CHAPTER ONE

INTRODUCTION

1.1 Heavy metals and their toxicity

The presence of heavy metals in aqueous streams, air, soil and food have become a problem due to their harmful effects on human health even at low concentration in the environment. Heavy metal pollutants in wastewater is one of the problems facing human beings; heavy metal can be toxic to the life (Table 1.1). For example in Malaysia a particularly vexing problem is the presence of toxic and hazardous heavy metals in industrial effluents. When such metal bearing waste streams are insufficiently treated before discharge, they find their way into the environment and subsequently into the food chain. According to the world health organization, the metals of most immediate concern internationally are aluminum, chromium, manganese, iron, cobalt, copper, zinc, cadmium, mercury and lead (WHO, 1984). Economic development has become synonymous with industrial progress in Malaysia. This is in line with federal government's policy, the industrial master plan, to promote growth in the nation's industrial sector (Yeoh, 1993).

Table 1.1
Classification of elements according to toxicity and availability (based on Wood, 1974).

Non-critical Accessible	Toxic but rare	Very toxic
Al	Ba	Ag
Br	Cs	As
C	Ga	Au
Ca	Hf	Be
Cl	Ir	Bi
F	La	Cd
Fe	Nb	Co
H	Re	Cu
K	Rh	Hg
Li	Ru	Ni
Mg	Ta	Pb
N	Ti	Pd

Na	Zr	Pt
O	W	Sb
P		Se
Rb		Ti
S		Te
Sr		Zn
Si		

The element (metal) that is relevant to present study is lead. It can be noted that this metal belongs to the group that is very toxic.

As a fact, metals are elements and they have been an intrinsic component of the environment to which human and animals are adapted. Therefore, a "natural" exposure to all metals may thus be harmless to a human being. Many "trace metals" are even fundamental for growth and metabolism at low concentrations as they serve a biological function. Heavy metals only exert their harmful effect when exposure is excessive. The United States Environment Protection Agency (EPA, 1987) has recommended the maximum permissible concentrations of some toxic metals for the protection of human health as given in (Table 1.2). Lead is primarily of interest because of its toxic effect and it therefore may cause either acute or sub acute health effects in a human population.

Table 1.2
Maximum permissible concentration of various metals in natural water for the protection of human health (based EPA, 1987).

Metals	Maximum permissible level	
	mg/m^3	µmol/m^3
Mercury	0.144	1.72
Lead	5	24
Cadmium	10	89
Selenium	10	127
Thallium	13	64
Nickel	13.4	228
Silver	50	464
Manganese	50	910
Chromium	50	962
Iron	300	5372
Barium	1000	7281

1.2 Lead

Lead has been known since ancient times. It is naturally present in the earth's crust in small concentration, but for centuries it has been mined and disseminated throughout the environments from where it has gradually become incorporated into the structural tissue of animals, plants and humans. Lead exists as Pb^{+2} ion during the chemical reaction. Lead is a well known highly toxic metal and a cumulative poison. The common industries that deal with lead are the battery manufacturing, motor vehicle repair, cable making and metal grinding industries and it is also used in piping, conducting materials, accumulators, lead chambers, printing characters, soldering, anti-knock substances, colored pigments, radiation shielding, wrappings for food, tobacco and as an additive in gasoline.

1.2.1 Toxicity of lead

Lead is among the majority toxic heavy metal ions affecting the environment (Alloway and Ayres, 1993). Lead poisoning results from ingestion of lead-containing materials such as paint or water which has stood in lead pipes. Poisoning can also occur from inhalation of fumes from burning storage batteries or solder.

However, there is no doubt that lead is seriously poisonous to human beings and evidence is accumulating that considerable differing effects result in different human beings who have absorbed similar amounts. Most of the absorbed lead is stored in the bones, blood or brain. Lead colic (painter's cramps) is characterized by severe abdominal pain. Damage to the brain can occur in children it is known to cause convulsions, mental retardation and even death. It is also known that lead is harmful to the kidney and permanent neurological injury (Snyder *et al.*, 1971). Lead can reason several unwanted effects, such as disorder of the biosynthesis of haemoglobin and anaemia, a rise in blood pressure, declined fertility of men through sperm damage and behavioural disruptions of children, such as aggression, impulsive behavior and hyperactivity.

Lead can enter a foetus through the placenta of the mother and it can cause serious damage to the nervous system and the brains of unborn children. Lead is known to cause precipitation of protein, through the interaction of lead ions with the sulphydryl (-SH) groups of proteins.

1.2.2 Naturally occurring lead

The common magnitude of the lead content of the earth's crust is approximately 16 µg/g of soil (De Treville, 1964). Basic rocks have a concentration of lead up to 8 µg/g and acidic rocks have concentration up to 20 µg/g. Lead is usually found in ore with silver, zinc and (most abundantly) copper, and is extracted together with these metals. Lead ores are found in England, Spain, Mexico and U.S.A. The most abundant lead-containing ore is galena, in which it occurs as sulphide, PbS, and from which commercial lead is chiefly obtained. It is also found in crocoisite (chromate), and cerussite (carbonate). It is present in plants and soils in varying is amounts. Fixation by clay of normal soil converts added lead to insoluble inactive compounds, but very acid soils increase the solubility and soluble compounds are toxic to plants except in very low concentration (Browning, 1969). Other naturally occurring lead is the result of the decay of Uranium-238, which occur naturally in trace amounts in the soil. Uranium-238 decays through a series of radioactive elements until the stable nuclide lead ends chain (Snyder *et al.*, 1971).

1.2.3 Sources of lead

1.2.3.1 Lead in the atmosphere

Lead enters the atmosphere through both natural processes and as a consequence of activities of human. Natural processes include a continuous emission of radioactive precursors of elemental lead from the surface of all soils around the earth except those covered heavily with snow and ice and those saturate with water. Lead in several chemical forms is released by volcanic activity, forest fires, dust raised by the wind. Lead can be emitted into the atmosphere during the combustion of lead-bearing fuels such as coal containing lead and other impurities because coal and fuel oil contain lead. These contributions of natural processes appear to be very small when compared to the results of activities from human.

1.2.3.2 Lead in the water

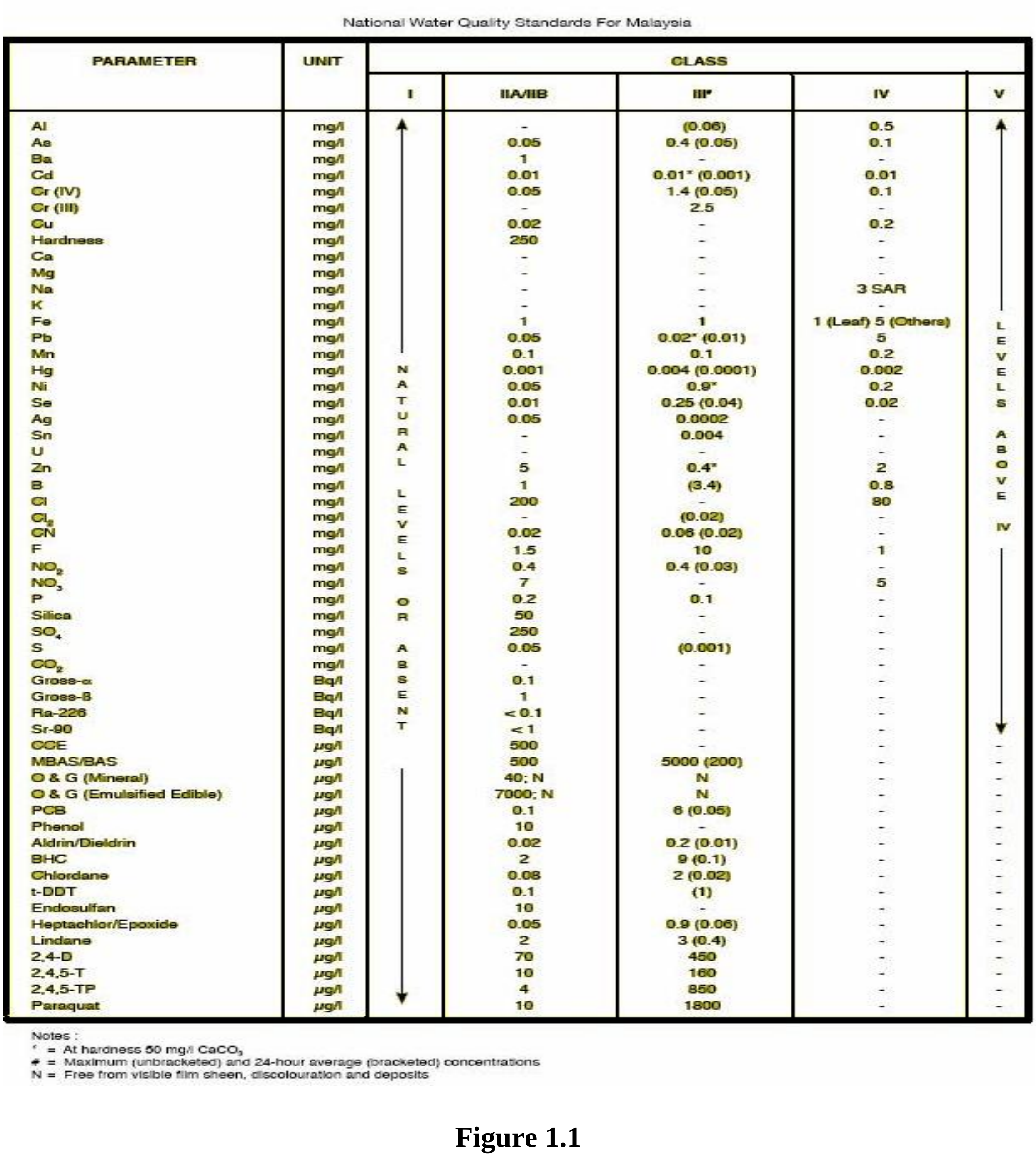
The natural sources for lead in water is the earth which the water comes in contact. The average value of lead in the earth's crust is approximately 16µg/g (de Treville, 1964).

One of the sources of lead is rainwater may contain significant increased lead as a result of washout of airborne lead in the precipitation process. Also the surface soils which are so affected by man's activities especially those near heavily-travel highways, represents a source of pollution to water courses draining the area.

Lead emissions from mining, smelting, and refining operation may cause local surface pollution. Lead products are widely used and may contribute in varying amounts to water pollution. Fortunately, much of the use of lead is in products such as batteries, lead shot, and sheet metal which are not readily mobilized in the environment. Another source of lead in water is from the use of lead pipe in older plumbing systems.

In Malaysia analysis of heavy metals in 5,613 water samples revealed that almost all samples complied with Class III, National Water Quality Standards for, lead (Pb) , zinc (Zn) mercury, (Hg), , chromium (Cr), arsenic (As)and cadmium (Cd), except iron (Fe) with 83 percent compliance (Figure 1.1). Lead contamination was evident in Perak (56%), Kelantan (78%) and Terengganu (86%), (DOE, 2006).

Heavy metals contamination was comparatively low with lead (Pb) exceeding the INWQS by 20 percent, followed by heavy metal mercury (18%) and copper (6%) (DOE, 2006).



National Water Quality Standards For Malaysia

PARAMETER	UNIT	CLASS				
		I	IIA/IIB	III*	IV	V
Al	mg/l	NATURAL LEVELS OR ABSENT	-	(0.06)	0.5	LEVELS ABOVE IV
As	mg/l		0.05	0.4 (0.05)	0.1	
Ba	mg/l		1	-	-	
Cd	mg/l		0.01	0.01* (0.001)	0.01	
Cr (IV)	mg/l		0.05	1.4 (0.05)	0.1	
Cr (III)	mg/l		-	2.5	-	
Cu	mg/l		0.02	-	0.2	
Hardness	mg/l		250	-	-	
Ca	mg/l		-	-	-	
Mg	mg/l		-	-	-	
Na	mg/l		-	-	3 SAR	
K	mg/l		-	-	-	
Fe	mg/l		1	1	1 (Leaf) 5 (Others)	
Pb	mg/l		0.05	0.02* (0.01)	5	
Mn	mg/l		0.1	0.1	0.2	
Hg	mg/l		0.001	0.004 (0.0001)	0.002	
Ni	mg/l		0.05	0.9*	0.2	
Se	mg/l		0.01	0.25 (0.04)	0.02	
Ag	mg/l		0.05	0.0002	-	
Sn	mg/l		-	0.004	-	
U	mg/l		-	-	-	
Zn	mg/l		5	0.4*	2	
B	mg/l		1	(3.4)	0.8	
Cl	mg/l		200	-	80	
Cl_2	mg/l		-	(0.02)	-	
CN	mg/l		0.02	0.06 (0.02)	-	
F	mg/l		1.5	10	1	
NO_2	mg/l		0.4	0.4 (0.03)	-	
NO_3	mg/l		7	-	5	
P	mg/l		0.2	0.1	-	
Silica	mg/l		50	-	-	
SO_4	mg/l		250	-	-	
S	mg/l		0.05	(0.001)	-	
CO_2	mg/l		-	-	-	
Gross-α	Bq/l		0.1	-	-	
Gross-β	Bq/l		1	-	-	
Ra-226	Bq/l		< 0.1	-	-	
Sr-90	Bq/l		< 1	-	-	
CCE	μg/l		500	-	-	-
MBAS/BAS	μg/l		500	5000 (200)	-	-
O & G (Mineral)	μg/l		40; N	N	-	-
O & G (Emulsified Edible)	μg/l		7000; N	N	-	-
PCB	μg/l		0.1	6 (0.05)	-	-
Phenol	μg/l		10	-	-	-
Aldrin/Dieldrin	μg/l		0.02	0.2 (0.01)	-	-
BHC	μg/l		2	9 (0.1)	-	-
Chlordane	μg/l		0.08	2 (0.02)	-	-
t-DDT	μg/l		0.1	(1)	-	-
Endosulfan	μg/l		10	-	-	-
Heptachlor/Epoxide	μg/l		0.05	0.9 (0.06)	-	-
Lindane	μg/l		2	3 (0.4)	-	-
2,4-D	μg/l		70	450	-	-
2,4,5-T	μg/l		10	160	-	-
2,4,5-TP	μg/l		4	850	-	-
Paraquat	μg/l		10	1800	-	-

Notes :
* = At hardness 50 mg/l $CaCO_3$
# = Maximum (unbracketed) and 24-hour average (bracketed) concentrations
N = Free from visible film sheen, discolouration and deposits

Figure 1.1
Interim National Water Quality Standards for Malaysia (INWQS),
(based on DOE, 2006)

1.2.3.3 Lead in food

The three primary means for the introduction of lead into the human body are through air, water and food. However, about 90 percent of lead uptake is due to food (Snyder *et al.*, 1971).

The highly unnatural lead levels on the modern diet result from the use of lead in food technology, e.g. from the rims of food cans as well as to some level from lead-glazed pottery, particularly if the glaze is chipped, cracked or improperly applied. However, human's activity has resulted in the lead level of food. Being about twenty times this value, 0.2μ/g (Snyder *et al.*, 1971). This value include not only the lead in the plant or its fruit but also that introduced in processing and handling of the food.

1.2.4 Properties of lead

Lead is a bluish-grey metal with a bright metallic. In moist air it becomes coated with a film, probably an oxide, ultimately converted into a basic carbonate. It is not tough enough to be hammered into foil or drawn into wire but can be pressed into pipes or rolled thin sheets. It is fairly rapidly dissolved by nitric acid and is soluble, to some extent, in organic acids such as food acids and acetic, and by water in pipes if the water holds carbon dioxide, ammonium salts and nitrates in solution. The presence of carbonate from chalk or limestone prevents this process by the formation of a film on the interior of the pipes which protects the lead from further action (Browning., 1969).

Atomic number of lead is 82 atomic, weight of lead is 207, specific gravity: 11.25-11.4, melting point: 327°C., boiling point: 1749°C.

Crystal structure, lead is a member of the cubic crystal system is clear. Elastic properties, elastic modulus of lead at room temperature between1.493kg/mm^2 and 2.040 kg/mm^2.

1.3 Waste treatment technologies for heavy metals

Physical-chemical treatment is the conventional technology employed to treat heavy metal waste. Therefore, method for heavy metal removal are continuously being developed and new technologies that are more practical and economically feasible are being explored by researchers. Conventional heavy metal removal techniques that are well established and widely employed include precipitation, ion-exchange, reverse osmosis, electrodialysis and adsorption.

1.3.1 Chemical precipitation

Unlike organic pollutants heavy metals are difficult to detoxify and cannot be degraded. The most common method employed to treat heavy metal waste is chemical precipitation. This done by adding lime or caustic soda to produce metal hydroxide precipitates. Alternatively sulphide compounds may be added to from metal sulphide precipitates. After de-watering the metal laden sludge is collected and disposed off in a landfill.

The main disadvantage of this method is in the separation of solids and the disposal of sludge. It has been shown that metal hydroxide sludges collected in clarifiers contain up to 3.4% solids.

To mitigate this problem anionic polyelectrolyte, alum and ferric chloride have been used as sludge conditioners and flocculating agents. The sludge produce has to be ultimately disposed in a landfill. This is not a good environmental practice because there is always the possibility that the heavy metals from the heavy metal sludge (classified as a hazardous waste) may leach out and pose a threat to the environment.

Furthermore, chemical precipitation is suitable only for wastewater containing high concentration (parts per thousand or higher) of metal pollutants. With dilute wastewater (in the range of ten parts per million or less), chemical precipitation is ineffective and costly.

1.3.2 Ion-exchange

In this method the ion exchange resins are used to remove and concentrate the heavy metal. These resins are polymeric beads that contain functional groups that act as binding site, depending on the resin type the appropriate heavy metal may be removed. Electrowining is then used to remove the metal ions from the wastewater by plating the metal onto cathodes of an electrochemical cell. The advantage of this method is that the heavy metals are recovered and may be reused. Although the ion-exchange/electrowining treatment method is environment friendly it is very expensive. The Method has also limited value and is not effective where the wastewater contains low concentrations of metal ions.

1.3.3 Adsorption technique

Adsorption is the accumulation of molecules from a material dissolved in a solvent onto the surface of an adsorbent particle. Adsorption techniques are used to take away soluble organic from wastewaters and drinking waters. The application of Adsorption technology for pollution control usually deals with the control of organic compounds. It is widely used in applications like wastewater purification, recovery of volatile organic compounds (VOCs), air-separation, drying of air, removal of bitter ingredients in fruit juices.

The substance on which adsorption takes place is known as the adsorbent and the substance which is adsorbed is known as adsorbate. Adsorption is a surface phenomenon in which the solutes are concentrated at the surface of adsorbent, and effective adsorbents which have a highly porous composition so that their surface area to volume ratio is very high. The solute particals is held in contact with the adsorbent by a combination of physical, ionic and chemical forces. When an adsorbent is left in contact with a solution the quantity of adsorbed solute increases on the surface of the adsorbent and decreases in the solvent. When the number of molecules of solute is equal in the solvent and on the adsorbent it represents the adsorption equilibrium

Generally the rate of adsorption is governed by the rate of diffusion of solute into the internal surface of porous of the adsorbent particle. The rate decreases with increasing particle mass and increases with increasing solute concentration and temperature. Low molecular weight solutes are more simply adsorbed than the high molecular weight solutes

1.3.4 Reverse osmosis

Heavy metal removal by reverse osmosis can be done by applying a pressure greater than the osmotic pressure of the solution and forcing the water to flow through the membrane whilst retaining the metal ions. In the treatment of wastewater, reverse osmosis may be used by itself or may supplement other methods. This technology is usually employed for the recovery of precious or common metals in the metal finishing industry. In principle, this is a simple technique but the development of suitable membranes is much more difficult. A membrane must possess selectivity for water over the metal ions and must have sufficiently high permeability to enable a reasonable flow rate of water across it without applying excessive high pressures.

Other operational constraints of this technology are caused by fouling of the membrane and the phenomenon of concentration polarization, which is caused by ion built-up, may shorten the service of the membrane. The use of high pressures and the need for sophisticated process equipment to adequately support the membrane give rise to high capital costs. Frequent membrane replacement and the associated high maintenance costs are additional factors which hinder the application of reverse osmosis to remove heavy metals.

1.3.5 Electrodialysis

Electrodialysis is another technique which utilizes membranes. In this case the metal ions move through the membrane by application of an electromagnetic force (emf). The treatment unit consists of a number of chambers made up of alternate cation-anion membrane located between two electrodes. When dilute solution is fed through the unit and an emf applied, the cations migrate towards the anode. Since the membrane only allows the transfer of one type of ions, one side of the membrane more concentrated with metal ions while the other become more diluted.

There are a number of intrinsic disadvantage by applying this technique. Dilute solution, particularly those produce in the unit, have very low electrical conductivity thus necessitating high power input to produce acceptable ionic transfer rates across the membrane. Consequently, accumulation of acidic and basic solution on opposite sides of the membrane will happen and precipitates may be formed which foul the membrane and it might lose its ion selective properties too.

Furthermore, electrodialysis involves high capital and operating costs even though the value of recovered metals may help to offset the costs in a small amount.

