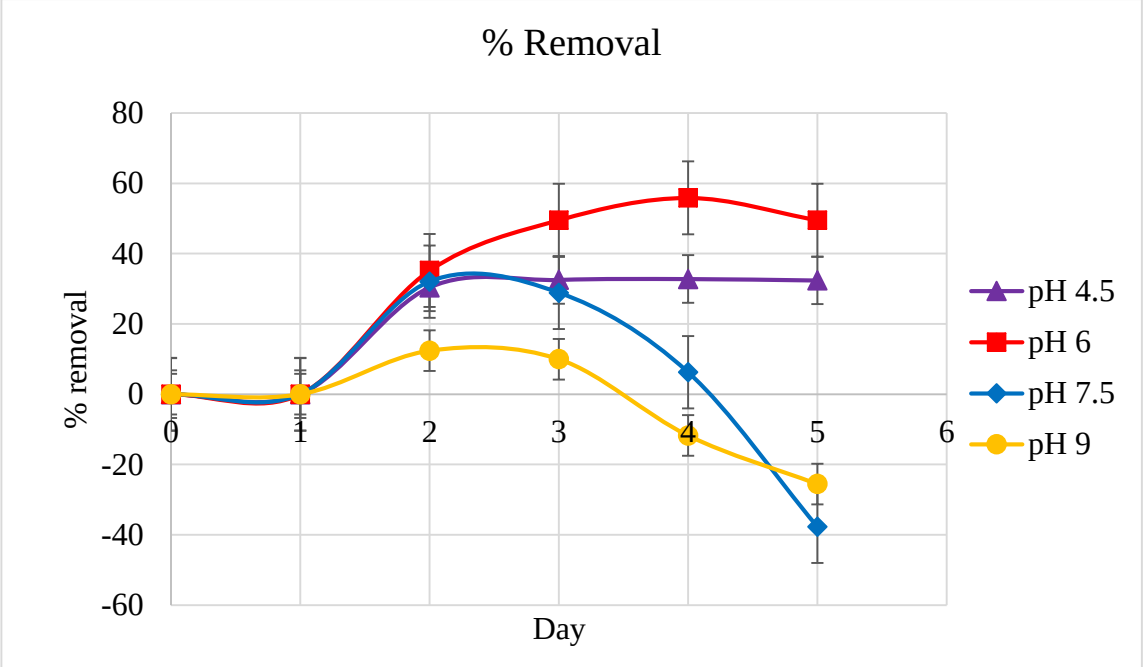

FIGURE 4.6 Percentage of removal of copper in various pH

Even though all experiment sets recorded poor growth, the biomass of *N. oculata* is still present in the growth medium and thus adsorption of copper would still occur. Based on the result obtained as shown in Figure 4.6, the adsorption quickly reached equilibrium when for sample at pH 4.5. In this experiment, large degree of precipitation of copper salt is observed at high pH values. For the experiment sets with pH 7.5 and pH 9, the results obtained are not accurate and might be due to high degree of experimental error as the percentage of removal is negative. Referring to Table 7.3 in the Appendices, we can find that the copper ions concentration for experiment at pH 9 do not differ much from day to day. Most probably the slight differences are caused by equipment error.

Figure 4.7 shows that when *N. oculata* grows, the percentage of removal increases. This is mainly due to the increase of adsorption sites as the microalgae grows. (It behaves similarly for samples at other pH values. Only the results at pH 6 is shown)

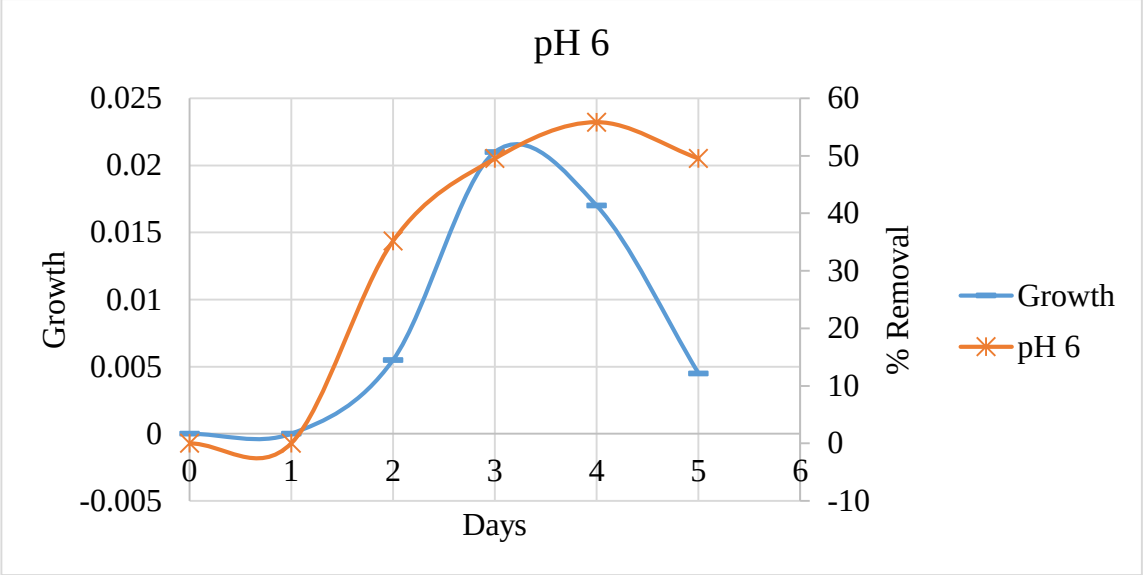

FIGURE 4.7 Graph of relationship of growth and percentage of removal of copper against time at pH 6

4.4 Copper Ionic Strength Experiment

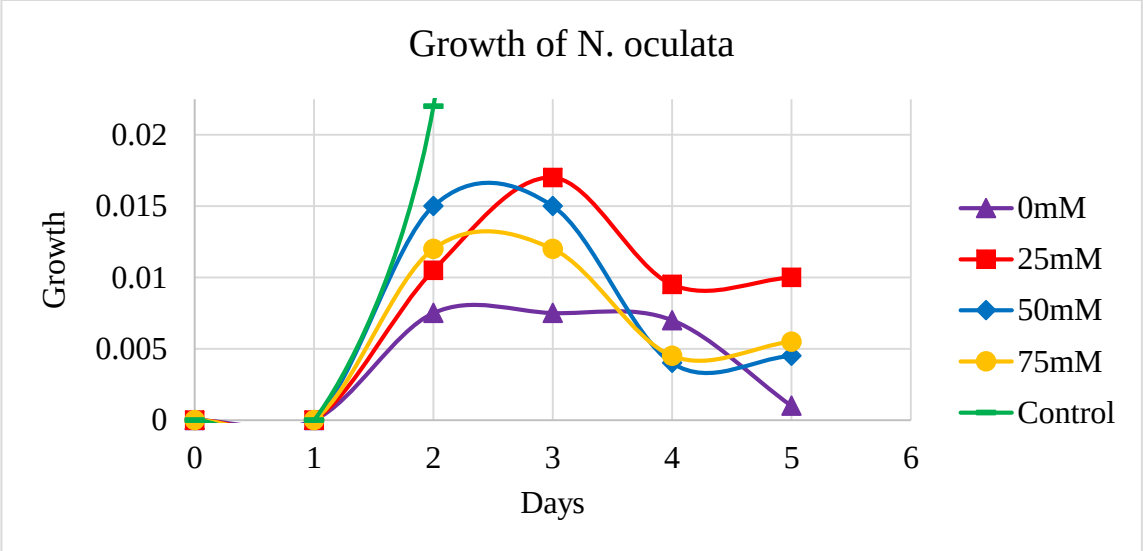

FIGURE 4.8 Growth of *N. oculata* in different ionic strength in copper

After having known that the *N. oculata* could not survive when exposed to 25 mg/l of copper in the pH experiment, the concentration of copper was reduced by half. Figure 4.8 shows the growth in the copper ionic strength experiment.

