B.Tech Thesis on

HEAVY METAL BIOSORPTION USING ALGAE

For partial fulfillment of the requirement for the degree of

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in

Chemical Engineering

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CERTIFICATE

This is to certify that the thesis entitled "**Heavy metal bio sorption using algae**" submitted by **Rajesh Kumar** Roll No.-110CH0467 in partial fulfillment of the requirement for the award of degree of Bachelor of Technology in Chemical Engineering at National Institute of Technology Rourkela; is an authentic work carried out by him under my supervision and guidance.

To best of knowledge, the matter included in this thesis has not been submitted to any other university or institute for the award of any degree.

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ABSTRACT

Heavy metal removal from water is essential as these toxic chemicals may enter to impasse food web and will cause severe disasters not only for human beings but also for all aquatic & non aquatic species. Many techniques have reported till date for removal of heavy metal especially from water but having their own advantages as well as disadvantages. In present work we report biosorption of Copper and Nickel by locally available contaminated mixed freshwater algae culture. In present work three algal samples were collected, in which two were from macro/sea weed origin where as another was *Senedesmus* species. In comparison study of Cu(II) removal it was found that, more than 95% adsorption was observed by sample 1, where as ~87% was from sample 2 and ~70% from sample 3. Approximately 25% of Nickel was also up taken by sample 3. As samples taken for observation were not isolated and purified so it is difficult to interpret best among macro/micro algal, bacterial or fungal biosorption capacity however, we conclude that even fresh water contaminated mixed algal species are a promising and cheap mean to utilize for heavy metal removal from various industrial or other wastes.

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CHAPTER -1

INTRODUCTION

INTRODUCTION

1.1. ALGAE DESCRIPTION

Algae are a very large and diverse group of eukaryotic organisms, ranging from unicellular genera such as *Chlorella* to multicellular forms such as the giant kelp, a large brown alga that may grow up to 50 meters in length. This is characterized by a high productivity per unit area when compared with other photosynthetic organisms of higher plants. Algae have outstanding photosynthetic efficiency, reduced number of internally competitive physiological functions, fast reproduction cycles, limited nutrient requirements, adaptation to a broad range of temporal and spectral irradiances. Furthermore, a few micro-algal cultures (e.g Dunaliella, Spirulina, and Chlorella spp.) are relatively prone to scale-up in photo bioreactors (PBRs), where it is possible to provide optimal nutrient levels on a continuous basis.

Algae are autotrophs, i.e. they can synthesize inorganic nutrients. A stoichiometric formula for the most common elements in algal cell is $\mathbf{C_{106}H_{181}O_{45}N_{16}P}$, and the elements should be present in these proportions in medium for optimal growth. The rate at which an algal cell takes up a specific nutrient depends on the difference between the concentration inside and outside the cell, and also on the diffusion rates through the cell wall. Microalgae have unique characteristics of CO_2 sequestration for the production of biofuels which allow them to be potentially utilized in broad and versatile ways in climate change technologies. Compared to other photosynthetic plants, microalgae are more productive carbon dioxide users and can fix larger amounts of carbon dioxide per unit area than terrestrial plants. Further, as a part of their metabolic processes, microalgae uptake nutrients (such as nitrogen and phosphorus), which occur in high level in secondary effluent from domestic as well as industrials wastewater treatment plants. Additionally they have the ability to adsorb heavy metals such as Ni, Cu, Co, Zn, Cd etc. onto their cell surfaces through a process called ‘bio sorption’. By removing both nutrients and heavy metals, microalgae provide significant treatment to the wastewater.

1.2 TOXIC HEAVY METALS

Most of the heavy metals are well-known toxic and carcinogenic agents and it represent a serious threat to the human population and the fauna and flora of the receiving water bodies. Heavy metals have a great tendency to bio-accumulate and end up as permanent additions to the environment. When wastewater is discharged to receiving water bodies without removal of heavy metals, heavy metals may be harmful to both human and aquatic life as they are non-degradable and persistent. Heavy metals are considered to be the following elements: Copper, Silver, Zinc, Cadmium, Gold, Mercury, Lead, Chromium, Iron, Nickel, Tin, Arsenic, Selenium, Molybdenum, Cobalt, Manganese, and Aluminum. The removal of heavy metals from waste water has recently become the subject of considerable interest owing to strict legislations. Industrial wastewater containing heavy metal should be treated before discharge to the water stream but its treatment is very costly. There are several techniques to remove heavy metals from wastewater such as filtration, electro coagulation etc but there is some limitation such as long treatment time. Various biological treatments, both aerobic and anaerobic can be used for heavy metal removal.

Heavy metals are major pollutants in the environment due to their toxicity and threat to creatures and human being at high concentrations. Copper is highly toxic because it is non biodegradable and carcinogenic. The effects of Ni exposure vary from skin irritation to damage of the lungs, nervous system, and mucous membranes. The following table shows the allowable amount of heavy metal in the water.

Table 1: Various Norms for heavy metal composition

Heavy Metal	W.H.O. [For Drinking] (mg/l)	U.S.E.P.A. [For wastewater] (mg/l)	C.P.C.B. [For wastewater] (mg/l)
Cu	0.05	1.3	1.5
Ni	0.05	1	0.05

Conventional methods of heavy metal removal such as ion exchange or lime precipitation are often ineffective or very expensive when used for the reduction of heavy metals at very low concentrations of 10 – 100 mg/L. Other methods include chemical precipitation, solvent extraction and adsorption which require high energy input, capital investment and operational costs and may not substantially decrease heavy metal concentrations in the desired amount. There has also been investigation into the use of electrochemical removal, photochemical degradation and oxidation. For the removal of many organic and inorganic contaminants however, adsorption is considered to be the preferred option as it is the most widely applicable.

As an alternative adsorption medium, heavy metal removal by microalgae is very efficient and economical.

While microalgae is a versatile and economic option for the treatment of effluent, and create a valuable byproduct, the suitability to serve these many purposes must be judged in part on the ability to remove heavy metals from effluent. As seen in **Table 2**, few of the studies related to algae and heavy metal removal have been completed using live algae and non-synthetic, domestic wastewater. Therefore, experiments described herein were designed to observe the influence of live microalgae on heavy metal removal, copper and nickel specifically, on effluent and the influence.

Table 2: Various Heavy Metal Removal Achieved by Microalgae

I	II	III	IV
Heavy Metal	**Algal strain and Condition**	**Process**	**Removal Efficiency**
Selenium	Combined algal-anaerobic bacteria Live algae	High rate ponds containing drainage water	94 to 100%
Chromium	*Spirogyra condensate* and *Rchizoclonium hieroglyphicum* Dried algae	Batch equilibrium in conical flasks Synthetic and Tannery Wastes	75% for low algae concentrations (<100 mg/L)
Chromium	*Chlorella miniata* Dried Algae cultivated in domestic wastewater	250 mL conical flasks, agitated at room temperature. Synthetic Solutions	At equilibrium, 75% and 100% removal for Cr(III) and Cr(VI) respectively
Chromium and copper	*Sargassum* sp. (macroalgae) and *Chlorococcum* sp.) Dried algae	Batch sorption in 500 mL polyethylene bottles Synthetic solutions	*Sargassum* removes up to 87% Cu (1.0 to 30.0 mg/L Cu) while *Chlorococcum* removed 43-75% Cu. *Sargassum* more efficient at higher Cr concentrations, while *Chlorococcum* removed 67% Cr at 2 mg/L solution.
Cadmium, copper, lead and zinc	*Haslea ostrearia, Phaeodactylum tricornutum, Skeletonema costatum* and *Tetraselmis suecica* Live algae	250 mL Erlenmeyer flasks at 14:10 light: dark cycle, 17°C Salt water or enriched seawater	Results show reduction in Cu and Cd