1.4 Biological treatment

1.4.1 Biosorption

Biosorption or biologically based treatment technology the ability of biological materials, both living and non-living to passively bind (adsorb) metal species dissolved in solution onto the cell surface is commonly known as biosorption.

The adsorption process can reversibly bind metal ions to the surface of non-living microbial biomass because the dead organisms are unaffected by the toxic nature of the metal ions. Although live cells can remove heavy metal, but the living cell may not survive under the conditions of point-source discharge of industrial wastewater which normally contain high levels of toxic metals.

A further limitation of the system is the inability to recover the metal ions from the metal-saturated cells while maintaining their viability. This makes living cell unsuitable for repeated use and eventually increase the cost of the process.

These problems and shortcomings have initiated the development of adsorption method which involved non-living biomass. A variety of biological materials have been used by researchers to study adsorption. These include bacteria, fungi, algae (Volesky, 1987) and oil palm waste (Hashim *et al.*, 1996). Biological treatment is excellent for treating streams with low concentration of heavy metal.

1.5 Objectives and scope of research

The main thrust of this research was to focus on the adsorption (removal) of lead by non-living biomass of algal species from aqueous solution. Lead(II) is selected as the model metal because is commonly found in industrial wastewater discharged by various industries.

The objectives of this study were:

1. To determine the effect of initial concentration, contact time, pH, and temperature on the adsorption of lead(II) by non-living algal biomass
2. To describe the thermodynamics of the adsorption of lead(II) by non-living algal biomass
3. To test the ability of non-living algal biomass in removing lead(II) from a polluted water sample.

CHAPTER TWO

LITERATURE REVIEW

2.1 Theory of adsorption

Adsorption is a process where molecules of a gas or liquid contact and adhere to a solid surface. The adsorption process occurs at an interface between any two phases, such as, liquid-liquid, gas-liquid, gas-solid, or liquid-solid interfaces. The interface of interest in water and wastewater treatment is the liquid-solid interface. The adsorption of various substances on solids is due to the increased free surface energy of solids to their extensive surface. According to the second law of thermodynamics, this energy has to be reduced. This is achieved by reduced the surface tension via the capture of extrinsic substance. From external surface of solids and liquids as well as from the internal surface of porous solid or liquids (Skoulikides, 1989). Adorption is one of the most widely used methods for potable and wastewater treatment (Ahmad-Asbchin *et al.*, 2008). By adsorption method most of the heavy metals are efficiently removed (Connell *et al.*, 2008). Depending on the type of bonding involved, adsorption can be classified as follows:

1. Physisorption

Physisorption or physical adsorption occurs as result of energy differences and/or electrical attractive weak forces such as the (Van der Waals forces), the adsorbate molecules (liquid contamination) are physically attached to the adsorbent molecules (solid surface). The reversibility of physisorption is dependent on the attractive forces between adsorbent and adsorbate. If these forces are weak, desorption is readily effected. The heat of adsorption for physisorption is at most a few Kcal/mole and therefore this type of adsorption is stable only at temperature below 150°C.

2. Chemisorption

Chemisorption or chemical adsorption occurs when a chemical compound is produced by the reaction between the adsorbent and the adsorbed molecule. Unlike physisorption, this procedure is one molecule thick and irreversible because energy is released to form the new chemical compound at the surface of the adsorbent and energy would be necessary to reverse the process.

Both processes take place when the molecules in the liquid phase are attached to the surface of solid as a result of the attractive forces at the adsorbent, overcoming the kinetic energy of the adsorbate molecules. The substance that is being removed from liquid phase at the interface is call adsorbate or sorbent. The adsorbent or sorbate is the solid, liquid or gas phase onto which the adsorbate accumulate, as show in (Figure 2.1)

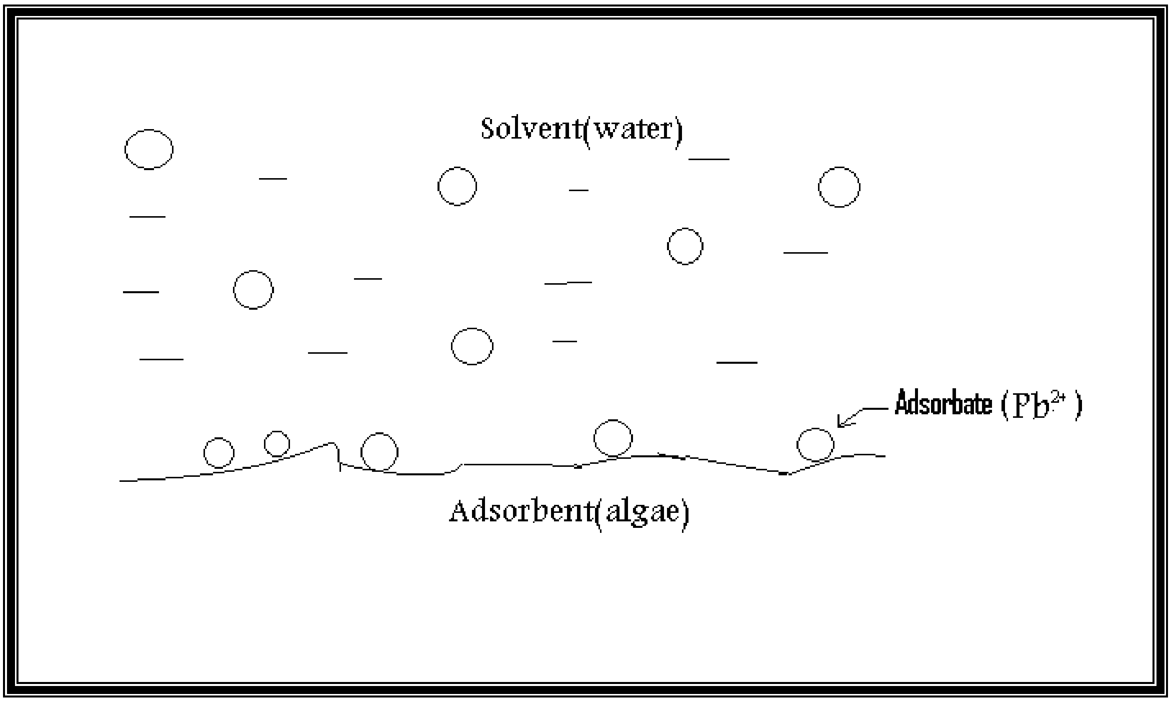


Figure.2.1
Mechanism of adsorption process

In general adsorption is the accumulation of molecules from a solvent onto the interior and exterior (i.e. pore) surface of the adsorbent.

The surface phenomenon is a manifestation of complicated interactions among three components involves, i.e. the algae, the lead(II) and the solvent (Figure 2.2) shows a schematic for the three components in adsorption model and their interactions. Normally, the affinity between the algae and the lead(II) is main interaction force controlling adsorption. However, the affinity between the algae and the solvent (i.e. solubility) can also play a main role in adsorption.

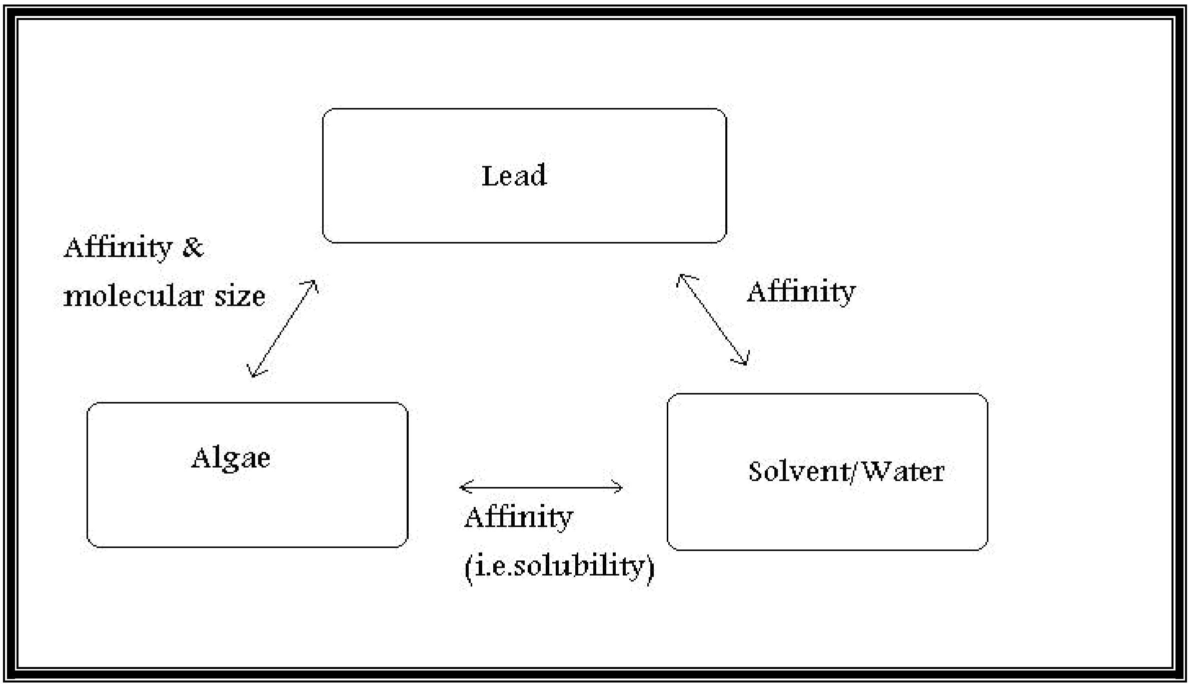


Figure.2.2
Schematic representation of adsorbate-adsorbent-solvent relationship.

There are four factors that effect an adsorption:

1.The physical and chemical characteristics of the adsorbate, that is molecular size, molecular polarity, concentration of the adsorbate in the liquid phase (solution) and chemical composition.

2. The physical and chemical characteristics of the adsorbent, that is pore size, surface area and chemical composition.

3. The characteristics of the liquid phase pH and temperature.

4. The residence time of the system.

2.2 Biological treatment methods for heavy metal removal

In view of the toxicity of heavy metals on human health and their high usage industries microbial adsorption of heavy metals has been employed to treat industrial effluents containing heavy metals before discharging into environment. The term adsorption is the process of accumulation substances that are in solution on a suitable interface. Another definition was a mass transfer operation in that a constituent in the liquid phase is transferred to the solid phase.

2.2.1 Algal adsorption

Algae are the main organisms that have been used in adsorption. This may be due to the rugged nature of algal biomass, making them easy to be processed into adsorbents. Furthermore, they can be cultivated easily and cheaply (Corder and Reeves, 1994). Their large surface area chelating potential are attractive for metal removal (Roy *et al*., 1993).

The ability of *Chlorella vulgaris* to remove copper, zinc and iron was shown by Aksu and Kutsal (1987). Further, it was shown to be a good adsorbent for lead(II), cadmium and copper removal by the brown alga *Fucus vesiculosus* (Mata *et al.*, 2008).

Macroalgae(seaweeds) also have good adsorption characteristics. The biomass of *Ascophyllum nodosum* has been shown to adsorb cobalt (Kuyucak and Volesky,1989). *Sargassum natans* and *Ascophyllum nodosum* were able to sequester lead(II) and cadmium to more than 20% of their biomass dry weight. The passive adsorptive uptake of lead(II) and nickel by some brown marine algae was also reported by Holan and Volesky (1994).

2.2.2 Bacterial adsorption

Bacterial cell wall possesses several metal-binding components contribute to the adsorption process. Shumate and Strandberg (1985) showed the ability of *Streptomyces elongateus* and *Pseudomonas aeruginosa* in the removal of uranium from seawater. *Zooglea ramigera,* which is commonly found in activate sludge, was shown to have chromium (VI) removal ability (Nourbakhsh *et al*., 1994).

In gram-positive bacteria such as *Bacillus subtilis*, metal adsorption was related to the carboxyl group of the glutamic acid of peptide glycan (Doyle *et al*.,1980). In addition, Beveridge and Fyfe (1985) suggested that teichoic and teichuronic acids were important binding sites in *Bacillus licheniformis.* On the other hand, most metal deposition gram-negative bacteria occurred at the phosphate group (Mclean and Beveridge, 1990).

2.2.3 Fungal and yeast adsorption

Chitin, a polymer of N-acetyl glucosamine which is an effective metal adsorbent, is the main chemical constituent in fungal cell wall (Tsezos and Volesky, 1981). This was confirmed by a study in which exposed chitin/chitosan after alkaline treatment exhibited better removal ability than untreated hyhae from *Mucor mucedo* and *Rhizomucor miehei* (Wales and Sagar, 1990).

Evidently, chitosan and other chitin derivatives have a significant adsorptive capability and it can be enhanced by chemical treatment. Niu *el al*. (1993) reported that *Penicillium sp.* exhibited high lead(II) removal within the pH range 4.0 to 5.0 . *Saccaromyces cerevisae* was also showed to be a good adsorbent to remove chromium (VI) (Nourbakhsh *et al*., 1994).

2.3 Adsorption of lead(II) by algal biomass

A variety of biological materials have been used by researchers to study adsorption utilized algae. Existing research on the adsorption of lead(II) by Spirogyra species biomass is still not well established and the availability of Spirogyra species in Malaysia with huge quantities. Furthermore, they can be cultivated easily and cheaply. Because of this the present research study was carried out to investigate the adsorption of lead(II) by algal of the principle mechanism of metallic ion sequestration involves the formation of complexes between a metal ion and functional groups present on the surface or inside the porous structure of the biological material.

On the other hand, this study might reduce the agriculture waste of *Spirogyra neglecta* which are widely available in Malaysia.

In this study is very important compared with others researchers because we are going to use this biological material to adsorb heavy metal-lead(II) which is very high effective to human health due to its highest concentration in water stream in Malaysia according to Department of Environment(2006) Also, we are going to use new species and size of algae with 0.05nm. Most researchers are using artificial solution, whereas we are going to use the artificial solution and natural solution to investigate ability of adsorption of a non-living algal biomass.

Several research have been completed to study the feasibility of using non-live algal. The ability of *Oedogonium* sp. and *Nostoc* sp to remove lead(II) ion from aqueous solutions in batch system was investigated. The biomass of *Oedogonium* sp. was found to be more appropriate than *Nostoc* sp. for the removal of lead(II) , as it showed higher values of adsorption capacity (145.0 mg/g for *Oedogonium* sp. and 93.5 mg/g for *Nostoc* sp.). Langmuir model fitted the equilibrium better than data the Freundlich isotherm. The spectrum of FTIR analysis showed included that amino and carboxyl groups on the surface of algal biomass were the main adsorption sites for lead removal.

Gupta and Rastogi (2008). Also, Denga *et al*.,(2007) it was shown to be a good adsorbent for lead(II) by green algae *Cladophora fascicularis* The adsorption kinetics followed the pseudo-second order model. The maximum adsorption capacity was 198.5 mg/g at 298K. The adsorption processes were endothermic from batch experiments.

Most algal species have unique metal binding properties. Both the living and non-living forms can reversibly bind metal ions from aqueous solution and different species exhibit binding characteristics at a given pH. The main reason for this is in their different cell wall structure. The cell wall is a fibrillar lattice of cellulose derivatives, pectins and proteins. This structure provides a large surface area for the rapid but reversible binding of cations. Table 2.1 lists the probably potential groups on the cell wall that are implicated for metal binding and their respective pK_a level (Segel, 1976).

Table 2.1
Ionisable groups in biological polymers capable of participating in metal binding (based on Hunt, 1986; Segel, 1976)

Functional group	Location	pK_a
Carboxyl	protein C-terminal	3.5-4.0
Carboxyl	Beta as partic	4-5
Carboxyl	Gamma glutamic	4-5
Carboxyl	Uronic acid	3-4.4
Carboxyl	N-Acetyl neuraminic	2.6
Carboxyl	Lactate	3.8
Suiphonic acid	cysteic acid	1.3
Phosphate	serine as ester	6.8,2.0
Phosphate	Polyol monoester	0.9-2.1
Phosphate	Polysaccharide diester	1.5,6.0
Hydroxyl	Tyrosine-phenolic	9.5-10.5
Hydroxyl	soccharide-alcol	12-13.0
Amino	protein N-terminal	7.5-8.0
Amino	Cytidine	4.11
Amino	Adenosine	3.45
Amino	Lysine	8.9,10.5
Imidazole	Guanosine	2.3
Imidazole	Histidine	6-7
Imino	peptide	13

2.3.1 Living biomass as bioaccumulator

Metabolically uptake or bioaccumulation of heavy metals was first put to practical use for the monitoring traces of heavy metals in the environment (Muraleadharan et al.1991). Several investigation have been made to examine the feasibility of using live algal cultures in lagoons or ponds to recover heavy metal ions from industrial effluents or processed water (Becker,1983; Filip *et al.*,1979).

In case of monitoring heavy metals in sea water *Fucus vesiculosus* and *Ascophyllum nodusum* have been suggested as bioindicator of metal contamination (Soderlund *et al.*, 1988). Karez *et al*. (1994) showed that phaeophyceae (brown algae) in general contained a greater concentration of metals such as zinc, cadmium and chromium adsorbed from sea water under aerated condition compared to Chlorophyceae (green algae) and Rhodophyceae (red algae). In addition to algae, some capsulated bacteria and N_2-fixing bacteria have been studied as adsorbent for heavy metals. Sag and Kutsal (1989) noted a very high metal uptake by *Zoogloea ramigera*.

Since these biomass actively accumulate metals, they can concentrate metals by a variety of mechanisms including ion exchange at the cell walls, complexation reaction at the cell walls, intra and extracellular precipitation and intra-extra cellular complexation reactions. Thus the kinetics of metal binding by living organisms may be affected the viability of the biomass.

As a result, the use of living organisms for metal removal and recovery is generally not feasible due to certain disadvantages. It is difficult to control and maintain the growth of microorganisms since wastewater normally contains high concentration of toxic metals and widely fluctuating pH condition. A further limitation is the incapability to recover the metal ions while maintaining their viability. This is because substantial pH adjustments or addition of specific complexing agents (which may be toxic) are necessary to strip bound metals from the biomass which require a narrow pH range for optimum growth. The acid or alkaline generates used for metal recovery will kill the organisms. Due to these limitations, more attention has been focused on the use of dead biomass as adsorbents.

2.3.2 Dead biomass as adsorbent

The main difficultly of using living biomass for the removal of heavy metals from water was to maintain the growth of the biomass in those polluted waters. The reason for this was that the pH of those water did not encourage the survival of species (Becker, 1983).

In addition, the marine algae(*Ecklonia radiate* and *Durvillaea potatorum*) showed that the maximum adsorption for lead and copper were 1.6 and 1.3 mmol/g, respectively .The heavy metal uptake process was found 90% of the adsorption completed in about 10 min in batch system(Jose *et al.*,1999).

The macro algae species (*Sargassum muticum, Bifurcaria bifurcata, Fucus spiralis* and *Laminaria hyperborea*) were shown to be effective for removing toxic metals (Pb(II), Zn(II) and Cd(II)) from aqueous solutions. Kinetic uptake with 75% of the first 10 min for all algal species. Experimental data were well fitted by a pseudo-second order rate (Olga *et al.*,2008).

The marine algae *Gracillaria* sp., *Ulva* sp., *Sargassum* sp. and *Padina* sp. were investigated for adsorption of lead, copper, nickel, zinc and cadmium from aqueous solutions. Experimental results were well to the Langmuir adsorption isotherm. FTIR analysis showed included amino groups and carboxyl(Sheng *et al*.,2004).

Further more, Ahmet and Mustafa (2008) used species *Ulva lactuca* of algae were investigated for adsorption Cd(II) and Pb(II) from aqueous solution. Langmuir isotherm fitted the equilibrium data better than the Freundlich model. The capacity of adsorption of *Ulva lactuca* biomass for Pb(II) and Cd(II) ions was found to be 34.7 mg/g and 29.2 mg/g, respectively. Adsorption processes of both metal ions followed pseudo-second-order kinetics.

The lack of organic substrates to maintain algal growth was another factor. Furthermore the adsorption of heavy metals itself would affect the viability of the algae. It would necessitate the addition of the complexing agents and the constant adjustment of the pH of the wastewaters. This involves cost and in some instances it would be impractical to do so. However, the use of dead cell systems would do away with these disadvantages in complicated and cut down costs. The disadvantage of course is that the process of active bioaccumulation would be sacrificed. Considering the fact 90% of total metal uptake by algal biomass can be attributed to adsorption it is a small price to pay. Moreover non-living adsorbents may also be reused repeatedly and the metal ions recycled (Harris and Ramelow, 1990). It also been shown that dead cells are capable of accumulation heavy metals as efficiently as their living counterpart (Sakaguchi *et al.*, 1979) because of these advantage, the main thrust of research into the field of adsorption has now been the use of dead biomass.

2.4 Adsorption kinetics and equilibrium

Evaluation of metal adsorption by non-living biomass as a unit operation often focus on two important physico-chemical aspects of the process, i.e. adsorption kinetics and equilibrium.