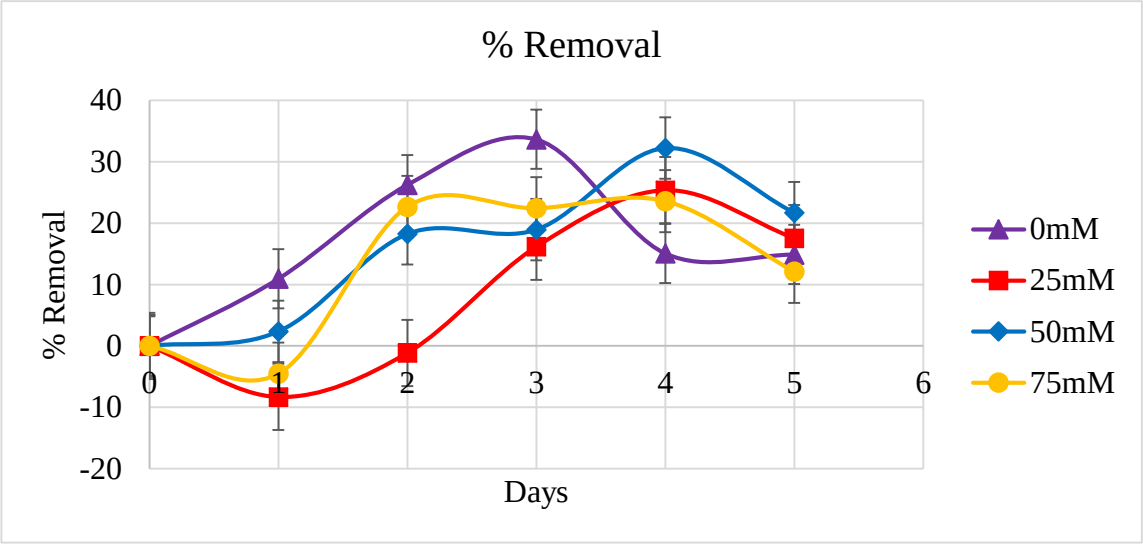

FIGURE 4.9 Percentage of removal of copper in copper ionic strength experiment

As shown in FIGURE 4.9, no distinctive trend could be determined from the bioremoval process. The hypothesis which states that at lower the ionic strength, the higher the percentage of removal is then defied. Bioremoval with living microalgae exhibits fluctuations [37]. Since no relationship can be observed from the graph of percentage of removal against time, then data obtained is plotted individually with the growth of the microalgae. (Only results of 0 mM and 75 mM are shown here)

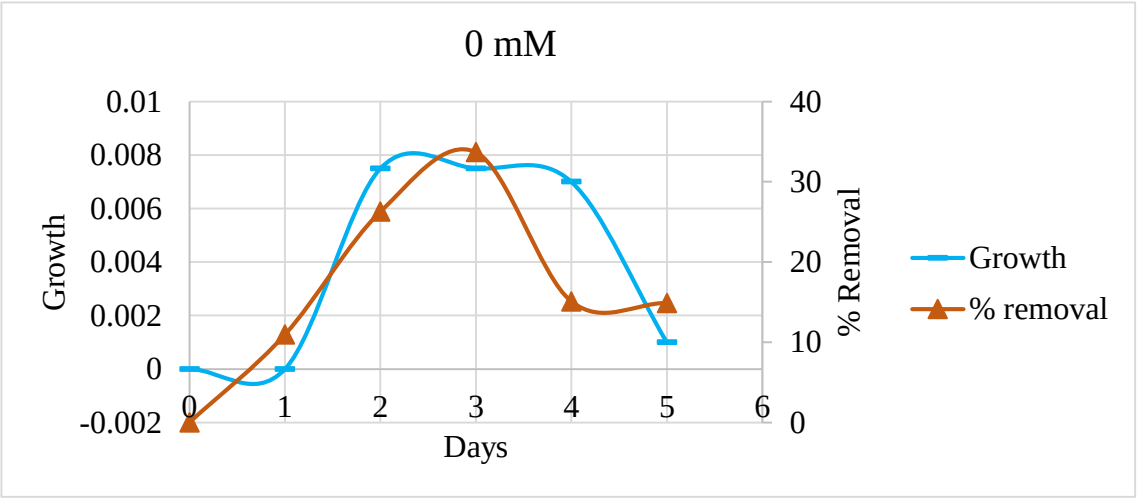

FIGURE 4.10 Graph of growth and percentage of removal of copper against time with ionic strength at 0 mM

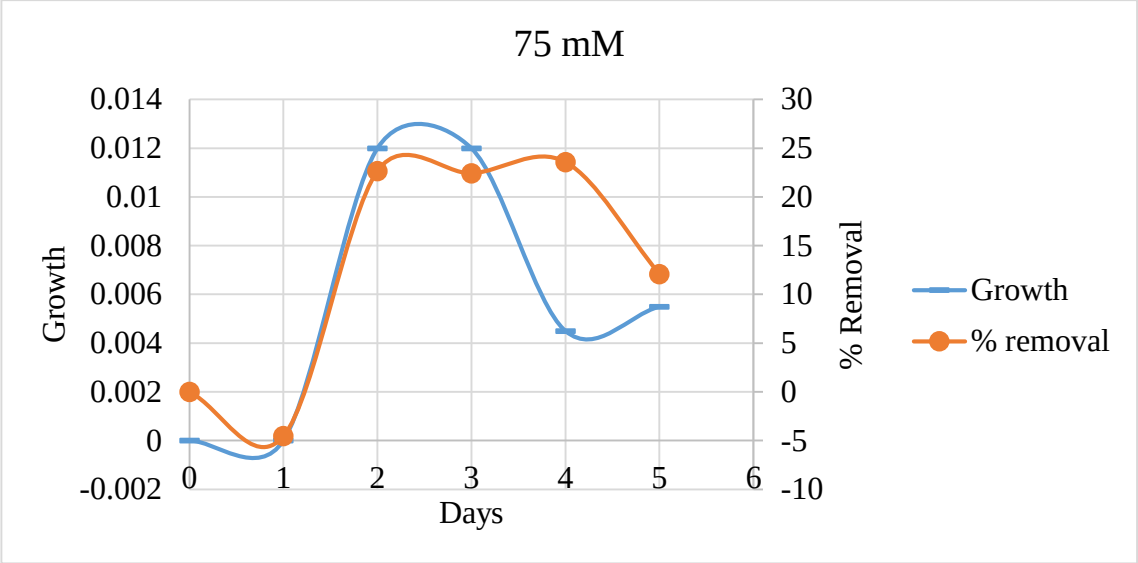

FIGURE 4.11 Graph of growth and percentage of removal of copper against time with ionic strength at 75 mM

The graphs plotted above show that the percentage of removal of copper increases when *N. oculata* grows. When it dies, the bioremoval decreases because the amount of biomass also decreases. In this way, we can say that the bioremoval with living *N. oculata* is more affected by the growth. Growth is also affected by the amount of nitrogen content in the medium [35]. There will be growth inhibition if the critical concentration is exceeded.

4.5 Mixture of Copper and Zinc pH Experiment

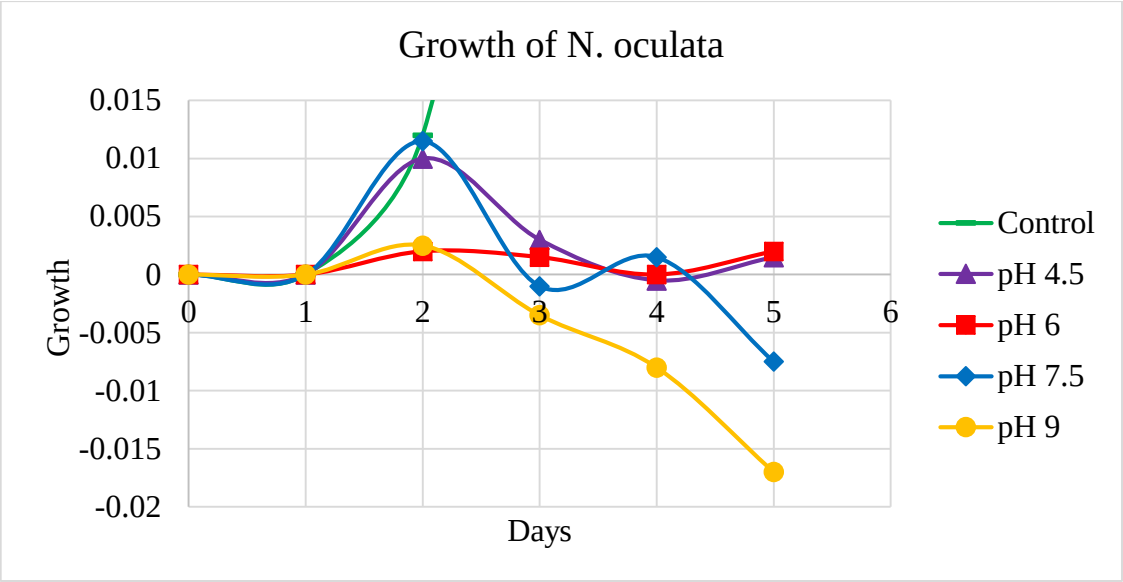

FIGURE 4.12 Growth of *N. oculata* in zinc and copper mixture in various pH

As shown in Figure 4.12, the toxicity effect of zinc and copper mixture is observed to be non-interactive if compared with the experiment of bioremoval of copper with variation in pH. Similar trend has been observed.

