POTENTIAL OF TEA FACTORY WASTE IN REMOVAL OF SELECTED HEAVY METAL IONS FROM SIMULATED CONTAMINATED WASTE WATER

BY

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DECLARATION

Declaration by the Student

This thesis is my original work and has not been presented for a degree in any other University. No part of this research can be reproduced without the prior knowledge of the author and University of Eldoret.

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Dedicated to my beloved wife Esther Biwott and Children; Mike Kiptoo, Emmanuel

Kirotich and Victor Kipkoech.

ABSTRACT

Pollution of water resources has increased with human activities world over and heavy metals are major contaminants. In this study, removal of Cd^{2+}, Cr^{3+}, Cu^{2+}, Pb^{2+} and Zn^{2+} ions from simulated contaminated water using tea waste has been investigated. The objective was to find out the efficiency of tea waste in removing these ions. Tea waste was collected from Nandi Hills Factory which processes tea leaves from Nandi Tea Estates farm, Eastern Produce Company farm and individual farms around Nandi Hills Town. The tea waste was then washed with hot water, air dried, ground and sieved through a mesh of size 10 (0.5 mm diameter). The ability of tea waste in removing the selected metal ions was investigated by studying from biosorption parameters namely pH, contact time, initial concentration and presence of other ions. A 0.3 g of the dry tea waste was used for 100 ml of model aqueous solution containing heavy metal ions. Solutions of 5 ppm which corresponds to the upper limit for the permissible concentration by World Health Organization (WHO) recorded in appendix 1 and 50 ppm which is far higher than the WHO permissible concentration limits of metal ions were used to represent low and high concentrations, respectively at optimum pH of 5 and room temperature, for single and mixed metal ions at both levels. Flame atomic absorption spectrometer (FAAS) was used to determine metal ion concentrations. The results show that biosorption is a two stage process, the first being rapid followed by a gradual one. Initial concentration affected the rate of biosorption for both single and mixed metal ions. Biosorption also differed significantly when the ions were mixed and especially in presence of Cr^{3+} ions. The data from the study fitted the Langmuir isotherm more than the Freundlich isotherm implying that the biosorption process took place at specific homogenous sites and the mechanism followed pseudo second order kinetics. Tea waste was effective in removing the selected ions with the highest percentages being; 66.8 % for Cd^{2+} single ions at low concentration, 75.5 % for Cr^{3+} mixed ions at high concentration, 87.6 % for Cu^{2+} single ions at low concentration, 93.7 % for Pb^{2+} single ions at low concentration and 86.7 % for Zn^{2+} single ions at low concentration. Tea waste was therefore found to be an efficient biosorbent for the selected heavy metal ions with the preference order being: $Pb^{2+} > Cu^{2+} > Zn^{2+} > Cr^{3+} > Cd^{3+}$.

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LIST OF SYMBOLS AND ABBREVIATIONS

AAS   Atomic Absorption Spectroscopy

BOD   Biochemical Oxygen Demand

COD   Chemical Oxygen Demand

EHT   Extra Heat Tension

FAO   Food and Agricultural Organization

FTIR   Fourier transform infra- red

KEBS   Kenya Bureau of Standards

L/min   Litres per minute

MH   mixed ions at high concentration

ML   Mixed ions at low concentration

mL   Millilitres

ppm   Parts per Million

RAM   Relative atomic mass

RFM   Relative formula mass

SH   single ions at high concentration

SL   Single ions at low concentration

TFW   Tea Factory Waste

WHO   World Health Organization

WMD   Weighted Mean Diameter

SES   School of Environmental studies (earth science)

ACKNOWLEDGEMENT

CHAPTER ONE

INTRODUCTION

1.1 Background information

In recent years, increasing awareness of water pollution and its far reaching effects has prompted concerted efforts towards pollution abatement. Contamination of aqueous environments by heavy metals is a worldwide environmental problem due to their toxic effects and accumulation through the food chain (Perez-Rama *et al.,* 2002; Sternberg &Dorn, 2002).

A heavy metal is a collective term for metals of high atomic mass, which that are toxic and cannot be processed by living organisms. Other definitions of heavy metals have been proposed based on density, atomic number and atomic mass. Any element that exhibits metallic properties, and belongs to the transition metals, metalloids or lanthanides and actinides can pass as a heavy metal. At one time, an IUPAC technical report described the term *heavy metal* as a "meaningless and misleading and incoherent term lacking coherent scientific basis and with unclear boundaries (Duffus, 2002).

Heavy metals have thus been defined collectively as metals of high atomic mass, particularly those transition metals that are toxic and cannot be processed by living organisms (Harrison and Waites, 1998).

Heavy metals can be broadly classified into three groups; those that are essential for certain biochemical processes, but is toxic when their concentration exceeds certain threshold limits. These include copper, zinc, cobalt, selenium and iron.

The second group consists of metals with no known biological function and toxic if present in concentrations above trace amounts. These include arsenic, bismuth, indium, antimony and thallium. The last and evidently the most dangerous group includes lead, cadmium and mercury which serve no known biological function and are toxic at all concentrations (Fernandez *et al.,* 1992). Within the European community the 11 elements of highest concern are As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Tl. Some of these elements are actually necessary for human health in trace amounts (Co, Cu, Cr, Ni) while others are carcinogenic or toxic, mainly affecting the central nervous system (Hg, Pb, As), the kidneys or liver (Hg, Pb, Cd, Cu), skin, bones or teeth (Ni, Cd, Cu, Cr), (Zevenhoven and Kilpinen, 2001).

The toxicity of trace metals arises from their interference with an organisms' uptake of essential metal ions such as sodium and calcium. For instance, cadmium and zinc block the uptake of calcium ions which is essential for bone and teeth development. The interaction of some heavy metals with enzymes and their tendency to bind to protein and other biological tissues also cause trace metal poisoning in organisms (Campbell, 1995).

The common results of trace metal toxicity to living organisms include brain disorder, gross deformities in development, carcinogenic effects and generally, disruption of biological processes. In most cases these elements find their way into the environment through human industrial processes such as mining, electroplating, battery manufacture, leather tanning, and manufacture of printing pigments and paints, among others. A high concentration of heavy metals in the environment is of great health concern because they are non-biodegradable and end up accumulating in food chains

in various forms such as organic, inorganic or organometallic species (Cordero *et al.*, 2004)

Heavy metals are major pollutants in marine, ground, industrial and even treated wastewaters (Valdman *et al.,* 2001). The presence of heavy metals in drinking water can be hazardous to consumers; these metals can damage nerves, liver and bones and block functional groups of vital enzymes. Metal ions in water can occur naturally from leaching of ore deposits and from anthropogenic sources, which mainly include industrial effluents and solid waste disposal (Ewan & Pamphlet, 1996).

1.2 Statement of the problem

Agrarian revolution and industrialization are recent developments that have solved many problems experienced by early man. Industrialization has given rise to urbanization and all these have led to excessive release of heavy metals to the environment. The rapidly increasing urban population has led to excessive demand for clean water. Industrial activities release heavy metals into the scarce water resources. Unlike organic pollutants majority of which are susceptible to biodegradation, heavy metal ions do not degrade into harmless   end-products. Heavy metal ions are a concern due to their toxicity to many life forms and their non-biodegradable nature results in   their accumulation in living organisms causing various diseases and disorders (Manaham, 2000; Yu, 2005).

Cancer has become one of the greatest human killers of our time. Heavy metals ingested by humans and animals are one of the major causes of these rising cases of

cancer and other related ailments. Heavy metals can be found in soil, water or organic matter. Industrial waste constitutes the major source of various kinds of metal pollution in natural water. This study aimed at finding methods of removing these heavy metals from contaminated water. The toxic metals of interest are Cd, Cr, Cu, Pb and Zn. There are, however, numerous methods currently employed in the removal and recovery of metals from our environment and many physicochemical methods have been proposed for removal of heavy metals from water. Most of the methods previously used are cost prohibitive and inefficient where the ions are in low concentration. In this research a concentration of 5 ppm for heavy metal ions was chosen to represent the lowest significant concentration corresponding to the highest permissible level of the five ions given by WHO (Appendix I). Previous researchers used a higher concentration of metal ions: Malkoc & Nuhoglu(2006a)-100ppm,Amarasinghe& Williams(2007)-100ppm, Wasewar *et al.(*2009)-50 ppm. Tea waste biosorbent was chosen for removing the chosen heavy metal ions because of its availability in large quantities in Kenya and because of its low cost. Previous researchers who used tea waste used one gram of biosobent tea waste per litre of aqueous solution on the lower side. In this thesis the amount of biosorbents used was 0.3 grams per litre which was lower mass of biosorbents than has ever been used. Insoluble cell walls of tea leaves are largely made of cellulose and hemicelluloses, lignin, condensed tannins and structural proteins. In this thesis the removal of heavy metals from simulated contaminated water by batch experiment was studied.

1.3 Rationale for the study

Kenya like all other developing countries suffers from water pollution. The high cost of proper waste disposal and effective treatment waste effluents constitutes a major problem in these countries. Heavy metal contamination exists in aqueous wastes of many industries such as metal plating, mining operations, ore refineries, paint and pigment factories, tanneries, sludge disposal, radiator manufacturing, smelting, alloy industries and storage battery industries, just to name a few (Kadirvelu *et al*., 2001). In view of the adverse effects caused by heavy metal pollution, research has been directed on solving this problem by removal or reduction of these metals especially from water which is viewed as a universal solvent. Conventional methods for the removal of heavy metals from water have been documented but many are cost prohibitive and have inadequate efficiencies at low concentrations, particularly in the range of 1-100 mg /L (Iqbal & Akhtar, 2005).