2.4.1 Adsorption isotherms models

Adsorption models are frequently used to describe the equilibrium between metal ions in solution and metal ions adsorbed on the surface. Equilibrium studies on adsorption give information about the capability of the adsorbent to remove a unit gram of metal ions under specific system report conditions.

Adsorption equilibrium is established when a certain amount of metallic species sequestered and bound by a solid phase, is in dynamic balance with remaining dissolved metal in the solution. When a metal ion in solution collides with a solid (adsorbent) surface, a limited number of outcomes are possible (Allen and Brown, 1995**)**. Positive adsorption in adsorbent-metal ion system results in the transfer of the metal ions to the surface of the algal cells where it increases in concentration until a dynamic equilibrium is reached between the metal ions and the adsorbent and with that of the interface. At this point of equilibrium position there is a define distribution of metal ions between the adsorbent surface (solid phases) and liquid (reaction solution). The distribution ratio is a calculate of the position of equilibrium in the adsorption process. It is usually represented in the form of an equilibrium isotherm. This isotherm is a efficient expression for the variation of adsorption with concentration of metal ion in the bulk solution at constant temperature. In other words, the isotherm plot is a graphical expression that represents metal adsorption by the adsorbent against the residual metal concentration in the contact solution.

Many researchers have used equilibrium isotherms to characterize the removal of heavy metals by adsorbent (Fourest and Roux, 1992; Avery and Tobin, 1993; Chang and Hong, 1994).

When a metal solution is contacted with algal cells, metal ions may collide with the cell surface and result in several incidences; they are as follows:

i. The metal ion may rebound from the surface.

ii. The metal ions may be adsorbed and the solid phase may referentially concentrate specific metal ion species from solution onto its surface.

iii. Reaction may take place between the incoming cation and functional group on the solid surface. The phenomenon is termed ion-exchange.

The two most commonly used equilibrium isotherms are the Langmuir isotherm model and the Freundlich isotherm model (Smith, 1981; Chang and Hong, 1994).

In this study, two adsorption isotherms: the Lungmuir and Freundlish were applied to fit equilibrium data of adsorption of lead(II) on the algal biomass.

Freundlich model is an empirical equation on sorption on a heterogeneous surface or surfaces supporting site of varied affinities. It is assumed that the increasing degree of site occupation (Freundlich., 1906)

Langmuir isotherm assumes monolayer adsorption onto a surface containing a limited number of adsorption sites of uniform strategies of adsorption with no transmigration of adsorbate in the plane of surface (Weber and Chakkravorti, 1974).

2.4.1.1 Langmuir adsorption isotherm

The Langmuir adsorption isotherm has been effectively applied to many pollutant adsorption processes and has been the most widely used isotherm for the adsorption of a solute from a liquid solution, on another word it has been employed to characterize the uptake of heavy metals by evaluating the adsorption capacities of the microorganisms. It is also useful to describe the equilibrium conditions for adsorption in different systems.

Basic assumptions of the Langmuir theory are:

1. Surface is homogeneous that is adsorption energy is unvarying over all sites.
2. Adsorption on surface is localized that is adsorbed molecules or atoms are adsorbed at definite localized sites.
3. Every site can accommodate only one atom or molecule.

This model can be written in non-linear form (Langmuir) as:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} K_L} + \frac{1}{q_{\max}} C_e \qquad (1)$$

Where

q_{max} = is the monolayer adsorption capacity of the adsorbent(mg/g) is the(maximum amount adsorbed)

K_L = is the Langmuir adsorption constant (L/mg)

C_e = the equilibrium metal ion concentration in the solution (mg/L),

q_e = is the equilibrium metal ion concentration on the adsorbent (mg/g)

The solid phase equilibrium metal concentration which is the amount of metal adsorbed on the biomass surface in a batch system was calculated using the following mass balance equation:

$$q = \frac{V(C_0 - C_t)}{W} \qquad (2)$$

Where

q = is the metal uptake (mg metal/g biomass),

C_0 = initial metal concentrations in the solution (mg metal/l fluid)

C_t = final metal concentrations in the solution (mg metal/l fluid),

V = the volume of solution,

W = is the dry weight of the added adsorbent (g).

2.4.1.2 Freundlich adsorption isotherm

The Freundlich model is the most popular adsorption model for a single solute system. The model has found broad acceptance because of its accuracy and broad applicability. The Freundlich model assumes a heterogeneous adsorption surface and dynamic sites with different energy. The Freundlich model (Freundlich equation) is

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e \qquad (3)$$

where

q_e = amount of the adsorbate per unit weight of adsorbent(mg/g).

K_F = is a constant relating the adsorption capacity (Freundlich constants)

$1/n$ = is an empirical parameter relating the adsorption intensity (which varies with the heterogeneity of the material).

2.4.2 Adsorption kinetics

The pseudo-first-order and pseudo second-order models were used to exam adsorption kinetic data to investigate the mechanism of adsorption. The kinetics of the metal sequestering process is a crucial parameter which determines the time required for the adsorption system to attain equilibrium, commonly referred to the contact time. The contact time is a critical factor especially in large-scale operation as it determines the size of the reaction which in turn affects the capital and operating costs of the process. A rapid metal binding process, i.e. short adsorbent-solution contact time is desirable.

The method of kinetic adsorption tests was identical to that of batch equilibrium tests. The lead(II) uptake at any time, q_t(mg/g), was calculated by equation (4).

$$q = \frac{V(C_0 - C_t)}{W} \qquad (4)$$

2.4.2.1 Pseudo-first-order-kinetic model

One of the major characteristics to define the efficiency of adsorption is its kinetics. The pseudo-first-order-kinetic model has been widely used to predict adsorption kinetics. The pseudo first-order model is presented by the following equation (Ho, 2004):

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \qquad (5)$$

Where

q_e and q_t = the amounts of lead(II) adsorbed on the algal biomass at equilibrium and time in mg/g

k_l = constant of pseudo first-order adsorption

2.4.2.2 Pseudo-second-order-kinetic model

The pseudo second-order model is presented by the following equation (Demirbas *et al.*, 2004):

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \qquad (6)$$

2.5 Factors affecting the adsorption process

Adsorption of heavy metal ions onto the surface of a microorganism is affected by many factors such as initial temperature, pH, biomass concentration, and initial metal ion concentration (Özer *et al.*, 1999).

2.5.1 Effects of initial concentration and contact time on adsorption of lead(II)

Contact time is one of the vital parameters for successful use of the adsorbents for practical application and rapid sorption is among desirable parameters (Akar and Tunali, 2005).

2.5.2 Effect of solution pH on adsorption of lead(II)

Adsorption process is dependent on the aqueous phase pH and the functional groups on the algal cell walls and their ionic states (at particular pH) determine the extent of adsorption (Crack, 1965; Wilde *et al.*, 1993; Genc *et al.*, 2003).

The effect of pH on adsorption can be explained by ion-exchange mechanism, in which the significant role is played by the functional groups of the biomass that have cation-exchange properties (Volesky *et al.*, 2000). Since adsorption is a chemical/physical reaction between positively charged metal ions and anionic group of cell surface, it is to be expected that metal adsorption is strongly influenced by pH which affects the speciation of metal and reaction groups. Initial adsorption rate and capacity are affected by pH.

The pH of metal solution is critical because of its effect on the formation of metal-adsorbent complexes. It not only affects the ionization of the function groups responsible for metal binding on the cell wall surface, but also regulates the solution chemistry affecting the chemical speciation of metal ions. This in turn determines the mobility and ability of the ions to adsorb onto the biomass surface (Kuyucak and Volesky, 1989, Zhou and Kiff, 1991). Each metal has its own individual optimal pH value, depending on its solution chemistry

2.5.3 Effect of temperature on adsorption of lead(II)

Temperature affects the adsorption rate by changing the molecular interaction and solubility of adsorbate. Temperature plays a vital part in biochemical reactions which hastens reactions if raised, since higher temperature increases the energy of the molecules, creating more collisions per unit time, and is an important factor influencing self-purification in streams. The metabolic rate of aquatic organisms is related to water temperature, and in warm waters, respiration rates increase leading to increase oxygen consumption. Growth rate will also increases. This can lead to increased decomposition of organic matter, water turbidity, macrophyte growth and algal blooms, especially when nutrient conditions are suitable (Jackson and Jackson, 1996). Toxic chemicals made more soluble by higher temperature may present an additional hazard to the organisms in the water (USEPA, 1986). Higher temperature increases the toxicity of many substances such as heavy metals or pesticides, whilst the sensitivity of the organisms to toxic substances also increases.

The temperature of the working solution usually play an important role in the adsorption processes because some of adsorption processes are endothermic, so the temperature increasing lead to increasing in adsorption capacity. This may be due to improved interaction or increased equilibrium constant between solute and the adsorbent.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Collection of algal biomass

Fresh algal biomass was collected from a shallow, slow-flowing stream in Rimba Bukit Kiara, Kuala Lumpur (Figure 3.1). The alga formed a dark-green filamentous mass floating the water surface or attached to submerged rocks and concrete surfaces.
The algal biomass hand picked with the aid of forceps and placed in a pail, was brought back to the laboratory.

In the laboratory the algal biomass was repeatedly washed with distilled water to remove dirt and other organisms such as zooplankton and epiphytes. A small portion of the algal biomass was kept aside for identification. The rest of the algal biomass was placed on a filter paper to reduce its water content. It was then dried for 72 hours in an oven with temperature set at 45 °C, this process killed the alga and therefore, the dried algal material is termed as “non-living algal biomass”.

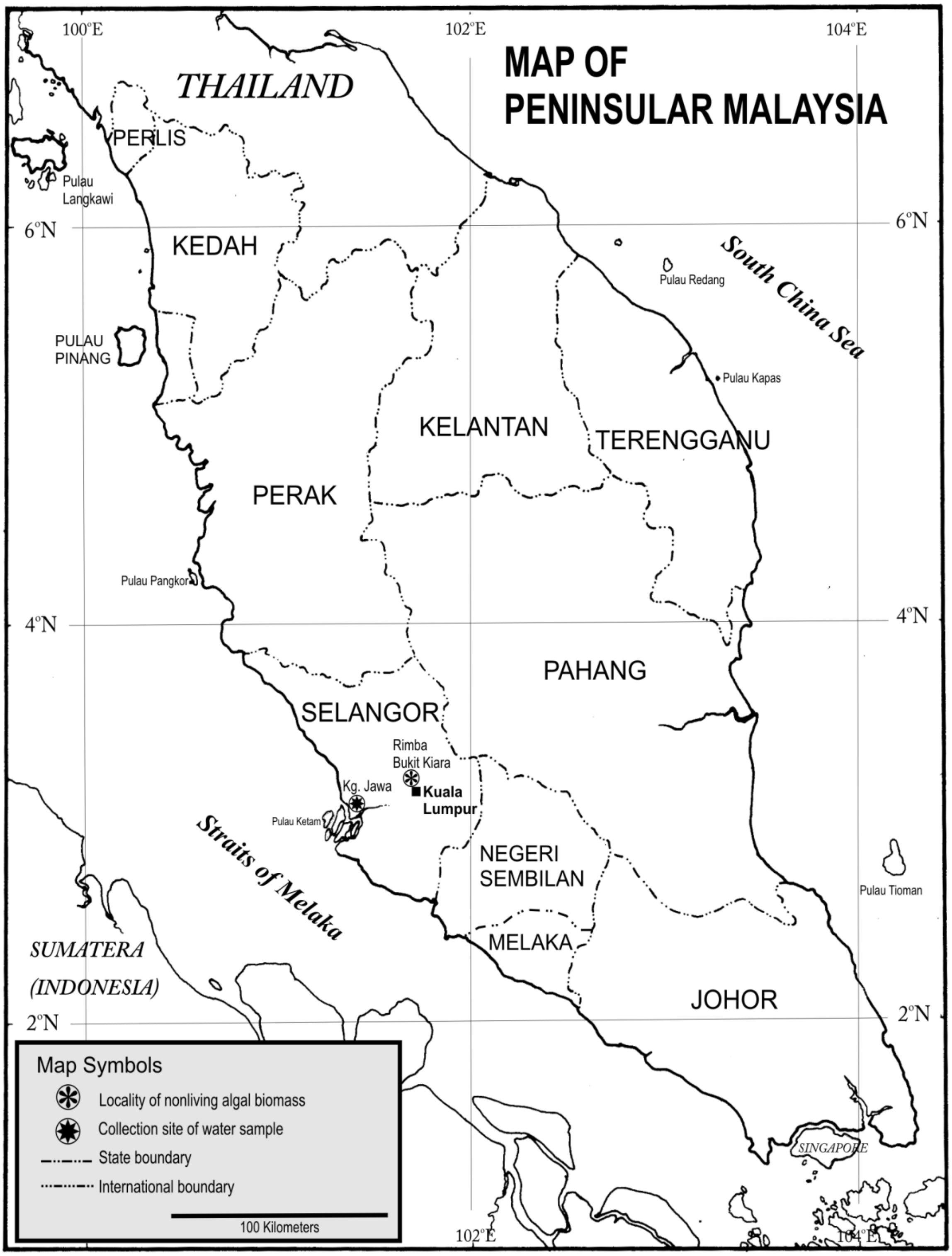


Figure 3.1 : Map of Peninsular Malaysia showing the locality of nonliving algal biomass and collection site of water sample

3.2 Identification of the alga

Filaments of the alga was placed and immersed in drop of distilled water on a glass slide. This was covered with a cover slip and then observed under a light microscope. Vegetative cell sizes were determined using a computer which was attached to the light microscope. The algae of chloroplasts in each vegetative cell were noted.

The characteristics of the alga were compared with those of other species of filamentous fresh water algae in the literature. The alga was identified as a species which was most agreeable that belong to the characteristics examined.

3.3 Preparation of sample algal and stock solution of lead(II)

The non-living algal biomass was ground to powder using an agate stone pestle and mortar. The non-living algal biomass powder was sieved using a standard testing sieve to sieved select the particles of 300 to 425 µm in diameter. Powder of the desired particle size was kept in an air-tight plastic container at room temperature. Samples of the non-living algal biomass for subsequent analysis were all obtained from the above.

Stock solution (2000 mg/L) of lead(II) was prepared by adding (3.19 g) powder lead nitrate (contain 2 of lead), to 1000 ml plastic volumetric flask.

3.4 Determination of baseline lead(II) concentration of the sample

The concentration of lead(II) in the sample of the non-living algal biomass was determined because high level of lead(II) in the sample interferes and affects the results of the subsequence experiments.

3.5 Effects of initial concentration, contact time, pH, temperature on the adsorption of lead(II) by the non-living algal biomass

3.5.1 Effect of contact time and initial concentration on adsorption of lead(II)

The effect of contact time and initial concentration on the adsorption uptake, were studied at room temperature 30±1. Through the following procedure: a series of initial concentrations of lead(II) solution (50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L and 250mg/L) were prepared from the stock solution according to the law of dilution(equation. 7). From each solution of the series mentioned above 50 ml was added to five vial culture containing (0.05g) of algae. Then put in a shaker and shaken at 150 rpm for 15 minute. No pH adjustments were made and all of the studies were carried out at natural pH. After predetermined time interval the content of vial culture were filtered by vacuum filtration through 0.45µm membrane filter. The filtrates were diluted ten times and analyzed for residual lead(II) using atomic absorption spectrometer (AAS) at wavelength of 283.3 nm. The same experiment was repeated at different times (30min, 45min, 60min, 75min, 90min, 120min, and 165min) to determine the final concentration of lead(II) solution.

$$M_1 \times V_1 = M_2 \times V_2 \qquad (7)$$

3.5.2 Effect of pH

The effect of pH on the lead(II) solution was examined by varying the pH of the solution from pH 3 to pH 6. The pH of the solution were adjusted to a desired pH using 0.1N HCl and 0.1N sodium hydroxide (NaOH), magnetic stirrer was used to agitate the solution continuously and pH measurements were done using EUTCH pH meter. The lead(II) initial concentration was fixed at 150 mg/L with biomass dosage of 0.05g agitation 1 hour, and rotation speed 150 rpm under room temperature.

3.5.3 Effect of temperature

The effect of temperature on the adsorption of lead(II) was studied by the varying of the adsorption temperature at 40, 50 and 60 °C by adjusting the temperature controlled at the shaking incubator(JEIO TECH, model SI-900r), while other operating parameters such as biomass dosage, rotation speed and initial concentration 150 mg/L were remained constant while the solution pH was original without any adjustment.

3.6 FTIR analysis of the non-living algal biomass

This was done by mixing approximately 1.0 mg dried sample of algae with 100 mg KBr (1:100), ground to fine powder and pressed under vacuum to a pellet. The instrument was switched on and background was obtained without placing the pellet. Then the pellet was placed and scan was obtained in the range from 370.0 to 4000.0 cm^{-1}. The data was plotted using standard software provided with the instrument.

3.7 Scanning electron microscopic examination of the non-living algal biomass

Scanning electron microscope examination of the adsorbent was done under SEM (JEOL 6400, Japan) to study the surface texture and morphology of the adsorbent which participated in metal adsorption. Sample of the adsorbent was prepared based on technique and procedure of Gabriel (1982).

3.8 Adsorption isotherms of lead(II) by the non-living algal biomass

Data obtained from the removal study of lead(II) were tested with Freundlich adsorption isotherm and Langmuir adsorption isotherm to have a satisfactory description of the equilibrium state between the two phases in order to successfully represent the dynamic behavior of lead(II) from the solution to solid (algal biomass) phase.

Batch mode adsorption studies were carried out at 30±1 °C by varying the concentration of lead(II) using atomic absorption spectrometer (AAS) at wavelength of 283.3 nm

3.9 Kinetics of lead(II) adsorption by the non-living algal biomass

Determination of effectiveness of adsorption process requires an understanding of kinetics of uptake of adsorbate or the time dependence of the time concentration distribution of solute in both bulk solution and solid adsorbent and identification of rate determining step. The study of kinetic of adsorption describes solute uptake rate.

In order to examine the order of adsorption process, several kinetic models were used to test the experimental data. In the present work following kinetics models were applied to check the applicability of the equations.

3.10 Thermodynamics of adsorption of lead(II) by the non-living algal biomass

In order to describe thermodynamic behavior of the adsorption of lead(II) ions onto *Spirogyra neglecta* biomass, thermodynamic parameters including the change in free energy (ΔG°), enthalpy (ΔH°) and entropy (ΔS°) were calculated from following equations

$$\Delta G^\circ = - RT \ln K_c \qquad (7)$$

Where

R = is the universal gas constant (1.987 cal/deg/mol)

T = is the temperature in kelvin. (K).

K_c (q_e/C_e) = is the distribution coefficient (Aravindhan *et al.*,2007, Sarı *et al.*,2007)

From the following equation:

$$K_c = (q_e/C_e) \qquad (8)$$

The enthalpy (ΔH°) parameters were obtained from Van't Hoof equation

$$\ln K_c(T_4) - \ln K_c(T_1) = \frac{-\Delta H^\circ}{R}\left(\frac{1}{T_4} - \frac{1}{T_1}\right) \qquad (9)$$

Where

T_4 and T_1 are two different temperatures

and entropy (ΔS°)from the following equation:

$$\Delta S^\circ = - \frac{\Delta G^\circ - \Delta H^\circ}{T} \qquad (10)$$

3.11 Application of non-living algal biomass to remove lead(II) from a polluted water sample

3.11.1 Collection and preservation of water sample

Water sample was collected from Klang River near Kampong Jawa, Selangor (Figure 3.1). Five samples were collected from under the surface of the river at intervals of 1 meter starting from the edge of the right bank. These collected samples were mixed in a plastic bottle and the solution was acidified with 1.5 ml of concentrated nitric acid (HNO_3). One liter of the acidified sample was stored in box containing ice and analyzed as soon as it reached the laboratory.

3.11.2 Analysis of water sample from a polluted river

150 ml of the sample bottled river water was filtered by vacuum filtration through 0.45 µm membrane filter. 50 ml of the filtered water was poured into a vial culture containing algae while another 50 ml was poured into vial culture without algae. Both were shaken for 1 hour and subsequently filtered by vacuum filtration through 0.45µm membrane filter. The filtered water was analyzed using atomic absorption spectrophotometer (AAS).

CHAPTER FOUR

RESULTS

4.1 Identification of the algae

The alga which was used in this study was identified as *Spirogyra neglecta* (Hasall) Kutzing. The alga was filamentous and unbranched. Its vegetative cells possessed spiral chloroplasts, 43.97µm to 47.90µm wide and 122.29µm to 186.25µm long. No reproductive cells were seen in the algae. (Appendix 1)

4.2 Baseline concentration of lead(II) in the non-living algal biomass

Percentage contamination of lead(II) in the algal biomass was 0.01%. This amount of contamination was considered negligible and therefore did not interfere with treatments in subsequent experiments.

4.3 Effects of initial concentration and contact time on adsorption of lead(II)

The adsorption of lead(II) increase with time and at certain point in time, it reached a content value beyond which no more lead(II) was further removed from solution. (Figure 4.1) shows that maximum adsorption taken place within the first 60 minute. The amount of lead(II) adsorbed at the equilibrium time reflected the maximum adsorption uptake of the adsorbent under the operating conditions applied. The information obtained from this experiment was further used successfully to estimate the kinetics of the adsorption process.

