BIOREMEDIATION OF HEAVY METALS PRESENT IN INDUSTRIAL WASTEWATER

(PhD Thesis)

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ABSTRACT

Numerous human activities (mining, agriculture, industries etc.) discharge huge amount of wastewater (WW) into water ecosystems without any purification or treatment that contained toxic heavy metals (THMs) such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn). Removal of these THMs is very essential to protect environment and human health. One of the promising and attractive technologies to remove THMs from WW or aqueous media is through various biomasses such as fresh water algae that can remove THMs very efficiently.

Four freshwater algae including *Cladophora glomerata*, *Oedogonium westii*, *Vaucheria debaryana* and *Zygnema insigne* were tested for their bioaccumulation capacity of selected THMs such as Cd, Cr and Pb in control environment with average temperature of 18℃, and light/dark duration of 12:12h. Experiments were performed in aqueous solutions containing selected toxic heavy metals (THMs) (ranged from 0.05 to 1.5 mg L^{-1}) with 0.5 g of living algae at 18°C and pH 6.8. The results indicated that *C. glomerata* was observed the most competent representative for the removal of Cr, Cd and Pb from aqueous solutions. THMs removal trends were in order of Cd > Cr > Pb, while removal efficiency of selected algae species was in order of *C. glomerata, O. westii, V. debaryana* and *Z. insigne*. Bioaccumulation capacity of *C. glomerata, V. debaryana* and *Z. insigne* was observed contrarily for different THMs. Removal of THMs was higher at low level of THMs in aqueous solutions. The results indicated that *C. glomerata, O. westii, V. debaryana* and *Z. insigne* had significant ($P \leq 0.01$) diverse bioaccumulation capacity for Cr, Cd and Pb.

Another experiment was conducted with green macroalgae present in freshwater ecosystems and famous for THM removal during wastewater treatment. In this study, the THMs uptake rate of *O. westti* was calculated. The equilibrium adsorption capabilities of *O. westti* were 0.974, 0.418, 0.620 and 0.261 mg g^{-1} Cd, Ni, Cr and Pb, respectively at 18°C and pH 5.0. The

removal efficiency was 55-95, 61-93, 59-89, and 61-96 % for Cd, Cr, Ni and Pb, respectively. The highest removal efficiency was observed for Cd and Cr from aqueous solution when the metal concentrations and pH were low, whereas for Ni and Pb the removal efficiency was highest at high pH and concentrations of metals in aqueous solution. The results summarized that *O. westti* are suitable to remove selected heavy metals from the aqueous solution and can be used in the treatment of wastewater (WW).

Freshwater algae biomasses have been studied for the biosorption potential of THMs using different concentrations and dosages from aqueous solution. The biosorption method is more effective in the removal and recovery of THMs. In this study four alga species biomasses such as *C. glomerata, O. westii, V. debaryana, Z. insigne* were used to remove Cd, Cr, Ni and Pb from the aqueous solution. Z. *insigne* showed high biosorption efficiency for the removal of Cr, Pb and Ni at low concentration and the biosorption reached to 78, 58 and 37 %, respectively from the aqueous solution at low concentration. The metal biosorption capability significantly ($P \leq 0.05$) changed with the biomass dosage, with the increase of biomass dosage, the metal biosorption capabilities slightly decreased. In this study all alga species at low dosage, the biosorption are highest for the Cr followed by Ni.

After using fresh biomass (living) and dead biomass of macroalgae for removal, bioaccumulation and biosorption of THMs. We conducted another research with objective to identify the most effective algae for the removal of Cd, Cr, Pb and Ni present in the industrial wastewater (IWW), collected at different outlet points of different main drain present in Hayatabad Industrial Estate (HIE), Peshawar, Khyber Pakhtunkhwa (KP),Pakistan. The bioaccumulation potential of selected indigenous alga such as *C. glomerata*, *O. westii*, *V. debaryana* and *Z. insigne* were within a range of 19.8-77.5, 19-66.5, 7.6-91 and 14.7-92 % for the removal of selected THMs, respectively. *C. glomerata*, *V. debaryana* and *Z. insigne* were recorded the hyper accumulating species for Cr 69-92 % followed by Ni, whereas the *O.*

westti showed high bio-removal capacity for Ni reached to 63-65 %. The *Z. insigne* showed hyper accumulation capacity for Cd and Pb. Based on results, it is concluded that these different algae species can be used to treat the IWW but long term field experiments are needed for further investigation of the effectiveness of this green technology, which is more environment friendly and needs low cost as compared to other traditional technologies.

Chapter-1

` Introduction

1.1 The Issue

Human and industrial activities discharge huge amount of waste and wastewater (WW) into nearest water ecosystems without any purification or treatment that contained toxic heavy metals (THMs) such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn). The industries such as mining, petroleum refining, metal plating, photographic processing are the main cause of water pollution that discharge potentially THMs (Mehta and Gaur 2005; Inyang et al., 2012; Abdel-Aty et al., 2013). The domestic effluents, land fill leachates, agricultural runoff, and acid rain also contribute THMs in WW (Abollino et al., 2003). Everyday 2 million tons of sewage and other effluents are drained into the world's water and more than 1.5 million children each year from waterborne diseases. This situation is worst in developing countries where 70 % of untreated industrial wastes and 90 % of raw sewage are dumped into surface water (UN water, 2010). Table 1.1 summarizes the list of industries contributing different THMs in the nearest water bodies.

It is considered that 40 THMs represent toxic potential for living organisms at all levels including human being due to their excessive accumulation in the food chain (Nalimova et al., 2005) and thus becoming a permanent burden on ecosystems (Bailey et al., 1999; Singh et al., 2007; Lim et al., 2010; Abdel-Aty et al., 2013). Industrial discharges, particularly those containing THMs are causing of serious threats to human health and other systems of life (Azza et al., 2013).

For the purpose of industrial WW pollution control national and international environment protection agencies have set standards and guidelines and it is of intense need to duly check the industrial discharges to bring it to the minimum permissible limits of many THMs (see Table 2.1). It required deep attention before discharge the industrial effluents into water bodies

and natural streams to make it sure that the metals concentration are within the permissible limits.

Table1.1 Major Industries associated with the discharge of THMs.

Industries	As	Cd	Cr	Cu	Fe	Hg	Pb	Ni	Zn
Organic chemicals, petrochemicals			√	√		√	√	√	√
Glass and ceramics				√			√		
Petroleum refining	√	√	√	√	√	√	√	√	√
Fertilizers	√	√	√	√	√	√	√	√	√
Leather tanning finishing			√						
Pulp ,paper mills, paper board			√	√		√	√	√	√
Steel works, metal works, foundries	√	√	√	√	√	√	√	√	√
Textile mill products			√						

Source :(Akhter, 2006)

The research is going onwards for this purpose to find out suitable and cost effective bio techniques. Biosorption promises to achieve the above mentioned objective (Voleskey, 2001; Ekmekyapar et al., 2006; Zhang et al., 2010; Azza et al., 2013).

The biosorption is the term used for the passive accumulation of THMs by biological materials usually dead biomass (Singh et al., 2012) serves as basis for biosorbents. Bio sorbents must be differentiated from the bioaccumulation which is an active process depending on the metabolic activity and occur only in the living organisms. There are diverse advantages in the using either dead or living biomass (Macaskie, 1999; Schiewer and Volesky, 2000; Brinza et al., 2007).

The living biomass is self-renewed and active biological transport into the cell may led to the high metal bioaccumulation (Afkar et al., 2010; Kumar and Gaur, 2011; Chen et al., 2012) however the use of dead biomass avoids problems with toxicity. Biosorption by dead biomass is faster often in comparison of bioaccumulation since only occurs the cell wall based binding

of metals and easily recovered the bound metals. This allows restoration of the bio sorbent materials. The THMs accumulated intracellular by living biomass can be recovered only when the cells are destroyed.

The published research focused on the development of biological techniques such as fungi, bacteria, and yeasts have studied for the removal of THMs from the industrial discharges (Seki and Suzuki, 2002). Microalgal biomass has been confirmed to be greatly effective for the elimination of the THMs from aqueous solutions (Garnham, 1997).

Lower plants such as algae have been used for removal of THMs present in contaminated water such as domestic sewage and industrial effluent (Gupta and Rastogi, 2008; Vogel et al., 2010). Different micro-algal strains such as *Chlorella fuscas, Chlorella vulgaris, Spirulina sp Spirogyra species, Cladophora fascicularis, Chaetophora elegans, Cladophora sp.* and *Enteromorpha sp.* have the potential to remove THMs from aqueous solutions and WW (Chojnacka et al., 2005; Andrade et al., 2005; Martins et al., 2006; Vijayaraghavan et al., 2006; Gupta and Rastogi, 2008; Kumar and Gaur, 2011; Chen et al., 2012; Apiratikul and Pavasant 2008; Akhtar et al., 2008).

The removal of THMs from WW is considered as a key area of research with respect to the economic and environmental consideration. There are numerous methods for removal of THMs from aqueous solutions such as membrane filtration including, adsorption on activated carbon, biochar, ion exchange, reverse osmosis and chemical precipitation (Yu et al., 2001). However, the practicability of these expensive techniques and some technical factors may limit the implementation of these methods (Sari and Tuzen, 2008; Turker, 2012).

One of the promising and attractive technologies to remove THMs from WW or aqueous media is through various biomasses such as algae (Gupta and Rastogi, 2009) that can remove THMs very efficiently.

1.2 Study objectives

This research work was conducted to achive the following objectives:

1. To compare different fresh water algae species for THMs bioaccumulation for the purpose of identifying a green technique for WW treatment and to remove THMs from industrial WW.
2. To compare different fresh water algae for biosorption of THMs present in WW.
3. To determine the optimal conditions such as THMs concentration and pH levels for metals uptake by green macro algae.
4. To investigate an economical and environmental friendly method for removal of THMs from industrial WW.

Chapter-2

Background Study

Industrialization and urbanization added huge amount of wastewater (WW) to natural water ecosystem, consisting of potentially THMs. The presence of these THMs in the natural environment is of great concern because of its toxicity, persistency and non-biodegradability properties (Lim et al., 2010; Abdel-Aty et al., 2013). When its concentrations exceed the permissible limits it can cause adverse health problems in organisms through bioaccumulation in food chain particularly Cd and Mercury (Hg). Some THMs are required by the organism in trace amount for balance growth such as Cu, Cobalt (Co), Zn, Selenium (Si), Iron (Fe), but the higher dosage can cause toxicity that is chronic, acute, synergistic, teratogenic/mutagenic (Singh et al., 2007; Krishna et al., 2009).

There are many processes including metal plating, finishing and metal process which originate THMs. Table 2.1 summarizes the list of the guidline limits for THMs in industrial WW set by different countries. In addition domestic WW, agriculture runoff, landfill leachate, atmospheric aerial deposition contribute THMs in WW (Abollino et al., 2003). Cd, Cr and Ni are THMs that are abundantly found in such a WW discharges.

Table 2.1 Maximum permissible limits for the THMs present industrial WW.

THMs	**Canada[a]**	**USA[b]**	**UK[c]**	**Pakistan[d]**
Cd	1.5	0.1	0.2	0.1
Cr	1.0	2.8		1.0
Cu	1.0	3.3	3.0	1.0
Pb	1.5	0.6	1.0	0.5
Ni	2.0	4.0	4.0	1.0
Zn	2.0	2.8	3.0	5.0

a=EPS (1997); b=UNEP (1989); c=ENDS (1992); d=Gazette of Pakistan (1993)

2.1 Application of THMs and its toxicity

THMs are considered to be very harmful and caused health problems because of its toxic nature that result from their exposure. Significant number of people have been exposed to the hazards of excess of THMs in the municipal water supplies according to the survey report from public health services of various countries (WHO, 1993). THMs bioaccumulated in the food web and stored in tissues (Deng et al., 2008; Lim et al., 2010; Zeng and Wu, 2013; Yangetal., 2014), for instance Cd has 10-30 years half-life in the human body (Moore and Ramamoorthy, 1984). Therefore their harm effects are mainly prominent in animals of high tropic levels predominantly human (Volesky and Schiewer, 1999). However these metal are very useful and applied in many industries. The following text will describe well the applications and harmful effects of THMs used in the present study.

2.1.1 Cadmium (Cd)

Cd is highly rust resistant and used as a protective coating for steel iron and copper. it is mostly used by electroplating, but hot dipping and spraying are also possible. Cd may be alloyed with gold (Au), silver (Ag), bismuth (Bi), Cu, and Ni to form easily fusible compounds. These alloys can be used as coating for other materials, welding, solders and electrodes. it can also be used as a neutron absorber in nuclear reactors, in electrodes of alkaline batteries, a stabilizer for polyvinyl chloride plastics, in making of fluorescent lamps, an amalgam in dentistry, a deoxidizer in Ni plating, semiconductors, jewelry and photocells, in process engraving, and in the aircraft and automobile industries (Sitting, 1981).

Cd can cause serious damage to kidney and bones in human and probably link with the Itai-Itai disease (Martins et al., 2006; Sari et al., 2008; Pathak et al., 2009). The main symptom of this disease is softening of the bones and led to painful skeletal malformations. Cd has been exposed to be an age-linked toxicant in animal, resulting in fetal malformations and other effects but no sure evidence present in humans (ATSDR, 1997).

The increase risk of lung cancer in human due to the Cd exposure has been reported from human studies but they are not sure due to confounding reasons. However in animals it is concluded that exposure to long term inhalation to Cd cause lung cancer. US EPA has declared this element as a possible carcinogenic to human as a Group B1.

2.1.2 Chromium (Cr)

The Cr is a steel gray solid and used mainly in manufacturing of steel and other alloys (ATSDR, 2001b). Cr present in the environment in the form of hexavalent Cr (VI) and trivalent Cr (III) which is used in manufacture of dyes and pigments, chrome plating, leather and wood preservation and treatment of cooling tower water. Small amount is used in textiles and toner for copying machines. Cr (III) are less toxic than Cr (IV) can target respiratory tract. The Cr (III) is an essential element in human, the human body detoxify some amount of Cr (IV) to Cr (III), but short and long term exposure to Cr (IV) can cause problems in respiratory tract. coughing, wheezing and shortness of breath were reported due to acute exposure to Cr (IV).while ulceration of the septum, decreased pulmonary function, perforations, pneumonia and other respiratory effects have been noted from chronic exposure. The human studies clearly shows that inhaled Cr (IV) cause lung cancer.

2.1.3 Lead (Pb)

The Pb is used in the manufacturing of cable covering, ammunition, paints, sheet lead, metal products, manufacture of batteries and ceramic glazes (ATSDR, 1999). Pb is emitted from vehicle fumes in atmosphere. Pb can cause a variety of effects even at low dose. Kidney, brain damage and gastrointestinal distress caused due to the acute exposure of Pb in humans (ATSDR, 1999). Chronic exposure to Pb in human can cause various effects on central nervous systems, blood pressure, vitamin D metabolism and kidneys (USDHHS, 1993). Children are very sensitive to chronic effects of Pb, with reduce growth and cognitive development (ATSDR, 2001a). The high exposure to Pb can also cause reproductive effects

such as reduced sperm count in men and unusual abortion in woman also linked with the high Pb exposure. The developmental fetus is at particular risks when the mother exposed to Pb, result in low birth weight and slow postnatal neurobehavioral development (USEPA, 1999). The studies regarding the Pb exposure in human are inconclusive of cancer.

2.1.4 Nickel (Ni)

Ni forms alloys with Mn, Cu, Zn, Cr and molybdenum. Stainless steel is broadly used Ni alloy. Ni and Cu alloy has tremendous corrosion resistance properties. Elemental Ni is used in anodizing aluminum, coinage, casting operations for machine parts, in the manufacturing of acid-resisting and magnetic alloys, Ni-Cd batteries, Ni soap in crankcase oils and glass. It is used as catalyst in the hydrogenation of oil, fats and other chemicals (Sitting, 1981). Ni is very important component of many enzymes (Reinhold, 1975).

However it is carcinogenic when present even at low concentrations. When the human exposed to chronic skin contact with Ni. It cause itching of the fingers, forearms and hands and respiratory effects. Human and animals studies revealed that exposure to Ni refinery dusts and Ni sub-sulfide, increased risk of nasal and lung cancers. Animal studies of soluble Ni compounds cause lung tumors. At high concentration Ni can react with DNA and result in DNA mutilation as shown in vitro mutagenicity (WHO, 1993).

Ni and its compounds shows irritants to mucous membranes of upper respiratory tract and conjunctiva of the eye (Sitting, 1981; Pathak et al., 2009).

2.2 Environmental criteria of THMs

National and international agencies has set the permissible limits for water quality criteria to protect the public health from the adverse effects of THMs. Table 2.1 summarize the Environmental Protection Agency (EPA) Pakistan and other countries have settled the water quality criteria in WW which highlight the necessity of an effective and low cost WW treatment technology to eliminate THMs.

2.3 Existing methods of THMs removal from WW.

Currently several methods are used for WW treatment to remove or separate THMs (Yetis et al., 1998; Chong et al., 2000). These method include:

- Chemical precipitation
- Ion exchange
- Membrane technology
- Adsorption
- Electrolysis

All of the above mentioned methods for the removal of THMs have some draw backs, despite it has the capability to remove metals up to some extent from the industrial discharges. The metals are usually changed to another form which itself to be disposed off. In addition they are costly in term of energy cost and capital (Brinza et al. 2007; Fu and Wang, 2011; Turker, 2012). Presently the existing clean-up technologies are being stretched to their operational limit and discharges limit should be followed. The environmental laws should be strictly imposed and for future essentially clean industrial process are required but it required modification, and in some cases the rebuilding of plants and factories and short term correction plan should be to introduce the amended clean up technologies as add-ons to the existing processes. Thus the search for innovative technologies in the area of removal of THMs from WW has focused the attention to bio sorption as an alternative.

2.4 Biosorption as an option

The biological materials to accumulate THMs from the environment is extensively recognized phenomenon that has a number of key implications. Biosorption is defined as the removal of metal or metalloid species, particulates and compounds from solutions by biological materials (Gadd, 1990).

Conventional methods for the removing of THMs from WW are costly when the metal

concentration are in the limits of 10-100ppm (Preetha and Viruthagiri, 2005; Sari et al., 2008; Azza et al., 2013; Weng et al., 2014). The biosorption experiment has conducted by several researcher which demonstrated the potential use of biosorption in metal removal and recovery from different types of solutions (Metha and Gaur, 2005; Afkar et al., 2010; Kumar and Gaur, 2011; Chen et al., 2012).

The microorganism has got much attention in the recent years to use in the biosorption process as potential application in industry to remove THMs (Romera et al., 2007; Rajfur et al., 2010). Many fungi, bacteria, yeast, and algae has the ability to remove metals from the aqueous solutions and to accumulate them within or on the surface of their cell structure (Kumar and Gaur, 2011; Chen et al., 2012). The main benefits of microbial biomass are (a) small and uniform size (b) the diversity of active binding sites (c) the possibility to regenerate and reuse the same biomass (Brinza et al., 2007). Microbial biomass be grown fast and effluent qualities with residual metal ions at concentration levels of the order of ppb can also be attained.

2.5 Mechanisms of biosorption

There are two types of binding that can occur in biological and biologically derived materials depending on whether the biomass is dead or living. Passive binding occurs in both nonliving and living cells and involves fast, reversible, ion exchange and physical adsorption with cell surface. Active binding occurs on living cell as a result of metabolic activity and involves slow, irreversible and this process is often termed as bioaccumulation.

Eccles (1995) defined bioaccumulation as “all the methods responsible for the uptake of metals by living cells” thus includes biosorptive mechanisms together with bio precipitation and intracellular accumulation mechanisms. The methods by which the biological materials removes the THMs from aqueous solutions depends on whether it is dead (non metabolism dependent) or living (metabolism dependent). The mechanisms involved in the process can be

classified as follow:

- **Complexion or cell surface sorption:** This process may occur both on dead and living biomass.
- **Precipitation /extracellular accumulation:** This process depend on metabolism and the THMs removal by this method may be assisted by biomass capability.
- **Intracellular accumulation:** This process also needed viable biomass. This process is very complex and required specific environmental condition to take bioaccumulation of THMs.

The complex mechanism involved in the biosorption are still not clearly understood, however the metal binding mechanism are explained as follow:

- **Chemisorption:** This may involve complextion, ion exchange, coordination, chelating and
- **Adsorption:** The physical forces such as electrostatic interaction.

The cell wall of biosorbent are mostly composed of polysaccharides and lipids (Schiewer and Volesky, 2000) Which offer many ways to bind metal ions at the surface of biomaterial through process via metal binding functional group such as amino, sulphate, carboxyl, hydroxyl and phosphate groups (Lesmana et al., 2009; Song et al., 2014). The binding of metal ions involves two mechanisms, ion exchange and the formation of complex compounds. Precipitation also occur both in solution and on the cell surface (Veglio and Beolchini, 1997).