This fact has necessitated the search for low cost methods for removal of heavy metals from aqueous solution. This search for low cost new technologies for removing toxic metals has directed attention to biosorption. Biosorption is a method of removal of heavy metals based on metal ions binding on various biological materials. A good percentage of these biological; materials are made of lignin and cellulose. Ligno-cellulosics have ion exchange capacity and general sorptive characteristics which are derived from their constituent polymers and structure. The polymers include extractives, cellulose, hemicelluloses, pectin, lignin and protein (Laszlo & Dintzis, 1994).

Among the many agricultural products suggested, (which was first investigated by Orhan & Buyukgungor (1993)), was tea waste, which has been used as a biosorbent in this study. The reason for choosing tea wastes was because apart from its ability to biosorb heavy metal ions the kind of process it undergoes is similar to thermal activation of carbon which gives tea waste additional adsorptive properties. Tea is the most widely consumed beverage in the world, second only to water as attested by the over 3.5 million tons of tea leaves produced annually (FAO, 2009) and therefore its wastes are abundant.

Tea beverage is obtained from the mild oxidation of the leaves from tea plant (*Camellia sinensis*) which once the beverage has been brewed becomes a waste that must be disposed of. Tea wastes present an unused resource. (Arvanitoyannis & Varzakas, 2008).

1.4 Objectives of the study

1.4.1 General Objectives

To investigate the efficiency of tea waste in biosorbing Zn^{2+}, Pb^{2+}, Cu^{2+}, Cr^{3+} and Cd^{2+} ions from simulated wastewater containing heavy metal ions to mitigate the effect these toxic metals in contaminated municipal and industrial wastewater.

1.4.2 Specific Objectives

1. To determine the rate of biosorption for single and mixed metal ions of Cd^{2+}, Cu^{2+}, Cr^{3+}, Pb^{2+} and Zn^{2+}.

2. To evaluate the effect of contact time and the presence of other ions on biosorption capacity of tea waste for each of the selected heavy metal ions.

3. To investigate the influence of initial concentration (of the selected heavy metal ions) on the rate of biosorption.

4. To investigate the nature of absorbing surface in tea waste biomass (using Langmuir and Freundlich models)

1.5 Null hypotheses

1. Tea waste is not an efficient biosorbent of the selected heavy metals

2. Initial concentration has no influence on the rate of biosorption

3. There is no difference in metal biosorption using tea waste whether the ions are present singly or in combined form.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Biosorption involves a solid phase (sorbent, biosorbent; adsorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (adsorbate, metal ions). Due to the high affinity of the adsorbent for the adsorbate species, the adsorbate is attracted and bound to the adsorbent by different mechanisms. The process continues until equilibrium is attained between the amount of solid bound (adsorbate species) and its portion remaining in the solution. The degree of the adsorbent affinity for the adsorbate determines its distribution between the solid and the liquid phases. The major advantages of biosorption over conventional waste treatment methods include: low cost, high efficiency, minimization of chemical and biological sludge, regeneration of the biosorbents and possibility of metal recovery (Kratchovil & Volesky, 1998).

This implies that the removal mechanism is not metabolically mediated. Bioaccumulation on the other hand, describes an active process where removal of metals requires the metabolic activity of a living organism (Lokendra S.T. & Mukesh P., 2013).

In recent years research on biosorption has intensified with a view to using biomass to remove heavy metals from industrial effluents or to recover precious metals from processing wastewater. Of the many types of biosorbents recently investigated, tea waste biomass has proven to be highly effective, reliable and predictable in the

removal of heavy metal ions from aqueous solutions (Lokendra S.T. & Mukesh P., 2013). It is particularly the spongy structure of processed tea waste and the available functional groups in the structure of the tea biomass which is responsible for this phenomenon (Dhanakumar *et al,.* 2007). While having a performance similar to ion exchange, biosorption is more advantageous because biosorbents are quite abundant, less costly and easily biodegradable. Additional cost reduction results from the possible recovery of heavy metals which have a high market value. The metal recovery process by tea waste is even economically viable as a metal recovery industry by itself for the more precious metals.

The disadvantages of biosorption include; early saturation when metal interactive sites are occupied, metal desorption is necessary prior to further use, the potential for biological process improvement (like through genetic engineering of cells) is limited because cells are no longer active in metabolic processes like excretion and there is no potential for biologically altering the metal valence states (Ahluwalia & Goyal, 2007).

The mechanism of metal biosorption is complicated because the status of biomass (living or non–living), types of biomaterials, properties of metal solution chemistry and ambient (environmental) conditions such as pH, influence the mechanism of metal biosorption. The metal biosorption process by living cells is a two step process. In the first step, metal ions are adsorbed to the surface of cells by interactions between metals and functional groups displayed on the surface of cells. All the metal ions before gaining access to the cell membrane and cell cytoplasm come across the cell wall. The cell wall consists of a variety of polysaccharides and proteins and hence offers a

number of active sites capable of binding metal ions. Difference in the cell wall composition among the different groups of micro-organisms which include; algae, bacteria, cyano-bacteria and fungi, cause significant differences in the type and amount of metal ion binding to them. Algal cell walls are mainly cellulosic. Cell walls of bacteria are principally composed of peptidoglycans. Fungal cell walls contain chitin and chitosan, which have been shown to sequester metal ions (Muzzarelli, 1972).

The first step involves passive biosorption, which is metabolism independent and proceeds rapidly by any one or a combination of the following metal binding mechanisms: complexation, ion exchange, physical adsorption (such as electrostatic) or inorganic microprecipitation. Passive biosorption is a dynamic equilibrium process of reversible adsorption-desorption. Metal ions bound on the surface can be eluted by other ions, chelating agents or acids. In the second step, due to active biosorption, metal ions penetrate the cell membrane and enter into the cells. Metal uptake by non-living cells is mainly in the passive mode (Volesky, 1990).

By investigating the biosorption of Cr (VI) and Fe (III) on *Streptococcus equisimilis, S. cerevisiae* and *Aspergillus niger,* Goyal *et al*. (2003) confirmed that the metal uptake by micro-organisms occurs in two stages: passive uptake which takes place immediately, and active uptake which takes place slowly. The first stage is thought to be physical adsorption or ion exchange at the cell surface, reaching the adsorption equilibrium within 30-40 min. Therefore, passive mode is independent of energy,

mainly through chemical functional groups of the material, comprising the cell and particularly cell wall whereas active mode is metabolism dependent and related to metal transport and deposition.

Aksu and co-workers (2002) reported that temperature does not influence the biosorption processes in the range of 20°C to 35°C. Temperatures higher than 40^oC affect biosorption because the kinetic energy of the ions increases which reduce the probability of the ions to be captured by the biosorbents. pH seems to be the most important parameter in the biosorption processes. It affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of the metal ions (Galun *et al*., 1987).

Biomass concentration in the solution seems to influence the specific uptake because lower values of biomass concentration leads to interference between the binding sites. Fourest & Roux (1992) invalidated this hypothesis attributing the responsibility of the specific uptake decrease to metal concentration shortage in solution. Hence, this factor needs to be taken into consideration in any application of microbial biomass as biosorbent.

2.2 Biosorption Equilibrium Models

Preliminary testing of solid-liquid absorption system is based on two types of investigations namely equilibrium batch sorption test, and dynamic continuous flow sorption studies. The equilibrium of the biosorption process is often described by

fitting the experimental points with models usually used for the representation of isotherm adsorption equilibria. The two widely accepted and linearized equilibrium adsorption isotherm models for single solute system are Langmuir and Freundlich given by the following equations:

Langmuir;

$$q_e = \frac{q_{max} k_L c_{eq}}{1 + k_L c_{eq}}$$ ....Eqn. 2.1

Where;

C_e is the equilibrium concentration of the adsorbate

q_e is milligrams of metal accumulated per gram of the biosorbent material;

C_{eq} is the metal residual concentration in solution;

q_{max} is the maximum specific uptake corresponding to the site saturation

k_L is the ratio of adsorption and desorption rates.

Langmuir equation can also be re-written as:

$$\frac{c_e}{q_e} = \frac{1}{k_L q_{max}} + \frac{c_{eq}}{q_{max}}$$ .... Eqn. 2.2

Another empirical model for monolayer adsorption is Freundlich, given as

$$q_e = k_f (c_e)^{\frac{1}{n}}$$ .... Eqn. 2.3

Where, k_f and n are constants and q_e is milligrams of metal accumulated per gram of the biosorbent material while C_e is the equilibrium concentration of the adsorbate.

$$\log q_e = \log k_f + \frac{1}{n} \log c_e .$$ .... Eqn. 2.4

These models can be applied at a constant pH, and used for modeling of biosorption equilibrium with reference to one metal. Recently, some biosorbents have emerged as an eco-friendly, effective and low cost material options. These biosorbents include some agricultural wastes, fungi, algae, bacteria and yeast. Studies using biosorbents have shown that both living and dead microbial cells are able to uptake metal ions and offer potential inexpensive alternative to conventional absorbents (Knorr, 1991).