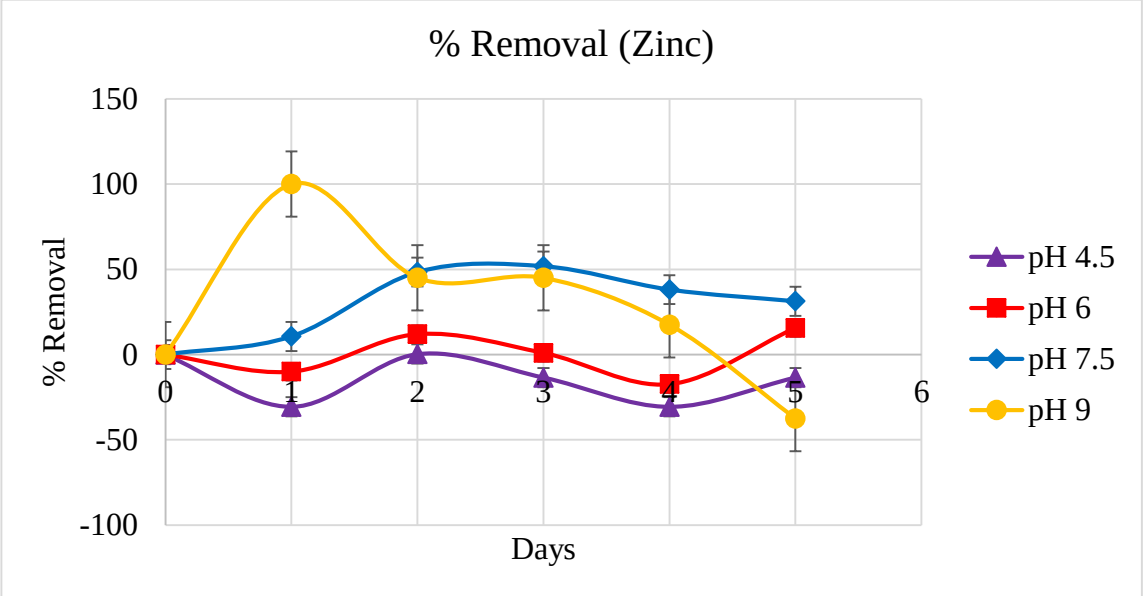

FIGURE 4.13 Percentage of removal of zinc in various pH in zinc and copper mixture

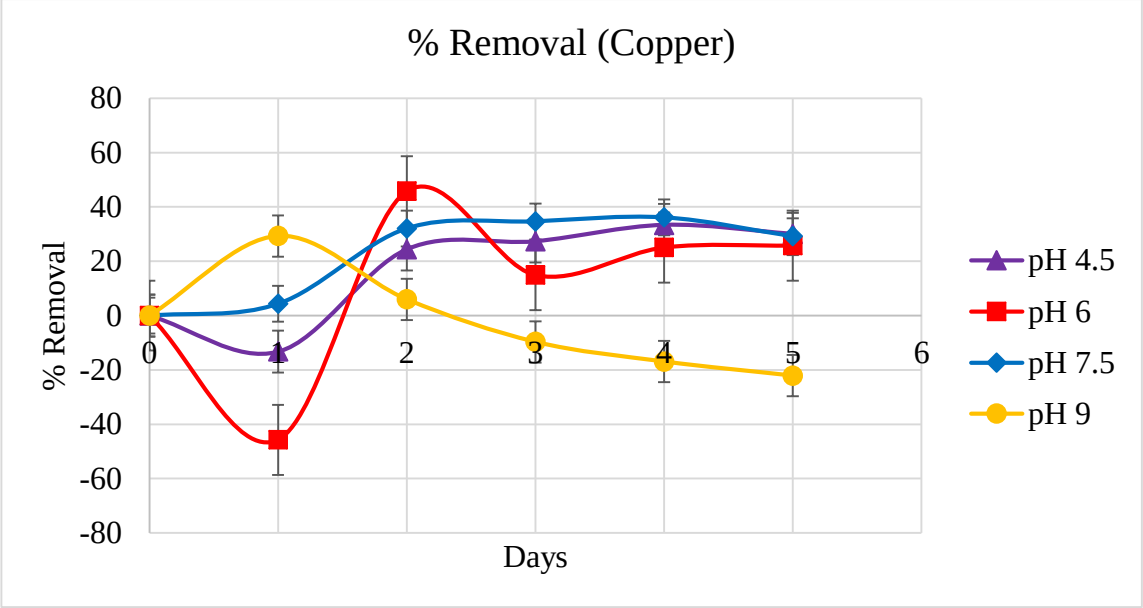

FIGURE 4.14 Percentage of removal of copper in various pH in zinc and copper mixture

No significant trend can be shown from Figure 4.13 and Figure 4.14. Bioremoval with living microalgae exhibits fluctuations [37]. Also, the interaction between heavy metal ions appear to be complicated and do not have a set pattern [29]. Some of the data points shown are negative and this must be due to experimental errors. Experiment would have to be carried out again to minimize the errors. Figure 4.15 and Figure 4.16 show the fluctuations and inconsistency of bioremoval by living *N. oculata*. (Only results of bioremoval of zinc and copper at pH 4.5 are shown)

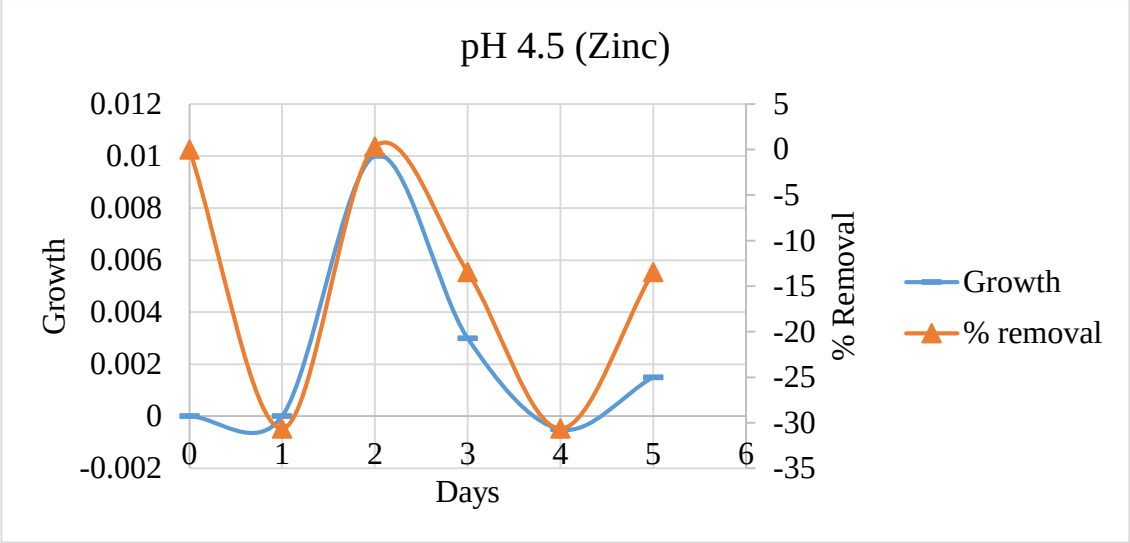

FIGURE 4.15 Graph of growth and percentage of removal of zinc against time in zinc and copper mixture at pH 4.5

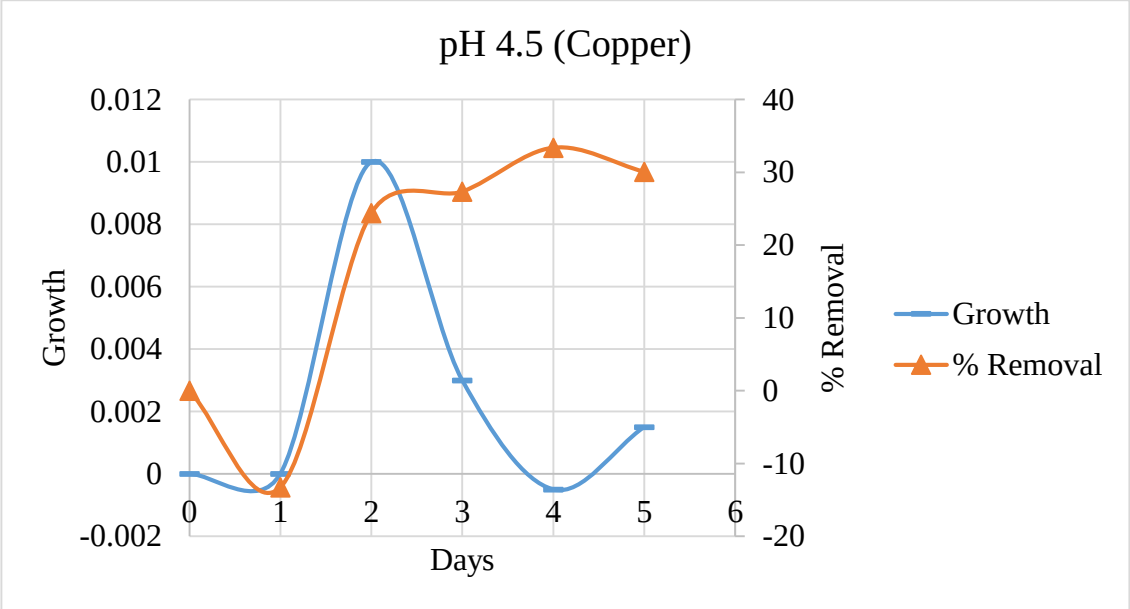

FIGURE 4.16 Graph of growth and percentage of removal of copper against time in zinc and copper mixture at pH 4.5