2.6 Binding sites

Different bio sorbent cell wall contain various macromolecules involved in the binding of HMs ions. In the biosorption method various chemical groups such as carbonyl, carboxyl, sulfonate, hydroxyl, sulfhydrl, amine, amide, imidazole, and phosphonats groups present in the cell wall of various microorganisms attracted the metal ions (Lesmana et al., 2009; Song et al., 2014). It depends on different factors that how the metals ions attracted toward these

groups such as: the accessibility of the site ,the number of sites in the biosorbent materials, chemical state of the sites and how much affinity present between site and metal (Vieira and Volesky, 2000). Sulfate, amino, carboxylate may be responsible for metal binding in freshwater algae (Crist et al., 1981; Song et al., 2014). Sulfate and carboxyl groups's participation were found by titration of marina alga biomass (Crist et al., 1992).

The fresh water alga *Chlorella* chelating ability was connected to their contents of uronic acids present in polysaccharides. There carboxyl groups would be negatively charged and could bind metal ions (Crist et al., 1981; Xue et al., 1988). Green et al., (1986) detected a complete loss of available sulfhydryl groups after binding of Au (III) to *Chlorella* fresh water algae, which show the involvement of this group, but only account for a small amount of total gold binding. But when the cell were modified with succinic anhydride, the sorption of tetrachloroaureate decreased by 50 % (Green et al., 1986). The modification of carboxyl group with acidic methanol resulted in decrease in binding of Cu and Al, but an insignificant increase in Au binding by five diverse algae (Gardea-Torresdey et al., 1990). The hard metal such as Al is expected to bind hard sites such as carboxyl groups, so that is why sorption decrease where as soft metals such as Cu bind sulfur or nitrogen group so the sorption increase. The binding of negatively charged Au increases possibly due to a decrease in the negative surface charges.

The brown algae were studied and assumed that alginates play an important role in metal-ion binding (Davis et al., 2003). Fourest and Volesky (1996) used *Sargassum fluitans* biomass studied the binding of Pb, Cd, and Hg by alginic acid before and after modification of carboxyl groups using propylene oxide and acidic methanol. A linear correlation between the contents of weak acid groups and the binding capacity for Cd resulting from different degrees of active site. The maximum metal binding capacity of different algae was linearly correlated with their weak acid groups.

2.7 Theoretical aspects of biosorption

Adsorption methods could be characterized by their equilibrium and kinetic isotherms. Different mathematical models are used during the batch process of adsorption. The adsorption isotherms are the equilibrium relationships between concentration of the adsorbed metal ions and metal solution at a given temperature. The degree of biosorption of a metal ion on a biosorbent has been found to be a function of equilibrium metal concentration in solution at constant pH and temperature conditions. To develop an equation the analysis of equilibrium data is essential that can be used for the design of adsorption systems (Aksu, 2001). Numerous isotherms used for the equilibrium modeling of biosorption systems. The most universally used isotherm is Langmuir isotherm:

2.7.1 Langmuir isotherm

The Langmuir model originally develop to describe the physical adsorption of gases by solids (Langmuir, 1918). It works on the assumption that the sites on the surface of the adsorbent occupied by the adsorbate from the solutions. The energy of adsorption for each molecule is the same and independent of surface coverage, adsorption take place isotherm only in localized sites with no interactions between adsorbed molecules and maximum adsorption occurs when the surface is shielded by a monolayer of adsorbate. This is the most widely used and written as follow:

$$Q_{eq} = Q_{max}\, b\, C_{eq} / (1 + bC_{eq}) \quad \text{---------- (Eq. 2.1)}$$

Q_{eq} = Amount of metal ion adsorbed by the biosorbent at equilibrium.

C_{eq} = Equilibrium metal ion concentration in the solution at constant temperature.

Q_{max} = Maximum amount of metal ions per unit weight of the biosorbent required for a complete mono layer bound to the surface i.e. provides the hypothetical monolayer saturation ability.

b = the constant b ($1\ mgg^{-1}$) of the binding sites

This equation is used for the homogenous sorption where every THM ions microorganism sorption has alike sorption activation energy and comply with the Henry, s law at little concentration.

The value of Q_{max} and b which are main features of Langmuir equation and explored from the equation (Eq. 2.2) for example:

$$C_e/Q = I/bQ_{max} + C_e/Q_{max} \text{----------(Eq. 2.2)}$$

A plot of $1/Q_{eq}$ versus $1/C_{eq}$ gives a straight line with a slope of $1/b\ Q_{max}$ and an intercept of $1/Q_{max}$.

The Langmuir fitted is considered to be an evidence that sorption stops at monolayer coverage consistent with the specific and strong sorption on to specific sites. This equation can be applied for biosorption equilibrium. Q_{max} support in comparison of adsorption performance particularly in cases where the sorbent didn't reach full saturation in experiments (McKay and Porter, 1997; Aksu, 2001). The shape of the isotherm curves will be different, may be explained with different mechanisms and compositional differences in biomasses used.

2.8 Factors affecting metal biosorption

Biosorption of THMs is effected by many experimental parameters including initial metal ion concentration, presence of different metallic ions in solution, biomass concentration, pH, ionic strength and temperature. If these factors are varied in the WW, it will mean that it will influence biosorption performance (Veglio et al., 1997). Hydrogen ion concentration affect the solubility of metal ions in solution and the ionization state of functional group present on the cell wall of the biomass (Tobin et al., 1984). Table 2.2 describes the sorption potential of different algae at different pH.

When the pH is very high or low,the hydronium ions (H_3O^+) present in the solution adsorbed on the surface of biosorbent giving positive charge to the biosorbent, As a result SO_4^{-2}, PO_4^{-2}, $HCr\ O^{4-}$, MnO^{4-} are sorbed to the surface of biosorbent , when pH of solutions increased the

metal ion more available for biosorption (Yu et al., 2001).

Temperature does not seem to influence the biosorption process in the range of 5-30 ℃ (Aderhold et al., 1996). The Marques et al., (1991) studied the bioaccumulation of metals ions by different biomass and found that temperature has no effect on the uptake metal ions from solution, however a few researcher found that if the temperature is above the 30 ℃, then they will play an important role in the interaction of metal with microbial biomass (Sag and Aktay, 2000; Aksu, 2001).

Biosorbent concentration is very important because it will determine the amount of metal ions sorbed from solution per unit of the sorbent. If the biosorbent concentration is less than 0.5 g per 50 ml that will be more suitable for removal of metal ions at concentration < 100 mg L^{-1}(Romero-Gonzalez et al., 2001).

The time is an important in the biosoprtion of metal ion to the bio sorbent, many researcher have found the time of between ten minutes and two hours for the biosorption methods (de Rome and Gadd, 1987). The rapid binding phase accounts for greater amount of metals ions taken by the biomass. The sources of the aqueous solution are numerous and effect the biosorption. For example, industrial effluents are unlikely to be homogenous, for metal or pollutants which may compete for the adsorption sites. The biosorbent could be used repeatedly with slight reduction up to 80 % recovery in metal biosorption capabilities (Williams et al., 1998). Desorption is very important method in which the metal ions can be thoroughly removed from the biosorbent and eventual disposal of the unusable biomass very easy if it remains contaminated.

Different biosorbent materials are used for biosorbent of THMs including algae, bacteria, yeast and fungi biomass (Malik, 2004). Other types of biosorbent include agriculture wastes and by product and waste from industrial process (Wase et al., 1997). Among all the microorganism algae are the most visible and easiest to grow, including around 70 % of the

earth biomass and classified into 21,000 different algal species (Robinson et al., 1986).

Table 2.2 Sorption potential of different algae at different pH.

Alga Species				
Green Algae	Species of Algae	pH	q_{max} (mmol/g)	References
Cd^{+2}	*Ulva sp.*	5.5	0.58	Sheng et al. (2004)
	Chaetomorpha linum	5	0.48	Hashim and Chu (2004)
	Codium vermilara	6	0.19	Romera et al. (2007)
	Caulerpa lentillifera	5	0.04	Pavasant et al. (2006)
	Ulva lactuca	5	0.25	Sari and Tuzen (2008b)
	Oedogonium sp.	5	0.79	Gupta and Rastogi (2008)
	Spirogyra insignis	6	0.2	Romera et al. (2007)
	Spirogyra sp.	-	0.006a	Rajfur et al. (2010)
Cu^{+2}	*Caulerpa lentillifera*	5	0.08	Pavasant et al. (2006)
	Codium vermilara	5	0.26	Romera et al. (2007)
	Spirogyra insignis	4	0.3	Romera et al. (2007)
	Spirogyra neglecta	4.5	1.8	Singh et al. (2007)
	Cladophora sp.	5	0.23	Lee and Chang (2011)
	Ulva fasciata	5.5	1.14	Karthikeyan et al. (2007)
	Ulva fasciata	5	0.42	
	Ulva sp.	5	0.75	Sheng et al. (2004)
	Spirogyra sp.	5	0.6	Lee and Chang (2011)
	Spirogyra sp.	5	0.53	Rajfur et al. (2012)
Ni^{+2}	*Ulva sp.*	5.5	0.29	Sheng et al. (2004)
	Codium vermilara	6	0.22	Romera et al. (2007)
	Ulva lactuca	4.51	0	Romera et al. (2007)
	Spirogyra insignis	6.01	0.29	Romera et al. (2007
Pb^{+2}	*Ulva sp.*	5	1.46	Sheng et al. (2004)
	Cladophora glomerata	4.5	0.35	Jalali et al. (2002)
	Codium vermilara	5	0.3	Romera et al. (2007)
	Caulerpa lentillifera	5	0.13	Pavasant et al. (2006)
	Cladophora sp.	5	0.22	Lee and Chang (2011)
	Ulva lactuca	4.5	0.61	Jalali et al. (2002)
	Spirogyra insignis	5	0.24	Romera et al. (2007)
	Spirogyra neglecta	5	0.56	Singh et al. (2007)
	Spirogyra sp.	5	0.43	Lee and Chang (2011)
Zn^{+2}	*Ulva sp.*	5.5	0.29	Sheng et al. (2004)
	Codium vermilara	6	0.22	Romera et al. (2007)
	Spirogyra insignis	6	0.29	Romera et al. (2007)
	Ulva lactuca	4.5	1.14	Zakhama et al. (2011)
	Ulva sp.	5.5	0.54	Sheng et al. (2004)
	Codium vermilara	6	0.36	Romera et al. (2007)
	Caulerpa lentillifera	5	0.04	Pavasant et al. (2006)
	Spirogyra insignis	6	0.32	Romera et al. (2007)
	Spirogyra sp.	-	0.02a	Rajfur et al. (2010)

Red Algae

Cd^{+2}	*Asparagopsis armata*	6.0	0.28	Romera et al. (2007)
	Gracillaria sp.	5.5	0.3	Sheng et al. (2004)
	Gracilaria changii	5.0	0.23	Hashim and Chu (2004)
	Gracilaria edulis	5.0	0.24	Hashim and Chu (2004)
	Gracilaria salicornia	5.0	0.16	Hashim and Chu (2004)
	Chondrus crispus	6.0	0.66	Romera et al. (2007)
	Ceramium virgatum	5.0	0.35	Sari and Tuzen (2008a)
	Corallina mediterranea	5.0	0.57	Ibrahim (2011)
	Mastocarpus stellatus	6.0	0.59	Herrero et al. (2011)
	Jania rubens	5.0	0.27	Ibrahim (2011)
	Pterocladia capillacea	5.0	0.29	Ibrahim (2011)
	Galaxaura oblongata	5.0	0.76	Ibrahim (2011)
	Hypnea valentiae	6.0	0.15	Rathinam et al. (2010)
Cr	*Corallina mediterranea*	5.0	1.35	Ibrahim (2011)
	Jania rubens	5.0	0.54	Ibrahim (2011)
	Palmaria palmate	4.5(Cr(III))	0.57(Cr(III))	Murphy et al. (2008)
	Polysiphonia lanosa	2(Cr(IV))	0.65(Cr(IV))	Murphy et al. (2008)
	Pterocladia capillacea	5.0	0.66	Ibrahim (2011)
	Galaxaura oblongata	5.0	2.02	Ibrahim (2011)
CO^{+2}	*Corallina mediterranea*	5.0	1.29	Ibrahim (2011)
	Jania rubens	5.0	0.55	Ibrahim (2011)
	Pterocladia capillacea	5.0	0.89	Ibrahim (2011)
	Galaxaura oblongata	5.0	1.25	Ibrahim (2011)
Cu^{+2}	*Gelidium*	5.3	0.51	Vilar et al. (2008)
	Gracillaria sp.	5	0.59	Sheng et al. (2004)
	Asparagopsis armata	5	0.33	Romera et al. (2007)
	Chondrus crispus	4	0.63	Romera et al. (2007)
Ni^{+2}	*Asparagopsis armata*	6	0.29	Romera et al. (2007)
	Gracillaria sp.	5.5	0.28	Sheng et al. (2004)
	Chondrus crispus	6	0.63	Romera et al. (2007)
Pb^{+2}	*Gracilaria corticata*	4.5	0.26	Jalali et al. (2002)
	Gracilaria canaliculata	4.5	0.2	Jalali et al. (2002)
	Gracillaria sp.	5	0.45	Sheng et al. (2004)
	Galaxaura oblongata	5	0.42	Ibrahim (2011)
	Asparagopsis armata	4	0.3	Romera et al. (2007)
	Chondrus crispus	4	0.98	Romera et al. (2007)
	Corallina mediterranea	5	0.31	Ibrahim (2011)
	Jania rubens	5	0.14	Ibrahim (2011)
	Polysiphonia violacea	4.5	0.49	Jalali et al. (2002)
	Pterocladia capillacea	5	0.16	Ibrahim (2011)
Zn^{+2}	*Asparagopsis armata*	6	0.33	Romera et al. (2007)
	Gracillaria sp.	5.5	0.4	Sheng et al. (2004)
	Chondrus crispus	6	0.69	Romera et al. (2007)

Brown Algae

Cd^{+2}	*Ascophyllum nodosum*	6	0.78	Romera et al. (2007)
	Ascophyllum nodosum	4.5	0.7	Lodeiro et al. (2005)
	Fucus vesiculosus	6	0.96	Mata et al. (2008)
	Fucus spiralis	6	1.02	Romera et al. (2007)
	Padina sp.	5.5	0.75	Sheng et al. (2004)
	Sargassum sp.	5.5	0.76	Sheng et al. (2004)
	Sargassum siliquosum	5	0.73	Hashim and Chu (2004)
	Sargassum baccularia	5	0.74	Hashim and Chu (2004)
	Sargassum vulgarie	4.5	0.79	Davis et al. (2000)
	Sargassum fluitans	4.5	0.71	Davis et al. (2000)
	Sargassum muticum	4.5	0.68	Davis et al. (2000)
	Sargassum filipendula	4.5	0.66; 0.70	Davis et al. (2000)
	Sargassum sp.	4.5	0.78; 0.90	Davis et al. (2000)
	Sargassum filipendula	5	1.17	Luna et al. (2010)
	Bifurcaria bifurcate	4.5	0.65	Lodeiro et al. (2005)
	Saccorhiza polyschides	4.5	0.84	Lodeiro et al. (2005)
	Laminaria ochroleuca	4.5	0.56	Lodeiro et al. (2005)
	Pelvetia caniculata	4.5	0.66	Lodeiro et al. (2005)
	Macrocystis pyrifera	3	0.89	Plaza Cazón et al. (2012)
Cr	*Fucus vesiculosus*	4.5 (Cr(III))	1.21(Cr(III))	Murphy et al. (2008)
		2 (Cr(VI))	0.82 (Cr(VI))	
	Sargassum muticum	2 (Cr(VI))	3.77(Cr (VI))	González Bermúdez et al. (2012)
	Sargassum sp.	2(Cr(VI)	0.60(Cr(VI))	Yang and Chen (2008)
Cu^{+2}	*Padina sp.*	5	1.14	Sheng et al. (2004)
	Ascophyllum nodosum	4	0.91	Romera et al. (2007)
	Sargassum sp.	5	0.99	Sheng et al. (2004)
	Sargassum fluitans	4.5	0.8	Davis et al. (2000)
	Sargassum vulgarie	4.5	0.93	Davis et al. (2000)
	Sargassum filipendula	4.5	0.89	Davis et al. (2000)
	Sargassum sp.	5.5	1.13	Karthikeyan et al. (2007)
	Sargassum filipendula	4.5	1.32	Kleinübing et al. (2011)
	Fucus vesiculosus	5	1.66	Mata et al. (2008)
	Fucus spiralis	4	1.1	Romera et al. (2007)
	Fucus serratus	5.5	1.6	Ahmady-Asbchin etal. (2008)
Ni^{+2}	*Cystoseria indica*	6	0.85	Pahlavanzadeh et al. (2010)
	Fucus spiralis	6	0.85	Romera et al. (2007)
	Fucus vesiculosus	3.5	0.39	Holan and Volesky (1994)
	Sargassum natans	3.5	0.41	Holan and Volesky (1994)
	Sargassum fluitans	3.5	0.75	Holan and Volesky (1994)
	Sargassum vulgare	3.5	0.09	Holan and Volesky (1994)
	Sargassum sp.	5.5	0.61	Sheng et al. (2004)
	Padina sp.	5.5	0.63	Sheng et al. (2004)
	Nizmuddinia zanardini	6	0.94	Pahlavanzadeh et al. (2010)
	Sargassum	6	0.94	Pahlavanzadeh et al. (2010)

	glaucescensand			
	Padina australis	6	0.46	Pahlavanzadeh et al. (2010)
	Ascophyllum nodosum	3.5	0.69	Holan and Volesky (1994)
	Ascophyllum nodosum	6	0.73	Romera et al. (2007)
	Sargassum filipendula	4.5	1.07	Kleinübing et al. (2011)
Pb^{+2}	*Ascophyllum nodosum*	3	0.86	Romera et al. (2007)
	Ascophyllum nodosum	3.5	1.31	Holan and Volesky (1994)
	Sargassum natans	3.5	1.22	Holan and Volesky (1994)
	Fucus vesiculosus	3.5	1.11	Holan and Volesky (1994)
	Sargassum vulgare	3.5	1.1	Holan and Volesky (1994)
	Sargassum hystrix	4.5	1.37	Jalali et al. (2002)
	Sargassum natans	4.5	1.14	Jalali et al. (2002)
	Sargassum sp.	5	1.16	Sheng et al. (2004)
	Padina pavonia	4.5	1.04	Jalali et al. (2002)
	Padina sp.	5	1.25	Sheng et al. (2004)
	Fucus vesiculosus	5	1.02	Mata et al. (2008)
	Fucus spiralis	3	0.98	Romera et al. (2007)
Zn^{+2}	*Ascophyllum nodosum*	6	0.64	Romera et al. (2007)
	Padina sp.	5.5	0.81	Sheng et al. (2004)
	Fucus spiralis	6	0.81	Romera et al. (2007)
	Sargassum sp.	5.5	0.5	Sheng et al. (2004)
	Sargassum filipendula	5	0.71	Luna et al. (2010)
	Macrocystis pyrifera	4	0.91	Plaza Cazon et al. (2012)

2.9 Algae

Algae are autotrophic, eukaryotic organisms and belongs to the kingdom Protista. Algae used in food since ancient time particularly in Asian coast. In addition it is also used as a raw materials in different cosmetics, pharmaceutical and food industries as additive products. In lately algae proposed to be used for the wastewater (WW) treatment due to their excessive THMs sorption attraction. The use of algae is very common in WW treatment due to THM removal efficiency, non-noxious chemical sludge and less charge. The algae have three main kinds such as red, brown and green that consist of carrageenan and cellulose that provides sites for binding such as amino, carboxyl, hydroxyl and sulfhydryl which remain the key reason for the choosiness of the above mentioned biomass as a biosorption and biosorbents for THMs removal.

2.9.1 Algae uses and their habitat

Algae are photosynthetic organisms that are not plants that found in the freshwater, marine, and some found in the terrestrial environments. Algae may be unicellular, multicellular or colonial. The epiphytic or benthic algae nurtured and attached to plants, docks, rocks and other substances (Lembi and Waaland, 1988; Raven et al., 1999).

The species more consumed are *Ascophyllum nodosum, Gelidium sesquipedale, Undaria pinnatifida, Palmaria palmate, Himantalia elongate* and *Porphydadioica*.The algae in the Eastern nations used in iodine extraction, glass manufacturing and fertilizers.

In the last decades the use of algae as biosorbent substance due to its more sorption ability for the removal of THMs and its abundant availability in oceans.

Algae various species are studied for its sorption properties. Table 2.3 shows different algae species maximum sorption uptake (q_{max}) for metals and constant b, connected with the affinity of biomass (green algae, red and brown), those were calculated from the Langmuir model.