However, living cells are subject to toxic effect of the heavy metals, resulting in cell death. Moreover, living cells often require the addition of nutrients and hence increase the BOD and COD in the effluent. For these reasons, the use of non-living biomaterials or dead cells as metal binding compounds has been gaining advantage because toxic ions do not affect them. In addition, dead cells require less care and maintenance before being used as biosorbents, and are cheaper (Mofa, 1995). Furthermore, dead biomass could easily be regenerated and re-used. Biosorption using tea waste has been done in Iran by Mahvi and co-workers (2005). This was a bench scale experimental type of research and analysis was performed using different amounts of adsorbent in solution with five different concentrations of each metal ion and also in combined form.

Besides the effects of various amounts of tea waste used in adsorption, efficiency was also investigated. Results indicated that removal efficiency was high for lead and low for cadmium. About 94 % and 100 % Pb^{2+} removal were achieved by using 0.5 and 1.5 g adsorbent for solutions having concentrations 5 and 10 ppm Pb^{2+} ions, respectively. The efficiency of cadmium was only 77.2 % at the same pH, 1.5 g mass

adsorbent and a concentration of 10 ppm. Mahvi and co-workers (2005) recommended from their findings that tea can absorb heavy metals with high efficiency. To increase the efficiency, they recommended that pre- treatment of tea waste with chemicals such as acids, bases and detergents be investigated and their effect on efficiency be established. However the concentrations and strengths of acids and bases used were not indicated.

Malkoc & Nuhoglu (2006) investigated the efficiency of waste tea in removing nickel from aqueous solution. By decreasing the particle size the performance of adsorption is improved but for column adsorption, reduced particle size results in high resistance to the movement of the aqueous solution through the column. In their results, they concluded that tea factory waste is an effective and inexpensive biosorbent for nickel (II) removal from aqueous solution.

Lavecchia and others (2010) performed experiments on the removal of Pb^{2+} from aqueous solution by spent tea leaves using batch adsorption unlike Malkoc & Nuhoglu (2006) who used column experiment. Batch adsorption experiments were carried out at temperatures between 4 ^{o}C and 40 ^{o}C and the results showed that the efficiency of tea waste was marginally affected by the temperature.

At low Pb^{2+} loading the biosorption equilibrium was well described by Langmuir equation with maximum biosorption capacity of 85- 100 mg/g. Their results showed that at 25 ^{o} C the amount of Pb removed in mg/g dry weight of biosorbent was 11.3 mg/g. This clearly supports the possibility of using spent tea leaves for the removal

of Pb^{2+} and possibly other heavy metals from contaminated water (Lavecchia *et al*., 2010).

2.3 Sources of tea factory waste

Kenya has more than 110,000 hectares of land devoted to tea. The tea plantations are found in the highland areas of Mau, Mount Kenya, Mount Elgon and Cherangany. Tea factories are situated within these regions. The main product from these factories is black tea. In 1995 Kenya became the largest exporter of black tea in Africa and the third largest in the world (Global Tea – Kenya, 1995).

Work on using tea factory waste as an absorbent of toxic heavy metal ions has been done in India, Turkey and Sri Lanka. The details are summarized in Table 2.1. Researchers studied the ability of tea waste to remove one metal ion, some two and some three metal ions which exist together in aqueous solution. In this thesis the number of metal ions studied was increased to five metal ions. Tea used in this study was collected from the Mau region in Nandi Hills.

Table 2.1: Source of TFW as an adsorbent for removal of toxic metals

	Reference	Source	Metal ion
1.	Cay *et al*.,(2004)	Tea processing plants located in Black Sea region in Rize, Turkey	Cu^{2+}, Cd^{2+}
2.	Malkoc and Nuhoglu (2005)	Tea plants located in Black Sea region in Giresun-Eynesil, Turkey.	Ni^{2+}
3.	Malkoc and Nuhoglu (2006a)	Tea plants located in Black Sea region in Giresun-Eynesil, Turkey.	Cr^{3+}
4.	Malkoc and Nuhoglu (2006b)	Tea plants located in Black Sea region in Giresun-Eynesil, Turkey.	Ni^{2+}
5.	Amarasinghe and Williams (2007)	Black tea from central highlands of Sri Lanka "high grown tea" was used for the experiments.	$Cu^{2+,}$ Pb^{2+}
6.	Malkoc and Nuhoglu (2007)	Tea plants located in Black Sea region in Giresun-Eynesil, Turkey.	Cr^{3+}
7.	Wasewar *et al*., (2009)	TFW was supplied from Tea Plants located in Palampur, Himanchal Pradesh, India.	Zn^{3+}

Adopted from; Wasewar (2010).

2.4 Methods used to prepare the adsorbent.

To improve the performance of plant materials in removing heavy metals, these plant materials are subjected to a process referred to as treatment. Treatment of biomass generally implies protonation of the biomass with a strong acid such as hydrochloric acid whereby the proton displaces the light metal ions from the binding sites like carboxylic and sulphonic sites. Alternatively, the biomass may be reacted with an aqueous solution of a given ion at high concentration so that the majority of sites are occupied by the ion. The term ion-exchange does not explicitly identify the binding mechanism, rather it is used here as an umbrella term to describe the experimental observations. The precise binding mechanism(s) may range from physical (electrostatic/ionic) to chemical binding (covalent). Furthermore, the term sorption refers to binding of a metal cation to a free site as opposed to one that was previously occupied by another cation. It is distinct from adsorption which strictly speaking, defines binding in terms of a physical rather than chemical surface phenomenon. In the case of biosorption of heavy metals by algal biomass, the mechanisms can be viewed, in principle, as being extra cellular, or occurring discretely at the cell wall. Intracellular sorption would normally imply bioaccumulation by a viable organism (Davis *et al.,* 2003).

Tea factory waste from Turkey was used by Cay *et al*., (2004). Before biosorption was done, hydrolysable tannins and other water soluble and coloured components were removed from the crushed tea waste by washing with hot water (80 ^{o}C) for one hour until a colourless solution of tea waste was spectrometrically observed at room

temperature. Cleaned and decolourized tea waste was dried in an oven at 105 ^{o}C and 60 − 170 mesh size particles were used in the adsorption experiment without further modification. A colourless solution of tea waste was observed in fifteen washing cycles. Since biosorption of heavy metal ions was carried out in relatively cold water (25 ± 0.3 ^{o}C) in experiments, washing of tea waste was complete in 5 minutes and no interfering colour was observed at room temperature (Cay *et al.,* 2004).

Mahvi *et al.,* (2005) washed tea waste at first step and then rinsed it with distilled water. After drying it in an oven at 100° C, it was ground and screened (using screen with mesh sized 10). Prior to the experiment, Malkoc & Nuhoglu (2005) removed other soluble dirt and soluble coloured components from the tea waste by washing with distilled water. The decolourized and cleaned tea waste was dried at room temperature for five days by spreading on wire gauze. Prior to biosorption experiments, hydrolysable tannins, coloured components and other water soluble components were removed from crushed tea waste by washing with hot water at 80 ^{o}C four times and washed with dilute sodium hydroxide solution until a colourless solution of tea waste was spectrometricaly observed at room temperature. It was found that using sodium hydroxide solution instead of pure hot water reduced the number of washing cycles.

2.5 Characterization of tea waste

The ability of tea waste to bind heavy metal ions is the result of a variety of mechanisms including chemisorptions, and physiosorption on surface and pores, ion exchange, microprecipitation, heavy metal hydroxide condensation and surface adsorption as define in appendix 6, (Demirbas, 2008). In order to understand how heavy metal ions are removed by tea waste, it is essential to identify the functional groups responsible for metal binding, a process known as characterization. Fourier transform spectroscopy was used to identify the functional groups in the tea waste before it binds to heavy metals ions and after it absorbs the metal ions. The difference in functional groups can indicate where the ions have been bound. Malkoc and Nuhoglu (2006a) characterized tea waste using FTIR spectrometer. The infrared spectra were obtained (and transferred to Microsoft Excel) using Perkin-Elmer Spectrum One FTIR spectrometer