4.6 Mixture of Copper and Zinc Ionic Strength Experiment

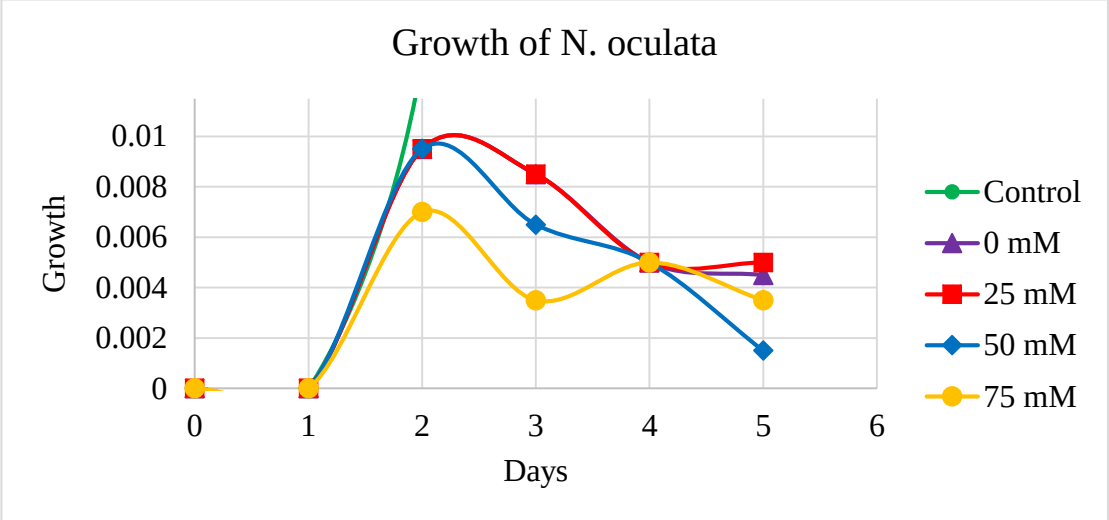

FIGURE 4.17 Growth of N. oculata at various ionic strength in zinc and copper mixture

From Figure 4.17, the toxicity effect of zinc and copper mixture is observed to be non-interactive if compared with the experiment of bioremoval of copper with variation in pH. Similar trend has been observed.

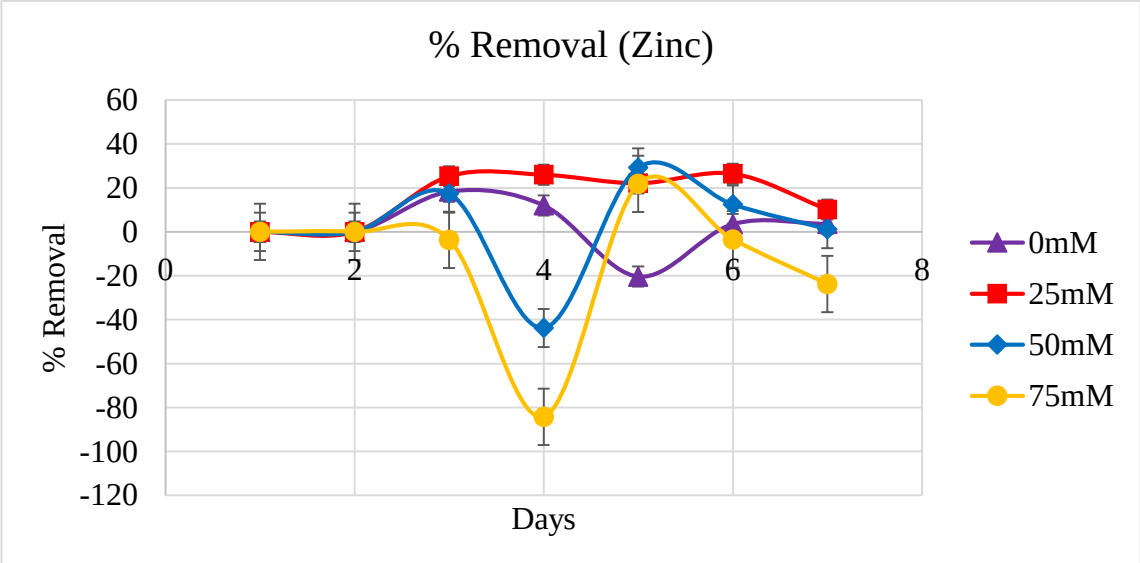

FIGURE 4.18 Percentage of removal of zinc at various ionic strength in zinc and copper

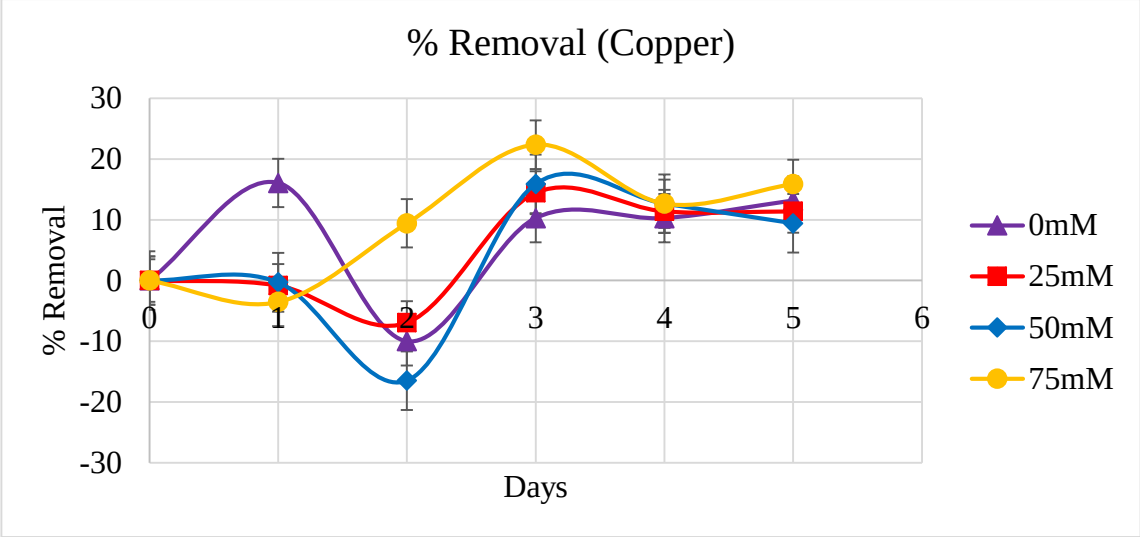

FIGURE 4.19 Percentage of removal of copper in different ionic strength in copper and zinc mixture

As in the pH experiment of zinc and copper mixture, the graph shown in Figure 4.18 and Figure 4.19 do not show distinct trend with the ionic strength. This could be due to fluctuations of bioremoval with living microalgae [37] and heavy metal ions do not have a fixed set of pattern of interaction [29].

Figure 4.20 and Figure 4.21 show how the bioremoval fluctuates and that it does not have a set pattern. (Only results obtained for 25 mM are shown)

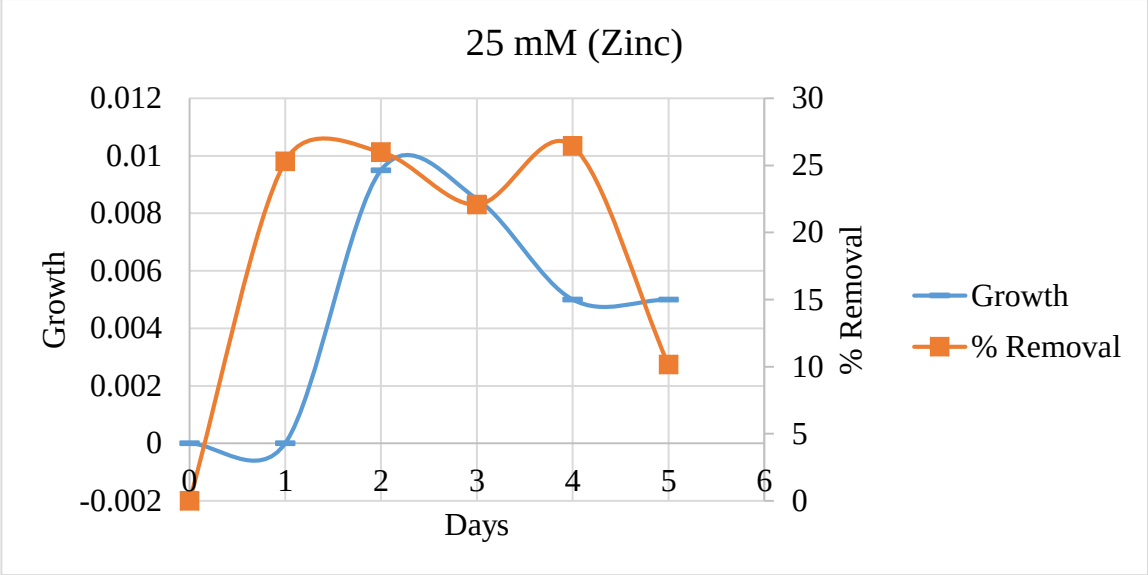

FIGURE 4.20 Graph of growth and percentage of removal of zinc against time of ionic strength experiment of mixture of zinc and copper at 25 mM