The use of algae as biosorption are very interesting for the reason that number of research group used algae for the biosorption of metals (Figueira et al., 2000a ; Aksu and Acikel, 2000; Al-Rub et al., 2006; Deng et al., 2007; Vilar et al., 2007; Han et al., 2007; Aksu and Donmez, 2006; Luo et al., 2006; Gupta et al., 2006; Vijayaraghavan et al., 2006; Martins et al., 2006; Akhtar et al., 2008; Grimm et al., 2008; Apiratikul and Pavasant 2008).

These researchers have studied the algae as biomass in column or batch experiments under the different condition such as metal initial concentration, pH, temperature, biomass dosage, and presence of anions and cations, thermodynamic and kinetic aspects, mathematical modelling and the biomass regeneration option using different extractant reagents.

Several remarkable reviews on the consumption of algae have been printed in the last decade (Davis et al., 2003; Mehta and Gaur, 2005; Brinza et al., 2007).

In three recent research publications give the additional interesting facts on the construction of

algae cell walls, directly linked to sorption mechanisms (Nilanjana et al., 2008; Wang and Chen, 2009; Lesmana et al., 2009).

Table 2.4 summarizes the main disadvantages and advantages of the use of algal biomass for HMs biosorbents from WW (Brinza et al., 2007). On the basis of advantages the algae may be used as alternate to conventional adsorbent substances in the treatment of domestic and industrial effluents contaminated with THMs.

Table 2.3 Sorption parameters of biomass using Algae (Romera et al., 2006).

Alga	Metal	B (l/m mol)	q_{max} (m mol/g)
Chlorella vulgaris (G)	Cu^{2+}	4.44–13–34	0.25–0.76
	Cd^{2+}	56	0.3
	Pb^{2+}	38	0.47
	Zn^{2+}	6	0.37
Chaetomorha linum (G)	Cd^{2+}	1.43	0.48
Chlorella miniata (G)	Ni^{2+}	2.93–16–63	0.21–1.02
	Cu^{2+}	26.586	0.366
Cladophora glomerata (G)	Pb^{2+}	447.53	0.355
Chondrus crispus (R)	Pb^{2+}	3.315	0.941
	Ni^{2+}	12.92	0.443
Codium fragile (G)	Cd^{2+}	1.124	0.0827
Codium taylori (G)	Pb^{2+}	5.38	1.815
	Ni^{2+}	29.06	0.099
Ascophyllum nodosum (B)	Ni^{2+}	3.28–9.16	1.35–2.32
	Cd^{2+}	6.29–31.24	0.34–1.91
	Pb^{2+}	21.75–42.06	1.31–2.31
Corallina officinalis (R)	Cd^{2+}	21.356	0.2642
Fucus vesiculosus (B)	Pb^{2+}	32.11–62.77	1.10–2.90
	Ni^{2+}	16.14	0.392
	Cd^{2+}	2.36	0.649
Galaxaura marginata (R)	Pb^{2+}	9.11	0.121
	Ni^{2+}	4.638	0.187
Gracilaria salicornia (R)	Cd^{2+}	9.04	0.16

Gracilaria changüí (R)	Cd^{2+}	9.65	0.23
Gracilaria edulis (R)	Cd^{2+}	4.82	0.24
Gracilaria corticata (R)	Pb^{2+}	327.36	0.260
Gracilaria corticata (R)	Pb^{2+}	480.68	0.201
Sargassum baccularia (B)	Cd^{2+}	4.67	0.74
Sargassum fluitans (B)	Ni^{2+}	17.26	0.409
	Pb^{2+}	44.34	1.594
Sargassum natans (B)	Pb^{2+}	54.07–116.03	1.15–1.22
	Cd^{2+}	23.49	1.174
	Ni^{2+}	6.98	0.409
Sargassum vulgare (B)	Pb^{2+}	19.27	1.1
	Ni^{2+}	30.88	0.085
Sargassum hystrix (B)	Pb^{2+}	89.09	1.375
Sargassum sp. (B)	Cu^{2+}	0.236	1.08
Sargassum siliquosum (B)	Cd^{2+}	6.52	1.4
Porphira columbina (R)	Cd^{2+}	6.6	0.73
Polysiphonia violacea	Cd^{2+}	4.496	0.404
	Pb^{2+}	2,734.91	0.492
Padina gymnospora (B)	Pb^{2+}	108.98	0.314
	Ni^{2+}	3.933	0.17
Padina tetrastomatica (B)	Cd^{2+}	4.65	0.53
	Pb^{2+}	149.177	1.049
Padina sp. (B)	Cd^{2+}	5.37	0.53

R= red, G = green and B = brown,

Table 2.4 Advantages and disadvantages for the removal of THMs from WW by consuming algal biomass (Brinza et al., 2007).

Advantages	Disadvantages
• High proficiency of THMs removal. • High bioaccumulation ability. • Selectivity for THMs ions. • It be used in WW with higher THMs concentrations than for membrane processes. • Biomass may be reapplied in many adsorption/desorption cycles. • Biomass can be regenerated. • No noxious chemical sludge produced. • Macroalgal biomass does not need to be immobilized. • Algae may be applied all year round. • If dried alage biomass used, no nutrient or oxygen quantity desired. • Few chemicals needed for regeneration and desorption of biosorbent. • Appropriate for anaerobic and aerobic effluent treatment units. • Little cost	• Energy needed for drying dead algae biomass. • Microalgae need to be immobilized. • Microalgae have limited applicability in batch systems.

2.9.2 Classification and types of algae

In classification of algae numerous aspects may considered such as color and habitat which depends on receiving radiation and living sites. Other aspects such as, the cell wall chemistry, flagellation, cell morphology, reproductive structures, life story patterns can be consider in the classification of the algae. Further features used to classify algae have been the type of motility, the nature of chlorophyll(s) present, the cell wall structure, and the carbon reserve polymers produced (Wang and Chen, 2009). Algae can be classified into five groups as follows:

2.9.2.1 Green algae

Green algae have at least 7000 species. They are both freshwater and marine species and consists of the pigments such as chlorophyll and carotenoids in plants and the same storage product (starch). Green algae form filaments, complex moss or may be unicellular, sheets, spheres nets like structure. Specific species of green algae can grow on snow, or in synergetic relations justlike lichens, or with sponges.

2.9.2.2 Diatoms

Diatoms have over one hundred thousand species and considered the most attractive of the algae. The diatoms consist of cell wall made of glass with explicit shape of lines and dots it is found in the open sea and accountable for round one quarter of the O_2 gas produced on the earth/year and grow well in spring and provide food for zooplankton.

2.9.2.3 Red algae

There are 4-6000 species of red algae. Red algae are large, complex sea weeds. The species are edible and economically important source of agar and carrageenan which are applied as food thickener and stabilizers.

2.9.2.3 Brown Algae

Brown algae have about 1500 species and completely found in sea and comprise most complex and the biggest seaweeds.

2.9.2.4 Dinoflagellates

Dinoflagellates have 2-4000 species. Dinoflagellates are unicellular algae, set up in both freshwater and marine ecosystems with shield of cellulose and flagella that cause them to swim.

2.9.3 Algal metal binding sites

In biosoption method various algae are used as biosorbents for removal of THMs. Biosoprtion mostly depends on the spatial structure and the surface of the cell wall. Several polysaccharides, including chitin, glycan, cellulose, , alginate, etc. present in algae have been shown to play a important role in metal binding for algae biomass. Certain functional groups such as O^-, N^-, S^-, or P^-, have to bind metal ions, particularly carboxyl group. The hydroxyl, amino, carboxyl, sulphate groups and polysaccharides act as binding sites for THMs (Lesmana et al., 2009). Treated alage with acid may dissolve polysaccharide composites in the external layer of the algae cell wall to a certain degree, thus generating extra binding sites (typically amino groups).

The most important of these groups have been summarized by Volesky (2007) and contain: carbonyl (ketone), carboxyl, sulfhydryl (thiol), sulfonate, thioeter, amine, secondary amine, amide, imine, imidazole, phosphonate, and phosphodiester (Volesky, 2007). Some explicit examples of THMs biosoprtion with diverse algae are illustrated in the below paragraphs.

It has been desired to sustain the classificaion of alage broadly considering four main groups: marine green macroalgae, fresh water green microalgae marine red macroalgae, and marine brown macroalgae (Lesmana et al., 2009).

2.9.3.1 Marine green macro algae

Marine green macroalgae such as *Cladophora fascicularis* or *Caulerpa lentillifera* are considered as biosorbents for cations such as Pb^{+2}, Cu^{+2}, and Zn^{+2} (Deng et al., 2006; Pasavant et al., 2006).

The functional groups such as amides, sulfonate, carboxylic acids, amines and sulfonyl used in the THMs sorption properties in case of *Caulerpa lentillifera*. Where as in case of *Cladophora fascicularis*, carboxyl, and phosphate and amino were the key groups used in biosorption processes.

2.9.3.2 Fresh water green microalgae

Various green microalgae have also been used for the removal of THMs from the aquous solutions (Lesmana, 2009). Divalent THMs like Cu, Hg, Pb, Cd, Ni, and Zn have been experienced with *Chlorella miniata*, *Chlamydomonas reinhardtii*, *Cladonia rangiformis*, *Chlorella vulgaris*, and *Fucus spiralis*. Trivalent like Cr, and Fe and hexavalent cations like Cr have removed by using algae such as Spirulina *platensis*, *Chlorella vulgaris*, and *Chlorella miniata* respectively. The adsorption of Ni^{+2} and Cu^{+2} onto *Sphaeroplea* algae were recorded by Rao et al. (2005).

Chlamydomonas reinhardtii is sole cell green algae and cell wall of this algae is composed of hydroxyproline-rich glycoproteins. Tuzun et al. (2005) used this as a biosorbent for THMs removal and found that the functional groups such as amino, carbonyl, and carboxylic acid are responsible for biosorption onto *Chlamydomonas reinhardtii* cell walls.

Chlorella belong to the phylum *Chlorophyta* and a single cell green algae. *Chlorella vulgaris* was as a biosorbent originating from its porous cell wall, letting the free passage of THMs ions and molecules in aqueous solution (Lesmana et al., 2009).

2.9.3.3 Marine red macroalgae

The red marine macroalgae *Gelidium sesquipedale* has been studied for the complex process used in the biosorption (Vilar et al., 2008).

2.9.3.4 Marine brown macroalgae

The brown algae cell walls largely comprise three constituents (Lesmana et al., 2009): (1) alginic acid, a polymer of guluronic and mannuronic acids and the conforming salts of K, Na, Ca, and Mg; (2) cellulose, the structural support; and (3) sulphated polysaccharides. Thus, sulphate and carboxyl are the active groups in Marine brown macroalgae. An example of the biotechnological use of the mentioned alga is linked to *Laminaria japonica*. A modified variety was applied for the biosoption of Pb due to the rising amount of carboxylic groups exposed after the modification method (Luo et al., 2006).

Chapter-3

Removal and bioaccumulation of heavy metals from aqueous solutions using freshwater algae

ABSTRACT

Four freshwater algae, including *Cladophora glomerata*, *Oedogonium westii*, *Vaucheria debaryana* and *Zygnema insigne*, were tested for their bioaccumulation capacity for cadmium (Cd), chromium (Cr) and lead (Pb) in a controlled environment with an average temperature of 18 WC, and light/dark duration of 12:12 h. Experiments were performed in aqueous solutions containing selected heavy metals (HM) (ranging from 0.05 to 1.5 mg L^{-1}) with 0.5 g of living algae at 18 °C and pH 6.8. The results indicated that *C. glomerata* was observed to be the most competent species for the removal of Cr, Cd and Pb from aqueous solutions. HM removal trends were in the order of Cd > Cr >Pb while the removal efficiency of selected algae species was in the order of *C. glomerata*, *O. westii*, *V.debaryana* and *Z. insigne*. The bioaccumulation capacity of *C. glomerata*, *V. debaryana* and *Z. insigne* was observed for different HM. Removal of HM was higher with low levels of HM in aqueous solutions. The results indicated that *C. glomerata*, *O. westii*, *V. debaryana* and *Z. insigne* had significant ($P \leq 0.01$) diverse bioaccumulation capacity for Cr, Cd and Pb.

Keywords: bioaccumulation, *Cladophora glomerata*, heavy metals, *Oedogonium westii*, *Vaucheria debaryana*, *Zygnema insigne*

3.1 INTRODUCTION

Industrialization and urbanization have generated huge amounts of wastewater (WW) that is frequently discharged into surrounding environments such as the aquatic environmnet without prior treatment. WW contain high concentrations of toxic heavy metals (THMs) which are highly persistent in nature. HM can be accumulated in the tissues of living organisms through transport and transformation and resulted in long-term adverse effects on biological systems (Mehta and Gaur 2005; Singh et al., 2007; Deng et al., 2008; Lim et al., 2010; Abdel-Aty et al., 2013).

The HM- like Cr is essential element and plays an important role in biological systems and causes toxic effects when its bioaccumulated concentration exceeds the permissible limits. Cd and Pb are the non-essential elements and are highly toxic even present in trace amount in environmental matrixes (Krishna et al., 2009). So it is very important to overcome this problem by investigating a suitable and economically feasible technique to remove these contaminants from biological systems.

Algae are frequently available and can be easily cultivated in aquatic ecosystem. Previous research has illustrated that algae absorb nitrate, phosphorus and HM which led to improved the water quality (Li et al., 2010).

It is well recognized that various fresh and marine water algae are capable of sequestering HM selectively from aqueous solutions and storing metals such as calcium (Ca), cobalt (Co), magnesium (Mg), selenium (Se),Cu, Zn, Crand Pb within their cells by active biological transport (Afkar et al., 2010; Kumar and Gaur, 2011; Chen et al., 2012).

Blue green algae (*cyanobacteria*) were listed in the top 10 natural methods of removing HM form aquous solutions (Chojnacka et al. 2005; Patrick, 2009). Freshwater filamentous green algae (*Stigeoclonium sp.)* have a relatively higher sorption efficiency for Zn and mostly live in mining WW (Pawlik-Skowronska, 2001). Metal sorption efficiency remained high for algae

biosorbents even with very low concentrations of metal in aqueous solutions (Mehta & Gaur, 2005; Tastan et al. 2012; Piotrowska-Niczyporuk et al. 2012).

Algae,s biosorbent advantages can compensate for the disadvantages related to the commercial treatment of WW, in which the sorption efficiency reduces at very lower metal concentrations (Eccles, 1999). Most of the published research works are related to the removal efficiency using nonliving biomass, which showed that dead cells may sorbs more metals than living cells (Mehta and Gaur, 2005; Singh et al., 2012).

Previously, different algae species (living and nonliving) were studied for their ability to remove HM from aqueous solutions (Ajjabi & Chouba, 2009; Tuzen & Sari, 2010; Abdel-Aty et al. 2013).

In this study, benthic filamentous macro algae such as *C.glomerata, O. westii, V. debaryana* and *Z.insigne* were selected as testing materials due to their high adaptability, wide distribution, ease of cultivation all over the world to investigate their removal efficiency for Cd, Cr and Pb at different HM concentration and to compare the HM bioaccumulation capacity of these algae species to determine a feasible environmentally friendly method to treat contamined water such as industrial WW. Futhermore, using algae, this study will help to achieve the HM target limits as set by different organizations for the discharge of WW into the aquatic ecosystem.

3.2 MATERIALS AND METHODS

3.2.1 Sampling, identification and cultivation of algae

O.westii was collected from the pond ecosystem in Islamia College University, Peshawar, Pakistan, while *C.glomerata, V.debaryana and Z.insigne strains* were isolated from local freshwater ponds, present in Peshawar City, Pakistan. The collected strains were washed thoroughly with tap water to remove all visible soil particles and then finally washed with distilled water. These algae were examined under a microscope, taxonomically identified

using the published literature (Prescott, 1962) which was confirmed by a taxonomist in the Department of Botany, University of Peshawar, Pakistan. These algae were then cultivated in distilled water for 3 weeks at room temperature under natural light and used for the subsequent experimental work.

3.2.2 Preparation of solutions

Stock solutions of HM were prepared using Cd $(NO_3)_2$, Cr $(NO_3)_3.12H_2O$ and $Pb(NO_3)_2$ (analytical grade) in double deionized water (DDW). The initial concentrations of Cd, Cr and Pb were ranged from 0.05-1.5 mg L^{-1}. The culture medium was modified Gorham's medium dilute (1:5) consisting of nutrient solutions such as $NaNO_3$ (496 mg L^{-1}), K_2HPO_4(39 mg L^{-1}), $MgSO_4$ $7H_2O$ (75 mg L^{-1}), $CaCl_2$ $2H_2O$ (36 mg L^{-1}), Fe citrate (6 mg L^{-1}),$Na_2SiO_3.9H_2O$ (59 mg L^{-1}), Na_2CO_3(20 mg L^{-1}), citric acid (6 mg L^{-1}) and EDTA (1 mgL^{-1}) in DDW. Before use, the medium was sterilized by autoclaving at 121 °C for ten minutes and the pH was adjusted to 6.8 with 1M NaOH (Fitzgerald, 1968).

3.2.3 Experimental design

The experiment was carried out in Erlenmeyer flasks (250 mL) containing 180 mL of Gorham's medium to which 25 ml each of Cd, Cr and Pb solutions of desire concentrations 0.05,0.1,0.5,1.0 and 1.5 mg L^{-1} were added (pH 6.8). To avoid HM contamination, all glassware was soaked in 10 % HNO_3 for 24 h, washed with DDW and then oven dried at 80°C prior to use. Living algae such as *C.glomerata*, *O. westii*, *V. debaryana* and *Z. insigne* (0.5 g of each) were added to each flask. One set without HM was included as control treatment. Each experiment was conducted in quadrureplicate. During the incubation period of 7 days, the temperature was kept at 18℃, while light/dark duration was 12:12 h in a controlled and clean environment with continuous aeration and where 3000 flux light intensity was continuously provided using fluorescent tube lamps.

3.2.4 Heavy metal analysis

After 7 days of incubation, algae samples were removed from each culture, washed three times with 5mM EDTA then with DDW to remove superficially bounded metals.The samples were then shade dried followed by drying in oven at 80°C for 1 h. HMs were extracted from dried algae biomass using a modified method of Rybak et al. (2012). Briefly, 0.5 g of powdered algae was taken in a beaker containing 10 mL solution of HNO_3 (65 %) and H_2O_2 (30 %) (3:1). Then beakers were placed on a hot plate at 110°C untill the acid solution become colorless.it was then filtered through millipore filter paper (0.40 µm) into volumetric flask (50 mL) and the final volume was made using DDW. HMs concentrations in algae biomass were measured using atomic absorption spectrometer (Analyst 700 PerkElmer,Waltham, Massachusetts,USA) in the Centralized Resource Laboratory, University of Peshawar, Pakistan.

3.2.5 Quality control

For accuracy and precision, reagent blanks and standard reference materials were used in each batch. Plant reference materials (GBW10015(GSB-6)) was obtained from the National Research Center for Standards in China. The recovery rates of these metals were satisfactory and ranged from 92.4±8.2-103±10.7 %.The highest recovery was obtained for Cd, while the lowest recovery was achieved for Cr and followed by Pb.

3.3 Data analysis

3.3.1 Bioaccumulation measurement

Bioaccumulation capacity (q) (mg metal ions g^{-1} active biomass or living cells) was determined using the following equation (Flouty & Estephane, 2012):

$$q = \frac{C_{int}}{m} \times V \text{ ----------(Eq. 3.1)}$$

Where C_{int} is the intercellular metal concentrations (mg L^{-1}) after bioaccumulation; m is the amount of the active biomass (g) and V is the volume of the aqueous solutions (L).

3.3.2 Statistical analysis

The data were statistically analyzed using the statistical package SPSS 16.0, while graphs were prepared with the Sigma Plot (10.0 version) presenting the mean values using independent cultures of freshwater algae.

3.4 RESULTS AND DISSCUSSION

3.4.1 Removal efficiency

Table 3.1 summarizes the uptake of Cd, Cr, and Pb by *O.westii*, *C.glomerata*, *V.debaryana* and *Z. insigne* grown at different concentrations (0.05-1.5 mg L^{-1}). In *O.westii*, the uptake rates of Cd, Cr, and Pb were ranged from 0.038-0.230, 0.015-0.261 and 0.027-0.261 mg L^{-1}, respectively during 1 week. The removal trend was in the order of Cr > Pb > Cd from aqueous solutions when the concentrations of selected metals were low but overall its efficiency decreased with increasing the concentrations of metals when compared to other algae species (See Fig. 3.1).