2.5.1 Fourier transform infrared spectra (FTIR Spectra)

The FTIR spectra before and after adsorption of tea factory waste are shown in Fig. 2.1a and 2.1b.
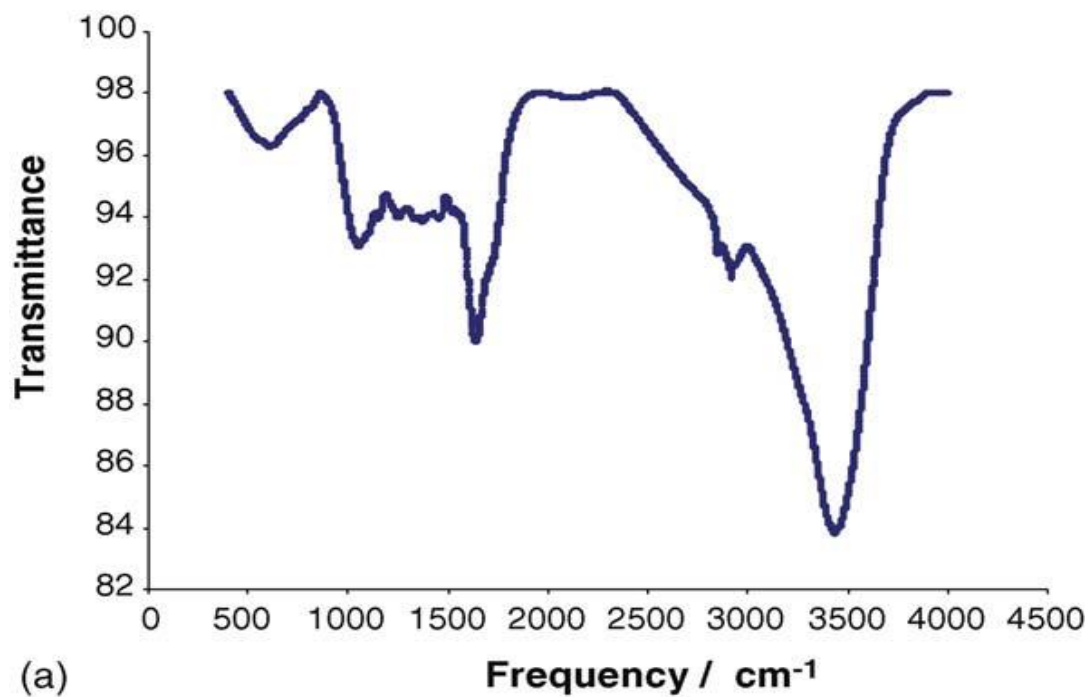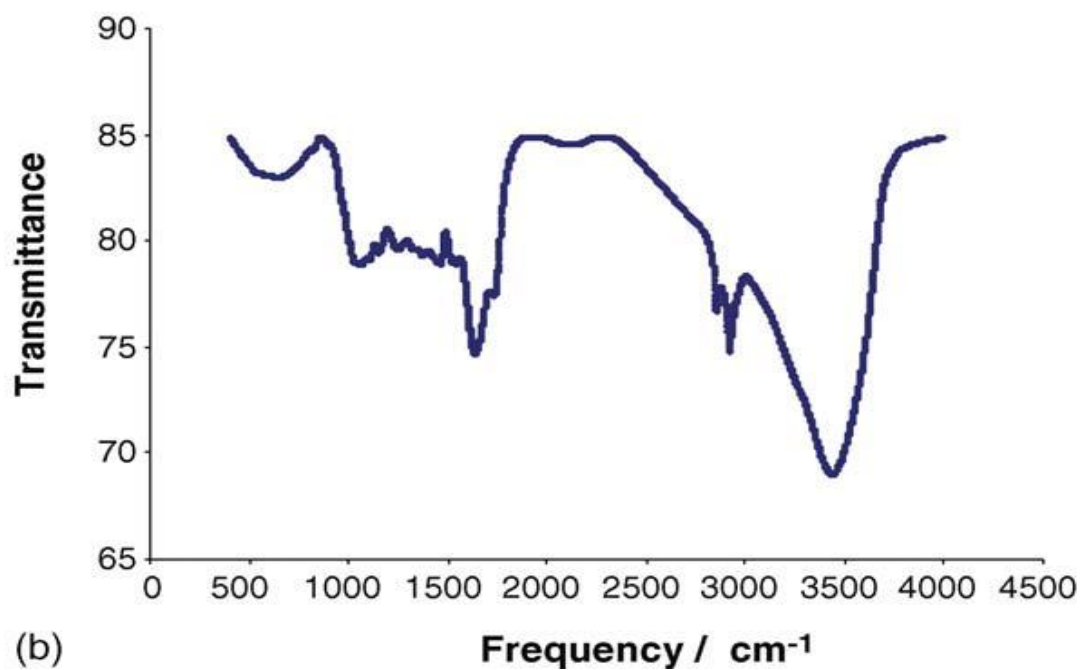
Figure 2.1 (a) and (b): FTIR spectra of waste tea before absorption and (b) FTIR spectra of waste tea after absorption

2.5.2   Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectra before and after were analyzed for the adsorption of tea factory waste were shown in figures 2.1 (a) and 1(b), and the FTIR spectroscopic characteristics are shown in table 2.2.

Table 2.2 FTIR spectral characteristics before and after absorption

IR Peak	Frequency (cm^{-1})			Assignment
	Before adsorption	After adsorption	Difference	
1	3429	3430	+1	Bonded –OH group
2	2924	2925	+1	Aliphatic C-H group
3	2855	2856	+1	Aliphatic C-H group
4	2361	2362	+1	1° amine stretching
5	1628	1626	-2	C=O stretching
6	1385	1389	+4	Aliphatic C-H group
7	1249	1262	+13	-CN stretching
8	1044	1040	-4	C-O str. of ether group
9	664	666	+2	-CN stretching
10	616	615	-1	-CN stretching

Adopted from Dhanakumar *et al.*, 2007.

From Figures 2.1(a) and 1(b), the spectra display a number of absorption peaks, indicating the complex nature of the material examined. The FTIR spectroscopic

analysis indicated broad bands at 3429 cm^{-1}, representing bonded –OH groups. The bands observed at about 2915–2848 cm^{-1} could be assigned to the aliphatic C–H group. The peak around 1631 cm^{-1} correspond to the -C=O stretch. The peaks at 2361 cm^{-1} correspond to primary amine group. The peaks observed at 1546 and 1511 cm^{-1} correspond to the secondary amine group. Symmetric bending of CH$_3$ is observed to shift to 1448, 1435 and 1345 cm^{-1} (Malkoc and Nuhoglu, 2006a).

Thus, the methyl functional group would likely have participated in metal binding. The peaks observed at 1232, 1141 and 634 cm^{-1} could be assigned to –SO$_3$ stretching, C–O stretching of ether groups and –CN stretching, respectively. As shown in table 2.2, the spectral analysis before (figure 1a) and after metal absorption (figure 1b) indicated that the bonded –OH groups, C=O stretching, secondary amine group and symmetric bending of CH$_3$ were involved in nickel biosorption. There were clear band shifts and intensity decrease of the band at 3420, 1631, 1546 and 1448 cm^{-1}. From FTIR study -OH, -C=O, secondary amine group, -CH$_3$ and -SO$_3$ groups were the main functional groups in tea (Malkoc and Nuhoglu, 2006).

Standard procedures used by different researchers to determine the physical and chemical characteristics of tea waste and were summarized in table 2.3

Table 1.3: Physical and chemical properties of various TFW

	Cay *et al*. (2004)	Malkoc an(2005)	Amarasinghe (2007)	Wasewar *et al*. (2008)
Characteristics	values			
Proximate analysis				
Moisture (%)	11.01	11.01	—	4.5
Ash (%)	2.97	2.97	—	3.8
Fixed Carbon (%)	6.04 (Water solubles)	6.04 (Water solubles)	—	16.6
Bulk density (kg/m^3)	328	112	206	224.66
Ultimate analysis (dry basis)				
C	—	—	—	46.74
H	—	—	—	7.642
N	—	—	—	0.831
S	—	—	—	0.005
Chemical analysis of ash (%)				
Insoluble Matter	—	—	—	75.34
Silica	—	—	—	2.45
MgO	—	—	—	1.04
Surface area (m^2/g)				
Langmuir	—	—	—	2.5667

Adopted and modified from Wasewar (2010).

2.5.3 Chemical composition of tea

White tea, green tea, oolong tea and black tea come from the exact same plant (*Camellia sinensis*). However, the flavour and chemical content develops by "chemical oxidation" during tea processing. This oxidation is stopped by applying heat because heat inactivates the enzymes. Enzymes and small molecules (such as flavonoids) occupy many specific compartments inside the living cells. Both white and green tea do not undergo oxidation because heat is applied soon after picking. For black tea which was used in this research, oxidation continues for some time until the leaves are dry. White tea is made from young leaves and buds while green tea requires more mature tea leaves. The leaves are dried for about 1-2 days after harvesting to allow oxidation to take place before processing (Yamimoto *et al*., 1997).

Apart from caffeine, the most important components in white and green tea are called flavonoids which give live plants the various colours that attract insects and birds for pollination and also act as antioxidants to protect them from stress. Flavonoids are characterized by 3 rings labeled as A, B and C in flavones the first diagram in figure 2.2 below. Depending on small changes in the rings (coloured in red), they can be sub-classified into flavones, flavonols, flavonones, catechins, anthocyanidins and isoflavonoids as shown in figure 2.2.