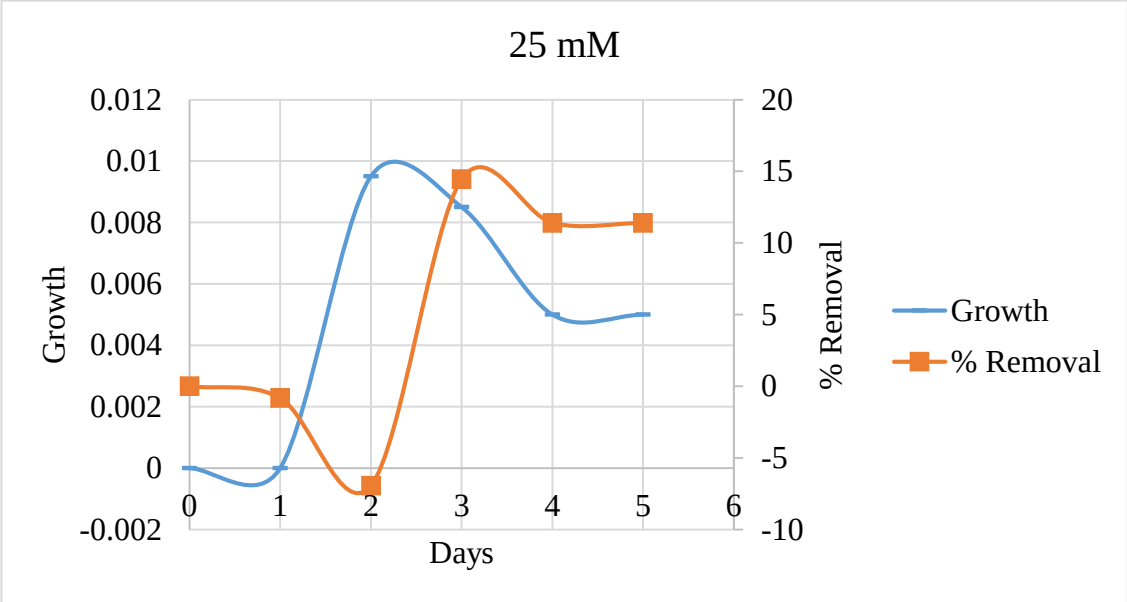

FIGURE 4.21 Graph of growth and percentage of removal of copper against time of ionic strength experiment of mixture of zinc and copper at 25 mM

CHAPTER 5
CONCLUSION AND RECOMMENDATION

It is proven that living *N. oculata* is able to uptake both zinc and copper. However, it lacks growth in the presence of copper. It is also found that bioremoval is more affected by growth of microalgae which is directly related to the pH value and ionic strength of the growth medium. Besides this, when there is growth, bioremoval increases which is due to increase of adsorption sites. Lastly, when both zinc and copper are present in the medium, the effect appears to be non-interactive because the growth trend behaves similarly like the one which has copper only.

This project can be further developed by introducing different heavy metals and organic wastes into the solution simulating real world pollution sites. The parameter temperature can also be included. The combination of several types of microalgae can also be experimented. Bioremoval experiments based on combination of non-living microalgae species should also be investigated.

Besides this, before starting the experiment, it is also very important to find out the heavy metal tolerance of the microalgae used and how sodium nitrate affects its growth. The duration of experiment can also be extended to investigate the equilibrium point of adsorption. Microalgae species which can tolerate large pH difference can be used to find out how pH affects the adsorption. Lastly, heavy metal salt which do not precipitate in f2 medium is recommended.

CHAPTER 6

APPENDICES

Experimental planning

TABLE 6.1 Summary of experiment design

Experiment set	Fixed variable	Manipulated variable	Value	Percentage of metal uptake (%)	
				Set 1	Set 2
pH value	• Concentration of microalgae • Concentration of metal • Ionic strength	pH value	4.5		
			6		
			7.5		
			9		
Ionic strength	• Concentration of microalgae • Concentration of metal • pH value	Ionic strength	0 mM		
			25 mM		
			50 mM		
			75 mM		

TABLE 6.2 Preparation of pH stock solution

pH	Volume (litre)	Target metal concentration (g/L)			Metal concentration achieved (g/L)		
		Zn	Cu	Zn+Cu	Zn	Cu	Zn+Cu
4.5	1						
6	1						
7.5	1						
9	1						

TABLE 6.3 Preparation of ionic strength stock solution

Ionic strength (mM)	Volume (litre)	Target metal concentration (g/L)			Metal concentration achieved (g/L)		
		Zn	Cu	Zn+Cu	Zn	Cu	Zn+Cu
0	1						
25	1						
50	1						
75	1						

TABLE 6.4 Experimental results data sheet

Experiment set	Value	Metal concentration (mg/L)									
		Set 1					Set 2				
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
pH	pH 4.5										
	pH 6										
	pH 7.5										
	pH 9										
Ionic strength	0 mM										
	25 mM										
	50 mM										
	75 mM										

TABLE 6.5 Growth record of *N. oculata*

Parameters	Absorbance value				
	Day 1	Day 2	Day 3	Day 4	Day 5
Control					
pH 4.5 / 0mM					
pH 6 / 25mM					
pH 7.5 / 50mM					
pH 9 / 75mM					

Raw data

TABLE 6.6 Raw data of bioremoval of zinc in various pH

Day	Concentration (mg/l)			
	pH 4.5	pH 6	pH 7.5	pH 9
1	0.4703152	0.363901244	0.4164334	0.371557
2	0.45466415	0.377831784	0.3941267	0.3772685
3	0.40182771	0.361258556	0.3862679	0.3740219
4	0.39609623	0.338823341	0.3808793	0.3685101
5	0.38461528	0.334723147	0.3785715	0.3729892

TABLE 6.7 Raw data of bioremoval of zinc in various ionic strength

Day	Concentration (mg/l)			
	0mM	25mM	50mM	75mM
1	0.514039	0.485769169	0.530772	0.591753
2	0.411928	0.411712653	0.395544	0.39398
3	0.384287	0.383026607	0.375518	0.369542
4	0.375917	0.375182438	0.372685	0.364557
5	0.389115	0.37962487	0.37314	0.371795

TABLE 6.8 Raw data of bioremoval of copper in various pH

Day	Concentration (mg/l)			
	pH 4.5	**pH 6**	**pH 7.5**	**pH 9**
1	3.59464143	0.413956184	0.1617291	0.1200106
2	2.50195987	0.268351423	0.1099066	0.1051577
3	2.42513825	0.209017333	0.115057	0.1080483
4	2.41632635	0.18280821	0.151634	0.1340998
5	2.43125575	0.208965434	0.222721	0.1506771

TABLE 6.9 Raw data bioremoval of copper in various ionic strength

Day	Concentration (mg/l)			
	0mM	**25mM**	**50mM**	**75mM**
1	0.18621091	0.187094138	0.1686831	0.1520431
2	0.15419595	0.174714251	0.1412095	0.1125084
3	0.13869352	0.144815598	0.1400017	0.1128387
4	0.17756168	0.128899849	0.1170336	0.1111655
5	0.17796909	0.142403878	0.1352262	0.1278704

TABLE 6.10 Raw data of bioremoval of zinc in various pH in mixture of zinc and copper

Day	Concentration (mg/l)			
	pH 4.5	**pH 6**	**pH 7.5**	**pH 9**
1	1.16449314	0.299542672	0.1213642	0.0567224
2	0.77744034	0.111393338	0.0862269	0.0754588
3	0.74698162	0.174903072	0.0829347	0.0880213
4	0.68499711	0.154021631	0.0810974	0.0938248
5	0.71902001	0.152569152	0.0898795	0.0980007

TABLE 6.11 Raw data of bioremoval of copper in various pH in mixture of zinc and copper