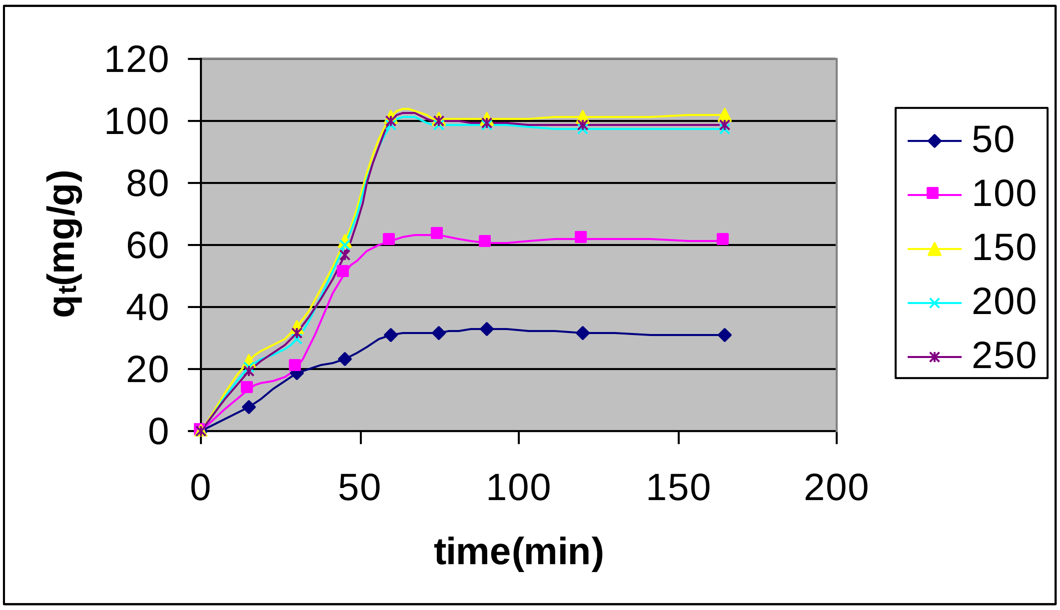


Figure 4.1
Effect of contact time and on initial concentration adsorption of lead(II) by non-living biomass of *Spirogyra neglecta*

4.4 Effect of pH on adsorption of lead(II)

The pH of a solution is an essential parameter in adsorption process. The adsorption of lead(II) ions by Spirogyra neglecta as a function of pH are shown in Figure 4.2. In all cases, metal uptake by the biomass will increases with increasing pH until it reaches a maximum pH at 6.0 g (Appendix 4). The pH of the solution decreased after adsorption the metal uptake. The adsorption at above pH 6 was not investigated because precipitation of this metal start in that pH range.

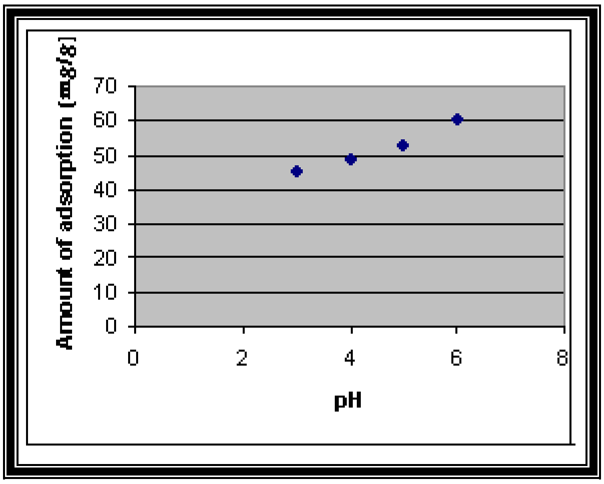


Figure. 4.2
Effect of pH on adsorption of lead(II) by non-living biomass *Spirogyra neglecta*

4.5 Effect of temperature on adsorption of lead(II)

Temperature has essential effect on adsorption process as it can increase or decrease the amount of adsorption. In the present experiment the adsorption rate of lead(II) on the adsorbent, at three different temperatures (40,50 and 60 ˚C) have been analyzed and adsorption capacity as a function of temperature are shown in Figure 4.3. The adsorption of lead(II) ion increase with increase of temperature from 40 to 60 ˚C, (Appendix 5). This effect is the characteristic of a chemical reaction or bonding being involved in the adsorption process with the increase in temperature. The fact that the adsorption increases with an increase in temperatures indicates the raise in the mobility of large metal molecules with increasing temperatures and the ongoing adsorption process is endothermic. Suggesting that the removal of the lead(II) from aqueous solutions to adsorbent is faster at high temperature than at low temperature.

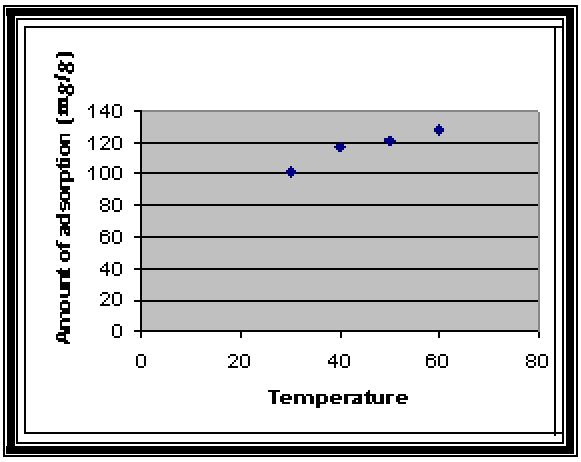


Figure 4.3
Effect of temperature on adsorption of lead(II)
by non-living biomass *Spirogyra neglecta*

4.6 Scanning electron microscopic examination of the non-living algal biomass

Figure 4.4(a-d) showed that it was evident from the micrographs that the adsorbent sample before and after adsorption. The micrograph (the surface texture and morphology of algae) is one of the principle characteristics affecting the adsorptive capacity of an adsorbent since the adsorption process result in a concentration of solutes at the surface.

In Figure 4.4(a-b) before the adsorption shown the algae exhibits a caves-like, or pores which increase capacity of adsorption. In contrast, Figure 4.4(c-d) after adsorption noted uneven surface texture along with lot of irregular surface because full the pores by heavy metal ion belong to the function group on surface of adsorbent which are playing important role in almost all potential binding mechanisms.

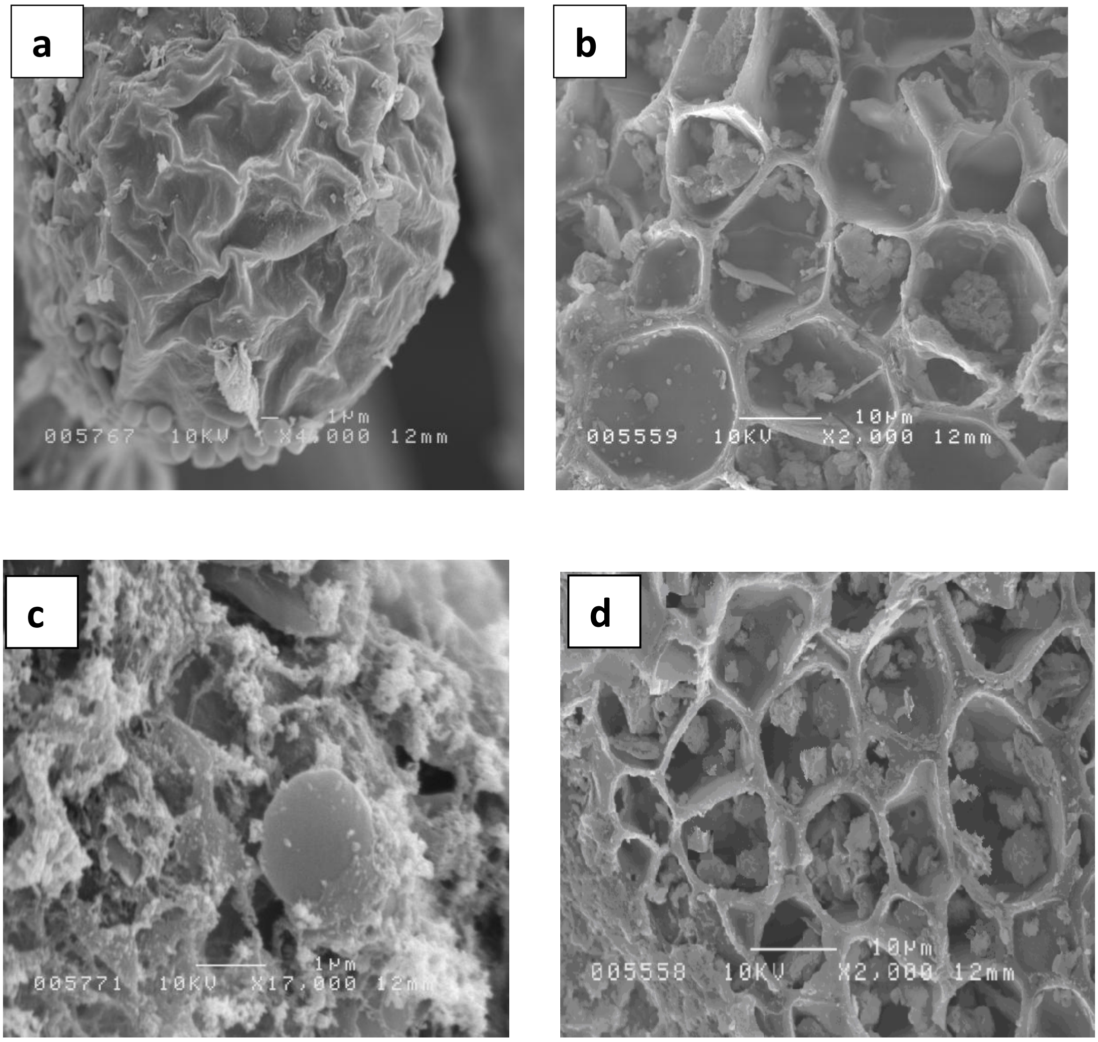
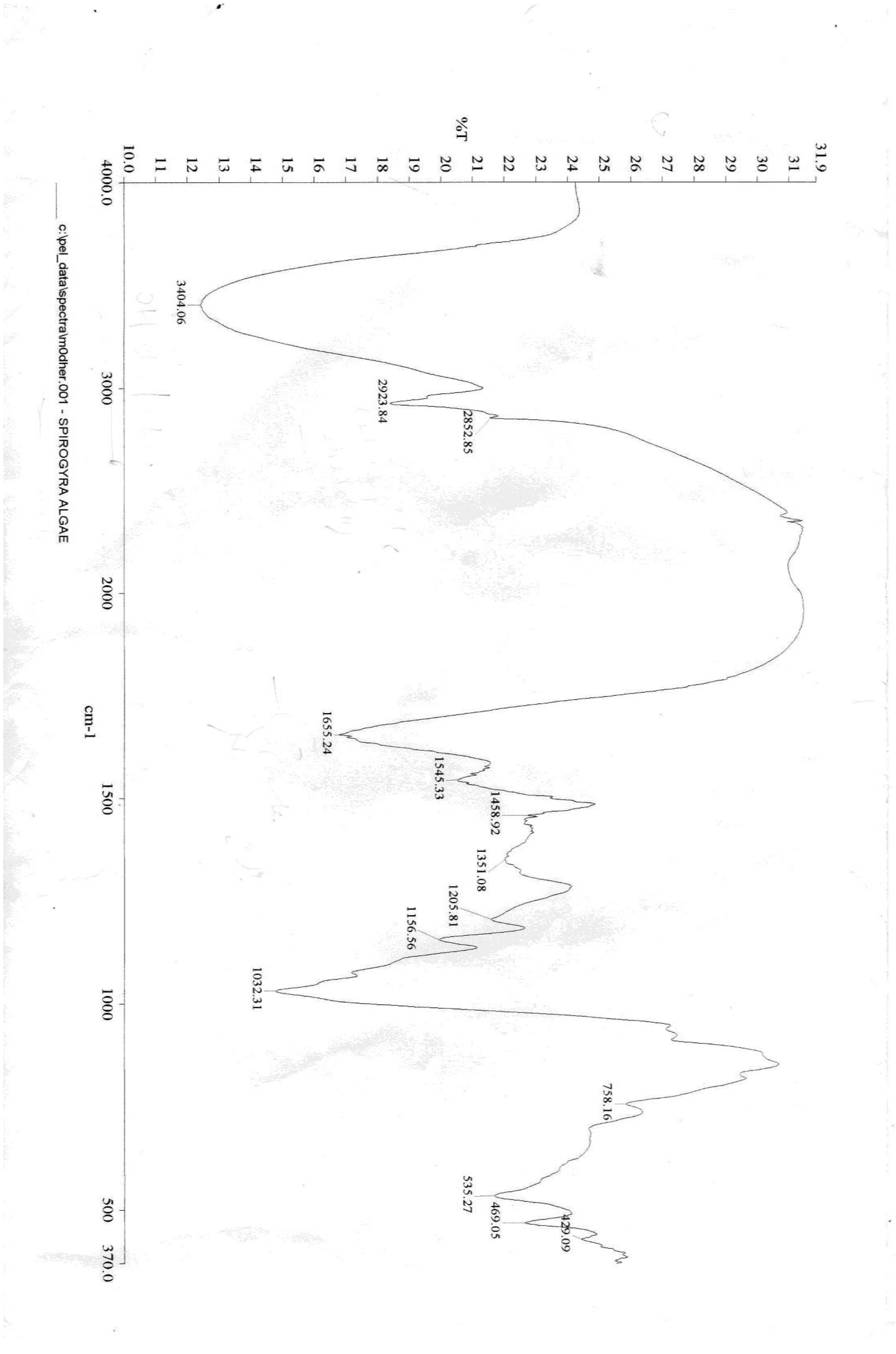


Figure 4.4(a-d)
SEM image the biomass particles, with and without adsorbed metal

4.7 The Fourier Transform Infrared Spectrometer (FTIR) analysis for the adsorbent

The spectrum of FTIR analysis are shown in (Figure 4.5). The FT-IR of the native biomass displays a number of adsorption peaks, indicating the complex nature of the biomass examined. The broad adsorption peak around 2923 cm^{-1} is indicative of the existence of bonded alkanes group (C-H). The peak observed at 1655 cm^{-1} can be assigned to the to the carbonyl group (C=O). The peak observed at 3250 cm^{-1} can be assigned to the carboxyl group (COOH). The peak observed at 3404.06 cm^{-1} can be assigned to the amine group (N-H).

The results indicated that the algae biomass has a variety of functional groups, such as carboxyl, and amine and these groups are involved in almost all potential binding mechanisms.



Fig*ure* 4.5
Peaks for non-living biomass of *Spirogyra neglecta* obtained from FTIR analysis (range 370.0-4000.0 cm^{-1}).

4.8 Adsorption isotherms of lead(II) by the non-living algal biomass

The adsorption of lead(II) by native biomass was rapid and attained equilibrium within the first 60 minutes and the maximum adsorption capacity (q_{max}) of *Spirogyra neglecta* was found 132 mg/g for lead(II) ions.

In Figure 2.3 shown the C_e versus C_e/q_e were drawn to calculate the value of Langmuir constant.

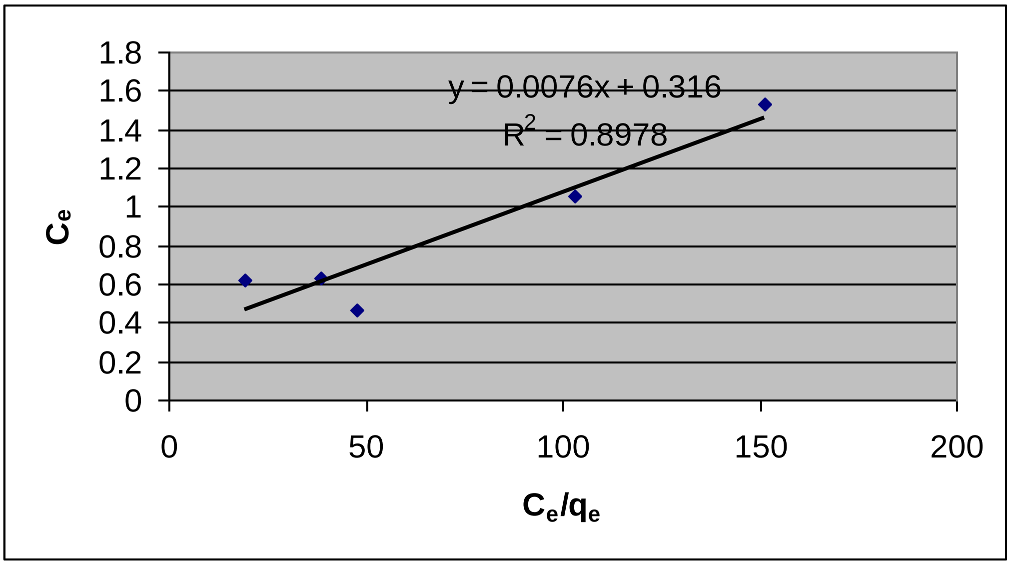


Figure 4.6
Langmuir adsorption isotherm of lead(II) by the non-living biomass of *Spirogyra neglecta*

In Figure 2.4 shown the ln/q_e versus ln/C_e were drawn to calculate the value of Freundlich constants.

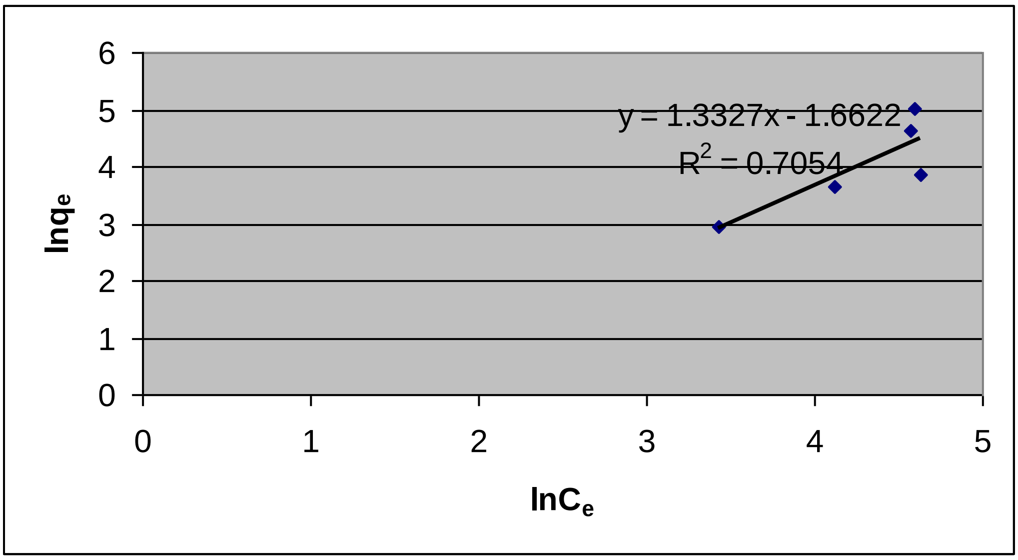


Figure 4.7
Freundlich adsorption isotherm of lead(II) by the non-living biomass of *Spirogyra neglecta*

All the correlation coefficient, R^2 values and the constants obtained from the two isotherms models applied for the adsorption of lead(II) on algal biomass are summarized in (Table 4.1).

Table 4.1
Langmuir and Freundlich constants at room temperature (30±1°C)

Models	**Parameters**	**Values**	**Calculation**
Langmuir	q_{max} (mg/g)	132	Appendix 2
	K_L (mg/L)	0.023	
	R^2_L	0.8978	
Freundlich	k_F (mg/L)	0.1897	Appendix 3
	N	0.75	
	R^2_F	0.7054	

4.9 Adsorption kinetics

In order to understand the adsorption kinetics of lead(II) ions, two kinetic models, which are the pseudo-first-order and pseudo-second-order have been applied for the test experimental data.

4.9.1 Pseudo-first-order-kinetic model

Result based on pseudo-first-order-kinetic model is shown in (Figure 4.6) explained the plot of log(q_e-q_t) versus t(time). The constants K_1 and q_e obtained from the slope and intercept of this plot respectively. The value of correlation coefficients R^2_1 obtained from the plot (Figure 4.6) for adsorption of lead(II) on the algal biomass at room temperature 30±1 °C are listed in (Table 4.2).