Copper, zinc, cadmium, mercury	*Cladophora fracta* Live algae	Batch testing in 100 ml Erlenmeyer flasks with synthetic solutions	Cu^{2+}, ,Zn^{2+}, Cd^{2+}, ,and Hg^{2+} were 99, 85, 97, and 98%, respectively
Lead, cadmium, copper and arsenic	Cyanophyta *(Oscillatoria princeps 92 %, Oscillatoria subbrevis 2%,*and *Oscillatoria formosa 1 %)* and Chlorophyta *(Spirogyra aequinoctialis 3%,Mougeta sp. 1 %,* and others 1 %) Dried algae	Batch testing in 250 ml Erlenmeyer flasks with synthetic solutions	Metals were removed
Cadmium, mercury. lead, arsenic and cobalt	*Spirogyra hyaline* Dried algae	Batch testing in 250 ml Erlenmeyer flasks with synthetic solutions	The order of metal uptake for the dried biomass was found to be Hg>Pb>Cd>As>Co
Cadmium, nickel, lead, zinc, copper	*Spirogyra neglecta, Pithophora oedogonia, Hydrodictyon Reticulatum, Cladophora calliceima, Aulosira fertilissima* Dried algae	Batch testing in 100 ml Erlenmeyer flasks with synthetic solutions	*S. neglecta* and *P. oedogonia* can remove >75% of Cu^{2+} and Pb^{2+} from the multi-metal solution
Nickel	*Oedogonium hatei* Untreated and acid - treated dried algae	Batch testing in 100 ml synthetic solution	Maximum adsorption capacity of untreated and treated algal biomass was found to have greater or comparable values compared to other similar biosorbents

1.3 OBJECTIVE OF PRESENT RESEARCH

The main objectives of present work are as follows:

4.1 Algae sample collection from N.I.T, Rourkela campus.

- Microscopy study for preliminary algae identification.
- Providing suitable condition and nutrient for collected algal growth.
- Study of Copper and Nickel adsorption.

CHAPTER-2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Introduction

Khoeyi et. al., 2011 described the effect of irradiances and photoperiods on the biomasses and fatty acid (FA) compositions of *Chlorella vulgaris* which was examined in the exponentials growth phase. Light regime had effects on the biomasses of *Chlorella vulgaris.* For the longer durations of light brought about increased biomass of *Chlorella vulgaris.* However, light intensities showed different effect on biomasses; increase in light intensities from 37.5 to 62.5 mol photon m^{-2} s^{-1} resulted in increased biomasses, but at 100 mol photon m^{-2} s^{-1}, biomasses decreased.

Carvalho et. al., 2010 in their study about light requirement in micro-algae photo-bioreactors, the constraints of light saturation may be overcomes by either of two approaches: increasing photosynthetic efficiencies by genetic engineering, aimed at changing the chlorophyll antenna sizes; or increasing flux tolerances, by tailoring the photonic spectrums, coupled with its intensities and temporal characteristic. This approach will allows increased controls over the illumination features, leading to maximization of microalgae biomass and metabolite productivities.

Walker et. al., 2005 discussed in their articles and compared the current commercially viable bioreactors system, outline recent progresses in micro-algae bio-technologies and transformations, and discuss the potentials of microalgae as bioreactor for the productions of heterologous protein. Bacterial fermentation is limited in application as bacteria are unable to performs posttranscriptional and posttranslational modification essentials for the productions of functional eukaryotic protein. Such modification includes intron-splicing, glycosylation and multimeric. An additional complication is that high intracellular level of heterologous proteins have a tendency to bring about the arrangement of protein totals as insoluble incorporation bodies.

Johnson et. al., 2009 expounded on, "Advancement of a joined microalgal development framework for biofuel handling". The destination of this study is to investigate a novel connected society framework for developing the alga chlorella sp. The biomass collected from the connected development framework (through scratching) had a water substance of 93.75%, like that gathered from suspended society framework (through centrifugation). On the whole, the connected algal society framework with polystyrene froth as a supporting material showed a great execution regarding biomass yield, biodiesel creation potential, simplicity to gather biomass, and physical power for reuse.

Fun et. al., 2011 talks about the unit methods needed for algal biofuels creation (i.e., developing the green growth, gathering, dewatering, extraction and transformation to biofuel). The processing of biofuels from microalgae, particularly biodiesel, has turned into a theme of incredible enthusiasm toward late years. Also there are two essential sorts of society frameworks were perceived and created around then: "open" lake frameworks and "shut" photobioreactors. Practically all business makers use open lakes where the green growth society is blended either by oar wheels (raceway lakes) or by a halfway rotated pivoting arm. The vitality held in the algal biomass could be recuperated in various ways including immediate thermo compound liquefaction. However the current primary enthusiasm toward green growth is the generation of fluid powers, particularly biodiesel and plane fuel, from the algal lipids.

Kumar et. al, 2010 in their examination, " Waste water medicine and metal (Pb^{2+}, Zn^{2+}) evacuation by micro-algal based adjustment lake framework", the development restraint in microalgae is identified with the measure of substantial metal particles bound to the algal cell surface, and additionally, to the measure of intracellular overwhelming metal particles. Instantly, the provision of routine wastewater medication system in country with low GNPs is limited because of high costs and technological complexity. Worldwide, there are continuous interest in algae-based waste stabilization ponds system that are inexpensive and are known for their abilities to achieve better removal of pathogen and organic pollutant.

2.2 Algae cultivation in municipal wastewater

The uncontrolled discharges of wastewater to the environment lead to “over-loads” and there may be a disruption of natural recycling process like photosynthesis, respirations and nitrogen (N_2) fixations.

Therefore the treatments of wastewater are important tasks which need to be performed in order to conserve the aquatic environment. Untreated wastewater contains potentially harmful substance such as (Rawat et al. 2010):

- Large amount of nutrient.
- Toxic compounds (heavy metals etc.).
- High levels of organic material.
- Pathogenic microorganisms.

2.3 Wastewater treatment processes

2.3.1 Conventional treatments technology

Conventional Wastewater treatments are combinations of process intended to generates water of sufficient, defined quality from Wastewater and other Wastewaters with a known compositions. It is important that the effluents from the Wastewater treatment plant can be discharged into a receiving bodies of water (mainly surface water like river), without deteriorating it. The complexity of the treatments is strongly dependents on the receiving water.

The following objective is to fulfilled by Wastewater treatment (Gray 2010):

- Conversions of potentially harmful substances in the Wastewater into product that can be safely disposed into receiving bodies of water without altering its qualities.
- Protections of public healths.
- Efficient and economic disposals of Wastewater.
- Recovery of valuables component in the Wastewater, like nutrients and energies.

In the wastewater treatments systems (Figure-1), the removal of biochemical oxygen demands (BODs), suspended solid, nutrient, coliform bacteria and toxicity are the main goals for getting purified wastewater. BODs exploit the ability of microorganisms to oxidize organic material to Carbon dioxides and water using molecular oxygen as oxidizing agents. Therefore, BODs can depletes the dissolved oxygen of receiving water leading to fish kill and an aerobiosis, and hence it removal is a primary aims of wastewater treatments. Suspended solid is removed principally by physical sedimentations.