Day	Concentration (mg/l)			
	pH 4.5	**pH 6**	**pH 7.5**	**pH 9**
1	-13.2513	-45.72372	4.3904941	29.31364
2	24.39101	45.808551	32.071317	5.964715
3	27.35323	14.911869	34.664886	-9.69034
4	33.38146	25.070426	36.112291	-16.9226
5	30.07261	25.777038	29.193829	-22.1265

TABLE 6.12 Raw data of bioremoval of copper in various ionic strength in mixture of zinc and copper

Day	Concentration (mg/l)			
	0mM	25mM	50mM	75mM
1	0.06840304	0.062251585	0.0559766	0.0601936
2	0.07368393	0.061679229	0.0975326	0.1069947
3	0.10080596	0.064937849	0.047973	0.0454071
4	0.08080692	0.061299471	0.0593929	0.0600911
5	0.08071295	0.074879899	0.0670083	0.071883

TABLE 6.13 Raw data of bioremoval of zinc in various ionic strength in mixture of zinc and copper

Day	Concentration (mg/l)			
	0mM	25mM	50mM	75mM
1	18.29713	25.303235	17.467768	-3.64206
2	11.98946	25.990014	-43.8025	-84.2248
3	-20.406	22.07994	29.268392	21.81749
4	3.48153	26.445692	12.430804	-3.46556
5	3.593769	10.1503	1.2026923	-23.769

Experimental results

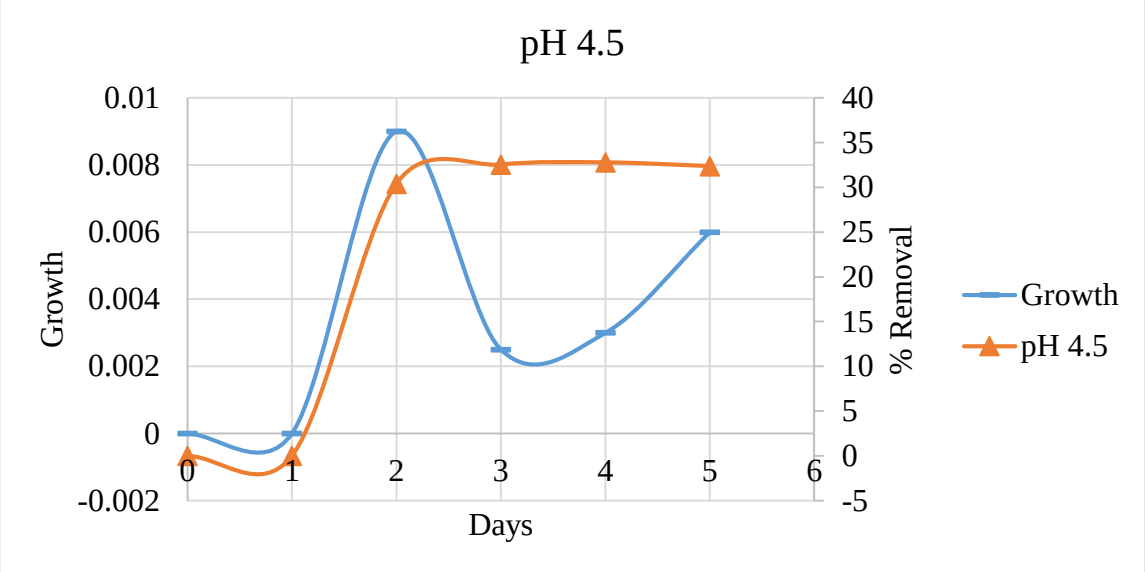

FIGURE 6.1 Graph of relationship of growth and percentage of removal of copper against time at pH 4.5

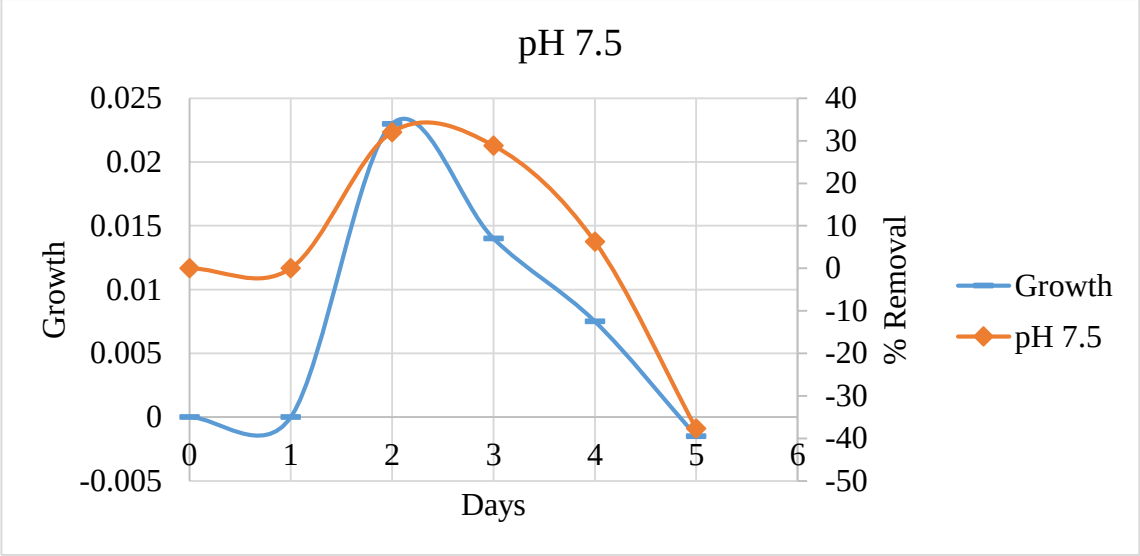

FIGURE 6.2 Graph of relationship of growth and percentage of removal of copper against time at pH 7.5

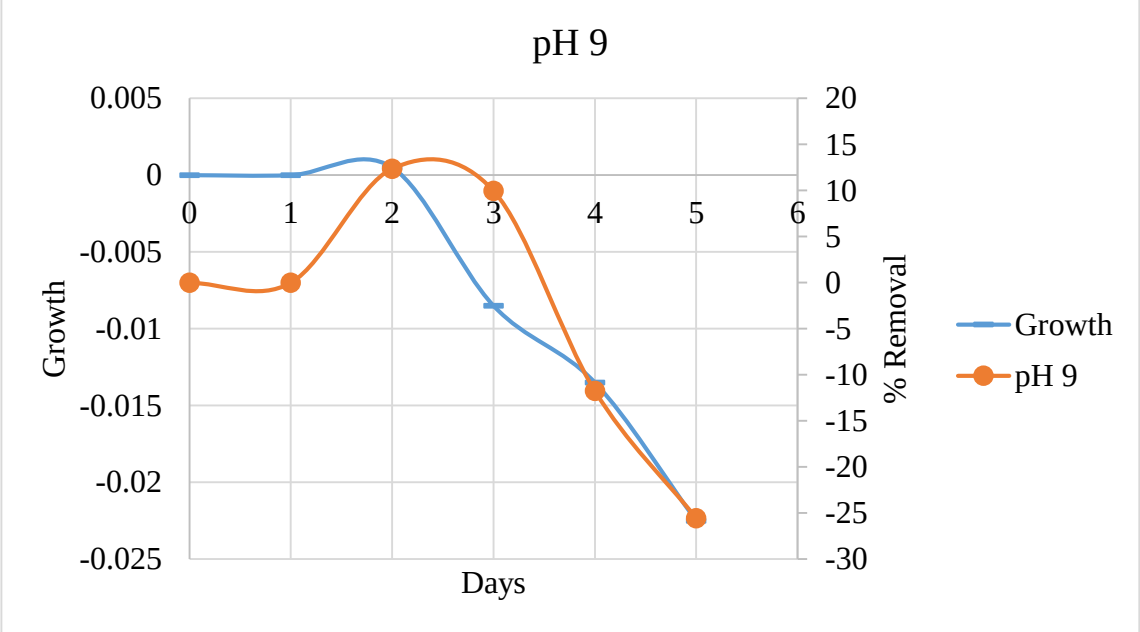

FIGURE 6.3 Graph of relationship of growth and percentage of removal of copper against time at pH 9