Flavone
(Apigonin)

Flavonol
Quercetin

Flavonone
(Eriodictyol)

Catechins
(Catechin)

Anthocyanidins
(Cyanidin)
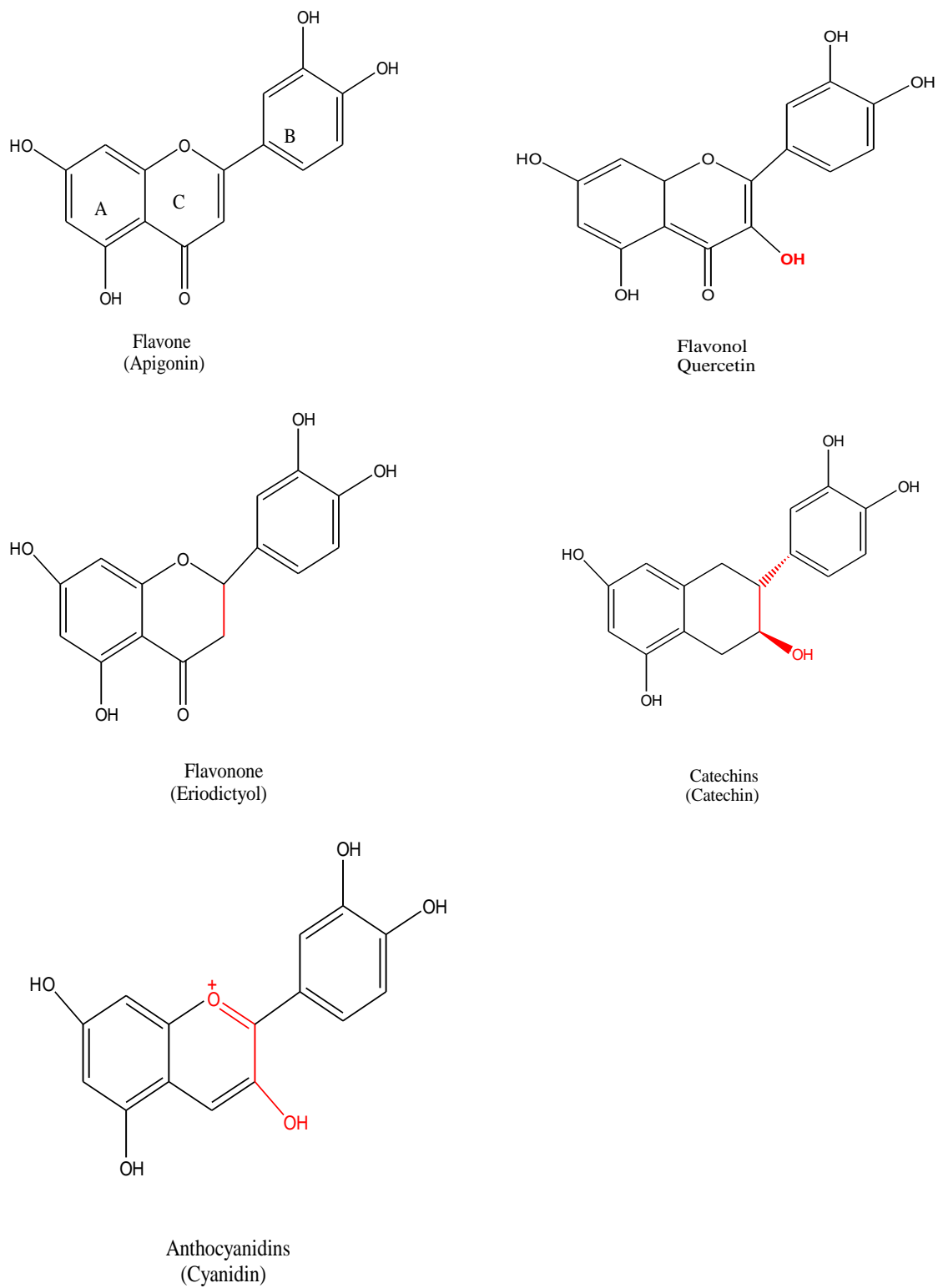
Figure 1.2: Flavonoids Present in White and Green Tea (Graham, 1992)

In the case of black tea, the leaves are allowed to oxidize 100%. The main difference is that the leaves are cut and bruised disrupting the cell structures so all the leaf juices (containing flavonoids) and enzymes (like polyphenol oxidase) mix up allowing a complete oxidation. The oxidation process takes up to three hours under a high humidity and an optimal temperature of between 25 and 27 ^{o}C for enzyme function. Once the process is complete, the broken leaves are dried and shaped for packing. Black tea contains very few flavonoids and mostly theaflavins and thearubigins (Graham, 1992).

The reaction scheme 2.1 shows an example of oxidation of catechins (a flavonoid also shown above) to theaflavin and thearubigin by the enzyme polyphenol oxidase. Thearubigin is what gives black tea its characteristic colour and taste. White and green tea contains a mixture of all kinds of flavonoids. The scheme shows some of the functional groups present in tea before and after processing. This scheme helps in understanding the adsorption process by the functional groups present in the processed tea. The heavy metal ions are removed by the ions replacing hydrogen in any of the compounds depicted in the scheme 2.1
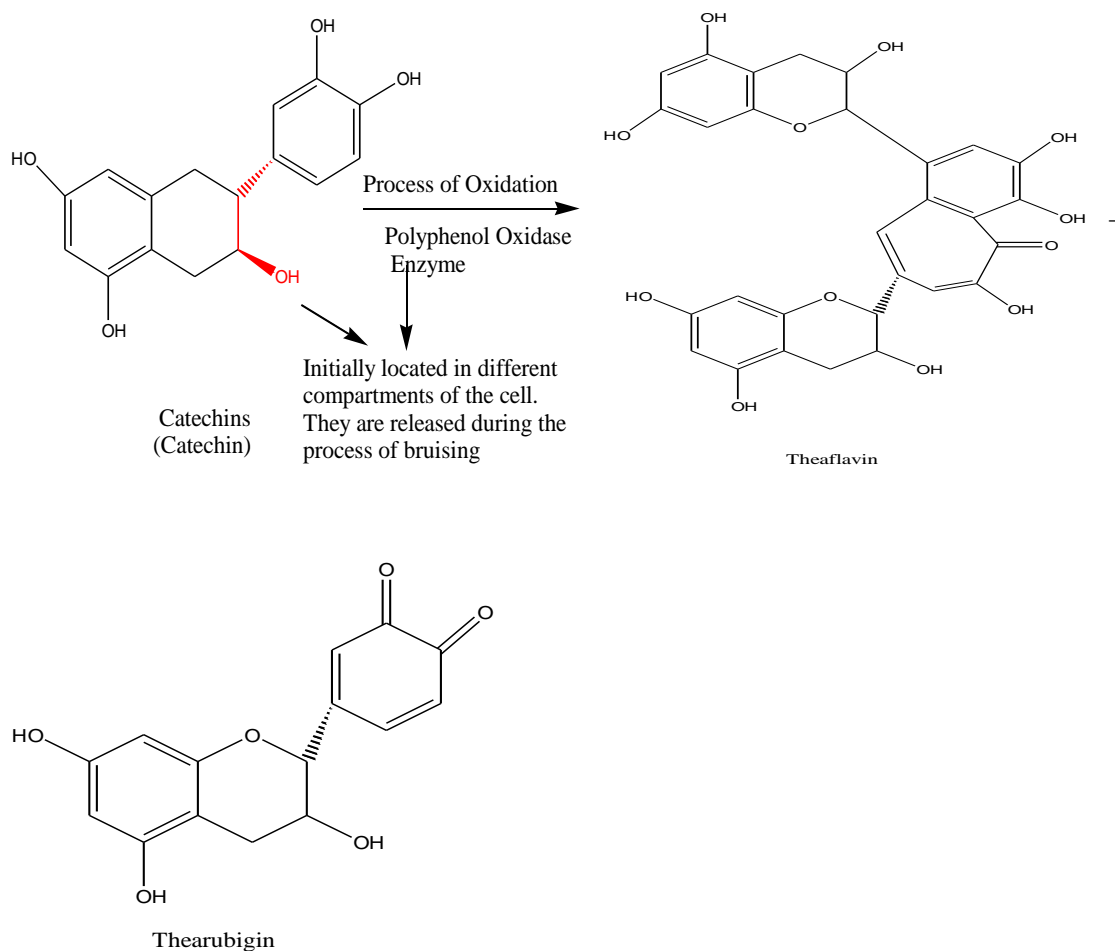
Reaction scheme 2.1: Production of Theaflavin and Thearubigin in Black Tea (Yamimoto *et al.*, 1997)

2.6 Effect of pH of solution on biosorption

The most important single parameter influencing the sorption capacity is pH. pH of adsorption medium is related to the adsorption mechanisms onto the adsorbent surface from water. pH determines the nature of physicochemical interaction of the species in solution and the adsorptive sites of the adsorbent (Aksu and Gonen, 2004).

The pH of feed is an important controlling parameter in the heavy metal adsorption process and thus the role of hydrogen ion concentration in the mixture of adsorbent

and aqueous solution is critical. It is well known that pH besides various physicochemical effects is an important variable in the ion exchange governed biosorption process, by which surface charges may be changed or modified (Cay *et al.*, 2004).

pH influences both the adsorbent surface metal binding sites and the metal chemistry in water . The pH of feed solution was examined by Malkoc & Nuhoglu from solutions at different pH, values covering a range of 2.0–5.0. In Ni (II) adsorption on waste tea, the highest maximum bed capacity and the longest breakthrough time was obtained at pH 4.0. At pH < 3.0, H+ ions compete with Ni(II) ions for the surface of the adsorbent which would hinder Ni(II) ions from reaching the binding sites of the sorbent caused by the repulsive forces. At pH > 5.0, the Ni (II) ions get precipitated due to hydroxide anions forming a nickel hydroxide precipitate. For this reason, the maximum pH value was selected to be 5.0. The hydroxyl groups of waste tea are effective binding sites for metal ions, forming stable complexes by coordination. The interaction between the functional groups in waste tea and Ni (II) ions may be responsible for pH increase in the initial stages and as the saturation of the bed proceeds, the pH decreased. For the influent solution with pH 2.0, 3.0, 4.0 and 5.0, the effluent pH was sharply increased to 3.1, 3.8, 4.7 and 5.6, respectively. It was that adsorption of hydrogen ions from the solution and dissolution of some impurities from the adsorbent surface could have resulted in increase in the effluent pH. As continued to flow through the column, the effluent pH dropped to the influent pH value (Malkoc & Nuhoglu 2005).