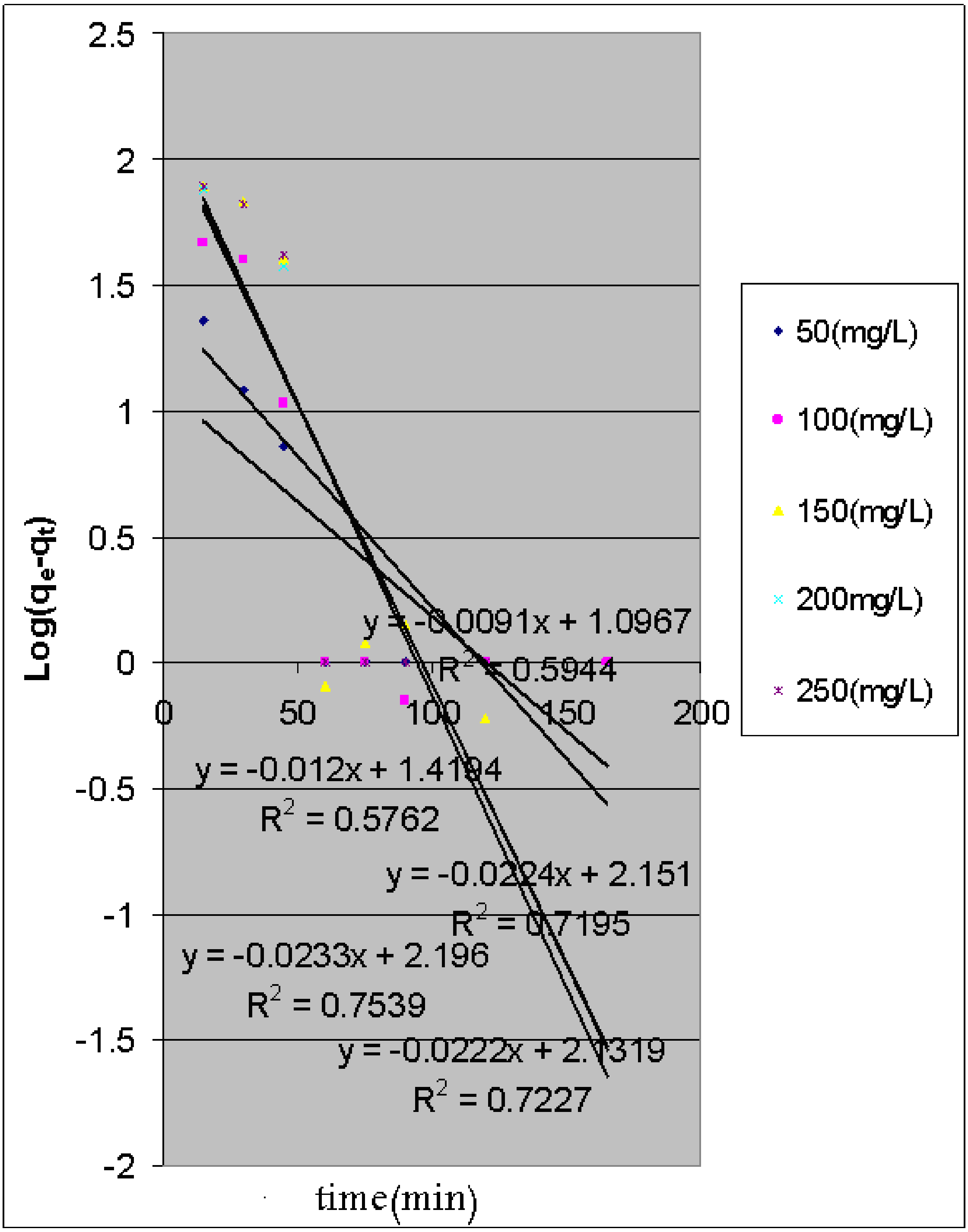


Figure 4.6
Pseudo-first-order kinetic adsorption of lead(II) by the non-living biomass of *Spirogyra neglecta*

In Table 4.2 lists the computed results obtained from pseudo- first -order kinetic model.

Table 4.2
Pseudo-first-order kinetic model

Concentration (mg/L)	**Pseudo-First-order kinetic model**			
	q_e (mg/g) experiment	**q_e1(mg/g)cal**	**K_1**	**R^2_1**
50	30.8	12.49	0.021	0.5944
100	61.4	26.26	0.028	0.5762
150	102.1	157.03	0.054	0.7539
200	97.2	135.49	0.051	0.7227
250	98.8	141.58	0.052	0.7195

4.9.2 Pseudo-second-order-kinetic model

The validity of the adsorption order were based on two main criteria, first based on the correlation coefficient and secondly based on the calculated q_e values.

Result based on the second-order-kinetic model is shown in Figure.4.7 explained the plot of t/q_t versus t(time) were used to calculate the second-order rate constants K_2 and q_e. The values of parameters K_2, q_e and correlation coefficients R^2_2 are summarized in Table 4.3.

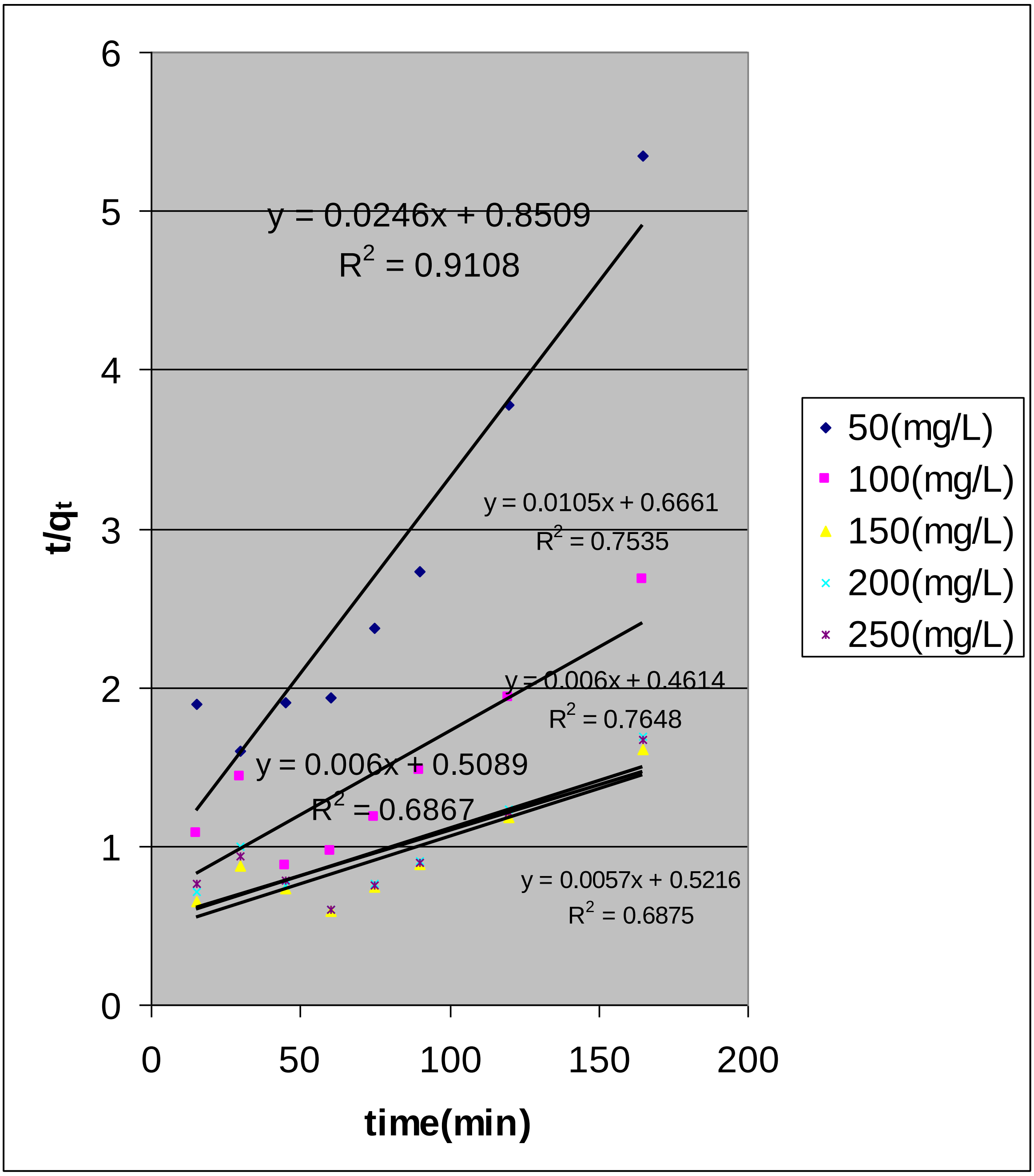


Figure 4.7
Pseudo-second-order kinetic adsorption of lead(II) by the non-living biomass of *Spirogyra neglecta*

In Table 4.3 lists the computed results obtained from the second-order kinetic model.

Table 4.3
Pseudo-second-order kinetic model

Concentration (mg/L)	**Pseudo- second –order kinetic model**		
	q_e2	K_2	R^2_2
50	40.65	1406.04	0.9108
100	95.23	2834.22	0.7535
150	166.66	12815.64	0.7648
200	166.66	14134.98	0.6867
250	175.43	16052.59	0.6875

The straight lines in plot of t/q_t versus t showed good agreement of experimental data with the second-order kinetic model for different initial concentration for adsorption kinetics of lead(II) on algae biomass at room temperature 30 ±1 °C.

4.10 Adsorption thermodynamics

The result obtained from the calculation is shown in Table 4.4.

Table 4.4
Thermodynamic parameters for the adsorption of lead(II) on algal biomass (*Spirogyra neglecta*) at different temperatures.

Temperature (K)	**Thermodynamic parameters**		
	ΔG° (cal mol^{-1})	ΔH°(cal mol^{-1})	ΔS°(cal mol^{-1} K^{-1})
303	- 457.6	6613.3	16.36
313	- 777.4		
323	- 930.6		
333	-1164.5		

Table 4.4 shows the positive value of ΔS° meaning entropy was increase and the positive value of enthalpy ΔH° meaning reaction had taken more calories. The value of $\Delta H^{\circ}$was

positive, indicating that the adsorption reaction is endothermic. This is also supported by the increase in value of uptake capability of the adsorbent with the rise in temperature. Positive value of enthalpy ΔH° assumes that the entropy is responsible for making the ΔG° value negative. The negative value of ΔG° confirms the feasibility of the process and the spontaneous nature of adsorption of lead(II) on *Spirogyra neglecta*.

4.11 Analysis of water sample from a polluted river

The concentration of lead(II) in the untreated water sample was analyzed using FAAS (flame atomic absorption spectrometer) at wavelength of 283.3 nm under room temperature (30 ± 1°C) was found 5.673 mg /L. After treatment of water sample with non-living algal biomass the concentration of lead(II) in treated water sample was analyzed and found 4.472 mg /L.

The percentage e of removal was calculated by using following equation:

$$\text{Percentage of removal (\%) of lead(II)} = \frac{\text{initial concentration} - \text{final concentration}}{\text{initial concentration}} \times 100\%$$

$$\text{Percentage of removal (\%) of lead(II)} = \frac{5.673 - 4.472}{5.673} \times 100\%$$

Percentage of removal lead(II) from polluted water was found 21.2%

CHAPTER FIVE
DISCUSSION

5.1 Effects of initial concentration and contact time on adsorption of lead(II)

The amount of lead(II) adsorbed at the equilibrium time reflected the maximum adsorption uptake of the adsorbent under the operating conditions applied.

The results revealed that the lead(II) adsorption was fast at initial stage of the contact period and then became slower near the equilibrium, which showed that no more lead(II) was further removed from the solution.

The amend in the rate of removal might be due to the fact that initially all adsorbent sites were vacant and the solute concentration gradient was high. So, the rate of adsorption was also high. Later, the lead(II) uptake by adsorbent was decreased significantly, due to the decrease in number of adsorption site as well as lead(II) concentration. As time passes, the number of sites on the adsorbent filled up by the adsorbate also increases. At equilibrium, when all the sites are filled, the rate of adsorption is equal to the rate of desorption. So, after equilibrium, it is found that there was no increase in removal of adsorbate with increase in contact time. Decrease removal rate, particularly towards the end of experiments, indicate the possible monolayer formation of lead(II) on the outer surface. Similar trend was observed for adsorption of lead(II) onto modified fly ash (Wooland *et al*., 2000), activated phosphate (Moufliha *et al*., 2005) and agricultural by-products (Tiemann *et al.*, 2002).

5.2 Effect of pH on adsorption of lead(II)

The effect of the pH of metal solution is one important parameter that influence the adsorption process. The results show that adsorption of lead(II) was increasing with increasing of pH of the solution. At low pH values, the inactivated cell exterior becomes more positively charged, leading to reduce the hold between metal ions and functional groups at the cell wall. In contrast, when the pH increases, the cell surface is extra negatively charged and the process of retention is favored (Pardo *et al.*, 2003; Volesky and Holan, 1995) until a maximum is reached around pH 6. However, at pH higher than 6, the precipitation of insoluble metal hydroxides takes place restricting the true adsorption studies according to the formula:

$Pb(NO_3)_2 + NaOH \rightarrow Pb(OH)_2\downarrow + NaNO_3$

In other words, adsorption decreases at higher pH.

Lead(II) adsorption is maximized at pH 6.0, a value which is in similar to the results obtained by Pardo *et al*. (2003), who found that the maximum pH for lead(II) by *Pseudomonas putida* is 6.0. Moreover, Seki *et al*. (1998) studied the function of pH on adsorption of lead(II) by *Rhodobacter sphaeroides* and reported that the maximum pH is around 6.0. This is not only related to the formation of soluble hydroxylated complexes of the metal ions, but also to the ionized nature of the cell wall surface of the biomass under the studied pH (Chojnacka, 2005).

5.3 Effect of temperature on adsorption of lead(II)

The adsorption reaction for the endothermic processes could be because of the increase in temperature increase the rate of diffusion of the adsorbate molecules across the external boundary layer and in the internal pores of the adsorbent particle, owing to the decrease in the viscosity of the solution. Wang and Zhu (2007) noted similar observations and they suggested that the increase in adsorption uptake with increase in temperature might be due to the possibility and in the total pore volume of the adsorbent, an increase of number of active sites for the adsorption as well as an increase in the mobility of the adsorbate molecules. In addition, Meena *et al.* (2005) reported the adsorption of metal ions has been found to increase with increase in temperature from 20 to 60 °C, and they suggested the increase in adsorption with temperature may be attributed to the increase in the number of active surface sites available for sorption on the adsorbent. The increase in metal adsorption with increase temperature could be due to dissociation of some compounds available in the adsorbent, which may provide more sites from metal adsorption (Sammer and Duvnjak, 1999). In other words, the rise in adsorption capacity with temperature is caused by the rise in the kinetic energy of adsorbent particles. Firstly, the collision rate between adsorbent and adsorbate increases this results in the enhanced adsorption on to the surface of the adsorbent. Secondly, at high temperature due to bond break of functional groups on adsorbent surface there may be an increase in number of active adsorption sites, which may also lead to enhance adsorption with the rise in temperature. The increase of the uptake capacity at high temperature indicated that the adsorption of lead(II) ions onto *Spirogyra neglecta* is endothermic in nature.

Temperature could effects the desorption and consequently the reversibility of the adsorption equilibrium also (Özer *et al.*, 2006). In other words, more increase in temperature will be followed by a decrease in adsorption capacity meaning with the further rise in temperature may be due to either the damage of active binding sites in the biomass (Ozer and Ozer, 2003).

5.4 Scanning electron microscopic examination of the non-living algal biomass

The morphology of adsorbent have most effect on selection of the mode application of the adsorption process using that particular adsorbent. Some morphology such as surface area and size and distribution of pores in particles direct affect adsorption performance by determining the amount of adsorbent capacity available and the individual ions or molecules of an adsorbate which can be adsorbed.

These pores (caves-like) in the biomass algal showed the increase surface area adsorption for the heavy metal-lead(II). After adsorption lead(II) was distributed uniformly over the algal surface independently of the initial morphology. In other words, it is noted that the electron-micrograph of the biomass algal after lead(II) adsorption Figure 4.4(c-d) was more dense than that of the biomass algal which not in contact with lead(II)-Figure4.4(a-b). The change in structure of the surface adsorbent was probably influenced by lead(II). Thus, it can be assumed that dense area contains most of the adsorption metal.

5.5 The Fourier Transform Infrared Spectrometer (FTIR) analysis for the adsorbent

The FTIR analyses of the sample provided a better understanding of the possible functional groups associated with the heavy metal binding.

The results indicated that the biomass has a variety of function group, including carboxyl and amine function groups. These groups are involved in almost all potential binding mechanism. Therefore, *Spirogyra neglecta* provides more adsorption sites for lead(II) ions. The similar FT-IR result were observed by Ruhan *et al*., (2009) on biosorption of lead(II) by *Lactarius scrobiculatus* biomass.

5.6 Adsorption isotherms of lead(II) by the non-living algal biomass

The equilibrium adsorption isotherms are known as one of the most important data to understand the mechanism of the adsorption. The value of the correlation coefficient for Langmuir model was higher compared to correlation coefficient for Freundlich model indicating that the adsorption of lead(II) onto *Spirogyra neglecta* biomass was unfavorable at studied conditions for Freundlich model. However, these results indicate that the Freundlich model did not describe adequately the relationship between the amounts of adsorbed metal ions and their equilibrium concentration in the solution. Therefore, it can be concluded that the Langmuir isotherm model was best fitted the equilibrium data since it presents higher R^2 value. In other words, the adsorption of lead(II) onto *Spirogyra neglecta* took place at the functional groups/binding sites on the surface of the biomass which is regarded as monolayer adsorption coverage on the surface of algal biomass with lead(II) and localized adsorption. In addition, all sites having equal adsorption energies. Therefore, the adsorption is homogeneous.

Similar results agreed with the works carried out by previous researchers which reported that Langmuir model gave a better fit than the Freundlich model on the adsorption of lead(II) using different adsorbents such as adsorption isotherms of lead(II) was obtained on brown seaweed *Turbinaria conoides* (Luo *et al.*, 2006) and pretreated biomass of Australian marine algae *Durvillaea potatorum* (Matheickal *et al.*, 1999) and *Ecklonia radiate*.

5.7 Adsorption kinetics

According to the correlation coefficients R^2_2 obtained for pseudo-second-order kinetics were greater than correlation coefficients R^2_1 for pseudo-first-order kinetics it can be concluded from the R^2 values in Table 4.2 and Table 4.3 that the adsorption mechanisms of lead(II) ions onto *Spirogyra neglecta* biomass does not follow the pseudo-first-order kinetic model. Moreover, from Table 4.2, it can be seen that the experimental values of q_e, exp are not in good agreement with the theoretical values calculated (q_e1,cal) from equation (5). Therefore, the pseudo-first-order model is not appropriate for modeling the adsorption of lead(II) onto *Spirogyra neglecta.* In the view of these outcome, it can be said that the pseudo-second-order kinetic model provided a excellent correlation for the adsorption of lead(II) onto *Spirogyra neglecta.* In addition, the pseudo-second-order model which further suggests that chemisorption involving valency forces through the sharing or exchange of electrons between the adsorbent and adsorbate. Therefore, it has been concluded that the pseudo-second-order adsorption model is more appropriate to describe the adsorption kinetics of lead(II) by algal biomass.

Similar results agreed with the works carried out by previous researchers which reported by Ozgul and Ferdi (2007) on adsorption of lead(II) ions by activated carbon prepared from biomass plant material of *Euphorbia rigida,* and Dandan *et al.* (2009) on removal of lead(II) using the modified lawny grass. Moreover, Vandana *et al.* (2007) also reported the removal of from lead(II) by using Cassia grandis seed gum-graft-poly(methyl methacrylate).

Also, similar results have been reported by Musa *et al.*(2008) on adsorption characteristics of lead(II) ions onto the clay/poly(methoxyethyl)acrylamide (PMEA) composite.

5.8 Adsorption thermodynamics

The positive value of ΔS° indicate some structure changes in the adsorbent and also reflect the affinity of the adsorbent therefore, increase in the randomness at the solid/solution interface during the fixation of the lead(II) on the active sites of the sorbent. Since the adsorption method is endothermic, it follows that under these situations the process becomes spontaneous because of the positive entropy change. Positive value of enthalpy ΔH° indicates the endothermic nature of the adsorption therefore, increase in adsorption on successive increase in temperature. Further, negative value of ΔG° at each temperature indicates the feasibility and spontaneity of ongoing adsorption. Positive value of enthalpy ΔH° assumes that entropy is responsible for making the ΔG° value negative.

The positive ΔS° value had been report by (Tsezos, 1984) showed a slight increase in cation adsorption by seaweed biomass with increase in temperature from 4 to 55 °C. Similarly, (Aksu, 2002) recorded increase in Ni^{2+} adsorption by dried biomass of *Chlorella vulgaris* with enhancement of temperature from 15°C to 45°C. On the contrary, some studies show exothermic nature of metal adsorption by algae. For instance, it is stated that Cd^{2+} sorption by *Sargassum sp*. biomass decreased slightly with an increase in temperature (Cruz *et al.*, 2004).

In addition, Musa *et al*. (2008) also reported about the adsorption of lead(II) ions onto the clay/poly (methoxyethyl) acrylamide (PMEA) composite.