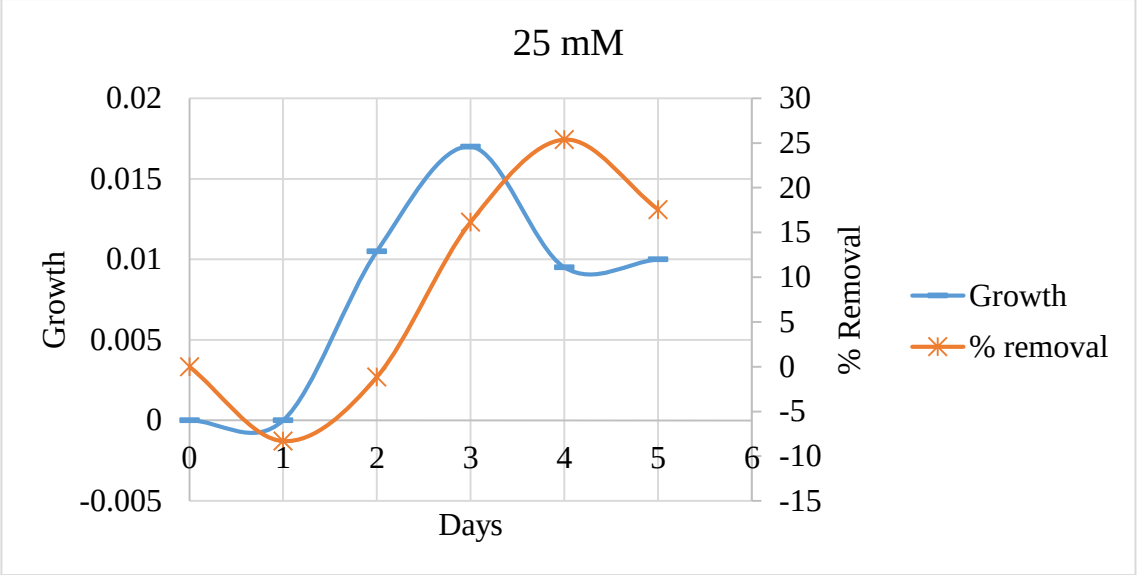

FIGURE 6.4 Graph of relationship of growth and percentage of removal of copper against time with ionic strength at 25 mM

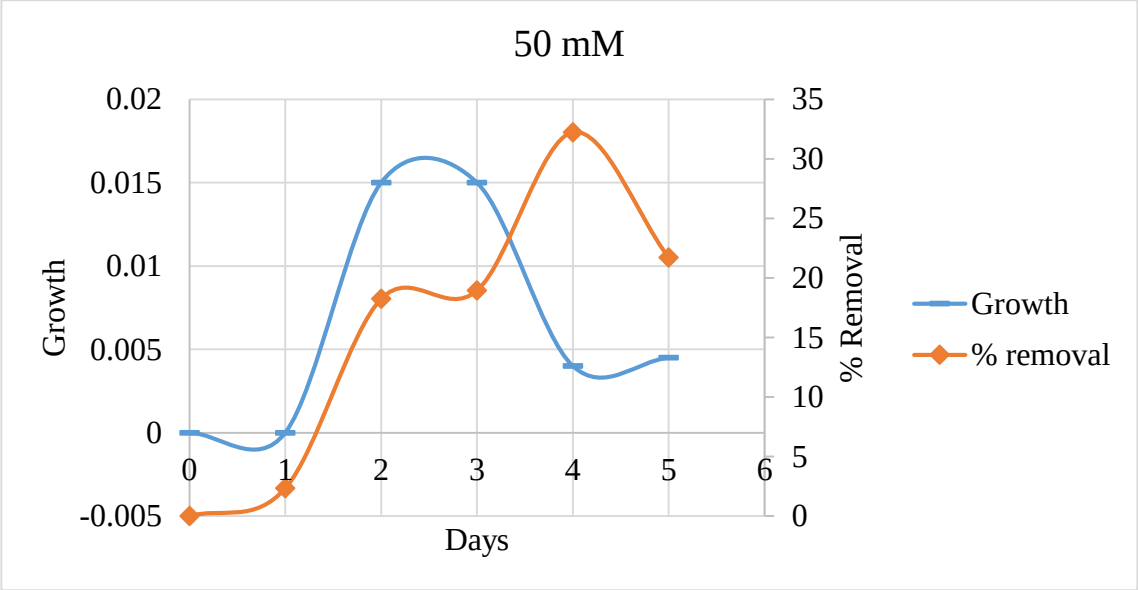

FIGURE 6.5 Graph of relationship of growth and percentage of removal of copper against time with ionic strength at 50 mM

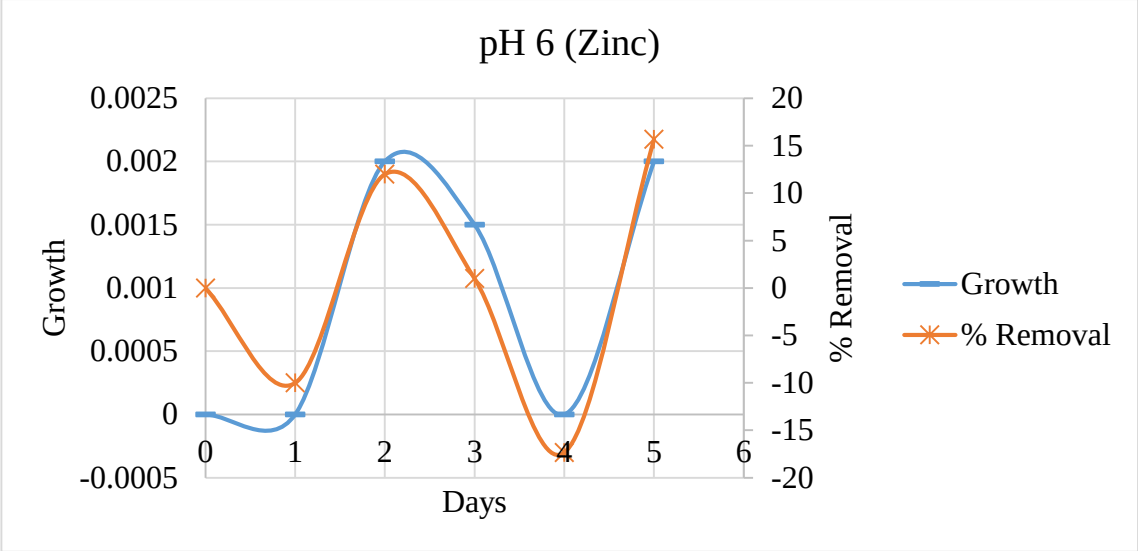

FIGURE 6.6 Graph of growth and percentage of removal of zinc against time of experiment of mixture of zinc and copper at pH 6

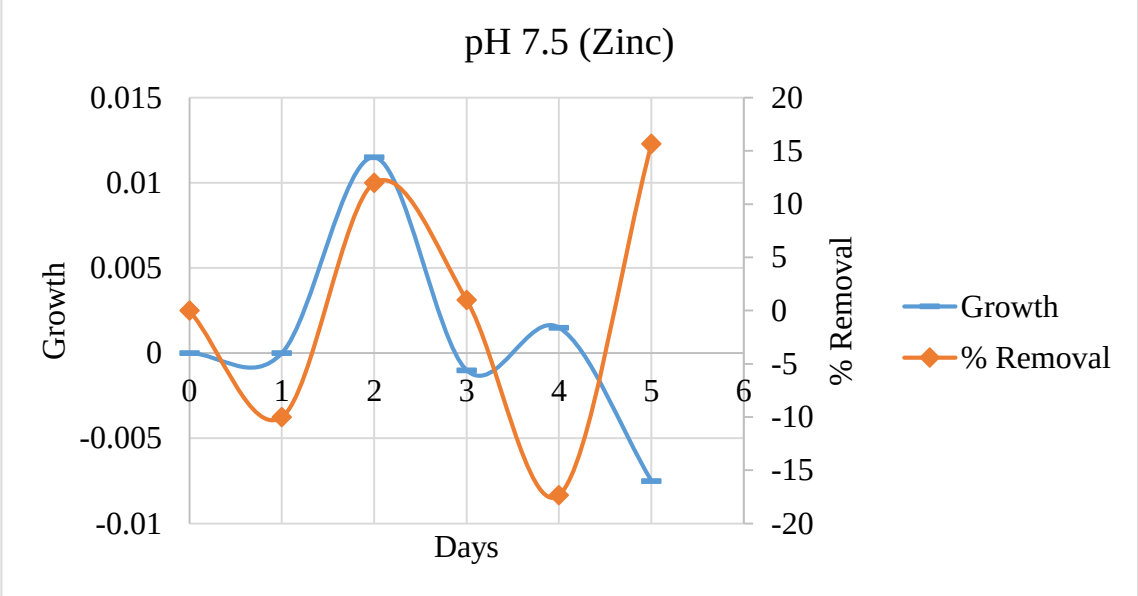

FIGURE 6.7 Graph of growth and percentage of removal of zinc against time of experiment of mixture of zinc and copper at pH 7.5