In wastewater treatments systems designed to remove nutrients, dissolved nitrogen, phosphorus and many heavy metals, is becoming an important steps of treatments. Discharge of those nutrient into sensitive water body lead to eutrophication by stimulating the growth of unwanted plants, for example, green growth and amphibian macrophytes. Different results of nitrogenous mixes in wastewater effluents are harmfulness of non-ionized alkali to fish and other amphibian creatures, impedance with purification where a free chlorine lingering is obliged and methemoglobinemia in influents because of exorbitant nitrate fixations (over 45 g/m3) in drinking water. It has been concluded that single unit processes is currently unavailable which may successfully and efficiently achieve all these requirements & consequently a combinations are required.

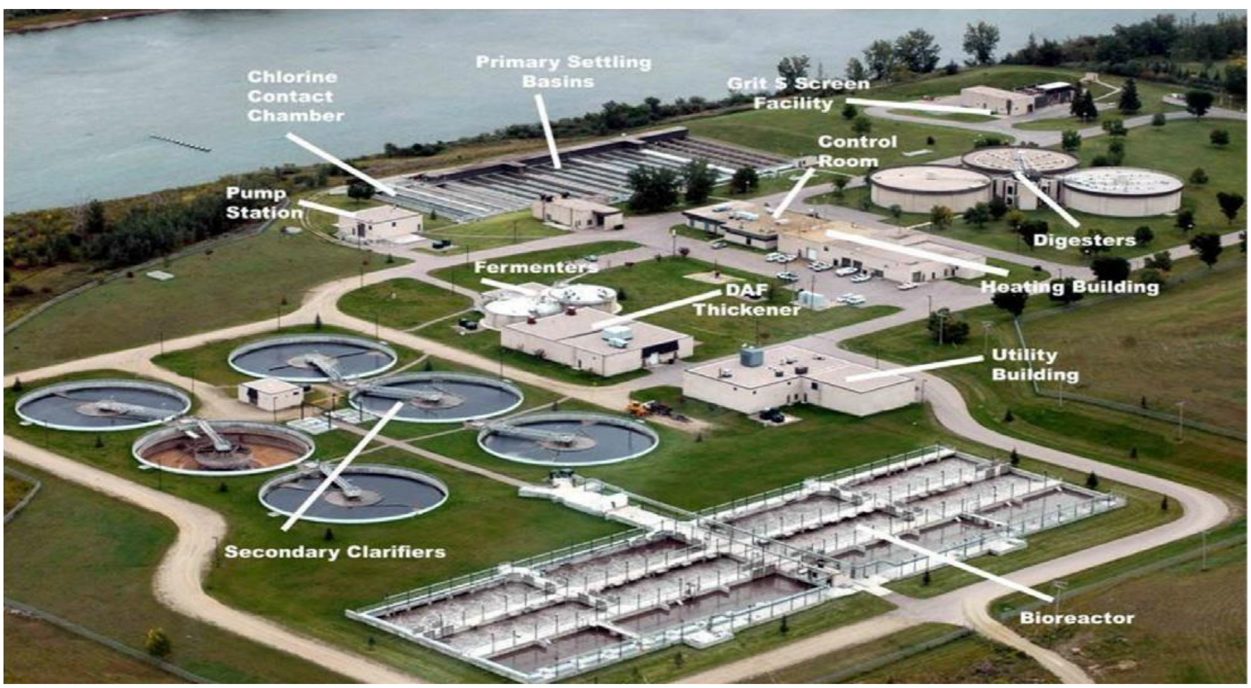


Figure 1: Wastewater treatment station model.

2.3.2 Aquatic systems for wastewater treatment

Serious interests in natural methods for wastewater treatment have reemerged. The using of aquaculture systems as engineered systems in wastewater (domestic and industrial) treatment and recycling has increased enormously over the past few years, these are designed to achieves specific wastewater treatments and can simultaneously solve the environmental and sanitary problems and may also be economically efficient.

Wastewater has been also used in a variety of aquaculture operations around the world for the production of fish or other biomass. Usually the production of biomass was a primary goal with marginal concern for wastewater renovation. The intensive growth and consequent harvesting of the algal biomass as a method for removing wastewater borne nutrients was first suggested and studied by Bogan et al. (1960). It was further investigated by Oswald and Golueke (1966) who proposed the removal of algae growth potential from wastewater by high-rate algal treatment. Large scale study in South Africa, reported by Bosman and Hendricks (1980) concerning the removal of industrial nitrogenous wastes with high-rate algal ponds concluded that a multi-stage algal system is required for exerting the full removal potential of nitrogen by algal biomass incorporation followed by algal harvesting.

Aquatic treatments system consists of one or more shallow pond in which one or more species of water tolerant vascular plant such as water hyacinth or duckweed are grown. Water hyacinth systems are capable of removing high levels of BOD, suspended solids (SS), nitrogen and refractory trace organic matter while phosphorus removal seldom exceeds 50–70% in wastewater, as it is mainly limited to the plant uptake.

Sea-going frameworks utilized for city wastewater the carbonaceous biochemical oxygen request (BOD) and the suspended solids (SS) are evacuated mainly by bacterial digestion system and physical sedimentation. In frameworks used to treat BOD and SS, the sea-going plants themselves realize almost no genuine medications of wastewater.

Many investigations have been conducted and concern the distribution and species composition of fresh water algal communities in different water supplies in Egypt in response to the impact of some environmental stresses (Abdel-Raouf et al., 2003). The polluted river, lake and sea, was aesthetically displeasing also by man which importantly were a public health hazards, since they harbored human pathogen and increased the risks of spreading excreta-related disease through the water-borne routes. In order to prevents such problem, the sewage treatments system was designed.

Through the majority of mankind's history, agribusiness has been in actuality a significant manifestation of biotic water medications through its utilization of the potential contaminations of human and creature squanders to help plant development. Metropolitan wastewater, for instance at times after medicine is connected as a wellspring of supplements over area involved by common vegetation or different products. Such squanders are still essential in world farming, particularly where business manures are not promptly accessible.

2.3.3 Microalgae for wastewater treatment

The history of the commercials use of algae culture span about 75 year with application to wastewater treatments and mass productions of different strain such as Chlorella and Dunaliella. An extensive amounts of work has been conducted on investigating the uses of algae to removes nutrient (mainly N_2 and P_4) in a controlled manners. Also removals of heavy metal, pathogen and other contaminant have been investigated. Wastewater treatment in combination with algae cultivation as a way to enhance the environmental and economic performances of the process. Algae cultivations serve two purposes by improving water qualities and producing biomasses for further utilization as e.g. biofuel.

Currently significant interest is developed in some advanced world nations such as Australia, USA, Thailand, Taiwan and Mexico. This is due to the understanding of the biologists in these nation for the biology and ecology of large scales algae culture, as well as in the engineering of large-scales cultures system and algae harvesting method, all of which are important to designs and operations of high rate algae culture to produce high-value product, such as Pharmaceuticals and genetically engineered product. These include anti-bacterial, anti-viral, anti-cancer, anti-histamine and many other biologically valuable product.

Bio-treatments with microalgae are particularly attractive because of their photosynthetic capability, converting solar energies into useful biomass and incorporating nutrient such as nitrogen (N_2) and phosphorus (P_4) causing eutrophication. These fascinating ideas launched some fifty-five year earlier in U.S. by Oswald and Gotaas in 1957 has since been intensively tested in many country.