5. 9 Analysis of water sample from a polluted river

The result indicated that the non-living algal biomass is able to adsorb 21.2% of lead(II) from a polluted water at room temperature(30 ±1°C). The percentage of removal is low may be because of competition between lead(II) and other heavy metals in polluted water. Also, speciation of lead(II) in polluted water is different from free lead(II) in artificial solution.

5.10 Comparison with other adsorbents

A comparison between the results of this work and others found in literatures are presented in Table 4.5. The value of lead(II) uptake found in this work is significantly higher than reported for other adsorbents. The adsorption capacity differences of metal uptake are because of the properties of each adsorbent such as functional groups, surface area, and structure. Thus, the comparison of adsorption capacities shows the alga *Spirogyra neglecta* biomass has the potential to remove lead(II) ions from aqueous solution.

Table 5.1
Uptake capacities for lead(II) of various adsorbents (at room temperature)

Adsorbent	q_{max} (mg /g)	Literature
Chlamydomonas reinhardtii	96.3	Tuzun *et al*. (2005)
Gelidium algae	64.0	Vilor *et al*. (2005)
Zoogloea ramigera	82.8	Sag *et al.* (1995)
Phanerochaete chrysosporium	134	Iqbal and Edyvean (2004)
Pseudomonas aerogenosa (bacteria)	23.00	Chang *et al*. (1997)
Cyclotella cryptica (diatom)	26.28	Gupta *et al.* (2001)
Bacillus sp.	92.27	(Tunali *et al*. (2006)
Arthrobacter sp.	130	Veglio *et al*. (1997)
Rhizopus arrhizus	76.4	Sag *et al*. (1997)
Spirogyra neglecta	132	This study

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

This study indicated that *Spirogyra neglecta* which are widely available, can be used as an efficient adsorbent material for removal of lead(II) from polluted water. The adsorption procedure was fast enough, as maximum removal took place within one hour of contact time for the studied alga. The values of lead(II) uptake found in this work were significantly higher than reported for other adsorbents.

The maximum lead(II) adsorption capacity has been found to be 132 mg lead(II)/g of dry weight of biomass at an algal dose of (0.05 g) with initial lead(II) concentration of 150 mg/L and optimum pH of adsorption is 6 and optimum adsorption temperature is 60°C .

The Freundlich and Langmuir adsorption model were used for the mathematical description of the adsorption of lead(II) ions onto algal biomass and it was found that the adsorption equilibrium data fitted well to the Langmuir model thus proving monolayer adsorption of lead(II) on algal biomass *Spirogyra neglecta*.

Analysis of data shows that the pseudo-second-order kinetic model agrees very well with the dynamic behavior for the adsorption of lead(II) ions on algal biomass *Spirogyra neglecta*.

Equilibrium data for thermodynamic treatment shows endothermic nature of the adsorption process and ΔG° (standard free energy) values indicated spontaneous nature of the adsorption. ΔS° (standard entropy) value suggests increase in the randomness at the solid/solution interface during the adsorption.

FTIR analysis suggested amino and carboxyl groups combine intensively with lead(II). The advantage of high metal adsorption capacity, the biomass of *Spirogyra neglecta* has the potential to be used as an efficient, effective methods and economic adsorbent material for the removal of lead(II) from polluted water.

6.2 Recommendation for future studies

All the results presented in this work had been derived from studies involving a single metal. In actual industrial wastewater, many inorganic and organic compounds are often present. Future work should investigate the behavior of the biomass in adsorption studies involving multi-component solution. The effect of the speed method (speed of the shaker) on the behavior of the biomass was not investigated in a comprehensive manner. More work has to be done in order to enhance the rate of uptake of lead(II).

Modification of the biomass by chemical methods such as (salt, acid and base) or physical methods such as (pyrolysis) to enhance activity of adsorption because of increase the surface area and functional group should be investigation in addition study effect of desorption agent to recovering the heavy metal from algae for reuse and at the same time does not damage the biomass also needed. Studies on the utilization of algae for removing organic pollutants such as dyes and phenolic compounds has to be done in order to reduce contamination in the environment. Study of physical properties such as odour and color of water after using an adsorbent in order to use biomass in home water-filter system in the future because it is cheap and naturally available.

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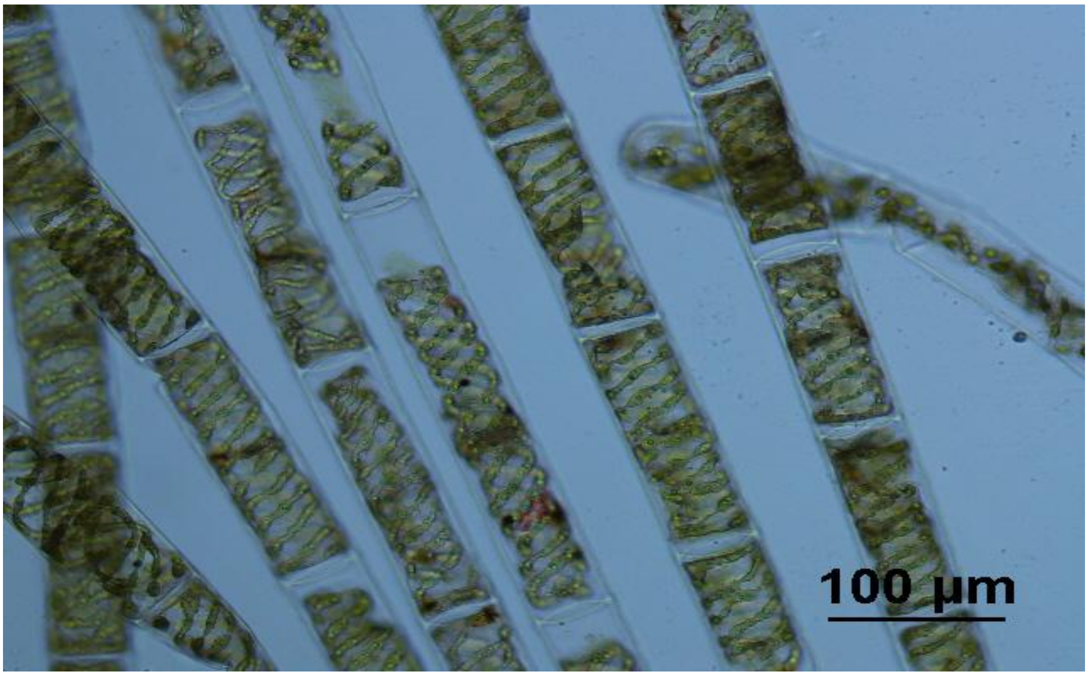
Appendices

Appendix 1: Filaments of *Spirogyra neglecta* (Hasall) Kützing(magnifications 20x scale 100µm).



Appendix 2: Calculation for Langmuir model

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} K_L} + \frac{1}{q_{\max}} C_e$$

Langmuir Model	Slope	Intercept	Correlation coefficient(R^2)
Values	0.0076	0.316	0.8978

Calculate of q_{max} :

$$\frac{1}{q_{max}} = \text{Slope}$$

$$\frac{1}{q_{max}} = 0.0076$$

q_{max} = 132 mg lead(II)/g of dry weight of biomass

Calculate of K_L :

$$\frac{1}{q_{max}\,K_L} = \text{Intercept}$$

$$\frac{1}{q_{max}\,K_L} = 0.316$$

$0.316 \times K_L \times 132 = 1$

$K_L = 0.023$

Appendix 3: Calculation for Freundlich model

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e$$

Freundlich Model	Slope	Intercept	Correlation coefficient(R^2)
Values	1.3327	1.6622	0.7054

Calculate of 1/n :

$$\frac{1}{n} = \text{Slope}$$

$$\frac{1}{n} = 1.3327$$

n = 0.75

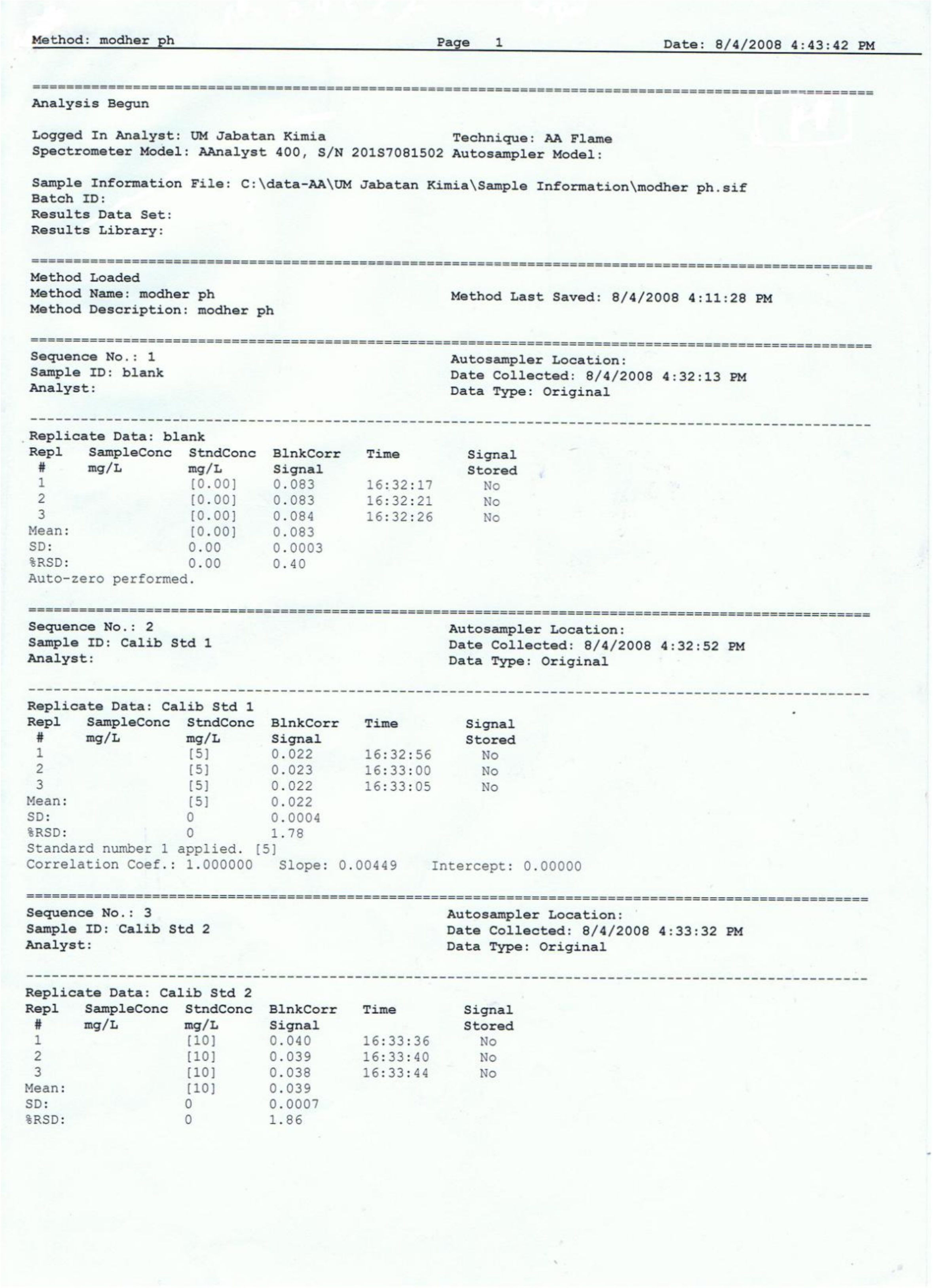
Calculate of ln K_F :

ln K_F = Intercept

ln K_F = 1.6622

K_F = 0.1897

Appendix 4: Atomic absorption spectrometer result showing effect pH on adsorption lead(II) by non-living biomass.



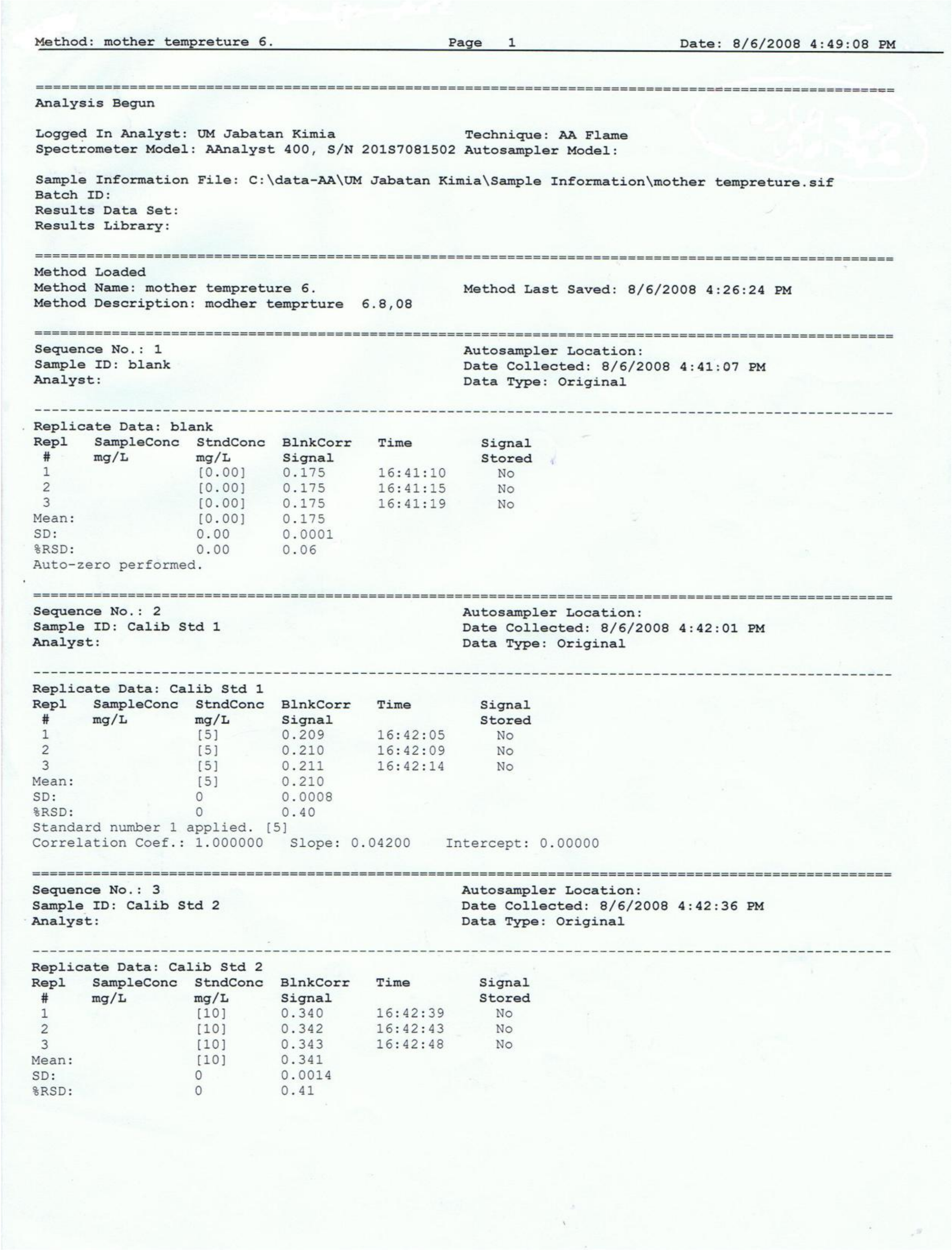
```
Method: modher ph                              Page   1              Date: 8/4/2008 4:43:42 PM

=============================================================================================
Analysis Begun

Logged In Analyst: UM Jabatan Kimia                 Technique: AA Flame
Spectrometer Model: AAnalyst 400, S/N 201S7081502 Autosampler Model:

Sample Information File: C:\data-AA\UM Jabatan Kimia\Sample Information\modher ph.sif
Batch ID:
Results Data Set:
Results Library:

=============================================================================================
Method Loaded
Method Name: modher ph                              Method Last Saved: 8/4/2008 4:11:28 PM
Method Description: modher ph

=============================================================================================
Sequence No.: 1                                     Autosampler Location:
Sample ID: blank                                    Date Collected: 8/4/2008 4:32:13 PM
Analyst:                                            Data Type: Original

---------------------------------------------------------------------------------------------
Replicate Data: blank
Repl    SampleConc  StndConc  BlnkCorr    Time         Signal
 #      mg/L        mg/L      Signal                   Stored
 1                  [0.00]    0.083       16:32:17      No
 2                  [0.00]    0.083       16:32:21      No
 3                  [0.00]    0.084       16:32:26      No
Mean:               [0.00]    0.083
SD:                 0.00      0.0003
%RSD:               0.00      0.40
Auto-zero performed.

=============================================================================================
Sequence No.: 2                                     Autosampler Location:
Sample ID: Calib Std 1                              Date Collected: 8/4/2008 4:32:52 PM
Analyst:                                            Data Type: Original

---------------------------------------------------------------------------------------------
Replicate Data: Calib Std 1
Repl    SampleConc  StndConc  BlnkCorr    Time         Signal
 #      mg/L        mg/L      Signal                   Stored
 1                  [5]       0.022       16:32:56      No
 2                  [5]       0.023       16:33:00      No
 3                  [5]       0.022       16:33:05      No
Mean:               [5]       0.022
SD:                 0         0.0004
%RSD:               0         1.78
Standard number 1 applied. [5]
Correlation Coef.: 1.000000   Slope: 0.00449    Intercept: 0.00000

=============================================================================================
Sequence No.: 3                                     Autosampler Location:
Sample ID: Calib Std 2                              Date Collected: 8/4/2008 4:33:32 PM
Analyst:                                            Data Type: Original

---------------------------------------------------------------------------------------------
Replicate Data: Calib Std 2
Repl    SampleConc  StndConc  BlnkCorr    Time         Signal
 #      mg/L        mg/L      Signal                   Stored
 1                  [10]      0.040       16:33:36      No
 2                  [10]      0.039       16:33:40      No
 3                  [10]      0.038       16:33:44      No
Mean:               [10]      0.039
SD:                 0         0.0007
%RSD:               0         1.86
```

```
Standard number 2 applied. [10]
Correlation Coef.: 0.981232   Slope: 0.00402    Intercept: 0.00000

==================================================================================================
Sequence No.: 4                                             Autosampler Location:
Sample ID: Calib Std 3                                      Date Collected: 8/4/2008 4:34:10 PM
Analyst:                                                    Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: Calib Std 3
Repl    SampleConc   StndConc   BlnkCorr    Time         Signal
 #      mg/L         mg/L       Signal                   Stored
 1                   [15]       0.057       16:34:14      No
 2                   [15]       0.056       16:34:18      No
 3                   [15]       0.055       16:34:23      No
Mean:                [15]       0.056
SD:                  0          0.0009
%RSD:                0          1.56
Standard number 3 applied. [15]
Correlation Coef.: 0.990382   Slope: 0.00383    Intercept: 0.00000

==================================================================================================
Sequence No.: 5                                             Autosampler Location:
Sample ID: Calib Std 4                                      Date Collected: 8/4/2008 4:34:47 PM
Analyst:                                                    Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: Calib Std 4
Repl    SampleConc   StndConc   BlnkCorr    Time         Signal
 #      mg/L         mg/L       Signal                   Stored
 1                   [20]       0.085       16:34:50      No
 2                   [20]       0.087       16:34:55      No
 3                   [20]       0.088       16:34:59      No
Mean:                [20]       0.086
SD:                  0          0.0017
%RSD:                0          2.02
Standard number 4 applied. [20]
Correlation Coef.: 0.985380   Slope: 0.00411    Intercept: 0.00000

==================================================================================================
Sequence No.: 6                                             Autosampler Location:
Sample ID: Calib Std 5                                      Date Collected: 8/4/2008 4:35:43 PM
Analyst:                                                    Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: Calib Std 5
Repl    SampleConc   StndConc   BlnkCorr    Time         Signal
 #      mg/L         mg/L       Signal                   Stored
 1                   [25]       0.096       16:35:47      No
 2                   [25]       0.097       16:35:51      No
 3                   [25]       0.012       16:35:56      No
Mean:                [25]       0.068
SD:                  0          0.0491
%RSD:                0          71.89
Standard number 5 not applied. [25]
   No calibration curve because standard absorbance and concentration values are not in the same order.

==================================================================================================
Sequence No.: 7                                             Autosampler Location:
Sample ID: Calib Std 5                                      Date Collected: 8/4/2008 4:37:48 PM
Analyst:                                                    Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: Calib Std 5
Repl    SampleConc   StndConc   BlnkCorr    Time         Signal
 #      mg/L         mg/L       Signal                   Stored
 1                   [25]       0.093       16:37:51      No
```