Similarly, *C.glomerata* has higher efficiency for taking up each HM from aqueous solutions. The metal removal trend was in order of Cd > Cr > Pb from aqueous solutions at low concentration, demonstrating tha *C.glomerata* has potential for removing Cd and Cr. However it performance was not very good at lowest concentration (0.05 mg L^{-1}) for removing Pb. *C.glomerata* worked better and showed more efficiency at higher concentrations for removing Pb from aqueous solutions (Fig. 3.1).

For *V. debaryana* the trend for metal removal was in the order of Cr > Pb > Cd from aqueous solutions when selected metal concentrations were high (See Fig. 3.1), *Z. insigne* uptake rates were in the order of Cd > Pb > Cr from aqueous solutions at high concentration, indicating that this algae has significant ($P \leq 0.01$) potential for removal of Cd. However, it performed better for Pb at high concentrations, at low concentration its efficiency was decreased (Fig.

3.1). The results of our study showed that the bioaccumulation in the selected algae was highly dependent on the initial metal concentrations. At low metal concentrations, these algae showed effectiveness for Cd, Cr and Pb bioaccumulation, but at high metal concentrations their bioaccumulation decreased. *Z. insigne C. glomerata* and *V. debaryana* were observed as the best Cd sorbents (Fig. 3.1). The results of this study are consistence with those reported by Tien (2002). However, Zhou et al. (1998) found that the sorption of Cd by *Sargassum kjellmanianum* and *Laminaria japonica* were not altered with the initial metal concentrations. Gupta and Rastogi (2008) studied green algae *Spirogyra* species, they mentioned that a high dose can result in high bioaccumulation capacity due to availability of more sorption sites. These results are in agreement with the results obtained in this study. Similarly, Bajguz (2011) observed that *C. vulgaris* achieves the highest reduction in the bioaccumulation of metal at high concentration. All metal ions present in the solutions would interact with the binding sites at lower concentrations and thus facilitate maximum adsorption. More ions are left unabsorbed in the solution due to saturation of binding sites at higher concentrations (Nirmal and Oommen, 2012).

3.4.2 Bioaccumulationcapacity (q)

Table 3.2 summarizes the bioaccumulation capacity of *C.glomerata* for Cd, Cr, and Pb which ranged from 15.5-78.5, 13-173.2 and 4.3-86.4 mg g^{-1}, respectively. The results indicated that *C.glomerata* was more efficiently in the bioaccumulation of Cd compare to other HM such as Pb and Cd. *C.glomerata* bioaccumulation rates were 36.2, 61.2 and 42.4 % for Cd, Cr and Pb, respectively at metal concentrations of 0.1 mg L^{-1} in aqueous solutions. The result showed that *C.glomerata* has significant ($P \leq 0.01$) bioaccumulation capacity for HM at 0.1 mg L^{-1} concentration in aqueous solutions.

The bioaccumulation capacity of *O.westii* for Cd, Cr, and Pb were ranged from 13.9-82.8, 5.4-94 and 9.72-94 mg g^{-1}, respectively (see Table 3.2). The results indicated that *O. westii* was

more efficient in bioaccumulation of Pb when compared to other HM such as Cd and Cr.The result showed that *O. westii* has significant ($P \leq 0.01$) bioaccumulation capacity for HMs at 0.1 mg L^{-1} and 0.05 mg L^{-1} concentrations in aqueous solutions as compared to other levels of metals.

The bioaccumulation capacity of *V.debaryana* for Cd, Cr, and Pb ranged from 10.4-74.2, 6.5-123.1 and 11.2-103.3 mg g^{-1}, respectively (see Table 3.2). The results indicated that the *V.debaryana* has the highest bioaccumulation for Cr and Cd exposed to different concentrations of aqueous solutions as compared to other algae species.

The bioaccumulation capacity of *Z.insigne* for Cd, Cr and Pb ranged from 10.1-69.8, 9-197.6, 5.04-82.4 mgg^{-1}, respectively (see Table 3.2). These results indicated that *Z. insigne* was more efficiently in bioaccumulation of Cr as compared to Pb and Cd at 1.5 mg L^{-1} concentration. *Z.insigne* showed maximum bioaccumulation capacity for Cd (30.2 %) at 0.1 mg L^{-1} followed by Cr (71.5 %) and Pb (42.4 %) at 0.5mg L^{-1} concentration, as compared to other algae species.

The HMs used in this study are frequently present in industrial and domestic WW which lead to contamination of aquatic ecosystems and raise several ecological and human health problems (Pribylet al., 2008; Shen et al., 2009). Lower concentrations of HMs were tested in these experiments because Cd usually occurs at very low concentration in the water (Shen et al., 2009). Pakistan EPA had established maximum allowable concentrations of 0.1, 1.0, 0.5 mg L^{-1} for Cd, Cr, and Pb respectively, for industrial WW. These metals Cd, Cr, and Pb or their compounds have been used in a number of industries such as plastic, cable batteries, paints, steel and glass industries. The effluent from these industries can cause the contamination of aquatic environment.

The results of this study confirmed that *C.glomerata*, *O. westii*, *V.debaryana and Z.insigne* were highly competent in metal uptake during incubation period (7 days). These algae species

survived in the stress environment caused by the presence of toxic HMs, therefore, this could be considered as a positive clue for algae to be used for phycoremediation of contaminated water.

According to the results obtained in this study *O. Westii* has significant removal effect for Cd from aqueous solution and this removal efficiency reached to 47.9 % as compared to other algae species in this study. This particular species of algae showed great effectiveness at low metal concentrations in aqueous solutions, therefore, this algae can be used as economically feasible option for metals removal from contaminated water.

In addition, the high adaptability, wide distribution, easy cultivation and harvesting are the major characteristics of *O. westii* which position it as a potential agent for the treatment of contaminated water.

All the freshwater algae grew well under the experimental conditions described above, with no apparent yellow and dead parts after 7 days culturing in the solutions containing HM. In conclusion, the absorption of HM from water body using freshwater algae was relatively stable. It can be used to develop high capacity biosorbent materials for the removal and recovery of HMs ions from WW streams.

The cell surface is the focal site of metal binding in algae (Andrade et al., 2005), and sorption of HM involves the exchange of metal ions with surface bound cations or protons (Mehta and Gaur 2005). Our research showed that *Z.insigne* has the maximum bioaccumulation capability for Cr (See Fig. 3.1) followed by *C. glomerata* even when the concentration increased to a very high level in aqueous solution. Raiz et al. (2004) reported that Cd binding by *Sargassum sp*. involves complexation, whereas Adhiya et al. (2002) stated that Cd biosorption to *Chlamydomonas sp*. involves chelation with carboxylic groups. However, most of the research mainly focused on the removal efficiency using dead biomass of algae. Deng et al. (2006;2008) used nonliving *Cladophora sp*. to adsorb Pb and Cd and it has been stated that

dead cells could adsorb more metals than living cells (Mehta and Gaur, 2005; Singh et al., 2007).

Our results are in agreement with those reported by Ji et al. (2012). They observed that living algae have higher removal capability of HM than nonliving biomass because dead algae may gradually fall to the bottom of the water body and result in an increase of sediment. In comparison, living algae are cheap, widely available and work better than dead algae biomass (Ji et al., 2012).

3.5 CONCLUSIONS

This experiment investigated the bioaccumulation capacities of *C. glomerata, O. westii, V. debaryana* and *Z. Insigne* for Cd, Cr, and Pb when tested as living material. The bioaccumulation capacities were influenced by the initial metal concentration in the aqueous solutions. Maximum bioaccumulation was recorded at low concentrations of selected metals in aqueous solutions. *C. glomerata, V. debaryana and Z. insigne* take up more Cr when the initial metal concentrations increased in the aqueous solutions. It is extremely important to note that these algae could not only survive up to a period of 7 days but also maintain their unique abilities to remove HM from aqueous solutions. The high removal efficiency of these living algae, low labor input, low transportation cost, and high yields of biomass under cultivation make this treatment technology suitable for removal of toxic HM from aqueous solutions.

Table 3.1

Uptake of HM by selected algae species cultivated at different concentrations.

	Uptake (mg L^{-1})			
Conc. ($mg\ L^{-1}$)	***O. westii*** (n=4)	***C.glomerata*** (n=4)	**V.*debaryana*** (n=4)	***Z. insigne*** (n=4)
Cd				
0.05	0.038±0.004	0.043±0.01	0.029±0.003	0.028±0.004
0.1	0.071±0.001	0.058±0.001	0.037±0.002	0.053±0.014
0.5	0.142±0.028	0.148±0.006	0.190±0.058	0.174±0.013
1.0	0.158±0.017	0.163±0.021	0.152±0.036	0.106±0.017
1.5	0.230±0.071	0.218±0.015	0.206±0.021	0.194±0.032
Cr				
0.05	0.015±0.001	0.036±0.001	0.018±0.002	0.025±0.002
0.1	0.093±0.009	0.067±0.006	0.035±0.012	0.026±0.002
0.5	0.135±0.013	0.325±0.007	0.211±0.001	0.474±0.022
1.0	0.189±0.080	0.385±0.006	0.295±0.004	0.541±0.139
1.5	0.261±0.061	0.481±0.001	0.342±0.003	0.549±0.013
Pb				
0.05	0.027±0.002	0.012±0.001	0.031±0.002	0.014±0.004
0.1	0.082±0.003	0.071±0.001	0.043±0.001	0.041±0.003
0.5	0.174±0.052	0.142±0.028	0.132±0.016	0.229±0.043
1.0	0.234±0.005	0.194±0.028	0.230±0.012	0.135±0.035
1.5	0.261±0.006	0.240±0.023	0.287±0.014	0.225±0.031

Algae biomass 0.5g; pH 6.8; Temperature 18°C

Table 3.2 Bioaccumulation capacity (q) of selected algae for HM at different concentrations.

	Bioaccumulation capacity (q) (mg g^{-1})			
	O. westii	***C.glomerata***	***V.debaryana***	***Z. insigne***
THMs (mg L^{-1})	(n=4)	(n=4)	(n=4)	(n=4)
Cd				
0.05	13.7±1.3	15.5±2.3	10.4±0.7	10.1±1.4
0.1	25.6±4.2	20.9±3.0	13.3±1.2	19.1±1.8
0.5	51.1 ± 4.9	53.3±3.9	68.4±6.3	62.6±4.3
1	56.9±5.4	58.7±5.7	54.7±3.2	38.2±3.6
1.5	82.8±7.3	78.5±7.03	74.2±5.5	69.8±7.6
Cr				
0.05	5.4±0.8	13.0±1.8	6.5±0.7	9±1.4
0.1	33.5±2.2	24.1±4.5	12.6±1.9	9.4±1.53
0.5	48.6±6.6	117±11.2	76.0±8.9	170.6±21.4
1	68.4±8.4	138.6±12.7	106.2±10.2	194.8±17.4
1.5	94.0±7.6	173.2±23.4	123.1±11.5	197.6±24.4
Pb				
0.05	9.7±1.5	4.3±0.9	11.3±1.3	5.0±0.73
0.1	29.5±3.0	25.6±3.8	15.5±1.2	14.7±1.2
0.5	62.6±4.5	51.2±4.3	47.5±4.3	82.4±6.7
1	84.2±7.6	69.8±3.4	82.8±6.2	48.6±5.2
1.5	94.0±9.0	86.4±7.6	103.3±14.5	81.3±7.5

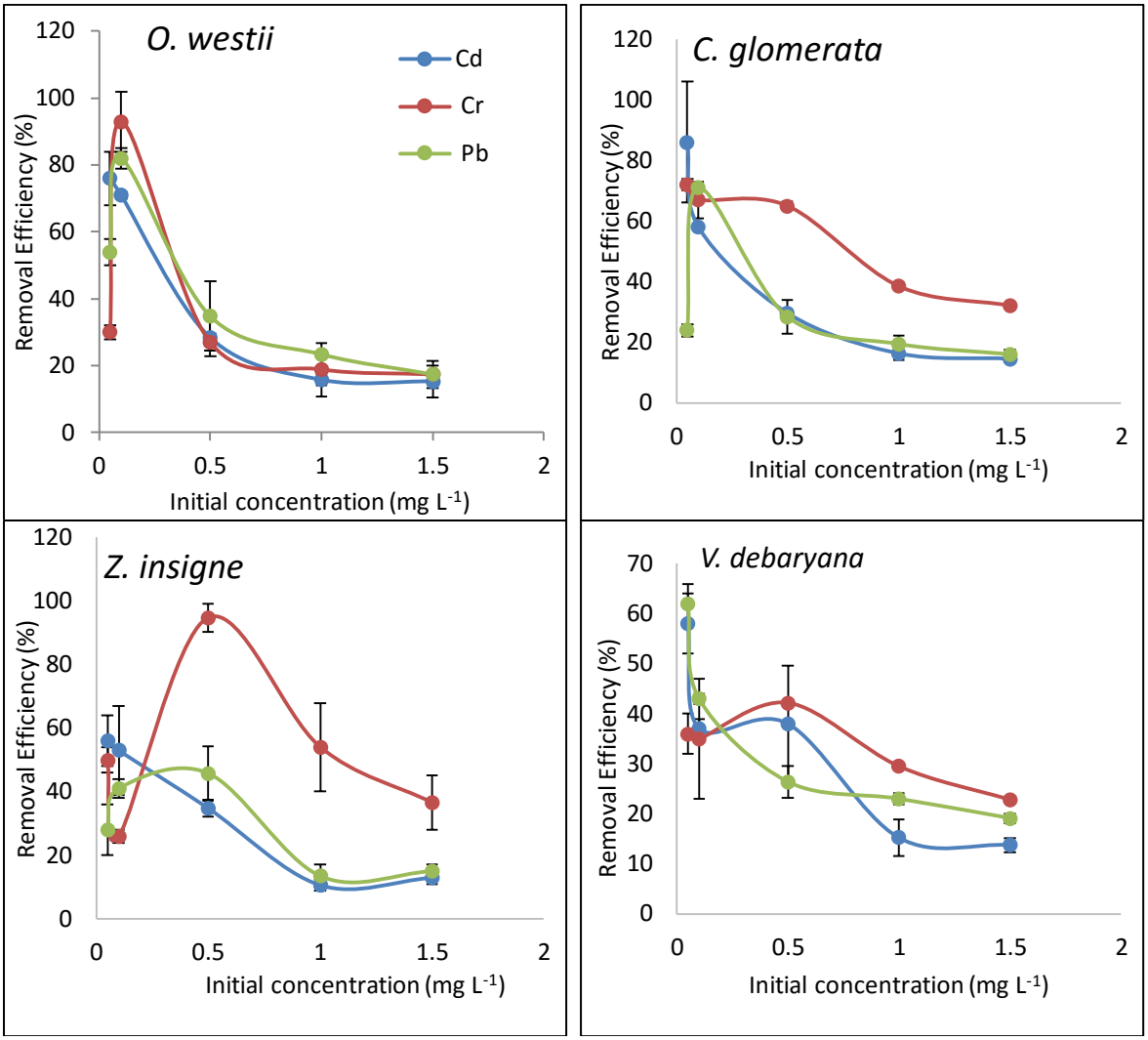

Fig. 3.1 HM removal efficiency (%) of different algae from aqueous solutions.

Chapter-4

Heavy metal uptake capacity by freshwater algae *O. westti* from aqueous solution

Abstract

The green macro-algae present in freshwater ecosystems have attracted a great attention of the world scientists due to heavy metal removal during WW treatment. In this study, the heavy metal uptake rate of *O.westti* was calculated. The equilibrium adsorption capabilities of *O.westti* were 0.974, 0.418, 0.620 and 0.261 mg g^{-1} for Cd, Ni, Cr and Pb, respectively at 18℃ and pH 5.0. The removal efficiency was 55-95, 61-93, 59-89, and 61-96 % for Cd, Cr, Ni, and Pb, respectively. The highest removal efficiency was observed for Cd and Cr from aqueous solution when the metal concentrations and pH were low, whereas for Ni and Pb the removal efficiency was highest at high pH and concentrations of metals in aqueous solution. The results summarized that *O.westti* are suitable to remove selected THMs from the aqueous solution and can be used in the treatment of WW.

Keywords*:* *Oedogonium westti;* Macroalgae; Uptake capacity; Wastewater treatment.

4.1 INTRODUCTION

Industrial, domestic and agricultural activities frequently generate massive amounts of wastewater (WW) that are mostly discharged into the water bodies without prior treatment. This practice has led to water contamination with organic and inorganic pollutants (Nguyen-Ngoc et al., 2009; Lim et al., 2010). The inorganic pollutants mostly present in WW are THMs including Cu, Cd, Cr, Ni, Fe, and As. These pollutants have great adverse impact on environment due to their high toxicity, persistency, and incremental accumulation in the living tissues through transformation and transportation resulting in possible severe and

toxicological effects on human health and ecological life in water ecosystem (Chen et al., 2008; Liu and Li, 2011; Zhang and Shao,2013; Zeng and Wu, 2013; Yangetal., 2014). The adverse effects of these inorganic pollutants on aquatic organisms have been attracted the world attention to find effective ways to mitigate THMs from water.

Chemical precipitation, adsorption, ion exchange, and purification by membrane techniques are the conventional methods used for water treatment to mitigate THMs from the aquatic environment (Chen et al., 2002; Pan et al., 2009; Fuand Wang, 2011; Turker, 2012). All these methods are mostly expensive, incompletely remove THMs, require high energy and their efficiencies are lower than biosorption techniques (Preetha and Viruthagiri, 2005; Sari et al., 2008; Azza et al., 2013; Weng et al., 2014). In addition, the production of secondary waste is also associated with conventional techniques (Volesky, 2001; Li et al., 2013), which is a great challenge for environmentalists. It is very desirable to find out alternative, low cost environmental friendly innovative technologies to remove THMs from WW. The freshwater algae, a renewable natural biomas show great potential towards different metal removal from the aqueous solutions because of their main constituents such as carbohydrates, proteins and phenolic compounds consisting of metal bonding groups such as amines, carboxylates and hydroxyls (Lesmana et al., 2009; Song et al., 2014) Fresh water algae are very common in fresh water ecosystems and present throughout the globe. These algae has closely linkages with human life (Lee and Chang, 2011). The previous research demonstrated that algae improve the water quality by absorbing THMs, nitrate (NO_3) and phosphorous (P) (Li et al., 2010) and *cyanobacteria* were identified first time in 2009 for the removal of THMs (Chojnacka et al., 2005; Patrick, 2009).

Algae has high metal removal efficiency at low level of metal ions in aqueous solution (Mehta and Gaur, 2005) and *Oedogonium rivulare* and *Cladophora glomerata* continuously uptake Cd, Cu, Co, Pb, Ni, Cr, Fe and Mn from WW (Vymazal, 1984). The trends in THMs

uptake vary greatly among different algae and generally observed in order of (green algae) *Chlorophyta* > *Phaeophyta* > (red algae) *Rhodophyta* (Al-Shwafi and Rushdi, 2008). The nonliving biomass of algae sorb more metals than living algae (Mehta and Gaur, 2005). Pawlik-Skowronska (2001) discovered that *Stigeoclonium sp*. fresh water algae can live in mining water having high concentration of Zn and had high uptake efficiency. In living algal cells, trace metals intracellular accumulated by active biological transport (Ajjabi and Chouba, 2009).

It is well well-known that several fresh water algae are able to uptake various THMs from aqueous solution and bioaccumulate these metals within their cells (Afkar et al., 2010; Kumar and Gaur, 2011; Chen et al., 2012). However, further research work is needed to investigate individual algae efficiency at different environmental conditions. This study aimed to assess the capacity of *O.westti* (freshwater macro algae) for removal of Cd, Cr, Ni, and Pb from aqueous solution at different conditions such as different concentrations of THMs and various ranges of pH. Adsorption equilibrium, adsorption isotherm and Langmuir model were used to study the potential of *O.westti* for removal of THMs from aqueous solution.

4.2 MATERIALS AND METHODS

4.2.1 Algae cultivation

Filamentous algae (*O.westti*) collected from fresh water ponds present in Islamia College University (ICU), Peshawar, Pakistan. Algal biomass was washed thoroughly with tap water to remove yellow ageing parts several times and finally with deionized water. The algae was cultured and acclimated for 14 d in distilled water at room temperature 18±1℃ and natural light. The acclimated algae were used for further experimental work.

4.2.2 Chemicals

All the chemicals were used of analytical grade. The Cd $(NO_3)_2$, Cr_2 $(NO_3)_2.12H_2O$, Pb $(NO_3)_2$ and Ni $(NO_3)_2$ salts were used to prepare the THMs stock solution of selected metals in deionized water. The THMs stock solutions were diluted to the desired concentrations.