Palmer (1974) surveyed microalgal genera from a wide distribution of waste stabilization ponds. In order of abundance, and frequency of occurrence the algae found were Chlorella, Ankistrodesmus, Scenedesmus, Euglena, Chlamydomonas, Oscillatoria, Micractinium and Golenkinia. A survey of algal taxa in six-lagoon systems in Central Asia was completed by Erganshev and Tajiev (1986). Their analysis of long term data revealed that the Chlorophyta was dominant both in variety and quantity followed by Cyanophyta, Bascillariophyta and Euglenophyta. Palmer (1969) listed the algae in the order of their tolerance to organic pollutants as reported by 165 authors. The list was compiled for 60 genera and 80 species. The most tolerant eight genera were found to be Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia, Navicula and Stigeoclonium. More than 1000 algal taxa have been reported one or more time as pollutions tolerant which include 240 genera, 725 species and 125 variety and form. The most tolerant genera includes eight green algae, five blue-green, six flagellates and six diatom.

Since the land-space requirement of microalgae wastewater treatment system are substantial, effort are being made to develops wastewater treatment system based on the use of hyper concentrated algae cultures. This proved to be highly efficient in removing N_2 and P_4 within very short period of time, e.g. less than 1 h (Lavoie and De la Nou¨ e, 1985).

The algae system can treats wastewater, livestock waste, agro-industrial waste and industrial waste. Also, microalgae system for the treatments of other waste such as piggery effluents, the effluents from foods processing factory and other agricultural waste have been studied. Also, algal based systems for the removals of toxic mineral such as lead, cadmium, mercury, scandium, tin, arsenic and bromine are also being developed.

The technologies and biotechnology of microalgae mass cultures have been much discussed. Algal system have traditionally been employed as a tertiary process . They have been proposed as a potential secondary treatment system. Tertiary treatments processes remove all organic ion. It may be accomplished by biology or chemistry. The biologicals tertiary treatments appear to performs well compared to the chemical process which are in general very much costly to be implemented in most place and which can leads to secondary pollution. However, each additional treatments step in a wastewater system greatly increases the total cost. The relative cost of treatment doubles for each additional step following primary treatment (Oswald, 1988b).

A complete tertiary processes aimed to remove ammonia, nitrate and phosphate will thus be about four times more expensive than primary treatment. Microalgal cultures offer an elegant solution to tertiary and quinary treatments due to the ability of microalgae to use inorganic nitrogen (N_2) and phosphorus for their growths. And also, their capacities to removes heavy metal, as well as some toxic organic compound, therefore, doesn't lead to secondary pollution. Amongst beneficial characteristic they produces oxygen, have a disinfecting effects due to increase in pH during photosynthesis.

Algae can be used in wastewater treatment for a range of purposes, some of which are used for the removal of coliform bacteria, reduction of both chemical and biochemical oxygen demand, removal of N and/or P, and also for the removals of heavy metal.

2.4 Heavy metals removal from wastewater

The term "heavy metals" is collectively applied to groups of metal (and metal-like element) with densities greater than 5 g/cm^3 and atomic number greater than 20. Microalgae are known to sequester heavy metals. Discharge of toxic pollutants to waste water collection systems has increased concurrently with society's progressive industrialization. Significant concentrations of heavy metals and toxic organic compounds have been measured in municipal wastewater. Consequently, the ability of wastewater treatment systems to tolerate and remove toxicity is of considerable importance. Microalgae are efficient adsorbers of heavy metals. Bioaccumulation of metals by algae may create a feasible method for remediating wastewater contaminated with metals. On the other hand advantages of algae are that it may be grown in ponds with little nutritional input or maintenance. Although the heavy metals content in some drainages system generally doesn't reaches the proportion found in industrials effluent, certainly not those of metals processing industry, the problem caused by their presence, particularly in area with dense populations, are of public concerns. It is well established that several marines and fresh water algae are able to take up various heavy metal selectively from aqueous media and to accumulate these metals within their cell.

Several author concluded that these methods, including the separations of the metals-saturated algae from the mediums, is an economic methods for removing heavy metal from wastewater, resulting in high qualities reusable effluents water. Numerous species of algae (living and non-living cells) are capable of sequestering significant quantity of toxic heavy metals ion from aqueous solution. Algal metals sequestering processes occur by different mechanism. This can be dependent on algae, the metals ions specie, the solution condition and whether the algal cell are livings or non-livings. In livings algal cells trace nutrients metal (such as Co, Mo, Ca, Mg, Cu, Zn, Cr, Pb and Se) are accumulated intracellularly by active biological transport.

Live photosynthetic microalgae have an effective role in metal detoxification of mine wastewater. By using cyanobacteria in a system of artificial pools and meanders, 99% of dissolved and particulate metals could be removed. Soeder et al. (1978) showed that Coelastrum proboscideum absorbs 100% of lead from 1.0 ppm solution with 20 h at 23 degree C and about 90% after only 1.5 h at 30 degree C. Cadmium was absorbed a little less efficiently, with about 60% of the cadmium being absorbed from a 40 ppb solution after 24 h.

Algae are also good accumulators of compounds such as organochlorides and tributyl tin. They have also been reported to break down some of these compounds have shown that the green alga Dunaliella bioculata produced an extracellular esterase which degrades the pyrethroid insecticide Deltamethrin. Algae have also been shown to degrade a range of hydrocarbons such as those found in oily wastes.

2.4.1 Cu^{2+} & Ni^{2+} removals from wastewater

A large numbers of technology have been adapted and practiced so far to ensures the environmental safeties against Copper in the industrial effluent. Each technologies has its own merit and demerit (Table 1). Bio-adsorption is a relatively new devised method for an efficient copper removals from the wastewater and still various bio-adsorbents are under testing phase for the removals of heavy metal.

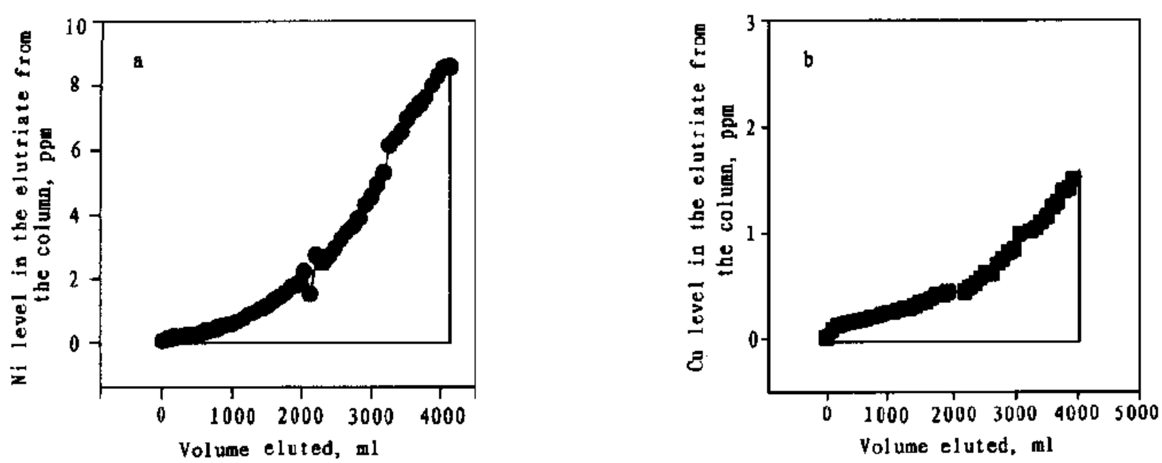
Table 3: Comparison of process technologies for copper and nickel removal (Bilal et al 2013).