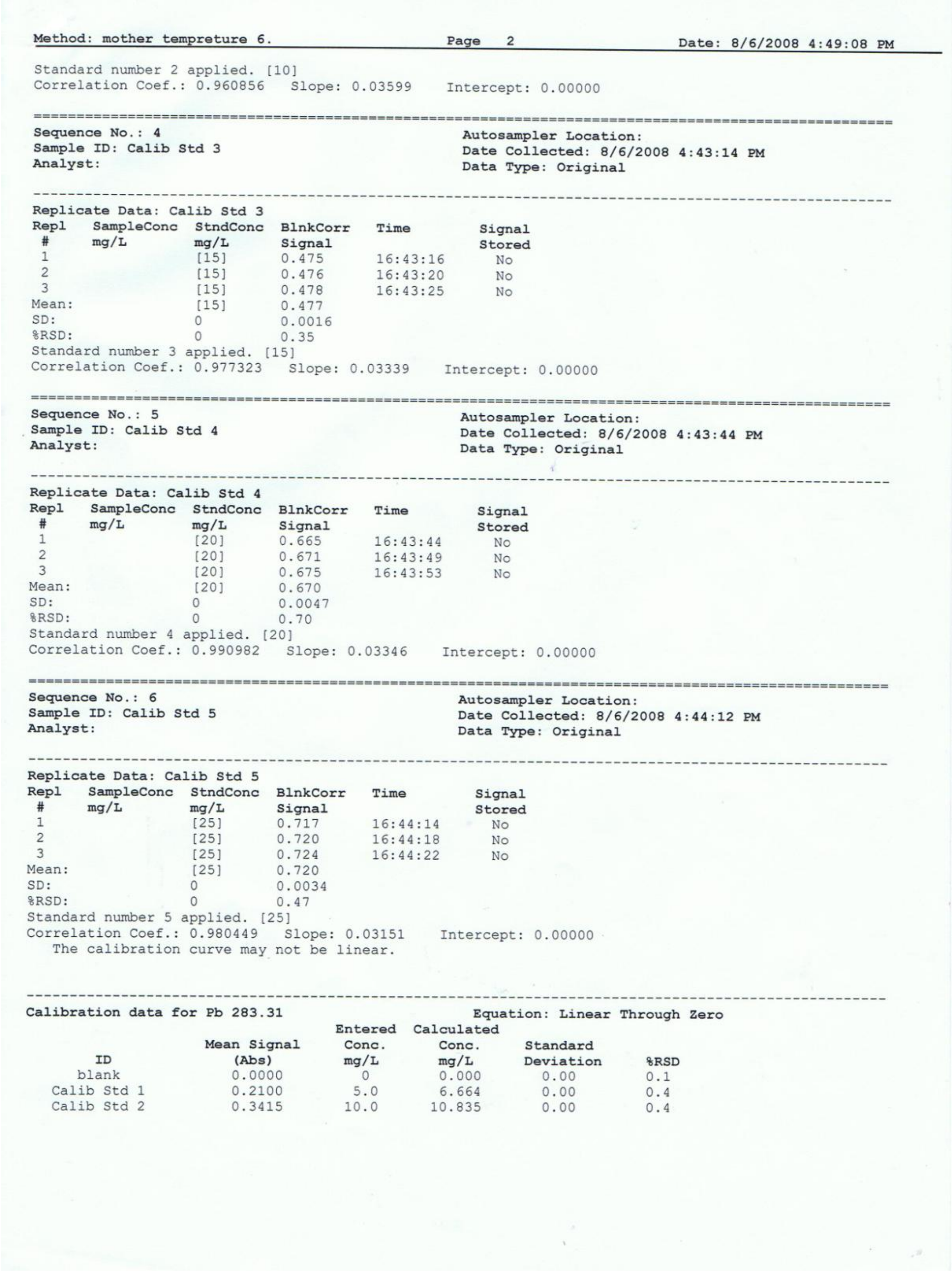
```
 2                 [25]      0.095       16:37:56       No
 3                 [25]      0.096       16:38:00       No
Mean:              [25]      0.095
SD:                0         0.0018
%RSD:              0         1.86
Standard number 5 applied. [25]
Correlation Coef.: 0.988056    Slope: 0.00397     Intercept: 0.00000
   The calibration curve may not be linear.


-------------------------------------------------------------------------------------------
Calibration data for Pb 283.31                        Equation: Linear Through Zero
                                     Entered  Calculated
                        Mean Signal   Conc.     Conc.     Standard
         ID                (Abs)      mg/L      mg/L      Deviation       %RSD
       blank              0.0000       0        0.000       0.00          0.4
     Calib Std 1          0.0225      5.0       5.665       0.00          1.8
     Calib Std 2          0.0388     10.0       9.788       0.00          1.9
     Calib Std 3          0.0558     15.0      14.066       0.00          1.6
     Calib Std 4          0.0864     20.0      21.796       0.00          2.0
     Calib Std 5          0.0945     25.0      23.838       0.00          1.9
Correlation Coef.: 0.988056    Slope: 0.00397     Intercept: 0.00000

===========================================================================================
Sequence No.: 8                                        Autosampler Location:
Sample ID: 1                                           Date Collected: 8/4/2008 4:38:45 PM
Analyst:                                               Data Type: Original

-------------------------------------------------------------------------------------------
Replicate Data: 1
Repl    SampleConc   StndConc   BlnkCorr    Time          Signal
 #      mg/L         mg/L       Signal                    Stored
 1      10.49        10.49      0.042       16:38:49       No
 2      10.51        10.51      0.042       16:38:53       No
 3      10.39        10.39      0.041       16:38:58       No
Mean:   10.46        10.46      0.041
SD:     0.066        0.066      0.0003
%RSD:   0.630        0.630      0.63

===========================================================================================
Sequence No.: 9                                        Autosampler Location:
Sample ID: 2                                           Date Collected: 8/4/2008 4:39:21 PM
Analyst:                                               Data Type: Original

-------------------------------------------------------------------------------------------
Replicate Data: 2
Repl    SampleConc   StndConc   BlnkCorr    Time          Signal
 #      mg/L         mg/L       Signal                    Stored
 1      10.47        10.47      0.042       16:39:23       No
 2      10.10        10.10      0.040       16:39:27       No
 3      9.844        9.844      0.039       16:39:32       No
Mean:   10.14        10.14      0.040
SD:     0.313        0.313      0.0012
%RSD:   3.089        3.089      3.09

===========================================================================================
Sequence No.: 10                                       Autosampler Location:
Sample ID: 3                                           Date Collected: 8/4/2008 4:39:54 PM
Analyst:                                               Data Type: Original

-------------------------------------------------------------------------------------------
Replicate Data: 3
Repl    SampleConc   StndConc   BlnkCorr    Time          Signal
 #      mg/L         mg/L       Signal                    Stored
 1      9.766        9.766      0.039       16:39:56       No
 2      9.283        9.283      0.037       16:40:00       No
 3      10.21        10.21      0.040       16:40:05       No
```

```
Method: modher ph                                   Page   4               Date: 8/4/2008 4:43:42 PM
Mean:  9.753          9.753      0.039
SD:    0.463          0.463      0.0018
%RSD:  4.746          4.746      4.75

=================================================================================================
Sequence No.: 11                                    Autosampler Location:
Sample ID: 4                                        Date Collected: 8/4/2008 4:40:25 PM
Analyst:                                            Data Type: Original

-------------------------------------------------------------------------------------------------
Replicate Data: 4
Repl   SampleConc     StndConc   BlnkCorr    Time        Signal
 #     mg/L           mg/L       Signal                  Stored
 1     9.143          9.143      0.036       16:40:25      No
 2     8.913          8.913      0.035       16:40:29      No
 3     8.934          8.934      0.035       16:40:33      No
Mean:  8.997          8.997      0.036
SD:    0.127          0.127      0.0005
%RSD:  1.413          1.413      1.41

=================================================================================================
Sequence No.: 12                                    Autosampler Location:
Sample ID: 5                                        Date Collected: 8/4/2008 4:40:58 PM
Analyst:                                            Data Type: Original

-------------------------------------------------------------------------------------------------
Replicate Data: 5
Repl   SampleConc     StndConc   BlnkCorr    Time        Signal
 #     mg/L           mg/L       Signal                  Stored
 1     4.387          4.387      0.017       16:40:59      No
 2     4.713          4.713      0.019       16:41:04      No
 3     4.449          4.449      0.018       16:41:08      No
Mean:  4.517          4.517      0.018
SD:    0.173          0.173      0.0007
%RSD:  3.830          3.830      3.83
```

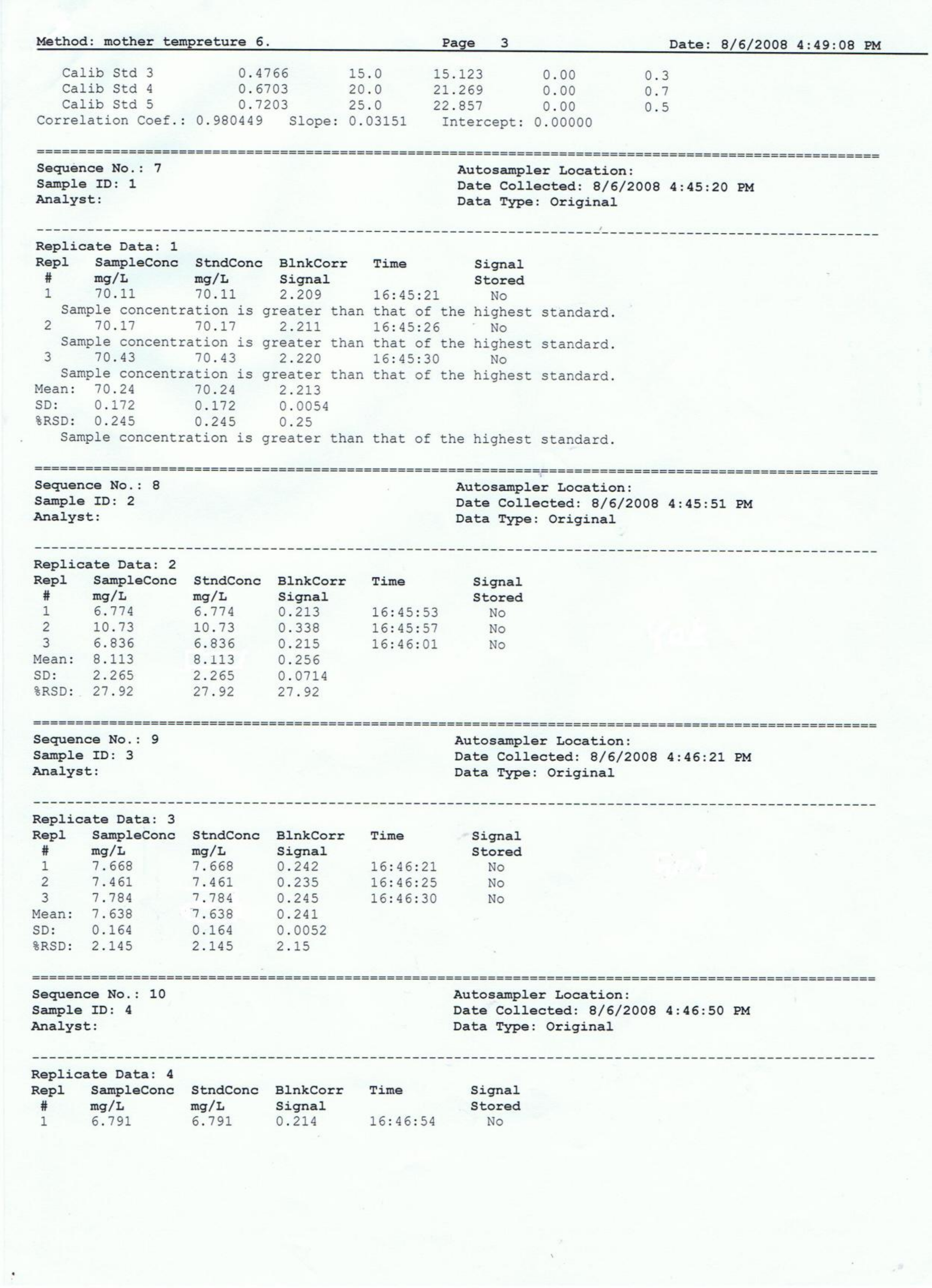
Appendix 5: Atomic absorption spectrometer result showing effect temperature on adsorption lead(II) by non-living biomass.



```
Method: mother tempreture 6.                    Page   1                Date: 8/6/2008 4:49:08 PM

==================================================================================================
Analysis Begun

Logged In Analyst: UM Jabatan Kimia                   Technique: AA Flame
Spectrometer Model: AAnalyst 400, S/N 201S7081502 Autosampler Model:

Sample Information File: C:\data-AA\UM Jabatan Kimia\Sample Information\mother tempreture.sif
Batch ID:
Results Data Set:
Results Library:

==================================================================================================
Method Loaded
Method Name: mother tempreture 6.                     Method Last Saved: 8/6/2008 4:26:24 PM
Method Description: modher temprture  6.8,08

==================================================================================================
Sequence No.: 1                                       Autosampler Location:
Sample ID: blank                                      Date Collected: 8/6/2008 4:41:07 PM
Analyst:                                              Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: blank
Repl    SampleConc   StndConc   BlnkCorr    Time        Signal
 #      mg/L         mg/L       Signal                  Stored
 1                   [0.00]     0.175       16:41:10      No
 2                   [0.00]     0.175       16:41:15      No
 3                   [0.00]     0.175       16:41:19      No
Mean:                [0.00]     0.175
SD:                  0.00       0.0001
%RSD:                0.00       0.06
Auto-zero performed.

==================================================================================================
Sequence No.: 2                                       Autosampler Location:
Sample ID: Calib Std 1                                Date Collected: 8/6/2008 4:42:01 PM
Analyst:                                              Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: Calib Std 1
Repl    SampleConc   StndConc   BlnkCorr    Time        Signal
 #      mg/L         mg/L       Signal                  Stored
 1                   [5]        0.209       16:42:05      No
 2                   [5]        0.210       16:42:09      No
 3                   [5]        0.211       16:42:14      No
Mean:                [5]        0.210
SD:                  0          0.0008
%RSD:                0          0.40
Standard number 1 applied. [5]
Correlation Coef.: 1.000000   Slope: 0.04200    Intercept: 0.00000

==================================================================================================
Sequence No.: 3                                       Autosampler Location:
Sample ID: Calib Std 2                                Date Collected: 8/6/2008 4:42:36 PM
Analyst:                                              Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: Calib Std 2
Repl    SampleConc   StndConc   BlnkCorr    Time        Signal
 #      mg/L         mg/L       Signal                  Stored
 1                   [10]       0.340       16:42:39      No
 2                   [10]       0.342       16:42:43      No
 3                   [10]       0.343       16:42:48      No
Mean:                [10]       0.341
SD:                  0          0.0014
%RSD:                0          0.41
```



```
Method: mother tempreture 6.                       Page   2                    Date: 8/6/2008 4:49:08 PM

Standard number 2 applied. [10]
Correlation Coef.: 0.960856   Slope: 0.03599    Intercept: 0.00000

=============================================================================================
Sequence No.: 4                                         Autosampler Location:
Sample ID: Calib Std 3                                  Date Collected: 8/6/2008 4:43:14 PM
Analyst:                                                Data Type: Original

---------------------------------------------------------------------------------------------
Replicate Data: Calib Std 3
Repl    SampleConc   StndConc   BlnkCorr    Time         Signal
 #        mg/L         mg/L      Signal                  Stored
 1                     [15]      0.475      16:43:16      No
 2                     [15]      0.476      16:43:20      No
 3                     [15]      0.478      16:43:25      No
Mean:                  [15]      0.477
SD:                    0         0.0016
%RSD:                  0         0.35
Standard number 3 applied. [15]
Correlation Coef.: 0.977323   Slope: 0.03339    Intercept: 0.00000

=============================================================================================
Sequence No.: 5                                         Autosampler Location:
Sample ID: Calib Std 4                                  Date Collected: 8/6/2008 4:43:44 PM
Analyst:                                                Data Type: Original

---------------------------------------------------------------------------------------------
Replicate Data: Calib Std 4
Repl    SampleConc   StndConc   BlnkCorr    Time         Signal
 #        mg/L         mg/L      Signal                  Stored
 1                     [20]      0.665      16:43:44      No
 2                     [20]      0.671      16:43:49      No
 3                     [20]      0.675      16:43:53      No
Mean:                  [20]      0.670
SD:                    0         0.0047
%RSD:                  0         0.70
Standard number 4 applied. [20]
Correlation Coef.: 0.990982   Slope: 0.03346    Intercept: 0.00000

=============================================================================================
Sequence No.: 6                                         Autosampler Location:
Sample ID: Calib Std 5                                  Date Collected: 8/6/2008 4:44:12 PM
Analyst:                                                Data Type: Original

---------------------------------------------------------------------------------------------
Replicate Data: Calib Std 5
Repl    SampleConc   StndConc   BlnkCorr    Time         Signal
 #        mg/L         mg/L      Signal                  Stored
 1                     [25]      0.717      16:44:14      No
 2                     [25]      0.720      16:44:18      No
 3                     [25]      0.724      16:44:22      No
Mean:                  [25]      0.720
SD:                    0         0.0034
%RSD:                  0         0.47
Standard number 5 applied. [25]
Correlation Coef.: 0.980449   Slope: 0.03151    Intercept: 0.00000
   The calibration curve may not be linear.


---------------------------------------------------------------------------------------------
Calibration data for Pb 283.31                            Equation: Linear Through Zero
                                     Entered  Calculated
                        Mean Signal   Conc.     Conc.     Standard
         ID                (Abs)      mg/L      mg/L      Deviation      %RSD
       blank              0.0000       0        0.000       0.00          0.1
    Calib Std 1           0.2100      5.0       6.664       0.00          0.4
    Calib Std 2           0.3415     10.0      10.835       0.00          0.4
```



```
Method: mother tempreture 6.                    Page   3                    Date: 8/6/2008 4:49:08 PM
   Calib Std 3           0.4766       15.0       15.123       0.00        0.3
   Calib Std 4           0.6703       20.0       21.269       0.00        0.7
   Calib Std 5           0.7203       25.0       22.857       0.00        0.5
Correlation Coef.: 0.980449   Slope: 0.03151    Intercept: 0.00000

==================================================================================================
Sequence No.: 7                                             Autosampler Location:
Sample ID: 1                                                Date Collected: 8/6/2008 4:45:20 PM
Analyst:                                                    Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: 1
Repl   SampleConc   StndConc   BlnkCorr    Time         Signal
 #      mg/L         mg/L       Signal                   Stored
 1      70.11        70.11      2.209       16:45:21      No
   Sample concentration is greater than that of the highest standard.
 2      70.17        70.17      2.211       16:45:26      No
   Sample concentration is greater than that of the highest standard.
 3      70.43        70.43      2.220       16:45:30      No
   Sample concentration is greater than that of the highest standard.
Mean:   70.24        70.24      2.213
SD:     0.172        0.172      0.0054
%RSD:   0.245        0.245      0.25
   Sample concentration is greater than that of the highest standard.

==================================================================================================
Sequence No.: 8                                             Autosampler Location:
Sample ID: 2                                                Date Collected: 8/6/2008 4:45:51 PM
Analyst:                                                    Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: 2
Repl   SampleConc   StndConc   BlnkCorr    Time         Signal
 #      mg/L         mg/L       Signal                   Stored
 1      6.774        6.774      0.213       16:45:53      No
 2      10.73        10.73      0.338       16:45:57      No
 3      6.836        6.836      0.215       16:46:01      No
Mean:   8.113        8.113      0.256
SD:     2.265        2.265      0.0714
%RSD:   27.92        27.92      27.92

==================================================================================================
Sequence No.: 9                                             Autosampler Location:
Sample ID: 3                                                Date Collected: 8/6/2008 4:46:21 PM
Analyst:                                                    Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: 3
Repl   SampleConc   StndConc   BlnkCorr    Time         Signal
 #      mg/L         mg/L       Signal                   Stored
 1      7.668        7.668      0.242       16:46:21      No
 2      7.461        7.461      0.235       16:46:25      No
 3      7.784        7.784      0.245       16:46:30      No
Mean:   7.638        7.638      0.241
SD:     0.164        0.164      0.0052
%RSD:   2.145        2.145      2.15

==================================================================================================
Sequence No.: 10                                            Autosampler Location:
Sample ID: 4                                                Date Collected: 8/6/2008 4:46:50 PM
Analyst:                                                    Data Type: Original

--------------------------------------------------------------------------------------------------
Replicate Data: 4
Repl   SampleConc   StndConc   BlnkCorr    Time         Signal
 #      mg/L         mg/L       Signal                   Stored
 1      6.791        6.791      0.214       16:46:54      No
```

Method: mother tempreture 6. **Page 4** **Date: 8/6/2008 4:49:08 PM**

```
 2     7.357        7.357      0.232      16:46:58      No
 3     6.825        6.825      0.215      16:47:03      No
Mean:  6.991        6.991      0.220
SD:    0.317        0.317      0.0100
%RSD:  4.539        4.539      4.54
```

Appendix 6: The Fourier Transform Infrared Spectrometer (FTIR)

Appendix 7: Scanning Electron Microscopy (SEM)

Appendix 8: Publication (http://www.scipub.org/fulltext/ajbb/ajbb5275-83.pdf)

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Characterization of the Adsorption of the Lead (II) by the Nonliving Biomass *Spirogyra neglecta* (Hasall) Kützing

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Abstract: Problem statement: Conventional techniques for removing dissolved heavy metals are only practical and cost-effective when applied to high strength wastes with heavy metal ion concentrations greater than 100 ppm. The possibility of using a nonliving algal biomass to solve this problem was carried in this study. Lead (II) was used in this study because it had been reported to cause several disorders in human. **Approach:** The nonliving algal biomass was obtained from a filamentous green alga *Spirogyra neglecta*. The effects of initial concentration and contact time, pH and temperature on the biosorption of lead (II) by the nonliving algal biomass were studied. The equilibrium isotherms and kinetics were obtained from batch adsorption experiments. The surface characteristics of the nonliving algal biomass were examined using scanning electron microscope and Fourier Transformed Infrared. The maximum adsorption capacity of the nonliving algal biomass was also determined. **Results:** Maximum adsorption capacity of lead (II) was affected by its initial concentration. Adsorption capacity of lead (II) increased with the pH and temperature of lead (II) solution. Langmuir isothermic model fitted the equilibrium data better than the Freundlich isothermic model. The adsorption kinetics followed the pseudo-second-order kinetic model. The nonliving algal biomass exhibited acaves-like, uneven surface texture along with lot of irregular surface. FTIR analysis of the alga biomass revealed the presence of carboyl, amine and carboxyl group which were responsible for adsorption of lead (II). The maximum adsorption capacity (q_{max}) of lead (II) by the nonliving biomass of *Spirogyra neglecta* was 132 mg g^{-1}. **Conclusion:** The maximum adsorption capacity for lead (II) by the nonliving biomass of *Spirogyra neglecta* was higher than reported for other biosorbents. Therefore, it had a great potential for removing lead (II) from polluted water. Its use will also need to consider the various factors that affect biosorption process.