Untreated biomass generally contains light metal ions such as K^+, Na^+, Ca^{2+} and Mg^{2+}. Treated biomass implies one or two chemical alterations. The first is protonation of the biomass with a strong acid such as HCl in which the proton displaces the light metal ion from the binding sites. Secondly the biomass is reacted with an aqueous solution of a given ion at high concentration so that the majority of sites are occupied by that ion hence the light metal ions are displaced (Malkoc & Nuhoglu 2005).

In order to establish the effect of pH on the adsorption of heavy metals onto tea waste, various batch and column experiments conducted by various researchers are summarized in table 2.4 (Wasewar, 2010).

Table 2.4: Effect of pH on adsorption of metals onto TFW

Reference	Metal ion	Time	Metal conc.	biosorbent Dose	pH Range	pH	Results
Cay *et al.* (2004)	Cu_{2+}, Cd^{2+}	1 hr	5 ppm	1 mg/L	1 to 8	5.5	After pH 5.5, recovery - constant 6.0-14.0. For pH values over 8.0 precipitation of heavy metals ions occurs.
Malkoc and Nuhoglu (2005)	Ni^{2+}	2 hr	100 mg/ lit	10 mg/L	2 to 5	4	Significantly decreased by reducing the pH values to 2.0 Effect related with zeta potential.
Malkoc and Nuhoglu (2006)	Cr^{3+}	1 hr	100 mg/ L	Bed depth: 30 cm, Bed dia: 2 cm	2 to 5	2	For pH 2.0, 3.0, and 5.0, 33.71, 28.07 and 23.76 mg g^{-1}, respectively were absorbed.
Amarasing he and Williams (2007)	Cu^{2+}, Pb^{2+}	1.5 hr	100 mg/ L	0.1 mg/L	2 to 7	5 to 6	At pH 2–3 range the adsorption was low and r increases between pH 4 & 5.
Malkoc and Nuhoglu (2006)	Cr^{2+}	1 hr	100 mg/ L	10 mg/L	2 to 5	2	Efficiency increased from 37% at pH 5.0 to 99% at pH 2.0. pH 2.0 to 5.0.

Adopted and modified from Wasewar (2010).

Cay *et al.* (2004) found out that the absorption percentages are very low at acidic medium. After pH 3, uptakes increase sharply up to pH 5.5 and thereafter they remained almost constant for greater pH values. No pH values greater than 8.0 were studied since precipitation of heavy metals occurs. Optimum pH for Cu (II) and Cd (II) in single and binary system was graphically determined as 5.5. Column adsorption studies at different pH values were carried out in the range of 2.0 - 5.0 for adsorption of Cr^{3+} onto tea waste. The highest maximum bed capacity and the longest breakthrough time were obtained at the lowest examined pH value. The maximum of Cr (III) absorption occurs at pH 2.0. It was observed that with decrease in the influent feed solution pH, the breakthrough curve shifted from left to right which indicated that more Cr (III) ions were removed. The amount of Cr (III) ions absorbed per unit weight of beds for pH 2.0, 3.0 and 5.0 were 33.71, 28.07 and 23.76 mg/L, respectively (Malkoc and Nuhoglu, 2006).

Amarasinghe and Williams (2007) studied the adsorption of Cu^{2+} and Pb^{2+} onto tea waste in the pH range 2.0 - 7.0. It was found that the maximum removal of metal is in the pH range 5.0 - 7.0. At pH 2.0 - 3.0 range, the absorption was very low and rapidly increased between pH 4.0 - 5.0. The surface charge of biomass materials was a strong function of the pH. At pH values above 10, the surface of the absorbent had a higher negative charge which resulted in higher attraction of cations. At very high pH values, the metal complex forms precipitates and hence separation may not be due to biosorption. The pH of the aqueous solution is an important controlling parameter in the heavy metal absorption process and thus the role of hydrogen ion concentration

was examined at different pH values covering the range of 2 - 12 (Wasewar *et al.*, 2008).

Zinc ion exists in different forms in aqueous solution and the stability of these forms is dependent on the pH of the aqueous solution. The optimum pH for tea waste for a solution containing Zn ions found to be 4.2 with removal of about 99% metal from solution with initial zinc concentration of 50 mg/dm^3,(Agrawal *et al.*, 2004)..   This may be because at pH values below 2.0, the electrostatic force of repulsion between absorbent and absorbate (Zn^{2+}) is prominent. At pH above 6, there is a possibility of zinc ions precipitating.   Between pH 2.0 and 4.2, there is a sharp increase in the percentage removal and almost constant for pH values larger than 4.2. This was due to the fact that metal ions start replacing hydrogen ions from absorbent surface (Gabaldon *et al.*, (1996).

The effect of pH on the absorption isotherm may be attributed to the interaction between ions in solution and complexes formed at the biosorbent surface. Bodek *et al.* (1995) reported the presence of different species of zinc in the aqueous solution depending on the pH of the solution. The speciation diagram in support of this has been reported by Carrot *et al.* (1997) which can be obtained using the reaction and equilibrium constants given below by Baes and Messmer (1976).

$$Zn^{2+} + H_2O \longrightarrow Zn(OH)^+ + H^+ \quad pK_1 = 7.94$$

$$Zn^{2+} + 2H_2O \longrightarrow Zn(OH)_2 + 2H^+ \quad pK_2 = 8.96$$

$$Zn^{2+} + 3H_2O \longrightarrow Zn(OH)_3^- + 3H^+ \quad pK_3 = 11.50$$

$$Zn^{2+} + 4H_2O \longrightarrow Zn(OH)_4^{2-} + 4H^+ \quad pK_4 = 12.80$$
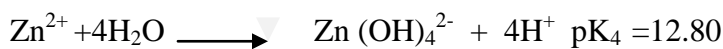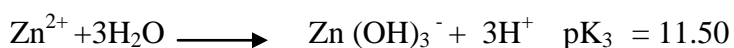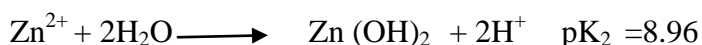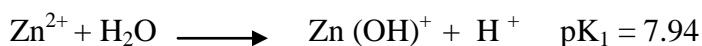
Thus the predominant ionic species is Zn $^{2+}$ at pH < 7. Zn (II) is present mainly as Zn $^{2+}$ at pH < 7, whereas Zn (II) is present mainly as Zn^{2+} and $Zn(OH)_2$ and in lesser quantity as Zn $(OH)^+$ at pH between 7 and 8. At lower pH values the H^+ ions compete with metal cations for the exchange sites in the system thereby partially releasing the metal ions, which compete with the M^{2+} ions for the adsorption site of waste tea. Decrease in biosorption at higher pH was due to the formation of soluble hydroxyl complexes. The heavy metal cations were completely released under circumstances of extreme acidic condition (Baes and Messmer, 1976).

Untreated biomass generally contains light metal ions such as K^+, Na^+, Ca^{2+} and Mg^{2+} (Aksu, 2002). Treated biomass implies one of two chemical alterations. The first is protonation of the biomass with a strong acid such as HCl whereby the proton displaces the light metal ions from the binding sites. In the second, the biomass is reacted with an aqueous solution of a given ion at high concentration so that the majority of sites are occupied by the light metals for example, calcium or potassium (Aksu, 2002).

2.7 Effect of absorbent dose

Effect of the amount of tea waste was studied at fixed pH by various researchers. The results of the effects of absorbent dose on the biosorption of various metals onto tea waste are summarized in table 2.5 below.

Table 2.5: Effect of absorbent dose on adsorption of metals onto TFW

Reference	Metal	Contact time	Metal conc.	pH	Dose range	Optimum dose	Results
Cay *et al.* (2004)	Cu , Cd	1 hr	5 ppm	5.5	0.2 - 4 gm/ L	1 gm/ L	Metal ions removed increases with the amount of absorbent. As the absorbent concentration increases, ratio of absorbent particles to metal ions increases, therefore, these particles attach more ions to their surfaces.
Mahvi *et al.* (2005)	Ni , Cd , Pb	1 hr	5 - 100 mg/L	–	5 - 15 gm/ L	—	94% removal of Pb from a 5 mg/L solution was possible by applying 0.5 g tea waste whereas the similar amount of absorbent was not enough to treat a 100 mg/L lead solution to more than about 76%. But by increasing the amount of tea waste to 1.5 g the efficiency of biosorption increased to about 96.5% for the same solution (100 mg/L Pb).
Amarasighe and Williams (2007)	Cu , Pb	1.5 Hr s	100, 200 mg/L	—	1.25 - 7.5 gm/ L	—	Percentage of lead ion removal increased from 37 to 94% when the biosorbent dose per 200 ml of solution was increased from 0.25 to 1.5 g.

Adopted from; Wasewar (2010).

The total amount of metal ions removed from the solution increases with the amount of absorbent. This is an expected result because as the absorbent concentration increases the number of particles surrounding the metal ions or ratio of absorbent particle to metal ions increases, therefore, these particles attach more ions to their surfaces. Increases in absorption with absorbent dose can be attributed to increased absorbent surface area and availability of more absorption sites (Cay *et al*., 2004).

In some cases, the amount of metal ions adsorbed per unit weight of adsorbent (q) decreases with the adsorbent dose. This is due to the fact that at higher adsorbent dose, the solution ion concentration drops to a lower value and the system reaches equilibrium at lower values of 'q' indicating the absorption sites remain unsaturated (Amarasinghe and Williams, 2007).