4.2.3 Experimental design

The uptake experiments were conducted in Erlenmayer flasks (500 mL) that were washed with dilute HNO_3 to remove metals and then washed with dionized water. Each flask contained 0.5 g of freshwater algae and 50 mL of metal solution of desire concentration. Two controls (One control without algae, while another with but without THMs were included to confirm metal contamination and effects of environmental conditions/factors on the growth of algae during the experiment. The initial concentrations of Cd, Cr, and Ni were observed within the range of 0.5 to 2 mgL^{-1}, while Pb was within the range of 0.1-0.8 mgL^{-1}. All treatments were prepared in five replicates and the experiment was performed for one week under natural light/dark 12:12h at temperature 18±1°C.

4.2.4 Effect of pH on THMs adsorption

To study the effect of pH on THMs adsorption, a series of experiments were conducted with 50 mL of selected THMs solution of desire concentrations ranging from 0.5 to 2 mgL^{-1} for Cd, Cr and Ni, while for Pb the concentration was within the range of 0.1 to 0.8 mgL^{-1} to investigate the effect of different pH range from 4 to 6 on the adsorption. The pH was adjusted with diluted NaOH or HNO_3. The pH was continuously checked with one hour interval and kept stable during the experiment.

4.2.5 THMs analysis

The extraction of THMs from algal biomass was done using a method mentioned by Rybak et al. (2012) with little modification. Briefly, 0.5 g of algae was taken in a beaker and dissolved in HNO_3 and H_2O_2 (3:1, v/v). Then beakers were put on a hot plate at 110℃ till the acid solution turn into colorless. After cooling, the extracts were filtered through whatman paper no. 42 into a 50 mL volumetric flask and final volume was made up to 50 mL with deionized water. THMs in algae biomass were analyzed using atomic absorption spectrometer (Analyst 700 Perk Elmer) in the Centralized Resource Laboratory, University of Peshawar, Pakistan.

4.2.5. Quality control

For precision and accuracy standard reference materials, and reagent blanks were used in each batch. Plant reference material (GBW10015 (GSB-6)) was purchased from the National Research Center for Standards in China. The recovery rates were ranged from 91.4 ±7.2-102± 9.7 %.

4.3 Data analysis

4.3.1 Adsorption equilibrium

The sorbed concentrations of THMs in *O.westti* were determined by the below mentioned formula:

$$q = (Ci - Ce)/W \text{ (Li et al., 2011)----------(Eq. 4.1)}$$

Where,

q = the adsorption amount at equilibrium (mgg^{-1}),

C_i = the initial concentration of THMs (mgL^{-1}),

C_e= the concentration remaining in solution at equilibrium (mgL^{-1}), and

W = the bio sorbent dosage (gL^{-1}).

4.3.2 Removal efficiency

The removal efficiency (%) was calculated by the following formula:

$$r = (\mathrm{Ci} - \mathrm{Ce})/\mathrm{Ci} \times 100$$ (Li et al., 2011)----------(Eq. 4.2)

Where,

r = the removal percentage at each testing time,

C_i = the initial concentration of THMs (mmol L^{-1}), and

C_e =the concentration remaining in solution at each testing time (mmol L^{-1}).

4.3.3 Adsorption isotherm

The equilibrium sorption isotherms well defined the capacity of an adsorbent. Which is described by certain constants whose values stated by the affinity of absorbent and surface properties. The Langmuir isotherms widely used to quantify the metal sorption by the test algae (Langmuir, 1918). The Langmuir model was expressed as follow:

$$Qe = \frac{Q_m b \mathrm{Ce}^{1-c}}{1+b\mathrm{Ce}^{1-c}}$$ (Li et al., 2011; Yalcin 2013)----------(Eq. 4.3)

Where,

Q_e = metal sorbed at equilibrium (mgg^{-1}),

C_e = equilibrium metal ion concentration (mgL^{-1}) and

Q_m = maximum amount of metal sorbed (mgg^{-1}) and b and c are Langmuir constants

4.3.4 Statistical analysis

The data were statistically analyzed using the statistical package SPSS 16.0, while graphs were prepared with Sigma Plot (10.0 version) presenting the mean values and standard deviation of five replicates.

4.4. RESULTS

4.4.1 pH effects on metal removal efficiency

Table 4.1 summarizes the equilibrium absorbing amounts of Cd, Cr, Ni, and Pb by *O.westt*. The metal sorption efficiency by *O.westti* was greatly affected with the initial concentrations of metals in the aqueous solution. Metal adsorption increased with increasing metal concentration in aqueous solution. The metal concentrations slightly decreased in the control treatment without algae. The adsorption amount of each control was determined by subtracting it from the equilibrium-absorbing amount of each concentration in the result. No metals were detected in the algae cultivated in control treatment. The equilibrium absorbing amount were 0.97, 0.42, 0.62 and 0.26 mgg^{-1} for Cd, Ni, Cr, and Pb respectively. The selected algae (*O.*westti) absorbed each of the selected metals from the aqueous solution at different pH and different concentrations, as shown in Fig. 4.1.

At pH 4 and 4.5, the Cd removal efficiency was observed 95 and 89 %, respectively at concentration of 0.1 in the solution. The increase in pH decreased the removal efficiency of Cd at low concentration. However, an increase in both pH and Cd concentration in aqueous solution slightly decreased (55.2 %) the removal efficiency of *O.westti* (Fig.4.1). *O.westti* performance was very well when the concentration of Cd was very low (0.1 mgL^{-1}). The initial Cd concentration remarkably influenced the equilibrium Cd sorption.

For Cr, at pH 4 the removal efficiency of *O.westti* was high at different concentrations of Cr in solution and ranged from 82-93 % (Fig. 4.1). When the pH increased, the removal efficiency of Cr by *O.westti* reduced to 61-87 % at low concentration.

For Ni, the removal efficiency by *O.westti* increased with the increasing pH and metal ion concentrations in the aqueous solutions. At pH 4, the removal efficiency was high even at high concentration (2.0 mgL^{-1}) reached to 86.6 % in aqueous solution. But when the pH increased the removal efficiency of Ni by *O.westti* slightly decreased (59.0-68.8 %).

O.westti sorbed Pb from the aqueous solution at different concentrations and pH. The removal efficiency increased with increasing the concentrations of metal ions. At pH 6, the removal efficiency ranged from 91-96 % even at high concentration of Pb in aqueous solution (Fig. 4.1). *O.westti* showed highest efficacy for sorption of Pb from aqueous solution even at high pH and Pb concentrations in aqueous solution. Similarly results has been reported by (Meitei and Prasad, 2014) and further increase of pH causes reduction of metal adsorption due to metal-hydroxide ions formation (Meitei and Prasad, 2013a)

Table 4.2 summarizes, the test of between- subject effects as applied on the removal efficiency with the concentration (A) and pH (B). Both factors A and B significantly affected the metal removal efficiency at ($p < 0.01$).

4.4.2 Adsorption isotherm and Langmuir model

The Langmuir model was applied for the adsorption isotherms. The basic postulated mechanism of this model is that the adsorption occurs at active site present on algae biomass within the aqueous solution of THMs. The Langmuir model was well fitted on the data (Fig. 4.2). The high correlation coefficient (R^2=0.913) was obtained, and constants Q_m, b and c were also calculated (see Table 4.3). The data indicated a high variation in the model constants for selected metals including Cd, Cr, Ni, and Pb sorption by*O.westti*. Obviously the Q_m value for Cr was highest 5.083 (see Table 4.3) followed by Ni. According to the results of Cr, Ni and Pb, a good metal sorbent should have high value of Q_m (Kratochvil and Volesky, 1998; Li et al., 2011) particularly for Cr.

4.5 DISCUSSION

The data summarized in this study that *O.westti* obviously removed Cd, Cr, Ni, and Pb from aqueous solutions. The removal efficiency varied because of different pH and metal concentrations. The Cd and Cr removal efficiency reached to 95 and 93 %, respectively when the metal concentrations were very low in aqueous solution and the pH was 4. The Ni removal efficiency reached to 89 % at pH 4, while Pb removal efficiency reached to 96 % at pH 6 at high concentrations of metal ions in the aqueous solution. The results indicated that *O.westti* are eco-environment friendly for the treatment of domestic and industrial WW because of their easy availability, wide distribution, easy cultivation and has low cost.

The metal concentrations used in this study were usually observed in the industrial WW streams. The Cd, Cr and Ni concentrations were different (0.1-2mgL^{-1}) than Pb concentrations (0.1-1mgL^{-1}) because of the reason that Cd and Pb usually occur in the water body at a low concentration (Shen et al., 2009). The natural Cd concentrations hardly exceeded the WHO (2006) permissible limit (3 µgL^{-1}). Cr is an essential species to mammals since it helps the body to control blood-sugar levels when present in trace concentrations, but hazardous to fish when its concentration in water exceed 5.0 mgL^{-1} (Alloway and Ayres, 1997). National environmental quality standard for maximum allowable concentration of Cd, Cr and Pb (0.1, 1.0 and 0.5 mgL^{-1}, respectively) were set by Pakistan EPA, 1993 for industrial WW.

In experiment we used freshwater algae *O.westti* which grow very well under the experimental condition mentioned above with no yellow and dead part apparently after 7 days culturing in the aqueous solution containing the selected THMs. This study results howed that *O.westti* metal sorption reached to high levels at low pH level and concentrations of Cd and Cr in aqueous solution, but for Pb and Ni the metal sorption was highest at high pH and metal concentrations in aqueous solutions.

When the pH is low, the whole surface charge on the algae cell wall will turn into positive,

which constrains the method of positively charged THMs cations. With the increase of solution pH, biosorbent surface is more negatively charged and the functional groups of the biomass were more deprotonated and thus available for metal ions (Lodeiro et al., 2006). Our research shows that *O.westti* has the highest uptake capacity for the Cr even when the concentration are very low and for Ni the uptake capacity are high even the concentration in aqueous solution are high. The algal species *sargassum sp*. acts as chelator for Cd (Raiz et al., 2004) whereas Adhiya et al. (2002) described that Cd sorb to *Chlamydomonas sp*. make complexation with carboxylic groups. Deng et al. (2008) studied dead *Cladophora sp*. to sorb the Pb and Cd. They mentioned that cell could sorb more metals (Mehta and Gaur, 2005; Singh et al., 2007). Our study clearly showed that high removal efficiency of selected THMs was obtained using living algae. Nonliving algae gradually subside in the bottom of the ponds, streams or rivers and increase the sediment, in contrast the living algae cost are affordable and work better than the dead materials. In conclusion the *O.westti* are very stable in the removal of toxic metals from WW streams and can be used to develop a high capacity bio-sorbents for the removal of THMs.

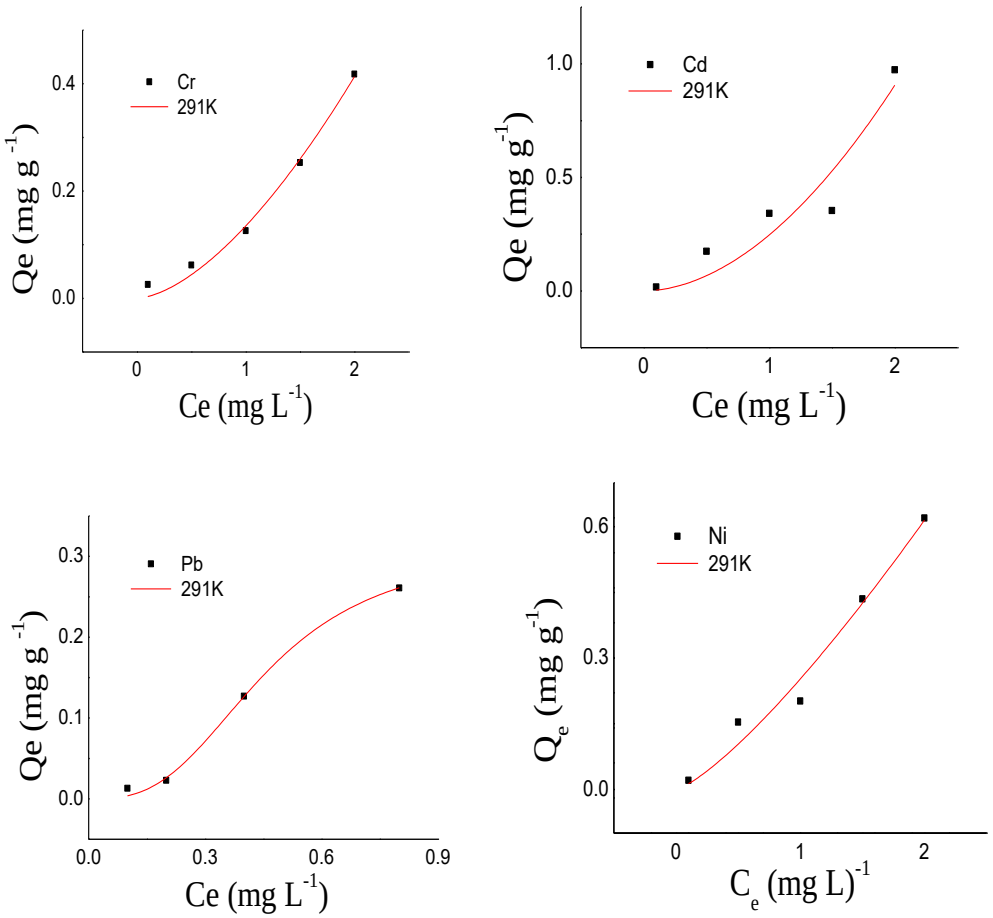

Fig. 4.1 Adsorption isotherm of Cd, Cr, Ni and Pb by *O. westti*

Table 4.1

Equilibrium absorbing amount of selected THMs in *O. westti*

Initial concentration (mg L^{-1})	Q_e (mg g^{-1})			
	Cd	Ni	Cr	Pb
0.1	0.017	0.021	0.026	
0.5	0.175	0.154	0.062	
1	0.342	0.202	0.126	
1.5	0.354	0.435	0.253	
2	0.974	0.620	0.418	
0.1				0.013
0.2				0.023
0.4				0.127
0.8				0.261

Algal biomass 0.5 g; pH 5.0; temperature 18℃

Table 4.2

Between-subjects effects of THMs dependent variable R

THMs	Factors	F	Sig.
Cd	A	11.44	0.000
	B	56.03	0.000
Cr	A	6.37	0.003
	B	119.6	0.000
Ni	A	5.54	0.005
	B	53.40	0.000
Pb	A	9.77	0.000
	B	52.21	0.000

R= removal, A= pH, B= Concentration

Table 4.3

Langmuir constant for the sorption of test metals

THMs	Langmuir constant			
	Q_m (mg g^{-1})	B	c	R^2
Cd	0.974	0.370	-1.417	0.731
Cr	5.083	0.128	-0.164	0.913
Ni	3.228	0.180	-0.326	0.834
Pb	2.185	0.421	-0.336	0.768

Chapter-5

Biosorption capacity of fresh water algae for THMs from aqueous solutions.

Abstract

Freshwater algae biomass have studied for the biosorption potential in the removal of THMs using different concentration and dosage from aqueous solution. The biosorption method is more effective in the removal and recovery of THMs. In this study four alga species biomass such as *C.glomerata, O.westii, V.debaryana, Z. insigne* were used to remove Cd, Cr, Ni and Pb from the aqueous solution. Z. *insigne* shows high biosorption efficiency for the removal of Cr, Pb, and Ni at low concentration and the biosorption reached to 78, 58 and 37 % respectively from the aqueous solution at low concentration. The metal biosorption capability is significantly ($P \leq 0.05$) changed with the biomass dosage, with the increase of biomass dosage, the metal biosorption capabilities slightly decreased. In this study all alga species at low dosage, the biosorption are highest for the Cr followed by Ni.

Keywords: Biosorption; Freshwateralgae; Heavymetals; *C. glomerata, O. westii, V. debaryana, Z. insigne*

5.1 INTRODUCTION

Over growth of urbanization and industrialization has significantly enhanced the degradation of our aquatic ecosystems through the release of domestic wastes and industrial effluents (Leghouchi et al., 2009; Nguyen-Ngoc et al., 2009) consisting of persistence and THMs such as Cd, Cr, Cu, Pb, Zn or their compounds have been widely used in variety of metal-finishing and chemical industries (Hamidi et al., 2008). The living organisms need trace amounts of diverse THMs including cobalt (Co), Cu, iron (Fe), strontium (Sr), manganese (Mg), molybdenum (Mo), vanadium (V), and Zn. Too much levels of essential HMs can be toxic for the organisms (Shanab et al., 2012).

Non-essential THMs such as arsenic (As), antimony (Sb), mercury (Hg), Cd, Cr, and Pb, are of particular concern and effect on metabolic or developmental process in all living organisms including hepatic injury, lungs damage, and renal dysfunction (Martins et al., 2006; Sari et al., 2008; Pathak et al., 2009). Beyersmann and Hartwig (2008) studied that Cd, Cr and Pb are carcinogenic to human.

Due to increasing discharges from the permissible limits, the scientists are in search for the cheap, environmental friendly and innovative methods for the removal of these toxic metals from the industrial effluents and WW. The current research shows that biosorption is the easily applicable method having great removal efficiency in comparison with the traditional methods for the treatment of WW (Ekmekyapar et al., 2006; Zhang et al., 2010).

Biosorption is an innovative method for the removal and recovery of THMs from the WW using dead and inactive biomass of different alga species. The researchers are taking keen interest because of the alternative methods for the treatment of WW. The scientist has focused to search for new biosorbent that has high sorption capacity especially on algae due to their abundance in various environmental systems, ability of adaptation to environmental conditions (Klimmek et al., 2001; Romera et al., 2007; Rajfur et al., 2010).

In the last decade, different biomasses such algae, fungi and bacteria are studied in the removal of THMs from WW with the aim to identify highly efficient biosorbent.

Algae can be found anywhere from soil to rocks and from ponds to oceans. Algae make up a large and diverse group of organisms. Some algae are single-celled microscopic organisms, while others are not. On the basis of color scientists identify and classify the algae species. Different algae species biomass are used for the biosorption such as red algae ((Holan and Volesky, 1994), brown algae (Chongand Volesky, 1995; Matheickal and Yu, 1999) and green algae (Donmez et al., 1999). The only difference between these algae types are the cell wall where biosorption take place. The cell wall of these algae consisting of different compounds such as cellulose, protein, the structural support and alginic acid. These compounds contain several functional groups such as carboxyl, hydroxyl amino and sulphate which play important role in the biosorption process (Romera et al., 2007).

The aim of the present study to evaluate the biosorption capacity of four fresh water algae such as *C. glomerata, O. westii, V. debaryana and Z. insigne* for the removal of Cd, Cr, Ni, and Pb from the aqueous solution at different concentrations and amount of dosage .

5.2 MATERIALS AND METHODS

5.2.1 Biomass preparation

O.westti was collected from ponds of Islamia College University (ICU), Peshawar and *C. glomerata, V. debaryana* and *Z. insigne* were collected from the natural ponds ecosystem in Pakistan. The collected algae samples were washed thoroughly with tap water several times to remove any adhering debris. The algae species were oven dried as mentioned by (Ibrahim, 2011) at 60 °C for 24 h ground and sieved through (< 0.5 mm) mesh. The biomass was kept in air tight polyethylene bottles until further needed.

2.9.3 Chemicals

All the standard chemicals were used of analytical grade. The Cd $(NO_3)_2$, Cr_2 $(NO_3)_2.12H_2O$, Ni $(NO_3)_2$ and Pb $(NO_3)_2$ salts were used to prepare the THMs stock solution of selected metals in deionized water. The THMs stock solutions were diluted to the desired concentration.

5.2.3 Experiment design

The biosorption experiments were performed in 250 mL Erlenmayer flasks that were rinsed with 10 % dilute HNO_3 to remove metal ions and then washed with distilled water. Two controls were run to confirm if something besides the algae could have caused the observed effects. One control which did not receive dry algae, and another used dry algae with no metal solution. The initial concentrations of Cd, Cr and Ni and Pb were 1, 2, 4, 8, 10 mgL^{-1}. Each flask contained 0.5 g of dry algae biomass. Flasks were shaken for 2 h at 200 rpm and room temperature 25±1°C (Ibrahim, 2011).

In the 2nd part of the experiment different dosage of algae biomass were used in the 250 mL Erlenmayer flasks that were rinsed as mentioned previously. Each flask contained 50 mL of metal solution. Different biomass dosage (0.5, 1.0, 1.5, 2.0, and 2.5 g) was used in this experimentand the pH was adjusted to 5. Flasks were shaken for 2 h at 200 rpm and room temperature 25± 1 °C (Ibrahim, 2011).

5.2.4 THMs analysis

The contents of the flask were filtered through filter paper and the filtrates were studied for THMs concentration. Briefly, filtrates was taken in a beaker and was dissolved in HNO_3 and H_2O_2 (3:1, V/V).Then beakers were then put on a hot plate at 110 ℃ till the acid solution turn into colorless then filtered through whatman paper no. 42 into a 50 mL volumetric flask and final volume was made up to 50 mL using deionized water. THMs in samples was analyzed by using Atomic Absorption Spectrometer (AAS) (Analyst 700 Perk Elmer) in the Centralized

Resource Laboratory, University of Peshawar, Pakistan.