Processes	Material	Advantages	Disadvantages
Chemical precipitation	Precipitants like lime, alkali, sulfide/flocculants, surfactants, acid, base, stirring, mixing and fluid handling	Low metal concentration in the effluent achieved. This approach can be adapted to handle large quantities of wastewater. Simple to use	High chemical requirement, pH maintenance at optimum level, handling of colloidal particle sludge disposal problem. A large number of factors such as temperature, pH, precipitant concentration etc. have to be monitored during this technique which is quite difficult
Ion exchange	Ion exchange resins either natural or synthetic	High treatment capability, higher rate of metal removal	Cannot be employed on a large scale, costly synthetic resins
Membrane filtration	Membranes, surfactants enhance and support the process	Reuse of wastewater, helpful in achieving stringent effluent limits, recovery of valuable material, prevention of environmental damages	Membrane fouling, capital cost, maintenance and operational cost, less efficient in case of lower metal ion concentration
Flocculation	Reagents like salts of aluminum, iron	Applicable to large scale wastewater	Costly reagents and production of sludge in large quantities and sludge

		treatment	disposal issues
Electrolytic recovery	Electrical energy	Lesser chemical consumption, recovery of pure metal, effective removal of the desired metal	Energy costs, high capital cost while designing and implementing, reduced efficiency at dilute concentration. This approach cannot be applied to higher quantity of wastewaters
Adsorption	Fluid handling unit/regenerating media, pumps for a constant and uniform flow	Highly effective for removing heavy metals to permissible limits	Chemical regeneration requirement, fouling and corrosion of treatment plant, disposal of exhausted adsorbents, preparation of adsorbent involve high cost such as in case of activated carbon, loss of adsorption capacity by the adsorbent at each cycle
Reverse osmosis	Resins supported with membranes	Effective removal of metals from wastewater	High costs of chemicals, fouling of membranes
Ion exchange	Resins supported by membranes	Selective heavy metal removal	Fouling and maintenance costs, high capital cost equipment and instrument, high operational as well as resin regeneration cost.

2.5 Metal removal efficiency by the algae

Fig. 1a and 1b show the Ni and Cu removal efficiency by the algae respectively. From the initial 4L 30 mg/L metal, over 97% of the Cu and 91% Ni loaded was taken by the algaebeads with a residual 1.76 mg/L Cu and 8.0 mg/L Ni in the effluent at the end. The results showed that algae had a stronger binding affinity for Cu than Ni. This is probably related to the fact that Cu is an essential element for normal algal growth and hence the cell surface possesses ligands or specific groups in holding Cu for assimilation. These working profiles provides means in assessing the working volume or time of algae with respect to the discharge limit required and hence information on working life of the algae for regeneration. Thus, if the discharge limit for nickel is 2 mg/L, the working volume would be 1880 ml and corresponding working life will be 12.5 hours.



Total Ni input: 120 mg
Amount of Ni discharged: 11.76mg(9%)
Ni removed by the algal column: 108.24mg (91%)

Total Cu input: 120mg
Amount of Cu discharged: 2.61mg(2.2%)
Cu removed by the algal column: 117.39mg (97.8%)

Fig 2: The nickel (a) and copper (b) removal efficiency by algae *chlorella vulgaris*

CHAPTER-3

Material

&

Methods

3. Material and methods:

3.1 Instruments:

The instruments and apparatu used throughout the experiments are listed in table below:

Table 4: List of Instruments used during the whole experiment.

S. No.	Instrument	Make/Model
1.	Atomic adsorption spectrophotometer	Perkin Elmer, AAnalyst 200
2.	Inverted Microscope	Olympus Corporation Tokyo, Japan
3.	Centrifuge	Indigenous
4.	Orbital shaker Incubator	Indigenous
5.	B.O.D. Incubator	Indigenous
7.	Autoclave	Indigenous

3.2 Glassware and Apparatus:

All glass wares (Conical flask, Pipette, Measuring cylinder, Beaker, Spatula, Petri plate and test tube etc.) used are of Borosil/ Rankem.

3.3 Chemical:

Analytical grades reagents were used for heavy metals solution. In all experimental works, Millipore water/Distilled water was used.

3.3.1 **Heavy Metals:**

$CuSO_4.5H_2O$ and $NiSO_4.6H_2O$ were obtained in analytical grades and was used without further purifications.

i) Copper solution: 1.5 ± 0.05 mg of $CuSO_4.5H_2O$ was added in the 50 ml of distilled water in 100 ml volume flask. It was dissolved by shaking and the volume was made up to the marks. Copper concentration of this solution was 7.635 mg/l.

ii) Nickel solution: 1.5 ± 0.05 mg of $NiSO_4.6H_2O$ was added in the 50 ml of distilled water in 100 ml volume flasks. It was dissolved by shaking and the volume was made up to the marks. Nickel concentration of this solution was 6.6989 mg/l.

3.3.2 Nutrient Media

Algae culture media is prepared using broth. Algae Culture Broth is recommended for the isolation and cultivation of algae. The composition of broth is following-

Table 5: Composition of broth

Sl.No.	**Ingredients**	**Gms / Litre**
1.	Sodium nitrate	1.000
2.	Dipotassium phosphate	0.250
3.	Magnesium sulphate	0.513
4.	Ammonium chloride	0.050
5.	Calcium chloride	0.058
6.	Ferric chloride	0.003
7.	Final pH (at 25°C)	7.0±0.2

To prepare the media, 0.0935 gm of broth is dissolved in 50 ml of distilled water. Sterilized flask is kept in autoclave for 15 – 20 min at temperature of 121 °C and pressure of 15 psi.

3.4 Sample Collection:

3.4.1 Algae samples collection

Three algae sample was collected. The sample was collected in a sterile plastic container with help of forceps or net. For precaution skin contact should be avoided.

Table 6: Sample collection details

Sample No.	Collection Location	Latitude	Longitude
Sample 1	NIT R Campus(Lake)	22.250800	84.907606
Sample 2	NIT R Campus	22.252993	84.903461
Sapmle 3	NIT R Campus(VSH)	22.252330	84.910533

3.4.2 Serial Dilution

The collected samples contains fine dust particles including algae. The samples was spread in a shallow tray so that the dirt can be settled down. Small pinches of algae was transferred with tweezers or a dropper to a separate container (any small dish or vial).

3.4.3 Microscopy

For analysis of algae inverted microscope was used. These microscopes shine a light through a sample sandwiched between a glass slide and a coverslip, usually with magnification options of 40X, 100X and often greater. We kept small amount of algae on glass slide for microscope analysis. The other advantage of spreading the sample out is that this helps to flatten the sample, which keeps more of the sample in focus at any one time. During the analysis it is important to keep the sample wet because many algae shrivel up quickly if they dry out. After getting the good microscope view of algae sample, identification of the algae was done.

3.5 **Growth Condition:**

Algae growing conditions must be optimized for maximum growth. The following table shows the different physical condition and parameter for optimized growth.

Table 7: Physical condition maintained

Sl. No.	Physiological condition	Parameters
1.	Light	Intensity 3000 lux
		(Light:Dark) 18:6(hr)
2.	pH	7.0±0.2
3.	Temperature	25±5

3.6 Analysis of Heavy Metal:

Estimation of metals: The metals were estimated using Atomic adsorption spectrophotometer

1. Copper as Cu (II) was determined at 324.75 nm.
2. Nickel as Ni (II) was determined at 232 .00 nm.

Heavy metal solution is prepared using $CuSO_4.5H_2O$ and $NiSO_4.6H_2O$ by dissolving 1.5 ± 0.05 mg of each in distilled water. The algal strain were transferred from nutrient broth to heavy metal solution and kept in the Orbital shaker incubator. A 18:6 hour light and dark cycle is maintained in the shaker. After every one week the adsorption of heavy metals by the algae is analyzed.