2.8 Effect of contact time

The percentage of Cu (II) and Cd (II) removed from single and binary system as a function of time was presented by Cay et al. (2004). The adsorbed amount of single and binary metal ions increased from 30 to 60 minutes after that a maximum removal was reached. The researchers selected 60 minutes as a suitable contact time for both metal ions in single and binary systems. Wasewar and co-workers (2009) studied the effect of contact time for absorbent and the metal ion to reach equilibrium was dependent on the initial concentration of the metal ion.

2.9 Effect of initial concentration of metal

The initial concentration provides an important force to overcome all mass transfer resistance of metal ions between the aqueous and solid phases. A high concentration difference provides a high driving force for the biosorption process (Malkoc and Nuhoglu, 2005)

Pb and Cu ion removal percentage increased when the initial ion concentration decreased

At low ion concentration, the ratio of surface active sites to the total metal ions in solution was high and hence all metal ions interacted with the adsorbent and were removed from the solution. However, amount of metal absorbed per unit weight of absorbent was higher at high concentrations. Adsorbent particles do not have many micro or macro pores, so its low surface area also results in lower sorption capacity (Aksu and Gonen, 2004).

Wasewar and co-workers (2009) studied removal of Zn (II) for various initial concentrations (25 – 200 mg/L) of zinc by tea factory wastes (0.2 g/50 cm^3) at different contact times.

The percent biosorption was decreased with increase in initial concentration but actual amount of Zn (II) absorbed per unit mass of absorbent (tea waste) increased with increase in metal concentration in the test solution. This was because of the decrease in resistance for the uptake of solute from solution with increase in metal concentration. All these findings clearly point to the fact that initial concentration affects the biosorption process.

2.10 Disposal of tea waste after adsorption

Disposal of used absorbent laden with heavy metal ions creates another environmental problem such as acid rains which can wash out the absorbed heavy metal ions (Cay *et al.,* 2004). This problem may be overcome to some extent by using one of the elimination methods such as elution, incineration and pyrolysis proposed by Gaballah and Kilbertus (1998).

The elution of heavy metals is the most common elimination method, allowing the recovery of solution of heavy metal ions at higher concentration for inertisation and recycling of the absorbent for subsequent uses. The percentage recoveries of the heavy metal ions were calculated by the elution method for single and binary systems. It was found out that the tea waste may be recovered for consecutive uses. Additional studies on the life-time of absorbent showed that tea waste may be efficiently used at least three cycles without changing retention capacity significantly (Cay *et al*., 2004).

Tea waste has a calorific value of about 21.69 mJ/kg (Wasewar *et al*., 2008). It can be used for making blended fuel briquettes which could be used as a fuel in furnaces. The bottom ash obtained from the furnace after its combustion can be blended with cementitious mixtures useful in building. Setting and leaching tests on these cementitious mixtures have shown that the bottom ash can be incorporated into the cementitious matrices to a great extent (75%) of total solid without the risk of unacceptable delay of cement setting as well as excessive heavy metal leacheability in solidified products (Mangialardi, 2003). However this mode of disposal requires

constant monitoring to ensure that the metal ions do not find their way back to the environment. In many parts of the world where tea waste is available at low or no cost, regeneration is not required and the metal laden biomass can be disposed of by incineration (Tunali *et al*., 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1   Research design

An aqueous solution with known concentration of specific heavy metal ion was used to represent contaminated water (5 ppm and 10 ppm to represent low and high concentration respectively). Tea waste of mass of 0.3g per 100 ml of the solution (which was the least mass for the same amount of solution ever used by researchers) was added to the aqueous solution for a specified period of time the then the tea waste filtered and the concentration of the aqueous solution determined. Difference in metal ion concentration implies that the tea waste has taken some of the metal ions with it.

3.2   Reagents and equipment

3.2.1 Reagents

The reagents that were used in the study were of analytical grade. The source of metal ions included lead (II) nitrate, $Pb(NO_3)_2$, copper (II) nitrate, $Cu(NO_3)_2.3H_2O$, zinc (II) nitrate, $Zn(NO_3)_2.6H_2O$, chromium (III) nitrate, $Cr(NO_3)_3.9H_2O$ cadmium nitrate, $Cd(NO_3)_2.5\ H_2O$. Acetate buffer: sodium acetate and acetic acid, phospate buffer: Na_2HPO4, HNO_3. All the reagents were obtained from Kobian chemical laboratory, Nairobi. Distilled water and deionised water were prepared at University of Eldoret chemistry laboratory. The analytical grade acetylene gas for AAS instrument was obtained from British Oxygen Company (BOC), Kenya. 1000 ppm pure standards of chromium, cadmium, copper, lead and zinc.

3.2.2 Equipment

The materials used were glassware (conical flasks, volumetric flasks, round bottomed flasks, beakers and stirring rods), plastic bottles, desiccators, analytical balance(UNIMAX 1010 DT made in Germany), pH meter (model Hanna GLP 110), mechanical shaker oven, plastic vials, labels, stop-watch, filter papers spatulas and washing detergent prepared in the laboratory. The AAS instrument used was a Varian SpectrAA 100/200. The instrument was interphased with computer software, which enables the results to be obtained in both hard and soft copy during and also after analysis. The fuel used was a mixture of air and analytical grade acetylene gas obtained from BOC, Kenya.

3.3   Source of tea waste

Tea waste was obtained from Nandi Tea Factory situated in Nandi Hills. After plucking, tea has five waste points giving five different types of waste. Four of these were used in this research. The first type of waste was removed before the tea leaves enter the factory and this type of waste was not sampled. Sampling was done at the different stages of waste within the factory and a pre-experiment done on which waste was the most suitable. The waste which came out after the final processing was the most suitable for the research mainly because it was the most porous with more pre spaces to absorb the metal ions. This type of waste has been referred to as black tea by researchers. Approximately 500 g of tea waste was collected at five different waste points where tea was being graded for packaging based on its fibre content. This mass was enough for the 200 sample of 0.3 g required for the experiment. The tea waste was

placed in a labeled polythene bag and transported to University of Eldoret chemistry laboratory where it was dried and kept in a desiccator to maintain a constant humidity awaiting preparation.

3.4 Sample preparation and measurement

3.4.1 Adsorbent

The tea waste was washed first with warm water (80 ^{o} C) to remove any dirt then rinsed with distilled de-ionised water. The tea waste was dried in an oven (model LR-271 C). After drying in the oven at 100 ^{o} C, the waste was ground and screened (using screen with mesh size 10). For preservation, the tea waste was kept in plastic stoppered bottles to minimize contact with humidity. All the bottles were preserved in desiccators before the time of use.

3.4.2 Adsorbate

A stock solution of 1000 ppm containing Cd^{2+}, Cr^{3+}, Cu^{2+}, Pb^{2+} and Zn^{2+} was prepared from their respective salts. The stock solution was prepared by dissolving; 1.599 g of $Pb(NO_3)_2$, 3.802 g of $Cu(NO_3)_2.3H_2O$, 2.9 g $Cd(NO_3)_2.5H_2O$, 7.695 g of $Cr(NO_3)_3.9H_2O$ and 2.86g of $Zn(NO_3)_2$, in distilled water in a 1000 ml volumetric flasks. The model solution was prepared containing the required metal ions under study and with pH meter (model Hanna GLP 110) the pH adjusted to 5.0 using commercial buffer tablets (pH 4 and 7). The acetate buffer was prepared using sodium acetate and acetic acid prepared as outlined in appendix 3B and the solutions made to the 1000 cm^3 mark with the distilled de-ionsed water.

Concentrations of 50 ppm and 5 ppm for both single and mixed metal ions were prepared as follows:

For 5 ppm single metal ions of each metal ion, 5 ml of the respective stock solution was put in a 1000 ml volumetric flask and distilled water was added to the 1000 cm^3 mark. The resultant solution contains ions with a concentration of 5 ppm. 100 ml of the 5 ppm solutions was put in 100 conical flasks prior to putting in the tea waste.

For 50 ppm single metal ions of each metal ion, 50 ml of stock solution was put in a 1000 ml volumetric flask and distilled de-ionised water was added to the 1000 cm^3 mark. The resultant solution contains ions with a concentration of 50 ppm. 100 ml of the 5 ppm solutions was put in 100 conical flasks prior to putting in the tea waste.

For 50 ppm mixed metal ions, 200 ml of each 250 ppm metal ion whose pH had been adjusted to 5 was mixed in a 1000 ml volumetric flask. Five replicate each having a total volume of 1000 ml mixed ions were prepared. 100 ml of the 50 ppm solution was put in 100, 250 ml conical flasks before the tea waste was added.

For 5ppm mixed metal ions, 200 ml of each metal ion of concentration 25 ppm previously prepared by diluting 25 ml of the 1000 ppm stock solution into a 1000 ml volumetric flask were mixed in a 1000 ml volumetric flask. 100 ml of the 5 ppm solution was put in 100, 250 ml conical flasks prior to adding the tea waste.

3.4.3 Biosorption procedure

A 0.3 g mass of dry tea waste was introduced to nine conical flasks containing 100 ml of the test solution. One conical flask with 100 ml test solution was left without the tea

waste to serve as the control. All the ten conical flasks were place in three mechanical shakers and their speeds was adjusted to be equal and the experiment was run concurrently. After ten minutes one conical flask was removed and the tea waste filtered off. One more was remove after an interval of ten minutes until the last one at ninety minutes. The solutions were taken immediately to the AAS to determine their concentration and result recorded.