5.2.5 Quality control

Standard Plant reference materials (GBW10015 (GSB-6)) were purchased from National Research Center for Standards from China to check precision and accuracy and reagent blanks were also run in each batch. A recovery rate was ranged from 92.4 ±6.2-103± 8.3 %.

5.3 Data analysis

5.3.1 Adsorption equilibrium

The amount of THMs ions sorbed by different algae biomass were calculated by the following formula:

$$q = (Ci - Ce)/W \text{ (Li et al., 2011)} \quad \text{----------(Eq. 5.1)}$$

Where,

q = the adsorption amount at equilibrium (mgg^{-1}),

C_i = the initial concentration of THMs (mgL^{-1}),

C_e = the concentration remaining in solution at equilibrium (mgL^{-1}), and

W = the biosorbent dosage (gL^{-1}).

5.3.2 Biosorption removal efficiency

The removal efficiency (%) was calculated by the following formula:

$$r = (Ci - Ce)/Ci \times 100 \text{ (Li et al., 2011)} \quad \text{----------(Eq. 5.2)}$$

Where,

r = removal percentage at each testing time,

C_i = initial concentration of THMs (mmol L^{-1}), and

C_e =concentration remaining in solution at each testing time (mmol L^{-1}).

5.3.3 Biosorption isotherm

The equilibrium sorption isotherms well defined the capacity of an adsorbent which is described by constants whose values expressed by the surface properties and affinity of absorbent. The Langmuir isotherms widely used to quantify the metal sorption by the test algae (Langmuir, 1918). The Langmuir model was expressed as follow:

$$Qe = \frac{Q_m b \mathrm{Ce}^{1-c}}{1+b\mathrm{Ce}^{1-c}} \text{---------- ---------} \quad \text{(Eq. 5.3)}$$

Where,

Q_e = metal sorbed at equilibrium (mgg^{-1}),

C_e = equilibrium metal ion concentration ($mg\ L^{-1}$) and

Q_m = maximum metal sorbed ($mg\ g^{-1}$) and b and c are Langmuir constants.

5.3.4 Statistical analysis

The data were statistically evaluated using the statistical package SPSS 16.0 and origin 8.0 while graphs were made with the Sigma Plot (10.0 version) presented the mean values and standard deviation of three replicates in order to assess biological and analytical variability.

5.4 RESULTS AND DISCUSSIONS

5.4.1 Biosorption of THMs by *C. glomerata* biomass

Different biomass dosage within the range of (0.5-2.5 g) of *C. glomerata* were studied for the biosorption of selected metals The metals biosorption capability are greatly changed with the biomass dosages, with the increase of biomass dosage the metal biosorption capabilities slightly decreased. *C. glomerata* sorb highest Cr at different biomass dosage followed by Ni. At low biomass dosage 0.5 g, it adsorbed more Cr ions from the aqueous solution (Fig. 5.5). High biosorption was recorded at less biomass dosage, when the biomass dosage increased its biosorption capabilities decreased. At 1.5 and 2.0 g dosage the biosorption potential of *C. glomerata* are almost the same for Cr and Ni followed by Cd > Pb (Fig. 5.5). This is because of the partial aggregation of biomass which decreases effective surface area for the

biosorption (Karthikeyan et al., 2007). *C. glomerata* biomass shows high biosorption capabilities for all the selected metals especially for Ni and Cr in comparison with other algae species in this study.

5.4.2 Biosorption of THMs by Z. insigne biomass

The *Z. insigne* different dosages (Fig. 5.5) were used to study the biosorption of THMs ions. With the increase in the biomass dosage, the metal biosorption capacity slightly decreased and reached to a point where it shows the same adsorption capabilities for the selected metal ions like *C.glomerata*. The biosorption of *Z. insigne* for the selected metal ions were in the order of Cr > Ni > Cd > Pb at different biomass dosage, but highly biosorption recorded at low biomass dosage and biosorption slightly reached to approximately constant for the selected metals at biomass dosage 2.0 and 2.5 g . But at 2.0 g the biosorption capability of this alga specie are more effective in comparison with 2.5 g. Because of the partial gathering of biomass which reduce effective surface area for the biosorption (Karthikeyan et al., 2007; Ibrahim, 2011). After *C.glomerata* the *Z. insigne* shows high biosorption for Cr > Ni followed by Cd > Pb.

5.4.3 Biosorption of THMs by *V. debaryana* biomass

The *V.debaryana* biosorption capabilities were tested for the selected THMs. This algal species shows high biosorption stability for Cr (16.4 mgL^{-1}) followed by Ni (14.9 mgL^{-1}) at 0.5g dosage. This algal biosorption capacity for Cr was highest at different dosage, with the increase in the biomass dosage the biosorption capacity decreased (Fig. 5.5). The biomass dosage is very important parameter during metal biosorption. The algae biomass at high cell densities, the biomass takes up more metal ions at a given equilibrium concentration (Mehta and Gaur, 2001; Romera et al., 2007) and electrostatic interactions is very significant factor in the relationship between metal sorption and biomass concentration. The lower the biomass concentration in suspension, the higher will be the metal biosorbent ratio and the metal hold

by sorbent unit, unless the biomass gets to saturation. High biomass can serves as shield protecting the active sites from being occupied by metal. This does not happen with Pb since this is the metal with the highest affinity for the biomass. Several scientists support such biosorption results (Modak and Natarajan, 1995; Sandau et al., 1996; Veglio and Beolchini, 1997; Donmez et al., 1999; Hammaini et al., 1999).

5.4.4 Biosorption of THMs by O.westti biomass

The effect of *O.westti* biosorption capabilities were studied for the selected THMs ions using different biomass dosage in the range of 1-2.5g. This algal species shows high biosorption stability for Cr (15.8 mgL^{-1}) followed by Pb (13.9 mgL^{-1}) at 0.5 g dosage. This algal bio sorption capacity for Cr was highest at different dosage. With the increase in the biomass dosage the biosorption capacity decreased. At high dosage 2.5 g the biosorption capacity of this algal for Cr decreased in comparison with 1.0, 0.5g , dosage followed by Pb > Cd > Ni (Fig. 5.5).

5.5 Biosorption isotherm Langmuir Model

The Langmuir Isotherms models are mainly used worldwide for biosorption because this model supposes that sorption process occur at specific sorption surface. This model basic postulated mechanism is illustrated by certain constants values which can applied to compare diverse biosorbent for various pollutants (Dursun et al., 2005) and the sorption process takes place at a specific sorption surface. The attraction between molecules decreasesas getting further from the sorption surface. In this study Langmuir equation were used to fit the experimental data (Fig. 5.1, 5.2, 5.3 & 5.4) for all algae biomasses in this study. The high correlation coefficients (R^2) are the evident, and constants Q_m, b and c were determined. The data in Table 5.2 summarized the high variation in the model constants for Cd, Cr, Ni and Pb sorption by *C.glomerata*. Obviously the Q_m value for Cd were high followed by Pb. The Cr, Ni, and Pb achieve the conditions that a good metal sorbent should have high value of Q_m

(Kratochviland Volesky, 1998;Li et al.,2011) particularly for Cd and for *O .westti* Q_m value for Ni were high followed by Cd > Pb > Cr. The Q_m value for *V.debrayana* was highest recorded for the Cd followed by Pb > Cr > Ni. Similarly the Q_m value for *Z.insigne* were recorded highest for Pb followed by Cr > Ni > Cd (see Table 5.2).

5.6 Biosorption removal efficiency

C. glomerata biosorption efficiency greatly changes with the initial metal concentration in the aqueous solution. At low concentration (1 mgL^{-1}) the biosorption efficiency for the pollutants such as Pb and Cd are decreased 36, 33 % respectively followed by Ni and Cr reduced upto 30 and 23 %. *C. glomerata* biosorbed each of the selected metals at different concentration from the aqueous solution. Table 5.1 summarizes the equilibrium absorbing amount of Cd, Cr, Ni and Pb by C. *glomerata*.

C. glomerata biosorption efficiency slightly decrease when the concentration of metals in the aqueous solution increase, at initial concentration of (2 mgL^{-1}), this algal biosorbed Pb (32%) followed by Cd (27 %) > Ni (25 %) > Cr (22 %). This algal species performed very well for Pb when the initial metal concentrations in the aqueous solution are less followed by Cd, when the initial metal concentration are reached to 10 mgL^{-1}, the biosorption efficiency was in order of Pb (23%) > Ni (22 %) > Cd (19 %) > Cr (12 %).

Table 5.1 summarizes the biosorption efficacy of the *O. westti*. At low concentration in aqueous solution (1, 2 mgL^{-1}) the biosorption are highly recorded for the toxic THMs such as Cd and Cr, and was in the range of 26-33 and 21-27 % respectively. At a low concentration of 1 mgL^{-1}, the biosorption was in order of Cr (25 %) > Ni (23 %) and at initial concentration 2 mgL^{-1} the biosorption was in order of Ni (19 %) > Cr (17 %). This species shows high biosorption effect for Cd and Cr. when the metal concentration in the aqueous solution increase , the biosorption capacity decrease, at initial concentration of 10 mgL^{-1}, the biosorption was in order of Pb (17 %) > Cd (13 %) > Ni (11 %) > Cr (9 %).

Table 5.1 summarizes the biosorption capacity of *V. debaryana*. This algal show high biosorption for the Cr and Ni at low concentration. The Cr biosorption was reached to 41 % followed by Ni (37 %) > Pb (34 %) > Cd (26 %) at low concentration 1 mgL^{-1}. At 2 mgL^{-1} the biosorption capacity was in order of Cr > Pb > Ni > Cd.

This species also shows the biosorption efficiency slightly decrease at high concentration in the aqueous solutions, at initial concentration 10 mgL^{-1}, the biosorption for the pollutants was in order of Pb (24 %) > Cr (19 %) > Ni (17 %) and Cd (16 %).

Table 5.1 summarize equilibrium biosorption amount for *Z. insigne* of each pollutant present in the aqueous solution, the removal efficacy of this algal was in the range of 38-78, 36-58, 27-49 and 22-37 % for Cr, Pb, Cd and Ni respectively at different initial concentration within the range of 1-10 mgL^{-1}. This algal shows high biosorption at a low concentration (1 mgL^{-1}) for Cr (78 %) followed by Pb (58 %) > Cd (49 %) and Ni (37 %). At low concentration 2 mgL^{-1}, the biosorption for Cr and Pb was 59 and 43 % respectively followed by Cd > Ni. But when the initial concentration of metals decreased in the aqueous solution, the biosorption slightly decrease but within the order of Cr > Pb > Cd > Ni.

WHO (2006) has set the maximum permissible limit for Cd (3 μgL^{-1}) concentration in the WW. Cr is very important element to mammals because it controls to help body blood-sugar levels when present in trace concentrations, but unsafe to fish when its concentration exceed 5.0 mgL^{-1} in water (Alloway and Ayres, 1997). Gazette of Pakistan (1993) has established National Environmental Quality Standard (NEQS) for maximum allowable concentration of Cd (0.1), Cr (1.0) and Pb (0.5 mgL^{-1}). This study results that *Z. insigne* metal biosorption reached to high levels for Cr and Pb at low level metal concentration in aqueous solution. Some biomass shows high affinity for a metal and less sorption capacity, linked with degree of affinity of a particular biomass for each metal. Total amount of metal adhered its surface; depend on number of the active sites and how easily it may reached (Hashim and Chu, 2004).

5.7 CONCLUSION

The algae species were studied for the biosorption of THMs from the aqueous solution at different concentration and dosage. The removal efficiency varied because of the different biomass dosage and concentration. *C. glomerata* biosorptions are very effective for the removal of Pb followed by Cd at low concentration from the aqueous solution. *O.westti* can be used in the removal of Cr and Cd from the WW, the biosorption efficiency of this alga reached to 33 and 27 % respectively for Cd and Cr. Similarly *V. debrayana* shows high biosorption for the pollutants such as Ni and Pb and remove these metal from the aqueous solution with the percentage of 37 and 34 % respectively. In this study Z. *Insigne* shows high biosorption efficiency for the removal of Cr, Pb and Ni at low concentration and the biosorption reached to 78, 58 and 37 % respectively from the aqueous solution at low concentration. The metal biosorption capability is greatly changed with the biomass dosage, with the increase of biomass dose the metal adsorption capabilities slightly decreased. In all algal species selected in this study at low dosage the biosorption was highest for the Cr followed by Ni.

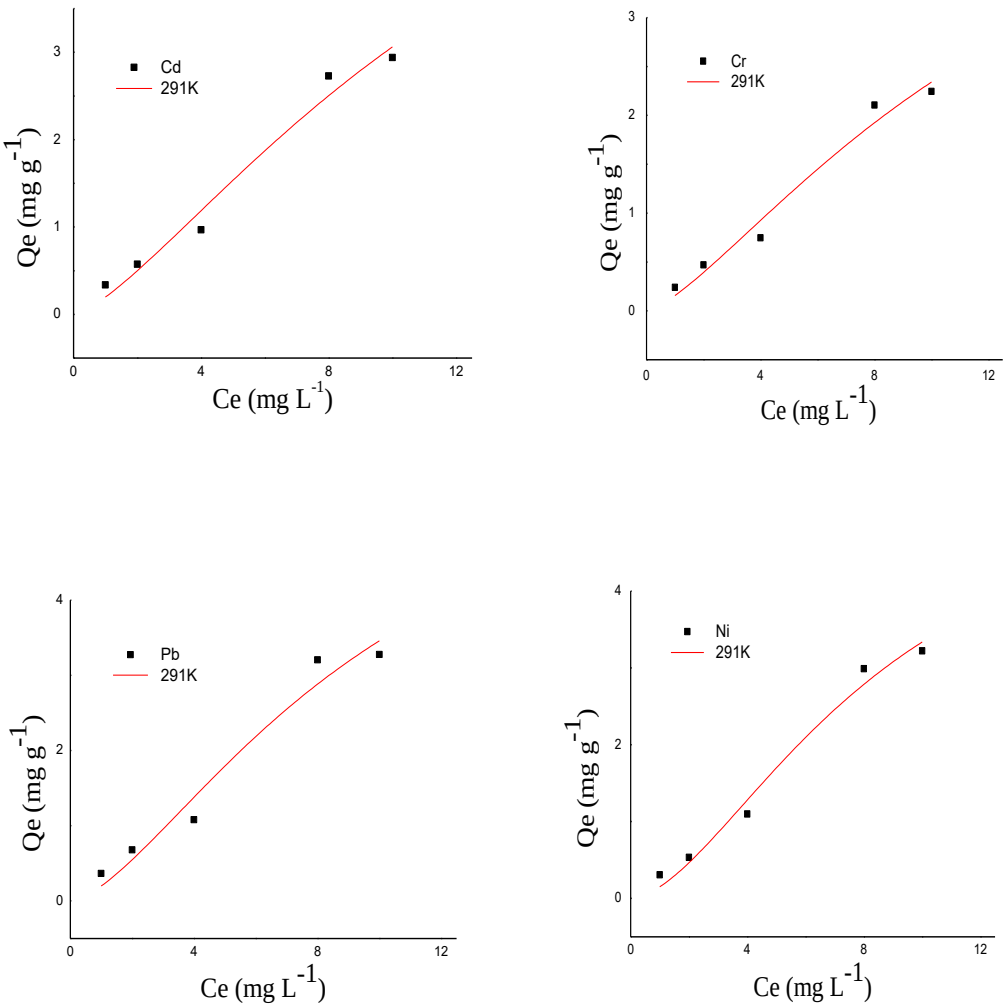

Fig. 5.1 Adsorption isotherms of Cd, Cr, Ni, and Pb by *C. glomerata*.

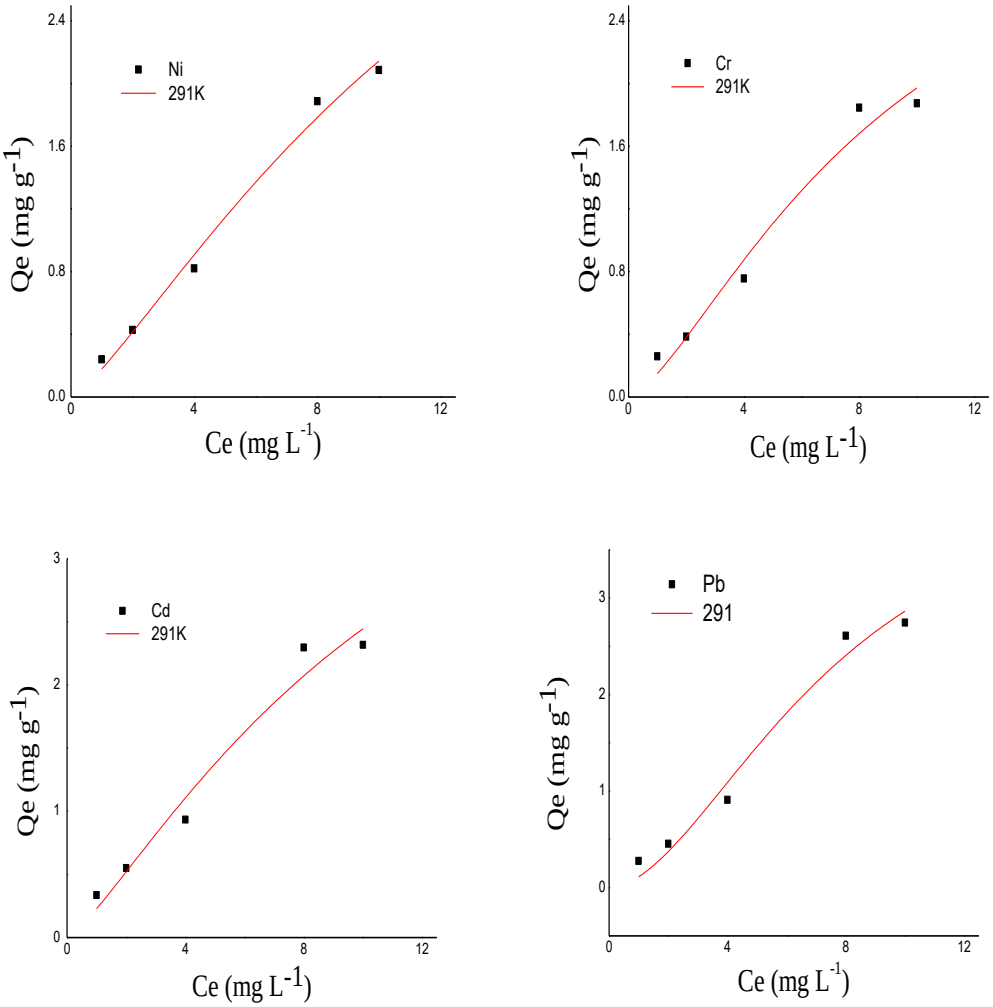

Fig. 5.2 Adsorption isotherms of Cd, Cr, Ni, and Pb by *O. westii*.