CHAPTER -4

Result

and

Discussion

4. Result and Discussion:

4.1 Algae identification (Microscopy)

All the three sample is identified using microscope. Small amount of algae sample is kept on the glass slide of the microscope and the sample is seen. After getting a good view of the sample, the structure is compared with the different structure of the algae. After comparison it is found that the sample 3 is *Scenedesmus Species* and other 2 samples were from microalgae/sea weed.

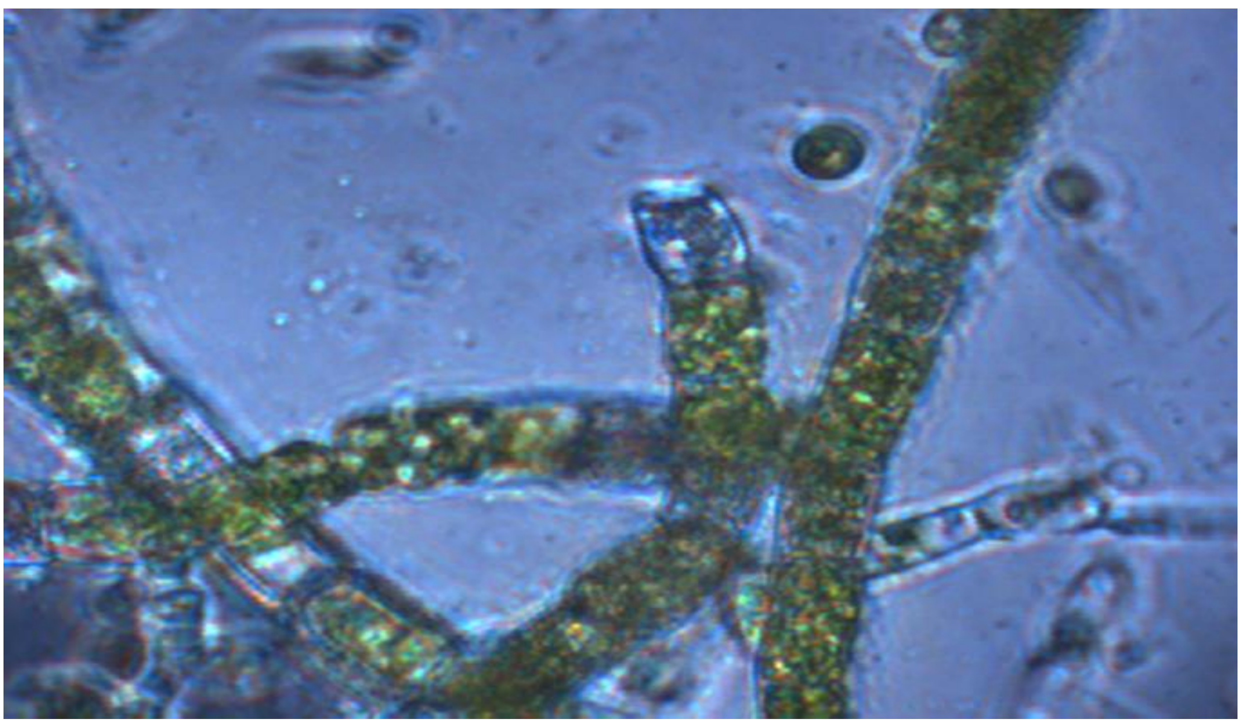


Fig. 3: Algae sample 1

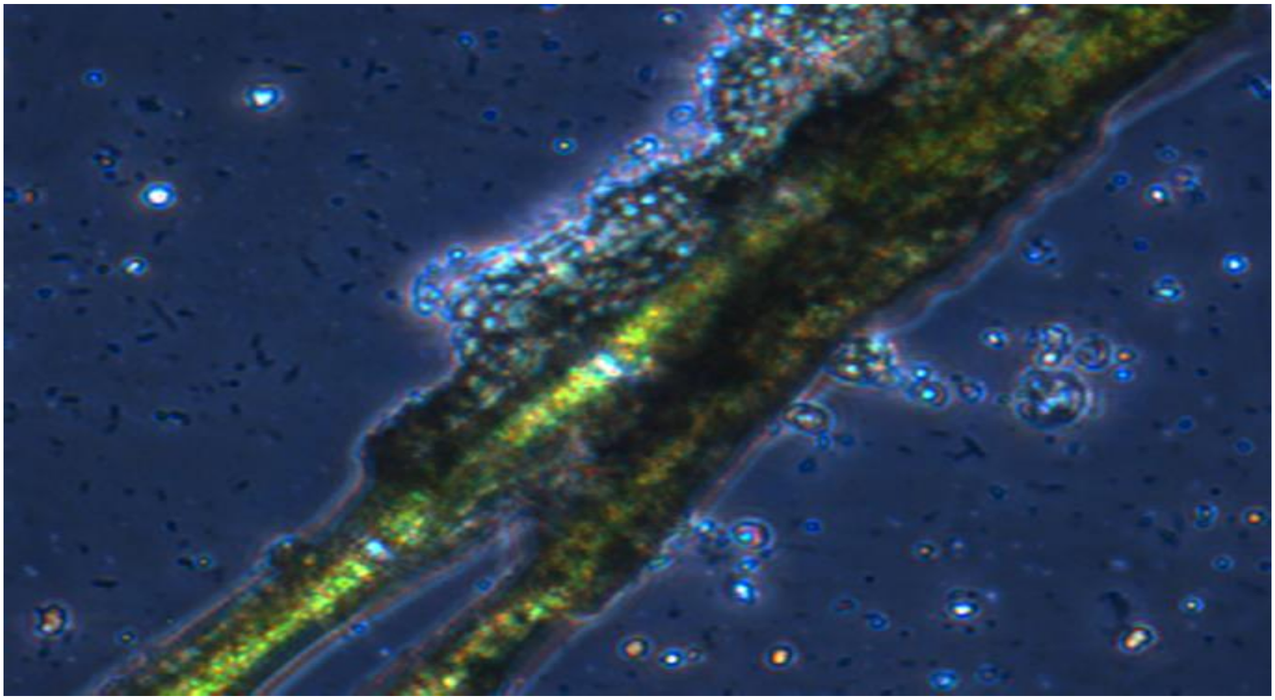


Fig. 4: Algae sample 2

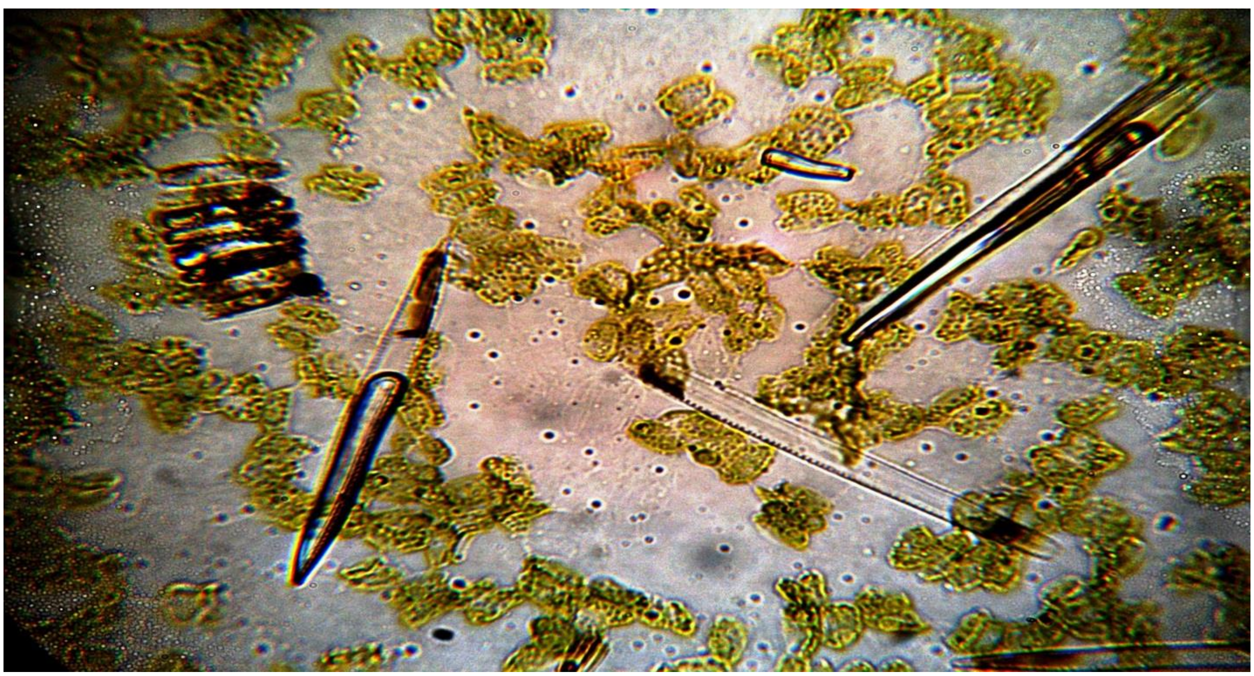


Fig. 5: Algae sample 3(*Scenedesmus Species*)

4.2 Adsorption of Heavy Metals

Adsorption of heavy metals of the entire three samples is analyzed after every one week. Initially the adsorption is more. As the passage of time, the adsorption is decreased. For the heavy metal Copper algae sample 1 is best. The total adsorbance of Copper by the algae sample 1 during the time period of 32 days is 97.34 % while for the sample 2 is 87.70 % and for sample 3 is 71.51%.