Key word: Adsorption, kinetics, *Spirogyra neglecta*, Langmuir model, second order model

INTRODUCTION

The presence of heavy metals in water systems has become a problem due to their harmful effects on human health even at low concentration in the environment. Lead (II) is among the most toxic heavy metal ion affecting the environment[1]. The current EPA and WHO drinking water standard for lead (II) is 0.05 mg L^{-1} and 10 μg L^{-1}, respectively. Lead (II) accumulates mainly in bones, brain, kidney and muscles and may cause many serious disorders like anemia, kidney diseases, nervous disorders and sickness even death[2]. It is therefore, essential to remove lead (II) from wastewater before disposal. Conventional techniques for removing dissolved heavy metals such as chemical precipitation, carbon adsorption, ion exchange, evaporation and membrane processes[3], are only practical and cost-effective when applied to high strength wastes with heavy metal ion concentrations greater than 100 ppm[4]. Low strength heavy metal containing wastewaters generally cannot be treated successfully with such methods.

Adsorbent materials (biosorbents) derived from suitable biomass, on the other hand, has been shown to remove and recover of heavy metal ions from wastewater streams even at low concentrations. The phenomena of adsorption has been described in a wide range of living biomass like fungi[5], bacteria[6,7], yeast[8], moss[9], aquatic plants[10] and algae[11,12]. Algae is one of the most promising biosorbents[13]. Different algal species often had different sorption characteristics. The sorption characteristics have shown to be influenced by pH, metal ion concentration, temperature, other metal ions[3].

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Langmuir isothermic model can be written in non-linear form as:

$$\frac{C_e}{q_e} = \frac{1}{q_{max} K_L} + \frac{1}{q_{max}} C_e \quad (2)$$

Where:

q_{max} = The monolayer adsorption capacity of the adsorbent (mg g^{-1}) is the (maximum amount adsorbed)
K_L = The Langmuir adsorption constant (L mg^{-1})
C_e = The equilibrium metal ion concentration in the solution (mg L^{-1})
q_e = The equilibrium metal ion concentration on the adsorbent (mg g^{-1})

The solid phase equilibrium metal concentration which is the amount of metal adsorbed on the biomass surface in a batch system is calculated using the following mass balance equation:

$$q = \frac{V(C_e + C_f)}{W} \quad (3)$$

Where:

q = The metal uptake (mg metal g^{-1} biomass)
C_e = Initial metal concentrations in the solution (mg metal L^{-1} fluid)
C_f = Final metal concentrations in the solution (mg metal L^{-1} fluid)
V = The volume of solution
W = The dry weight (g) of the added biosorbent

The Freundlich isothermic model is written as:

$$\ln q_e = \ln K_f + \frac{1}{2} \ln C_e \quad (4)$$

Where:

K_f = A constant relating the adsorption capacity (Freundlich constants)
1/n = An empirical parameter relating the adsorption intensity, (which varies with the heterogeneity of the material)

To describe the kinetics of the biosorption of lead (II) by the nonliving algal biomass, two models used: Pseudo-first-order-kinetic and Pseudo-Second-order-kinetic models. The pseudo first-order model is presented by the following equation [25]:

$$\log(q_e - q_t) = \log q_e - \frac{K_1}{2303} t \quad (5)$$

Where:

q_e and q_t (mg g^{-1}) = The amounts of lead (II)adsorbed on the algal biomass at equilibrium and time in mg g^{-1}
K_1 = Constant of pseudo first-order adsorption

The pseudo second-order model is presented by the following equation[26]:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e} + \frac{1}{q_e} \quad (6)$$

Morphology of biosorbent: Scanning electron microscope examination of the biosorbent was done under SEM (JEOL 6400, Japan) to study the surface texture and morphology of the biosorbent. Sample of the biosorbent was prepared based on technique and procedure of Gabriel[14].

Functional groups of biosorbent: Detection of functional groups in the biosorbent was done using the Fourier Transformed Infrared (FTIR). A sample of the biosorbent was mixed with KBr disc. The mixture was analyzed using FTIR in the range 370.0-4000.0 cm^{-1}.

RESULTS

Effects of initial concentration and contact time: The adsorption of lead (II) increased with time and at certain point in time, it reached a constant value beyond which no more lead (II) was further removed from solution (Fig. 1). Maximum adsorption took place within the first 60 min. The amount of lead (II) adsorbed at the equilibrium time was the maximum adsorption uptake by the adsorbent.

Effect of pH: The results of the adsorption lead (II) ions by the biosorbent are shown in Fig. 2. It shows that the process is a function of pH. In this study, adsorption of lead (II) by the biosorbent increased until at pH 6.0.

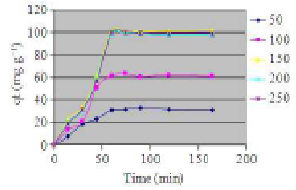


Fig. 1: Effect of initial concentration and contact time on adsorption of lead (II) by nonliving biomass *Spirogyra neglecta*

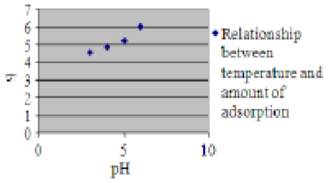


Fig. 2: Effect of pH on adsorption of lead (II) by nonliving biomass *Spirogyra neglecta*

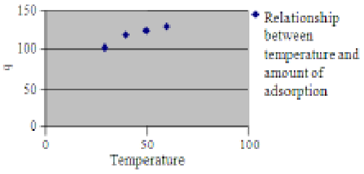


Fig. 3: Effect of temperature on adsorption of lead (II) by nonliving biomass *Spirogyra neglecta*

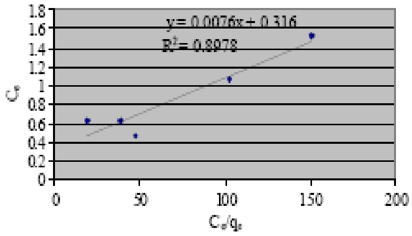


Fig. 4: Langmuir adsorption isotherm of lead (II) by the nonliving biomass of *Spirogyra neglecta*

Effect of temperature: Lead (II) adsorption uptake was found to increase with the increase in temperature (Fig. 3).

Determination of the adsorption capacity: The maximum adsorption capacity (q_{max}) of lead (II) by the nonliving algal biomass was 132 mg g^{-1}.

Biosorption isothermics and kinetics Figure 4 shows the plots of C_e versus $C_{e/qe}$ were drawn to calculate the values of K_L which are shown in Table 1.

The equilibrium relationship between metal adsorption and residual metal is typically hyperbolic[24].

Figure 5 shows the plots of $1/q_e$ versus $1/C_e$ were drawn to calculate the values of K_F and 1/n which are shown in Table 1

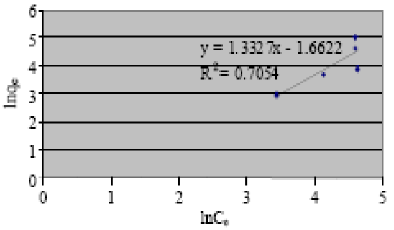


Fig. 5: Freundlich adsorption isotherm of lead (II) by the nonliving biomass of *Spirogyra neglecta*

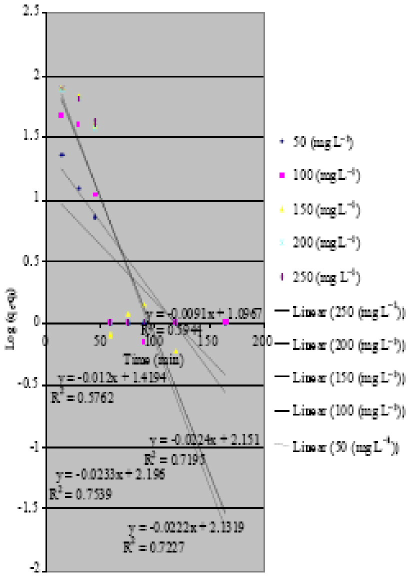


Fig. 6: Pseudo-first-order kinetic adsorption of lead (II) by the nonliving biomass of *Spirogyra neglecta*

Table 1: Langmuir and Freundlich constants

Parameter		
Langmuir	**Temperature**	
q_{max} (mg g^{-1})	132.0000	30±1
k_L (L mg^{-1})	0.0230	30±1
R_L^2	0.8978	30±1
Freundlich	**Temperature**	
k_f (L mg^{-1})	0.1897	30±1
n	0.7500	30±1
R_f^2	0.7054	30±1

The plot $\ln(q_e-q_t)$ versus t as shown in Fig. 6 gives the slop of K_1 and intercept of lnqe. The values of K_1 and correlation, R_1 obtained from the Fig. 6 for adsorption of lead (II) on the biomass algal at 30°C are shown in Table 2.

Table 2: First order kinetic model

	First order kinetic model			
Con mg L^{-1}	q_e(mg g^{-1}) exp	q_{e1}(mg g^{-1})	K_1	R_1
50 mg L^{-1}	30.8	12.49	0.021	0.5944
100 mg L^{-1}	61.4	26.26	0.028	0.5762
150 mg L^{-1}	102.1	157.03	0.054	0.7539
200 mg L^{-1}	97.2	135.49	0.051	0.7227
250 mg L^{-1}	98.8	141.58	0.052	0.7195

Table 3: Second order kinetic model

	Second -order kinetic model		
Con. mg L^{-1}	q_{e2}	K_2	R_2
50 mg L^{-1}	40.650	1406.040	0.9108
100 mg L^{-1}	95.230	2834.220	0.7535
150 mg L^{-1}	166.660	12815.640	0.7648
200 mg L^{-1}	166.660	14134.980	0.6867
250 mg L^{-1}	175.430	16052.590	0.6875

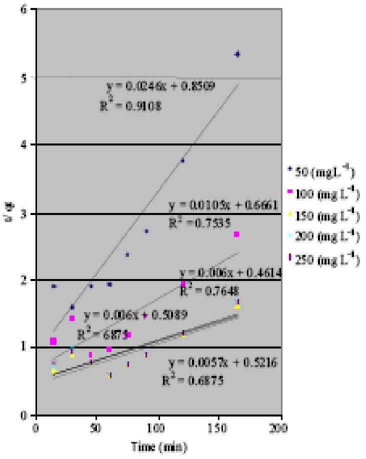
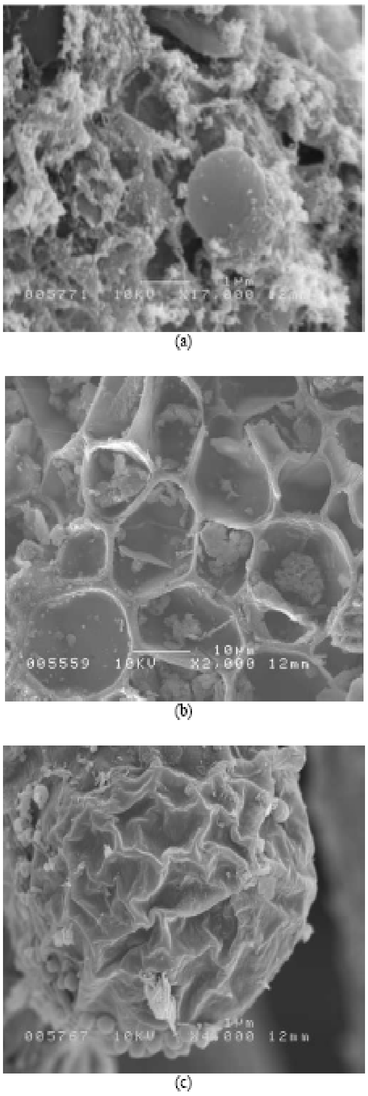


Fig. 7: Pseudo-second-order kinetic adsorption of lead (II) by the nonliving biomass of *Spirogyra neglecta*

Figure 7 showed the plots of t/qt versus t at various initial concentrations. K_2 is the second order adsorption rate constants. The values of parameters K_2, q_e and correlation coefficients were shown in Table 3. The correlation coefficients obtained were greater from R_1.

Morphology of nonliving biomass of *Spiroygra neglecta*: Fig. 8a-d. It was evident from the micrographs that the biosorbent sample after-before adsorption. The algae exhibits acaves-like, uneven surface texture along with lot of irregular surface.



(a)

(b)

(c)

Fig. 8a-c: SEM image the biomass particles, with and without adsorbed metal

The Fourier Transform Infrared Spectrometer (FTIR) analysis: The result is shown in Fig. 9. It displayed a number of absorption peaks, indicating the complex nature of the biomass examined. The broad absorption peak around 2923 cm^{-1} is indicative of the existence of bonded carboxyl group (C-H).

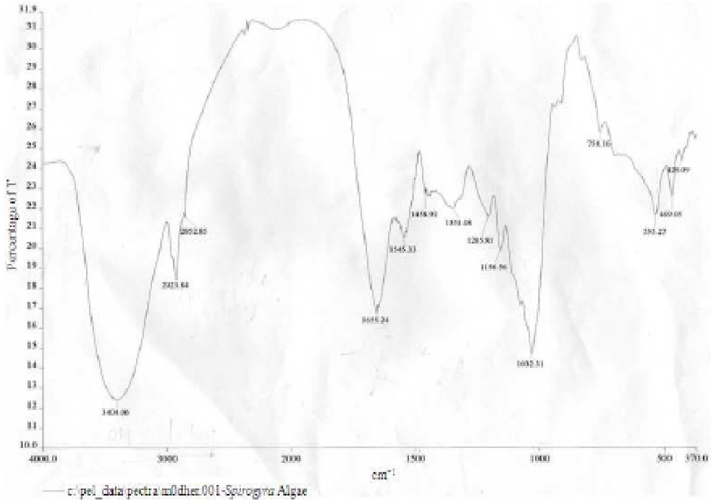


Fig. 9: Peaks for nonliving biomass of *Spirogyra neglecta* obtained from FTIR analysis

The peak observed at 1655 cm^{-1} can be assigned to the (C = O) carbonyl group. The peak observed at 3404.06 cm^{-1} can be assigned to the (N-H) amine group The peak observed at 1032 cm^{-1} can be assigned to the (C-O) carbonyl group.

DISCUSSION

Effects of initial concentration and contact time: The results revealed that the lead adsorption was fast at initial stage of the contact period and then became slower near the equilibrium. This phenomenon was due to the fact that a large number of vacant surface site were available for adsorption during the initial stage. Near the equilibrium the remaining vacant surface sites were difficult to be occupied due to repulsive forces between the solute molecules on the solid and bulk phase.

Effect of pH: These results suggest that the adsorption of metals on the biomass surface is controlled by ionic attraction. At low pH values, the inactivated cell surface becomes more positively charged, to reduce the attraction between metal ions and functional groups at the cell wall. In contrast, when the pH increases, the cell surface is more negatively charged and the process of retention is favored[16,17]. Until a maximum adsorption is reached around pH 6. At pH higher than 6, precipitation of insoluble metal hydroxides takes place restricting the true adsorption studies according to formula:

$$(NO_3)_2 + NaOH \rightarrow (OH)_2 \downarrow + NaNO_3$$

lead (II) adsorption is maximized at pH6.0, a value which agrees with the results obtained by[18] on their study of lead (II) by *Pseudomonas putida*. Seki *et al.*[21] studied the function of pH on adsorption of lead (II) by *Rhodobacter sphaeroides* and reported that the maximum pH is around 6.0. The pH of metal solution has been shown to play an important role in the adsorption process[19].

Effect of temperature: The adsorption reaction for the endothermic processes could be due to the increase in temperature increase the rate of diffusion of the adsorbate molecules across the external boundary layer and in the internal pores of the adsorbent particle, owning to the decrease in the viscosity of the solution[20] also noted similar observations and they suggested that the increase in adsorption uptake with increase in temperature might be due to the possibility and in the total pore volume of the adsorbent, an increase of number of active sites for the adsorption as well as an increase in the mobility of the adsorbate molecules. Meena *et al.*[21] reported that the increase in sorption with temperature may be attributed to either increase in the number of active surface sites available for sorption on the adsorbent or due to the decrease in the boundary layer thickness surroundings the sorbent, so that the mass transfer resistance of adsorbate in the boundary layer decreased. Temperature could influence the desorption and consequently the reversibility of the adsorption equilibrium also[22]. Increase in temperature will be followed by a decrease in adsorption capacity. This is because the rise in temperature damage active binding sites in the biomass[23].

Langmuir and Freundlich adsorption isotherms: The correlation coefficients were extremely high, as shown in Table 1. These values of the correlation coefficients strongly support the fact that the lead–algal biomass adsorption data closely follow the Langmuir model compared to the Freundlich correlation coefficients . The high degree of correlation for the linearized Langmuir relationship suggests a single surface reaction with constant activation energy is the predominant sorption step and possibly the predominant rate-controlling step.

Pseudo-first-order-and Pseudo-Second-order kinetic model: The values of R1 relatively small for the pseudo-first-order are not satisfactory. Therefore, it has been concluded that the pseudo-second-order adsorption model is more suitable to describe the adsorption kinetics of lead (II) by algal biomass. These results imply that the adsorption system adheres to the pseudo second-order kinetics which further suggests that chemisorption is the rate-controlling step[36]. Therefore, the pseudo-second-order rate kinetic model best described the experimental data.

Characterization of the biosorbent:

Scanning electron microscope: These caves in biomass evidence the increase surface adsorption of lead.

The Fourier Transform Infrared Spectrometer (FTIR) analysis: The results indicated that the biomass has a variety of functional groups, such as carboxyl and amine and these groups are involved in almost all potential binding mechanisms. Depending on the pH value of the aqueous solution, these functional groups participate in met ion bindings [15].

Comparison with other adsorbents: The monolayer adsorption capacity (q_{max}) obtained in this study is compared with those obtained for other organisms. This is summarized in Table 4. The q_{max} value obtained in this study is higher than the q_{max} value of other organisms.

Table 4: Uptake capacities for lead (II) of various adsorbents at room temperature

Adsorbent	q_{max} (mg g^{-1})	Literature
Chlamydomonas reinhardtii	96.30	[29]
Gelidium algae	64.00	[30]
Zoogloea ramigera	82.80	[31]
Pseudomonas aerogenosa	23.00	[7]
Cyclotella cryptic (diatom)	26.28	[32]
Bacillus sp.	92.27	[33]
Arthrobacter sp.	130.00	[13]
Phanerochaete chrysosporium	134.00	[28]
Rhizopus arrhizus	76.40	[34]
Arthrobacter sp.	130.00	[14]
Rhizopus arrhizus	76.40	[34]
Spirogyra neglecta	132.00	This study

CONCLUSION

This study shows that nonliving biomass of *Spirogyra neglecta* has the potential to remove heavy metals in polluted water. The adsorption for lead (II) was quick, as maximum removal took place 1 h of contact time. The maximum adsorption capacity for lead (II) is higher than reported for other biosorbents of lead (II) by the nonliving biomass of *Spirogyra neglecta* are characterized by the initial concentration of lead (II), temperature and pH of the solution, Adsorption isothermal models and functional group. These are useful in predicting the behavior of the biosobent under the different conditions so that it can be used effectively to remove heavy metals such as lead. Using nonliving biomass of *Spirogyra neglecta* has an advantage because the alga is one of most ubiquitous species of *Spirogyra* in Malaysia. However, the ability of the nonliving biomass of the alga to adsorb other metals will also need to be studied.

ACKNOWLEDGMENT

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