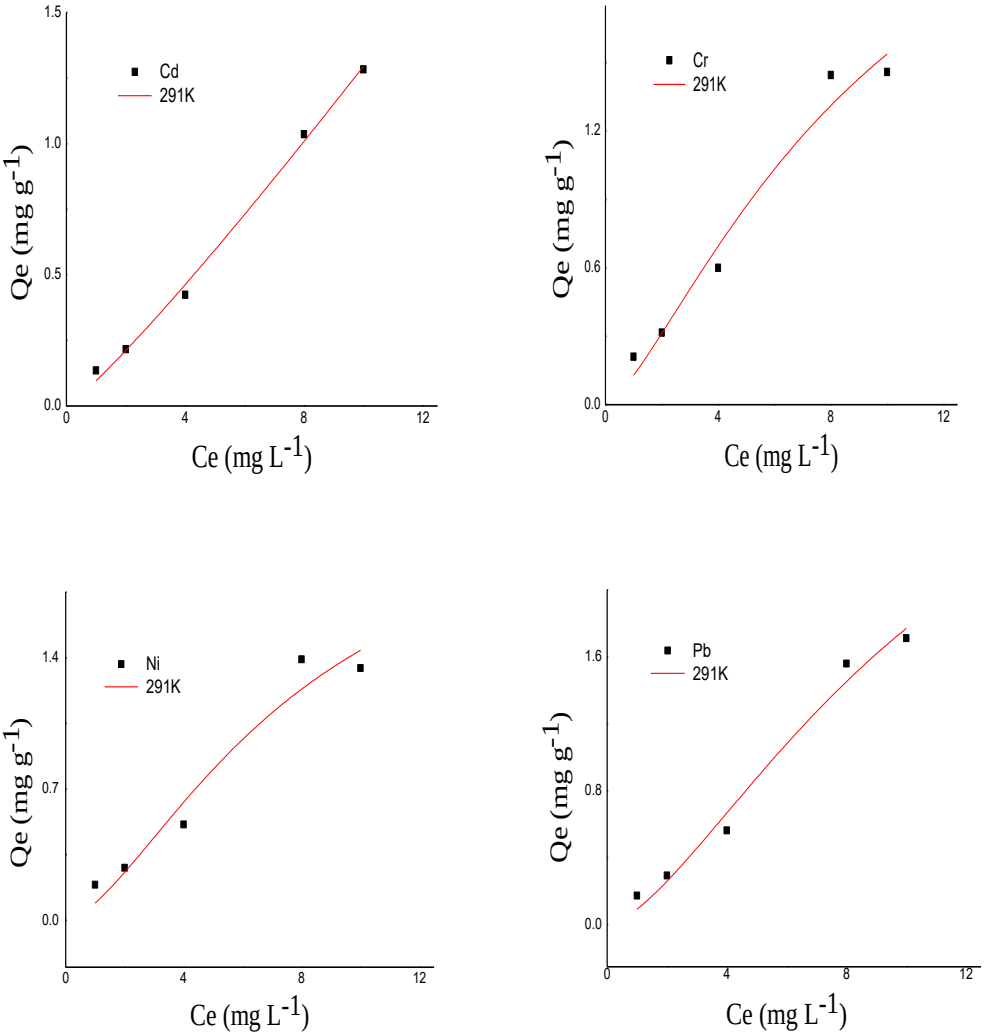

Fig. 5.3 Adsorption isotherms of Cd, Cr, Ni, and Pb by *V. debaryana*.

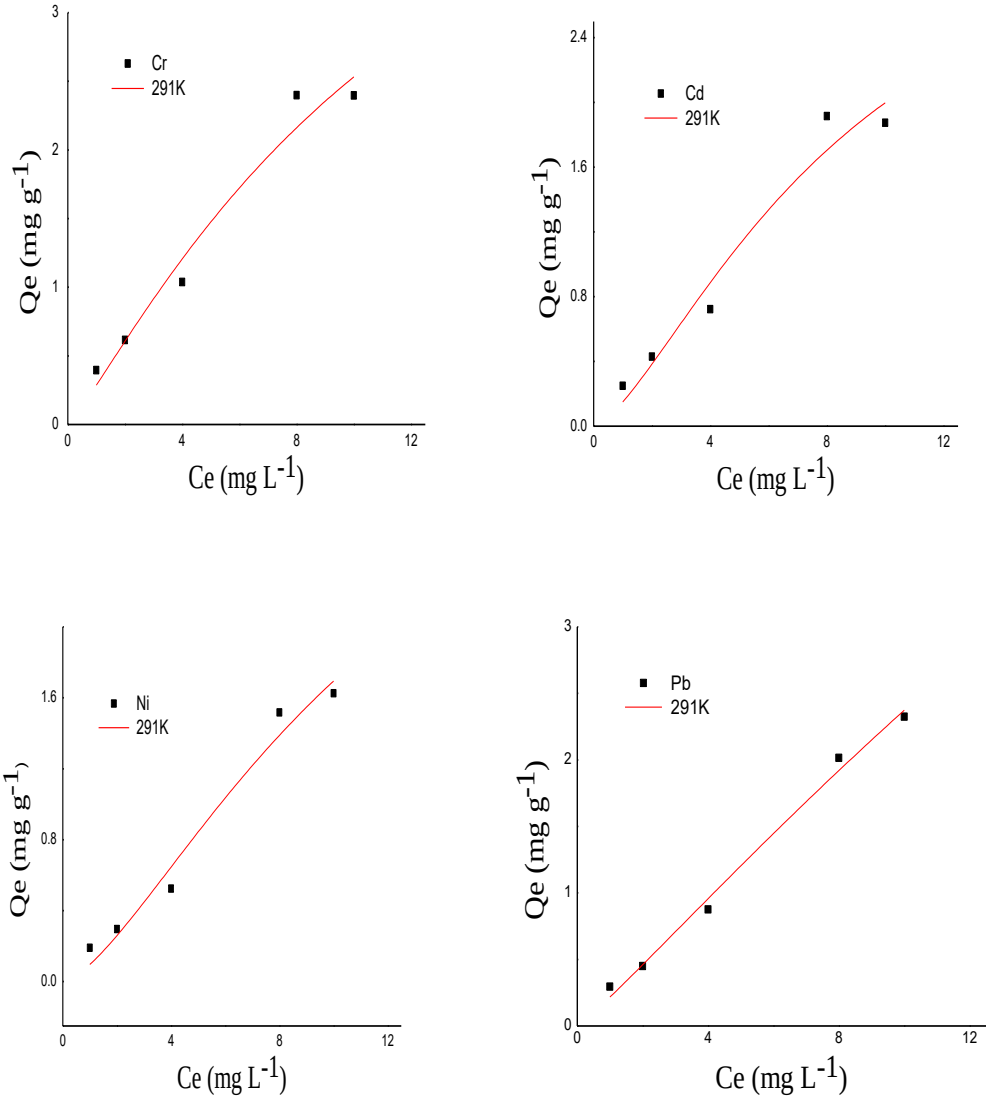

Fig.5.4 Adsorption isotherms of Cd, Cr, Ni, and Pb by *Z. insigne*.

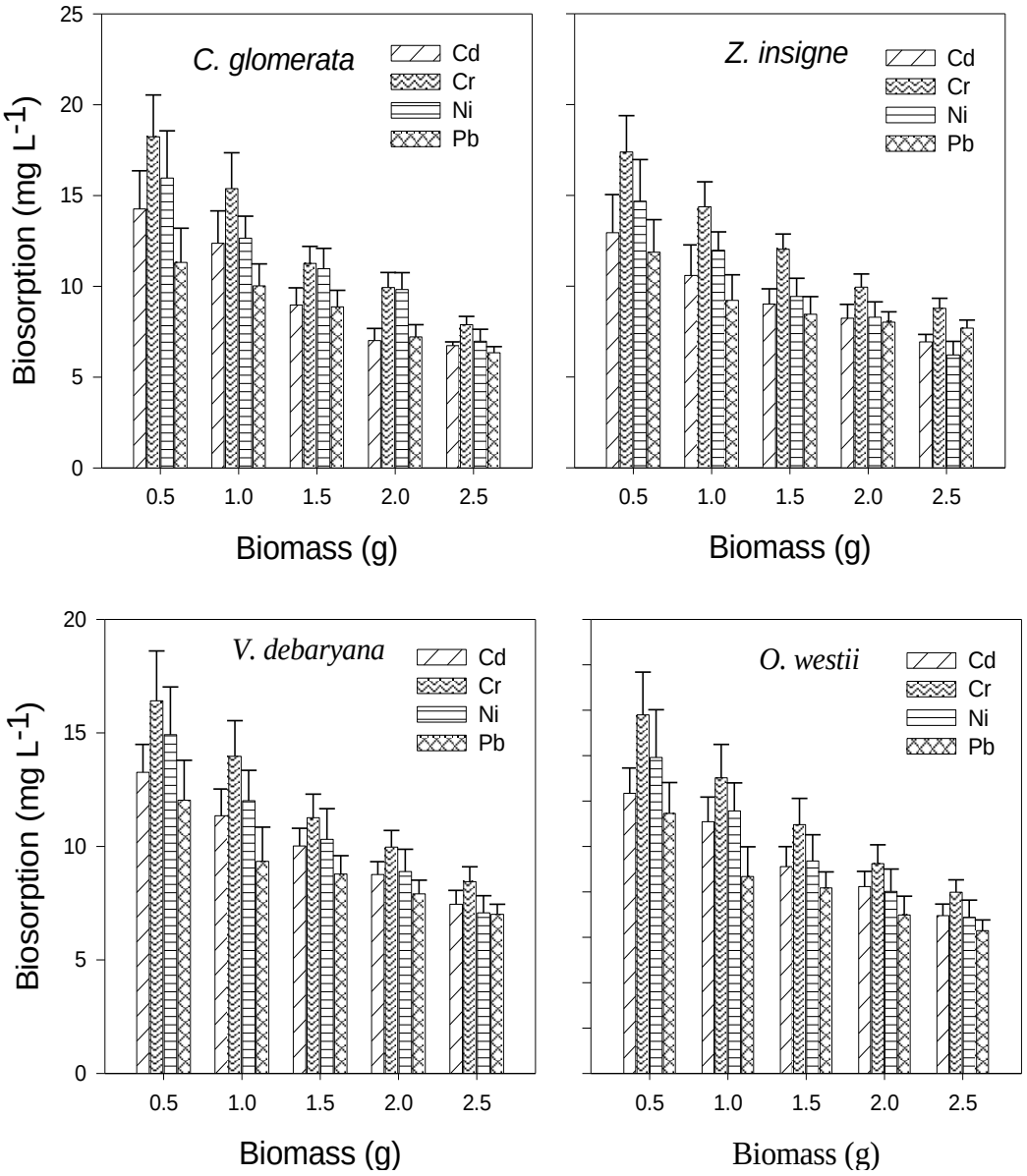

Fig. 5.5 Biosorption capacity of algal species for Cd, Cr, Ni, and Pb

Table 5.1 The equilibrium absorbing amount of selected THMs by algal species.

	Q_e (mgg^{-1})			
Algae species				
Initial concentration (mgL^{-1})	**Cd**	**Cr**	**Ni**	**Pb**
C. glomerata				
1	0.336	0.240	0.306	0.366
2	0.573	0.470	0.535	0.678
4	0.967	0.748	1.098	1.080
8	2.729	2.105	2.989	3.207
10	2.940	2.244	3.221	3.277
O. westii				
1	0.335	0.260	0.240	0.275
2	0.550	0.385	0.428	0.454
4	0.933	0.756	0.820	0.908
8	2.296	1.847	1.888	2.609
10	2.317	1.874	2.087	2.744
V. debaryana				
1	0.135	0.210	0.190	0.175
2	0.216	0.316	0.280	0.295
4	0.424	0.600	0.511	0.564
8	1.036	1.445	1.391	1.560
10	1.283	1.459	1.344	1.711
Z. insigne				
1	0.249	0.395	0.191	0.296
2	0.429	0.615	0.296	0.450
4	0.723	1.037	0.524	0.875
8	1.916	2.399	1.517	2.015
10	1.874	2.396	1.625	2.324

Table 5.2 Langmuir constant for the sorption of test metals by algal species

Algae species		Langmuir constant			
		Q_m (mg g^{-1})	B	c	R^2
C. glomerata					
	Cd	7.940	0.026	-0.389	0.954
	Cr	5.840	0.028	-0.384	0.951
	Ni	5.851	0.027	-0.699	0.969
	Pb	6.752	0.030	-0.539	0.932
O. westii					
	Cd	5.435	0.044	-0.269	0.940
	Cr	3.633	0.043	-0.440	0.948
	Ni	5.558	0.033	-0.276	0.982
	Pb	4.641	0.025	-0.809	0.957
V. debaryana					
	Cd	100.094	0.001	-0.132	0.993
	Cr	2.956	0.046	-0.377	0.946
	Ni	2.404	0.040	-0.569	0.914
	Pb	3.820	0.025	-0.533	0.968
Z. insigne					
	Cd	3.629	0.043	-0.453	0.923
	Cr	6.017	0.050	-0.162	0.939
	Ni	3.854	0.026	-0.485	0.951
	Pb	15.126	0.015	-0.106	0.986

Chapter-6

Bioaccumulation of THMs by freshwater algae from industrial wastewater (IWW), Hayatabad Industrial Estate, Peshawar Khyber Pakhtunkhwa (KP), Pakistan

Abstract

The present study objective were to identify a cost effective method for the removal of Cd Cr, Pb and Ni from the industrial wastewater (IWW), collected at different outletpoints of different industries into the main drain of Hayatabad industrial Estate (HIE), Peshawar, Khyber Pakhtunkhwa (KP), Pakistan. The bioaccumulation potential of selected indigenous alga such as *C.glomerata*, *O.westii*, *V.debaryana* and *Z. insigne* were within the range of 19.8-77.5, 19-66.5, 7.6-91 and 14.7-92 % for the removal of selected THMs respectively. *C. glomerata*, *V. debaryana* and *Z. insigne* were recorded the hyper accumulator for Cr 69-92 % followed by Ni, whereas the O. westti shows high bioremoval capacity for Ni reached to 63-65 %. The *Z. insigne* shows hyper accumulator for Cd and Pb was highly uptake by *C. glomerata* 57 % from the IWW collected from the HIE, Peshawar.

Keywords: Bioaccumulation; Freshwateralgae; Heavymetals; *C. glomerata, O.westii, V. debaryana, Z. insigne, Hyperaccumulator.*

6.1 INTRODUCTION

Rapid Industrialization water activities discharge organic contaminants in the nearest natural water flow without any prior treatments. According to an estimation 2 million tons day^{-1} of sewage and other effluents are discharged into the world waters bodies. In developing countries 70 % of untreated industrial wastes (UIW) and over 90 % of raw sewage are dumped into surface water sources (Anonymous 2010). The water are mostly used in agriculture sector (87 %), while domestic and industry supplies consume 8 and 7 %, respectively (El-Gohary, 2001; Samhan, 2008).

The water resources are eventually polluting due to increase use of THMs in the industries such as textile, mining, metal plating, fertilizer, storage batteries manufacturing, fuels, leather, and tanning materials via their discharges consisting of THMs such as arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), and Copper (Cu) (Unlu and Ersoz, 2006; Nguyen-Ngoc et al., 2009) which crossed the limits of wastewater (WW) discharge criteria. These metals are potentially persistence and toxic to the ecological system and human health (Sheg et al., 2004; Dahiya et al., 2008).The adverse health effects of these contaminants on the organisms including hepatic injury, hypertension, renal dysfunction, lung damage and cancer (Martins et al., 2006; Sariand Tuzen, 2008) has involved the world thinking towards the treatment of industrial wastewater (IWW).

Different conventional methods such as electroplating, evaporation, ion exchange, precipitation and membrane processes are applied to remove THMs when the concentration are high in aqueous solution (Deng et al., 2007; Pan et al., 2009; Lesmana et al., 2009; Turker, 2012).These methods are not effective and expensive when THMs concentration are less, within the range of 1–100 mgL^{-1} in aqueous solution (Volesky, 1990; Ahluwalia and Goyal 2007; Sari et al., 2008) and not so effective than biosorption (Preetha and Viruthagiri, 2005; Azza et al., 2013) and generates secondary sludge which are difficult to dispose(Volesky,

2001; Deng et al., 2007; Sud et al., 2008). It is very important to find environmental friendly new and economically attractive technologies WW treatment systems to tolerate and remove THMs ions from IWW.

Fresh water algae are widely available biological resource in many regions and closely linked with human life (Lee and Chang, 2011) and play an important rule to remove THMs, nitrate and phosphorous from aquatic ecosystems by improving the water quality (Li et al., 2010). Algae can be applied in WW treatment for a series of purposes, including elimination of N and P, reduce the biological oxygen demand (BOD), Inhibition of coliforms and reduction of THMs. The concentration of N and P are more in the WW so the WW is cheap possible nutrients sources for the algae biomass production also means that WW may be feasibly used as economical nutrient sources for algal biomass production. This algal biomass could be used for composting, methane production of liquid fuels, aquaculture or in animal feed and production of fine chemicals (Abdel-Raouf et al., 2012).

The previous research demonstrated that algae have high metal binding capacity due to the presence of lipids on the cell wall surface containing functional groups such asamino, sulphate, carboxyl, hydroxyl which acts as binding sites for metals(Ramelow et al., 1992; Deng et al., 2007).

The algae species such as *Cladophora glomerata* and *Oedogonium rivulare* continuesely uptake THMs such as Cd, Cr, Ni, Fe, Mn, Co, and Cu from WW (Vymazal, 1984). The green algae (*Chlorophyta* > *Phaeophyta*) in comparison with red algae (*Rhodophyta*) uptake more THMs (Al-Shwafi and Rushdi, 2008). The fresh water algae such as *Stigeoclonium sp*. can survive in mining water having high concentration of zinc (Zn) and high bioaccumulation efficiency(Pawlik-Skowronska, 2001).The trace metals accumulate intercellular in algae speciesin fresh and marine water by active biological transport(Afkar et al., 2010; Kumar and Gaur, 2011; Chen et al., 2012).

The water pollution caused by THMs, especially Cd, Cr, Ni, and Pb is a potential risk to the human health, welfare and aquatic organisms. The present research aimed to evaluate the THMs bioaccumulation efficiency of the selected freshwater water algae such as *C.glomerata*, *O.westii*, *V.debaryana* and *Z. insigne* and their survival in industrial wastewater (IWW) collected from the Hayatabad Industrial Estate (HIE) Peshawar.

6.2 MATERIALS AND METHODS

6.2.1 Algae cultivation

Fresh water algae, *C.glomerata*, *O.westii*, *V.debaryana* and *Z.insigne* were collected from ponds of Islamia College University (ICU), Peshawar, Pakistan. Algal biomass was washed thoroughly with tap water to remove yellow ageing parts several times. The algae was cultured and acclimated for 14 days (d) in distilled water at room temperature 18±1°C and natural light. After 14 d the acclimated algae were ready to be used in the experiments.

6.2.2 Industrial wastewater (IWW) collection

The IWW was collected from Hayatabad Industrial Estate (HIE) in sterilized screw capped 1 L bottles at different outlet points of different industries into the main drain and filtered through whatman paper no. 42 method mentioned by (Wang et al., 2009) to remove large particles and made a composite sample before using. The IWW were analyzed for physiochemical characteristics (see Table 6.1).

6.2.3 Chemicals

All the chemicals used in this experimentwere of analytical grade.

6.2.4 Physiochemical Characteristics

6.2.4.1 pH, electric conductivity (EC)(s cm^{-1}), temperature (°C), total suspended solids (TSS), and total dissolved solids (TDS) (mg L^{-1}) measurement

The pH and EC of the IWW was measured by using Accumet XL 60 meter equipped with both pH and EC electrodes. The water organisms desire the pH within the range of 6.5-

8.0.The pH and EC was 4.75±0.2 and 863±42 respectively. The Pakistan National Environmental Quality Standard (Pak-NEQS) for pH is 6-10 whereas for EC the Pak-NEQS are not yet available. The temperature was measured by using the glass thermometer. The temperature of the IWW recorded 35.7°C±4.4. The Pak-NEQS has set the maximum permissible limiting value for temperature, is 40°C. The TSS was determined by evaporating the IWW to dryness at 180 °C and weighing the residues (Peavy et al., 1985). The TSS value of the IWW was 43±5.4.The Pak-NEQS value for TSS was 150 ppm.The TDS is an important indicative parameters for aesthetic value of the drinking water and was determined by using gravimetric method (Peavy et al., 1985). The TDS value was 462±26, which is below Pak-NEQS value of TDS (see Table 6.1).

6.2.4.2 Alkalinity dissolved oxygen (DO), biological oxygen demand (BOD_5), chemical oxygen demand (COD) and hardness (mg L^{-1})

The alkalinity of the IWW was determined by titration method with an acid.The alkalinity value was 247±19.5. The Pak-NEQS limit are not yet mentioned. The DO was measured by using DO meter (Hanna, instrument model H19142). The DO value was 1.829±0.5. The DO value when in water drop from 5 mL^{-1}, the aquatic organisms become under stress (Aubrey, 1950). The BOD_5 value was determined by the standard BOD_5 method (Greenberg et al., 1992). The BOD_5 was 25±4.7.The Pak-NEQS for BOD_5 is 80 ppm.Reactor digestion method were used to calculate the COD of IWW (Jirka and Carter, 1975). The COD was 51±9.6.The Pak- NEQS value for COD is 150 ppm. Hardness is determined by the titration method with EDTA (Peavy et al., 1985). The hardness of IWW was 348±10.2 (see Table 6.1).

6.2.4.3 Total organic carbon (TOC), total phosphorus (P) and total nitrogen (TN)

The TOC, TP and TN was determined by using TOC analyzer in the IWW (Shimadzo, Japan). The concentration was 470, 3.4 and 31 mgL^{-1} respectively.

6.2.5 Experiment design

The bioaccumulation experiments were performed in 1 L plastic containers that were washed with HNO_3 diluted to remove metal ions and then washed with distilled water. Each plastic container contained 2 g of freshwater algae. Two controls were run to confirm if something besides the algae could have affected the observed effects. One control which did not receive algae, and another used algae with distilled water. The experiment was carried out for twelve days in five replicates under natural dark / light 12:12h at temperature 18±1°C.