In case of heavy metal Nickel, total adsorption by the sample 3 during the 32 days is only 25.44 % which is much less than the adsorption of Copper.

The adsorption readings of the different sample are as follows:

Table 8: Adsorption of Copper of Sample 1

Sl. No.	**No. of days**	**Concentration**
1.	0	7.8387
2.	8	3.5192
3.	15	2.0439
4.	24	1.1382
5.	32	0.208

Table 9: Adsorption of Copper of Sample 2

Sl. No.	**No. of days**	**Concentration**
1.	0	7.7369
2.	8	3.4386
3.	15	1.9876
4.	24	1.2556
5.	32	0.951

Table 10: Adsorption of Copper of Sample 3(*Scenedesmus Species*)

Sl. No.	**No. of days**	**Concentration**
1.	0	7.7878
2.	8	4.3594
3.	15	2.7618
4.	24	2.3891
5.	32	2.218

Table 11: Adsorption of Nickel of Sample 3(*Scenedesmus Species*)

Sl. No.	No. of days	Concentration
1.	0	6.9669
2.	8	6.1750
3.	15	5.5879
4.	24	5.3187
5.	32	5.194

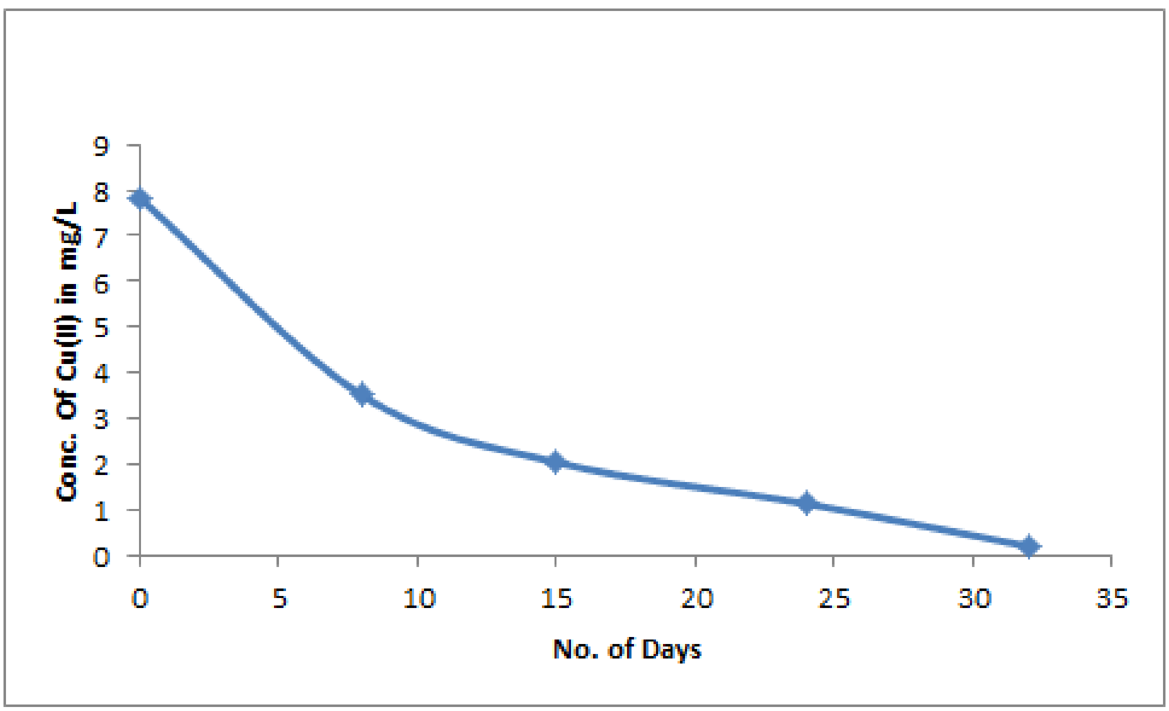


Fig. 6: Adsorption curve of sample 1 for Cu(II)