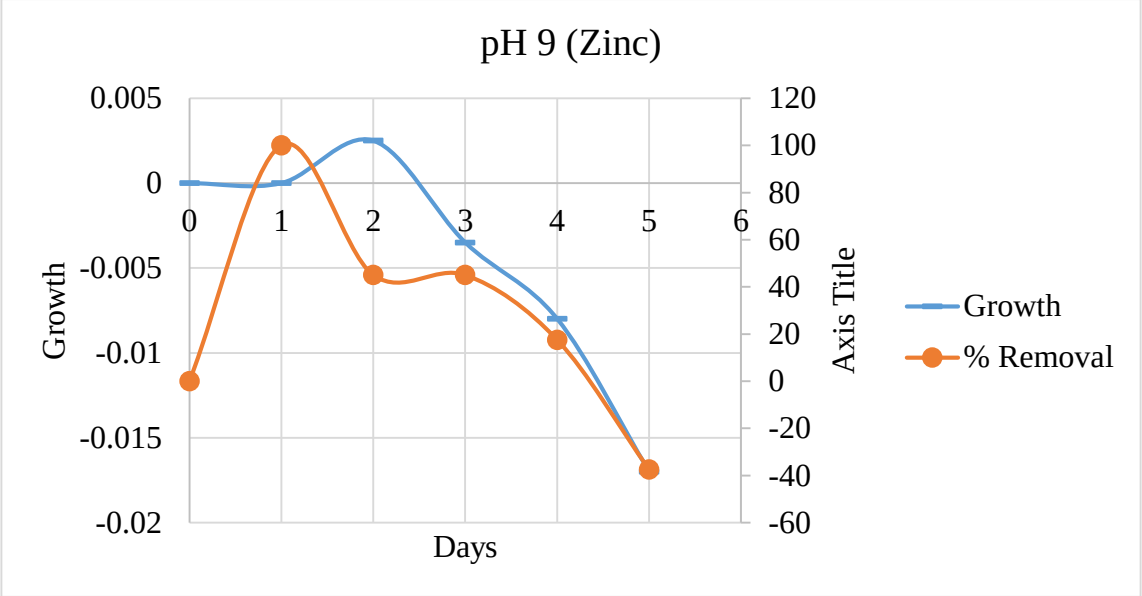

FIGURE 6.8 Graph of growth and percentage of removal of zinc against time of experiment of mixture of zinc and copper at pH 9

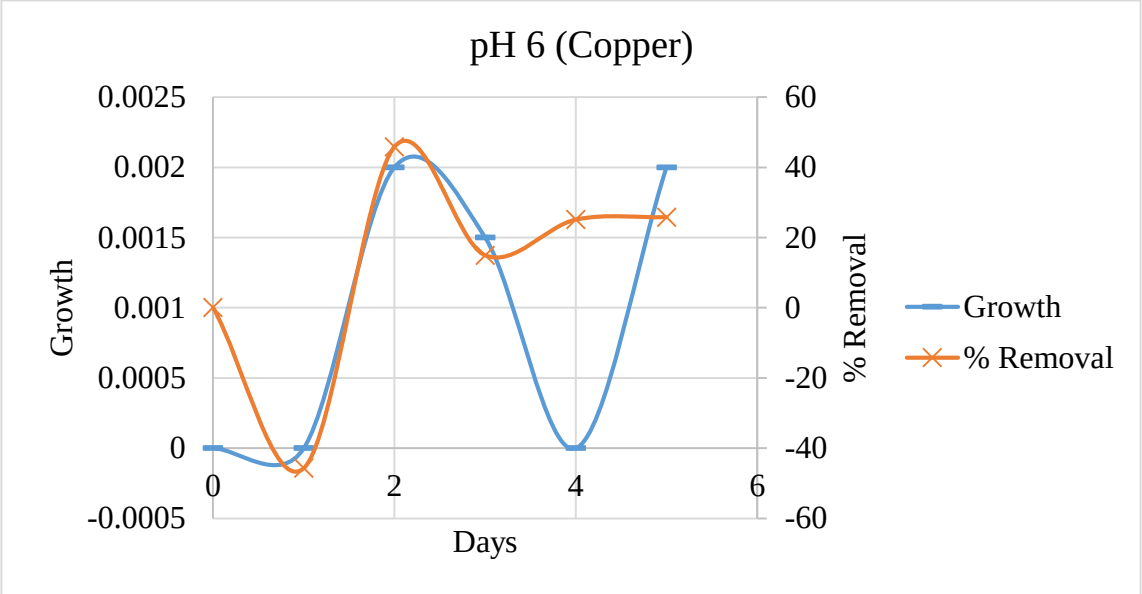

FIGURE 6.9 Graph of growth and percentage of removal of copper against time of experiment of mixture of zinc and copper at pH 6

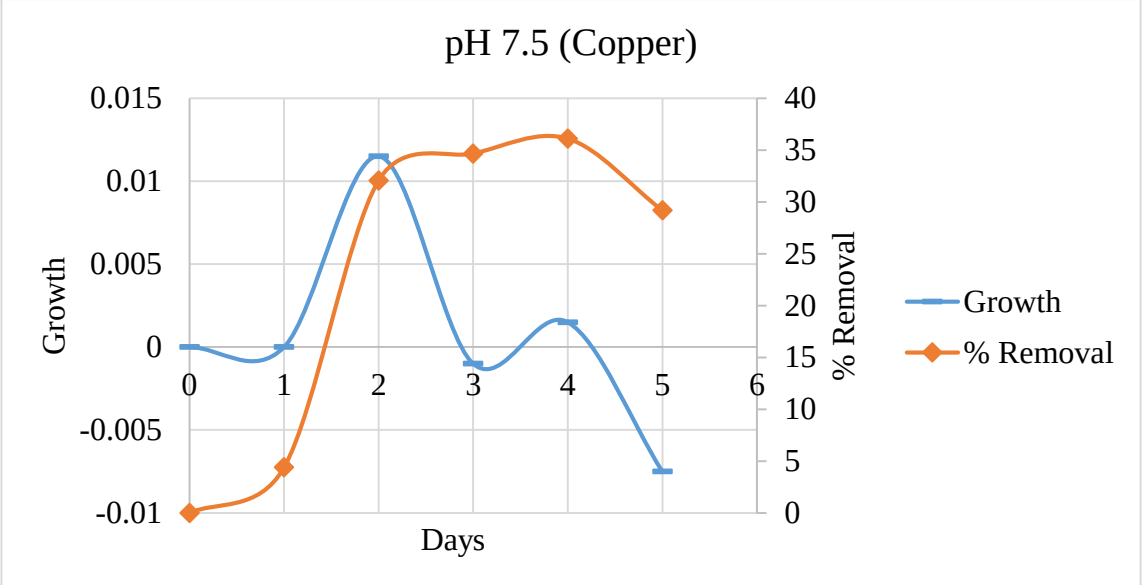

FIGURE 6.10 Graph of growth and percentage of removal of copper against time of experiment of mixture of zinc and copper at pH 7.5

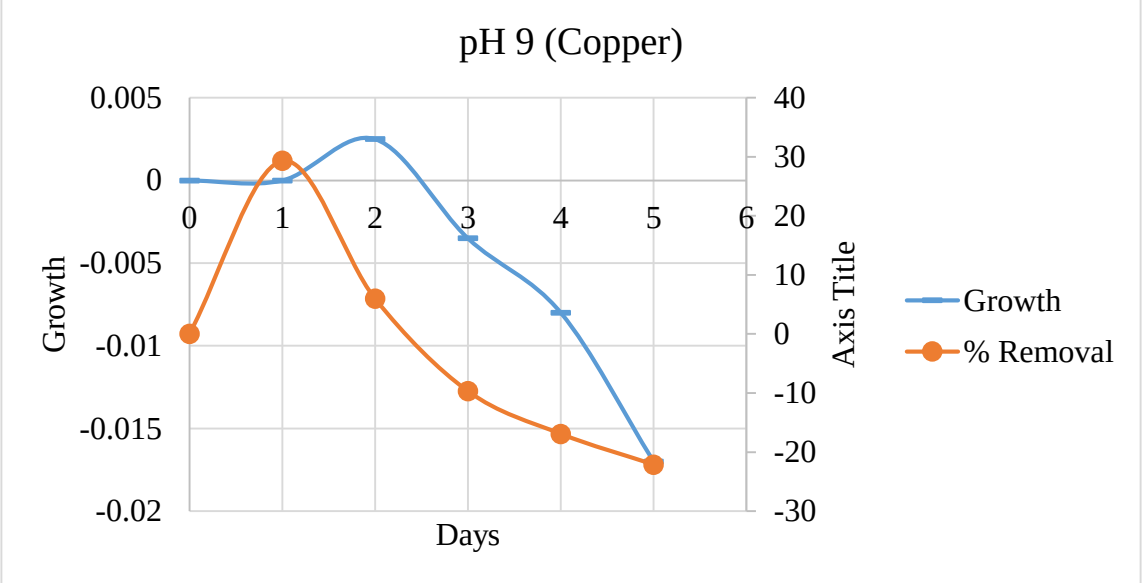

FIGURE 6.11 Graph of growth and percentage of removal of copper against time of experiment of mixture of zinc and copper at pH 9

CHAPTER 7

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