6.2.6 THMs extraction

A method mentioned by Rybak et al. (2012) was followed for the THMs extraction from algae biomass. Briefly, 0.5 g of algae was taken in a beaker and was dissolved in H_2O_2 and HNO_3 (1: 3, v/v). After that the beakers were put on a hot plate at 110 ℃ until the acid solution change into colorless then filtered through whatman paper no. 42 into a 50 mL volumetric flask and final volume was made up to 50 mL using deionized water. THMs in algae biomass were analyzed by using Atomic Absorption Spectrometer (Analyst 700 Perk Elmer) in the Centralized Resource Laboratory, University of Peshawar, Pakistan.

6.2.7 Quality control

Reagent blanks and standard reference materials were used in each batch for precision and accuracy. Plant reference materials (GBW10015 (GSB-6)) was obtained from the National Research Center for Standards in China. Recovery rates ranged from 93.4 ±6.2-103± 10.3 %.

6.3 Data analysis

6.3.1 Bioaccumulation

The metal bioaccumulation (q) of the selected algae were calculated by the following equation.

$$q = \frac{Ci - Cf}{M} V$$ (Volesky, 1992)----------(Eq. 6.1)

Where

q = Metal bioaccumulation (mgL^{-1})

M = dry mass of algae (g)

V = Volume of culture media (L)

6.3.2 Bioremoval efficiency (%)

The removal efficiency (R) of the selected algae species was calculated by the following mentioned equation:

$$R = \frac{Ci - Cf}{Ci} \times 100$$ (Zhang et al., 1998)----------(Eq. 6.2)

Where,

R = Bioremoval efficiency (%)

C_i = Initial conc. of metal in IWW (mgL^{-1})

C_f = Final conc. of metal in IWW (mgL^{-1}).

6.3.3 Statistical analysis

The data were statistically analyzed using the statistical package SPSS 16.0 while graphs were drawn with the Sigma Plot (10.0 version) presented the mean values and standard deviation of five replicates in order to evaluate analytical and biological variability, using independent cultures of freshwater algae.

6.4 Results

6.4.1 Bioaccumulation of Cd, Cr, Ni and Pb *C.glomerata*

The initial concentration of Cd, Cr, Ni, and Pb was detected in IWW collected from the HIE Peshawar (see Table 6.1). The concentration of Cr and Ni was within the permissible limit set by PAK-NEQS. Whereas Cd and Pb was above the permissible limits set by PAK-NEQS (see Table 6.1). The IWW was biotreated with freshwater green macro algae *C.glomerata*. The observation shows that this alga is grow very well and easily cultured in the IWW under the physiochemical properties (see Table 6.1).

The *C.glomerata* shows the significant bioaccumulation for the selected metal such as Cd, Cr, Ni, and Pb that was summarized in table 6.2. The bioremoval efficiency of *C.glomerata* were slightly decrease after 9 d. After 3 d the *C.glomerata* shows high bioaccumulation potential for Ni that was reached to 24.1 % whereas for the remaining three metal the bioremoval efficiency was 19.8-23.3 % (see Table 6.2)

After 6 d *C.glomerata* shows high bioaccumulation potential for Cr. The bioaccumulation potential was reached to 45 % whereas for the remaining three metal the bioaccumulation was in the range of 34.7-36.3 %.

After 9 d the bioremoval efficiency of *C.glomerata* for Cd, Cr, Ni, and Pb was 46.6, 78.5, 69.0 and 59.8 % respectively. The bioaccumulation after 9 d of the selected metal was in order of Cr > Ni > Pb > Cd.

After 12 d the bioremoval efficiency of *C.glomerata* was slightly decreased for the selected metals. The Cr and Pb was bioaccumulated 69.3 and 50.6 % respectively by this algal after 12 d.

6.4.2 Bioaccumulation of Cd, Cr, Ni and Pb *O. westii*

The table 6.2 summarize the bioaccumulation potential of *O. westii* for the selected THMs in IWW. The *O.westii* bioaccumulation capacity are less in comparison with *C. glomerata* but a competitive alga for the removal of selected THMs. The removal efficacy of this algal after 3 d was19.5-21.3 % for the selected THMs. After 6 d *O. westii* significantly uptake Cr from IWW and bioremoval efficiency reached to 41.8 %. The Cd, Ni and Pb bioaccumulation after 6 d was 26.9-34.9 %.

After 9 d algal shows high bioaccumulation for the Ni and reached to 65.5 % followed by Pb and Cr, 51.9 and 49 % respectively and the order of bioaccumulation potential capacity were Ni > Pb > Cr > Cd.

After 12 d the bioaccumulation of algal was slightly decrease in comparison with 9 d. After 12 d *O.westii* was hyper accumulator for the Ni (63.5 %) followed by Pb (44.3 %) (See Table 6.2). The *O.westti* are easily available and cultivated the data indicate that *O.westti* are eco environment friendly in IWW treatment collected from HIE.

6.4.3 Bioaccumulation of Cd, Cr, Ni and Pb*V. debaryana*

The metal bioaccumulation by *V. debaryana* are summarized in the table 6.2. The *V. ebaryana* has less bioaccumulation potential in comparison with *O.westti and C.glomerata*. The *V. debaryana* is hyper accumulator for Cr.

After 3, 6 and 9 d the bioaccumulation of algal was 22.2, 45.6 91.1 % and decrease slightly after 12 d for Cr upto 69.3 %.

The *V. debaryana* after 3 d bioaccumulate Cd, Ni and Pb within the range of 7.6-15 %.After 6 d the bioaccumulation potential of agal for Cd and Pb was almost uniform reached to 15.2 and 31.5 % for Ni. After 9 d the *V. debaryana* bioaccumulate more Ni 59.6 % and slightly decrease after 12 d reached to 51.5 %.The metal bioremoval efficiency of this algal was in order of Cr > Ni > Pb > Cd. The *V. debaryana* shows positive correlation and uniform

bioaccumulation for Cd and Pb from the IWW with the passage of time.

6.4.4 Bioaccumulation of Cd, Cr, Ni and Pb *Z. insigne*

The bioaccumulation of the *Z. insigne* for Cd, Cr, Ni and Pb are summarized in the table 6.2. After 3 d the bioaccumulation of *Z. insigne* for Cd, Ni and Pb was 14.7-17.5 % and for Cr 26.6 % respectively.

After 6 d the bioaccumulation of *Z. insigne* for Cd and Pb was almost 19 % and Cr was decreased to 54.2 % followed by Ni (32.6 %).

After 9 and 12 d the bioremoval efficiency of Cr was reached to 92 % and was slightly decrease reached to 80.7 % (see Table 6.2). This alga is very stable and shows high bioaccumulation potential for Cr and can be used for the IWW biotreatment that consist of Cr. After 9 d the bioaccumulation efficiency was in order of Cr > *Ni* >*Pb* > *Cd*.

6.5 DISCUSSION

The IWW are discharged in the nearest water streams consisting of THMs. This issue is one of great environmental concern in the developing countries like Pakistan. These IWW loaded with toxic metal ions are discharged in the environment without prior treatmentin developing countries. Chemical methods are used to remove THMs from IWW. Such conventional methods have many shortcomings, for instance, high reagent requirements, unpredictable metal ion removal, and the generation of toxic secondary sludge (Ciba et al., 1999). Recently, biological pycoremediation has emerged as an alternate technique to such traditional chemical methods (Brierley, 1990). It is inexpensive, nondestructive and pollution remains localized (Rise Roberts, 1998). These have been used to remove THMs from contaminated IWW on a large scale. Bioaccumulation of THMs by living cells has developed one of the best attractive means for bioremediation of IWW. Metal resistant algae have been reported in WW and metal polluted environment (Chojnacka et al., 2004; Rehman et al., 2007).

In the present investigation the data summarized that *C. glomerata* and *O.westti* obviously bioaccumulate more Cd, Cr, Ni, and Pb from the IWW in comparison with other algal species *V. debaryana* and *Z. insigne*. The bioremoval efficiency varied because of the initial concentration of the selected metal present in the IWW. The C. *glomerata* bioremoval efficiency for Cr after 9 and 12 d reached to 77.5 and 69.3 % respectively when the metal initial concentration in the IWW was 0.972 mgL^{-1}. The Ni bioremoval efficiency reached to 68.2 % and Pb removal efficiency reached to 57.1 % at constant pH 4.75 at initial metal concentration of 0.846 and 2.671 mg L^{-1}in the IWW. After 9 d the *O.westti* remove 65.5 % of the Ni from IWW. This algal remove the selected metals within the range of 35.1-65.5 %. The *V. debaryana and Z.insigne* shows high bioaccumulation ability for the Cr reached to 92 % after 9 d but for the remaining selected metal the bioremoval efficiency was less in comparison with C. *glomerata* and *O.westti*. The C. *glomerata* and *O.westti* can grow well in the IWW with no yellow and dead part apparently after 12 d culturing in the IWW and show high bioaccumulation potential for the selected metal. The data of this study indicate that these two algal species are eco environment friendly in the IWW treatment because of its easily cultivation, increase growth during 12 d experiment period, easily availability, and low cost.

The metal concentration determined in the IWW are within the permissible except for the Cd and Pb (see Table 6.1). The natural Cd concentrations hardly exceed the WHO (2006) permissible value 3 µg L^{-1}. Cr is an essential element to mammals since it helps the body to control blood-sugar levels when present in trace concentrations, but hazardous to fish when its concentration in water exceed 5.0 mgL^{-1} (Alloway and Ayres, 1997) cause cancer, embryo toxicity, teratogenicity and mutagenicity (Nair and Krishnamurthi, 1991; Junaid et al., 1995; Asmatullah et al., 1999; Bona et al., 1992; Kurosaki et al., 1995). Pb cause anthropogenic, phytotoxic effects and mutagenic carcinogenic in nature (Zelikoff et al., 1988; Alvarez et al.,

2003)

Gazette of Pakistan (1993) established national environmental quality standard for maximum allowable concentration of Cd, Cr, and Pb which are 0.1, 1.0, and 0.5 mgL^{-1}, respectively.

In algae the cell surface are the main site for binding metal (Andrade et al., 2005). The surface bound cations and protons on algae surface exchanged metal ions and sorb THMs on algae surface (Mehta and Gaur 2005). Our research shows that *C. glomerata* and *O.westti* has the highest bioaccumulation for the Cr and Ni even when the concentration are high in the IWW. The algal species *sargassum sp.* acts as chelator for Cd (Raiz et al., 2004) whereas Adhiya et al. (2002) described that Cd sorb to *Chlamydomonas sp.* make complexation with carboxylic groups. Most research published on the removal efficiency using dead biomass of algae. Deng et al., 2008 studied dead *Cladophora sp*. to sorb the Pb and Cd he mentioned that cell could sorb more metals (Mehta and Gaur 2005; Singh et al., 2007).

Our study clearly shows high bioremoval efficiency of selected THMs using living algae. The bioremoval efficiency of the selected contaminants in the studied algae was in order of *C.glomerata* > *O. westii* > *Z.insigne* > *V.debaryana.* Nonliving algae gradually subside in the bottom of the streams and river and increase the sediment, in contrast the living algae cost are cheap and work the best.

Table 6.1 Physiochemical properties of IWW collected from HIE, Peshawar, Pakistan

Parameters	value	PAK-NEQS (ppm)
pH	4.75±0.2	6-10
Conductivity (scm^{-1})	863±42	NA
Alkalinity(mgL^{-1})	247±19.5	NA
DO (mgL^{-1})	1.829±0.5	
BOD5 (mgL^{-1})	25±4.7	80
COD (mgL^{-1})	51±9.6	150
Hardness (mgL^{-1})	348±10.2	
Temperature (°C)	35.7±4.4	40
TSS (mgL^{-1})	43±5.4	150
TDS (mgL^{-1})	462±26.1	3500
TN	31±4	
TP	3.4±0.35	
TOC	470±21.3	
THMs(mgL^{-1})		
Cd	1.483	0.1
Cr	0.972	1.0
Ni	0.846	1.0
Pb	2.671	0.5

Table 6.2 Bioaccumulation of Cd, Cr, Ni and Pb by algae species growing in IWW.

Algae species	THMs in IWW (mgL^{-1})	Bioaccumulation(mgL^{-1}) 3 d	%	6 d	%	9 d	%	12 d	%
C. glomerata									
Cd	1.483	0.342±0.02	-21.6	0.561±0.01	-36.3	0.691±0.01	-45.1	0.545±0.02	-35.3
Cr	0.972	0.236±0.03	-23.3	0.452±0.03	-45.5	0.763±0.03	-77.5	0.683±0.03	-69.3
Ni	0.846	0.211±0.03	-24.1	0.309±0.03	-35.7	0.584±0.03	-68.2	0.435±0.01	-50.6
Pb	2.671	0.601±0.02	-19.8	0.998±0.03	-34.7	1.597±0.02	-57.1	1.286±0.03	-45.5
O. westii									
Cd	1.483	0.311±0.01	-19.5	0.421±0.02	-26.9	0.542±0.02	-35.1	0.445±0.02	-28.5
Cr	0.972	0.214±0.03	-21.0	0.416±0.02	-41.8	0.486±0.03	-49.0	0.394±0.02	-39.6
Ni	0.846	0.187±0.02	-21.3	0.298±0.03	-34.4	0.561±0.02	-65.5	0.544±0.01	-63.5
Pb	2.671	0.605±0.02	-20.0	0.921±0.01	-31.8	1.431±0.01	-50.9	1.254±0.03	-44.3
V. debaryana									
Cd	1.483	0.135±0.03	-7.6	0.247±0.01	-15.2	0.4705±0.01	-30.2	0.363±0.03	-23.0
Cr	0.972	0.225±0.02	-22.2	0.453±0.01	-45.6	0.895±0.02	-91.1	0.6831±0.03	-69.3
Ni	0.846	0.134±0.01	-15.0	0.274±0.01	-31.5	0.511±0.03	-59.6	0.443±0.01	-51.5
Pb	2.671	0.345±0.02	-10.2	0.481±0.03	-15.3	0.942±0.03	-32.6	0.765±0.01	-26.0
Z. insigne									
Cd	1.483	0.278±0.02	-17.3	0.293±0.01	-18.3	0.558±0.03	-36.1	0.485±0.03	-31.2
Cr	0.972	0.268±0.02	-26.6	0.536±0.01	-54.2	0.904±0.03	-92.0	0.784±0.01	-79.7
Ni	0.846	0.155±0.01	-17.5	0.283±0.03	-32.6	0.524±0.02	-61.1	0.425±0.02	-49.4
Pb	2.671	0.465±0.02	-14.7	0.577±0.03	-18.9	1.065±0.01	-37.2	0.855±0.01	-29.3

- Sign indicate reduction.

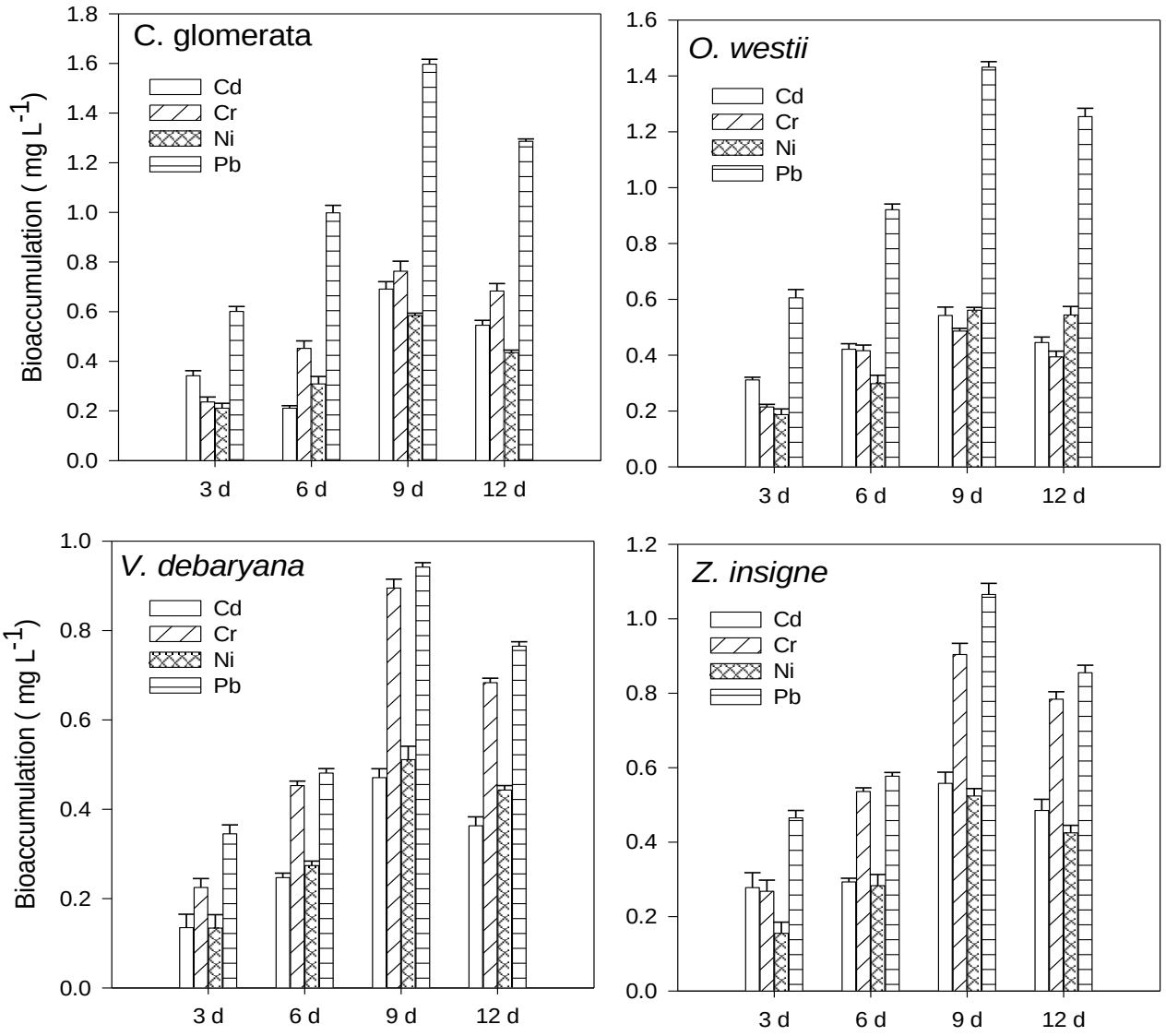

Fig. 6.1 Bioaccumulation of THMs by algal species from IWW collected from HIE, Peshawar KP, Pakistan

Chapter-7

Conclusions

The present research mainly focused on the removal of THMs from aqueous solution and WW of industries using indigenious algae apecies. Four types of algae species *C. glomerata, O.westii, V.debaryana and Z. insigne* were used for the bioaccumulation and biorsorption of Cd, Cr, Pb and Ni from the aqueous solution and IWW to develop an inexpensive and effective biosorption systems. The following conclusions are drawn based on the present research.

1. *C.glomerata* was recorded the most competent representative for the removal of Cr, Cd and Pb from aqueous solutions following by *O.westii*, *V.debaryana* and *Z.insigne respectively*. THMs removal trends were in order of Cd > Cr > Pb.
2. Bioaccumulation capacity of *C. glomerata*, *V. debaryana* and *Z. insigne* was recorded higher at low level of THMs in aqueous solutions.
3. The results shows that *C. glomerata*, *O. westii, V. debaryana* and *Z. insigne* had significant ($P \leq 0.01$) diverse uptake capacity for Cd, Cr, and Pb.
4. The equilibrium adsorption capabilities of *O.westti* were 0.974, 0.620, 0.418, and 0.261 mgg^{-1} for Cd, Cr, Ni, and Pb and the removal efficiency was 55-95, 61-93, 59-89, and 61-96 % respectively.
5. The highest removal efficiency was recorded for Cr and Cd, when the metal concentrations and pH were low, whereas for Ni and Pb the removal efficiency was highest at high concentrations of metals in aqueous solution and high pH.
6. The *O.westti* are appropriate to remove selected THMs from the aqueous solution and can be used in the treatment of wastewater (WW).
7. The biosorption method is more effective in the recovery and removal of THMs. Four

alga species biomass such as *C.glomerata, O.westii, V.debaryana, and Z.insigne* were used to remove Cd, Cr, Ni and Pb from the aqueous solution. Z. *insigne* shows high biosorption capacity for the removal of Cr, Pb and Ni reached to 78, 58 and 37 % respectively from the aqueous solution at low concentration.

8. The metal biosorption capacity is significantly ($P \leq 0.05$) altered with the biomass dosage. The metal biosorption capabilities slightly declined with the increase of biomass dosage.
9. The bioremoval efficiency of the selected algae in the IWW was in order of *C.glomerata* > *O. westii* > *Z.insigne* > *V.debaryana* for Cd, Cr, Pb and Ni.

Referenes

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