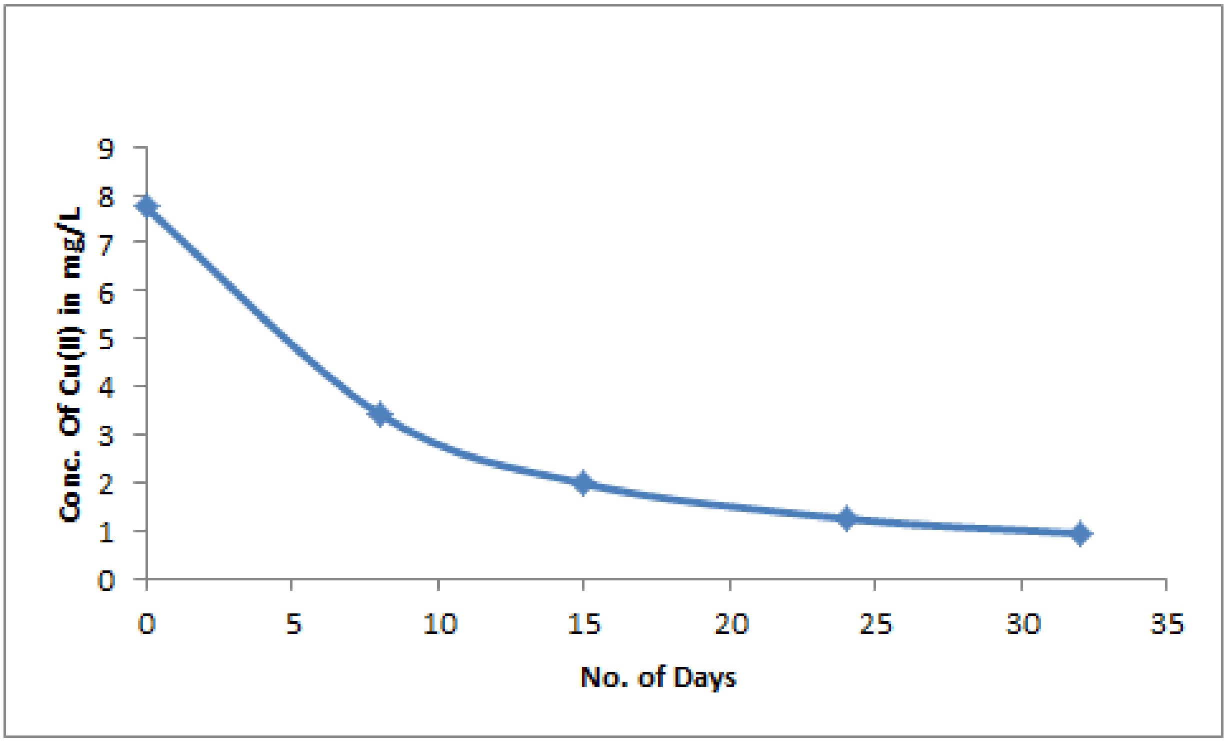


Fig. 7: Adsorption curve of sample 2 for Cu(II)

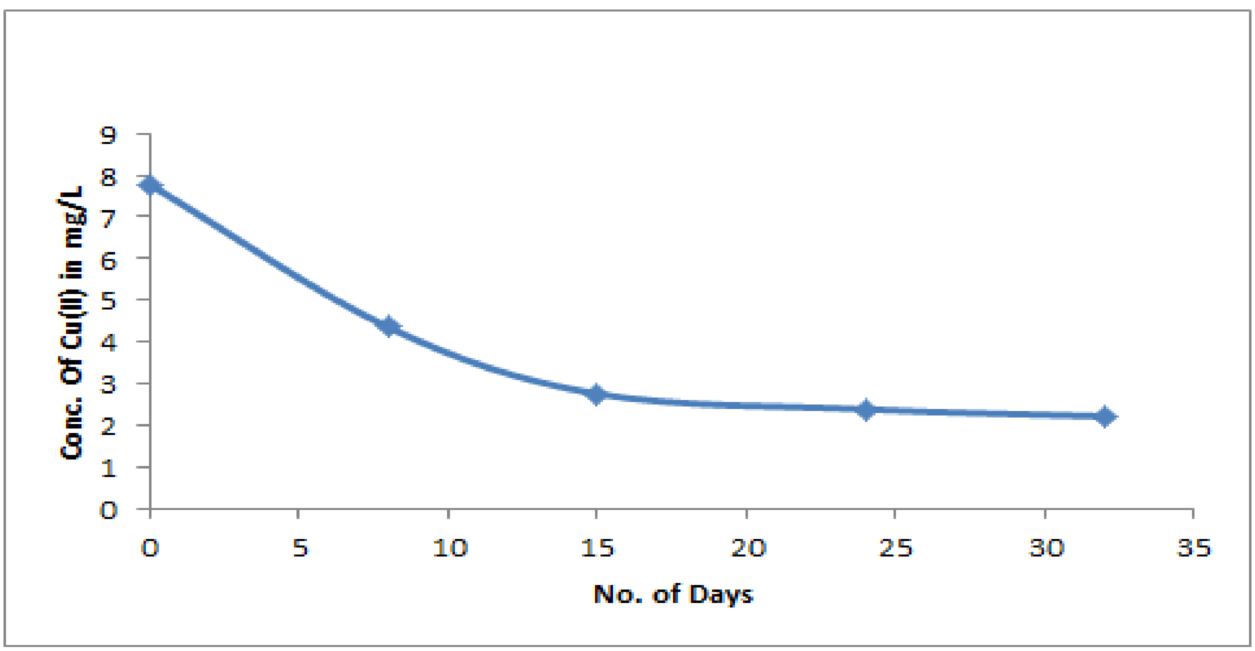


Fig. 8: Adsorption curve of sample 3 for Cu(II)

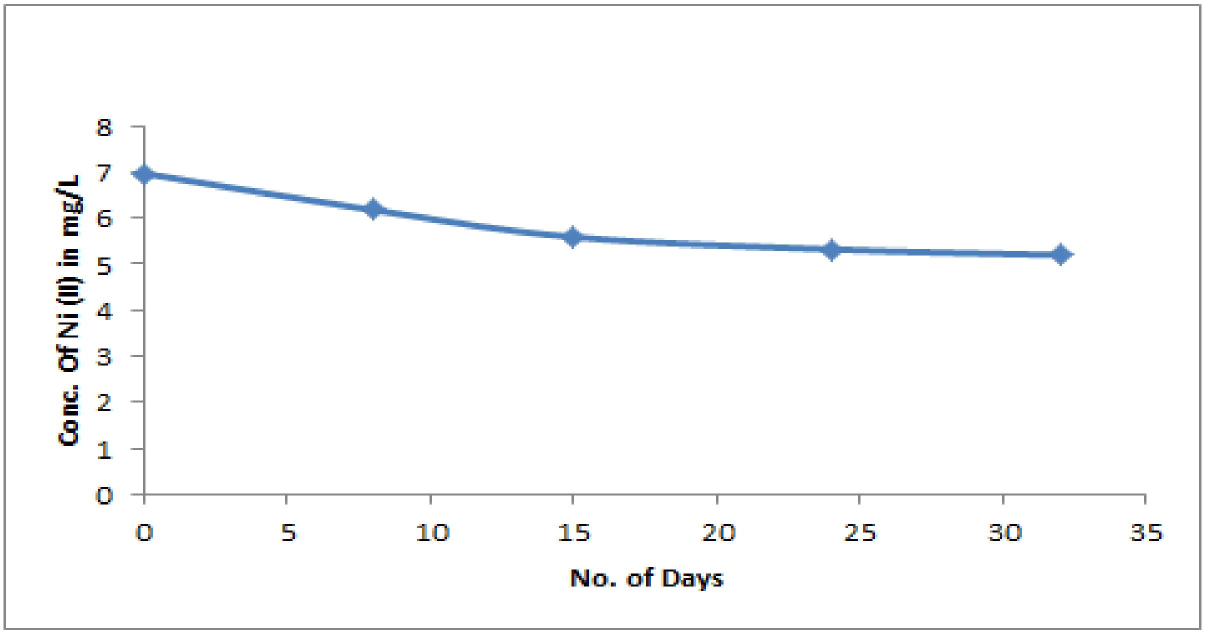


Fig. 9: Adsorption curve of sample 3 for Ni(II)

CHAPTER -5

Conclusions

5. Conclusions

Many investigators have tried various methods for removals of heavy metal from waste water. Biological method are found to be effective for heavy metals removals from wastewater. Algae are a cheap and effective adsorbents for the removals of Cu and Ni ion from wastewater. This experimental study on adsorbent would be quite useful in developing appropriate technologies for the removals of heavy metals ions from industrial/ domestic effluent. Using algae species for treating wastewater also have the ability to sustain growth of algae that produce oil/fats which can be used for biodiesel productions. Therefore the algae provides dual benefit of wastewater treatments and oil productions.

CHAPTER -6

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6. References

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