 The process was repeated to give duplicate results.

The same procedure was followed for each metal ion at each level the two different concentration levels (5 ppm for low and 10 ppm for high) and also at the two existence levels (single or mixed) differently. The concentration of each of the five ions (Cd, Cr, Cu, Pb and Zn) was determined by AAS. For each sample the corresponding wavelength was set and the instrument calibrated using appropriate standards. The samples from the biosorption experiment in each of the ten conical flasks, was divided into three to give three replicate concentrations which were averaged. The samples of the first conical flask which did not have tea waste gave the initial concentration of ions in that particular experiment hence serving as the control.

3.5   Analytical methods

3.5.1   Flame Atomic Absorption Spectrometer

Flame atomic absorption spectroscopy was used to determine the concentration of heavy metals. The Flame Atomic Absorption Spectrophotometer used was (210 VGP, UK) equipped with hollow cathode lamps as the light source and air - acetylene fuel

system and a Perkin Elmer Optima 5300 D ICP – OES spectrophotometer operating in

axial mode were used for metal determination).

The technique of flame atomic spectroscopy is summarized by the flow chart in Figure

3.1
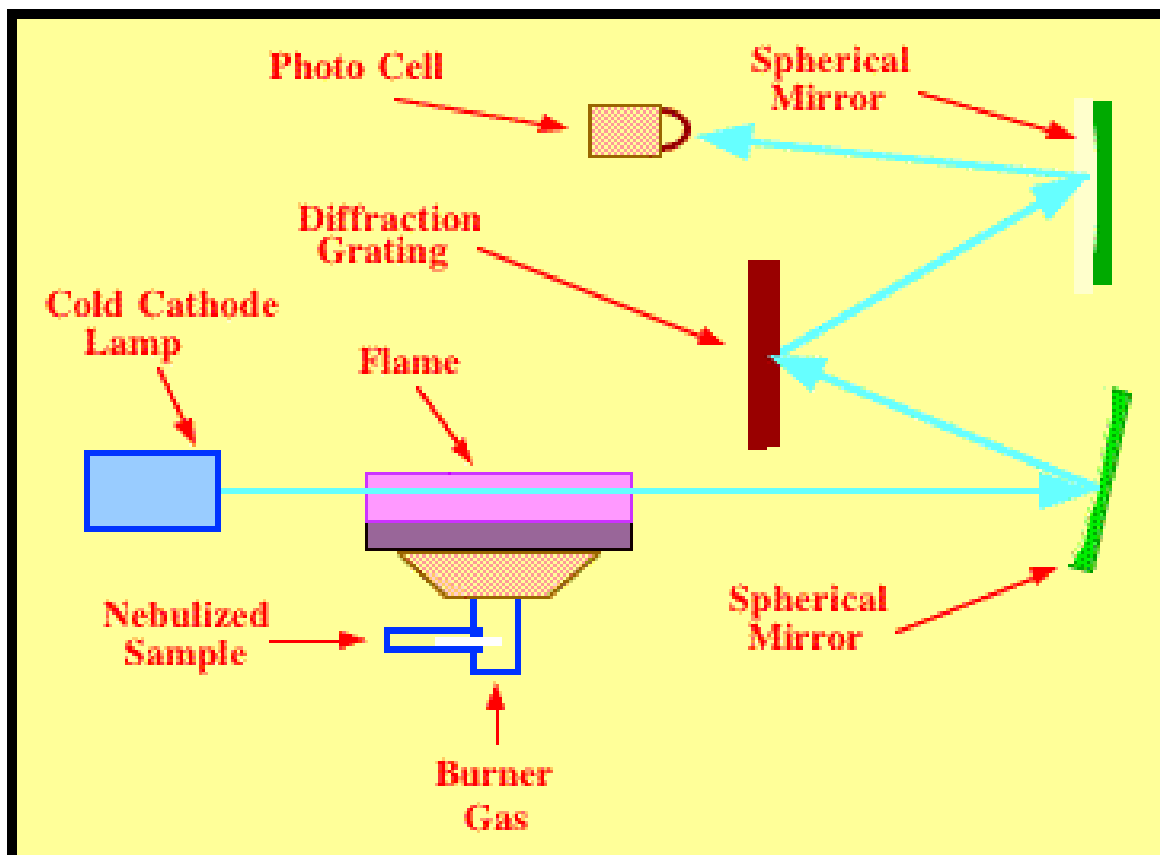
Figure 3.1: The Flame Atomic Absorption Spectrometer (chromatography-*online.org, 2012*)

3.5.2 Operation principle of an atomic absorption spectrometer

The characteristic wavelengths are element specific and accurate to 0.01-0.1 nm. To

provide element specific wavelengths, a light beam from a lamp whose cathode is

made of the element being determined is passed through the flame. A device such as

photomultiplier can detect the amount of reduction of the light intensity due to absorption by the analyte and this can be directly related to the amount of the element in the sample.

3.6 Analysis by AAS

3.6.1 Optimizing operating conditions

The instrument's operating conditions were optimized before the actual analysis. The fixed parameters were automatically set upon selection of the element to be analyzed. These parameters included; wavelength, lamp current, slit width and extra heat tension (EHT). The other parameters were set according to the manufacturers specifications in the manual. The acetylene and oxygen flow rate was set to attain maximum transparency of the flame. The optimized conditions for the AAS instrument during analysis are shown in table 3.1.

Table 3.1: Optimized conditions for AAS

Element	Wavelength λ (nm)	Lamp Current (mA)	Slit Width (nm)	Acetylene flow rate (L/min)	Oxidant flow rate (L/min)	BH (mm)	EHT (v)
Zn	213.9	5	1	2	13.5	13.5	800
Cu	324.8	4	0.5	2	13.5	13.5	494
Pb	217	10	1	2	13.5	13.5	485
Cd	228.8	4	0.5	2	13.5	13.5	562
Cr	357.9	7	0.2	2.9	13.5	13.5	594

Key: BH - Burner height,   EHT - Extra Heat Tension

3.7 Calibration Curves

Sensitivity and detection limit checks were carried out to ensure that they were in agreement with the operating parameters by running the stock solution before the actual analysis. Calibration curve for each element were prepared using the standard working solutions. The curves had absorbance in the y – axis and concentrations in the x – axis. For example copper is calibrated using pure copper standards and the percentage of copper ions in solution is calculated relative to the standard and the results presented in graphs like the one shown in figure 3.2
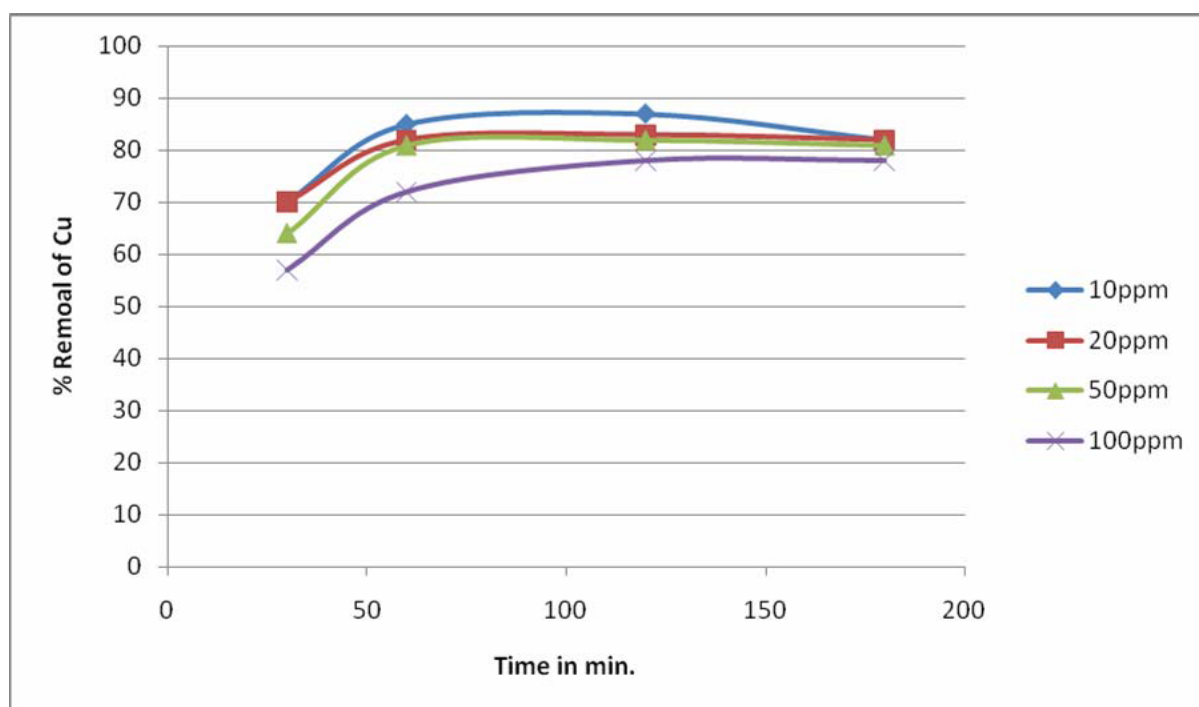
Figure 3.2: % removal of Cu^{2+} ions (Lokendra &Mukesh, 2013)

CHAPTER FOUR

RESULTS AND DISCUSION

4.1 Introduction

The potential of tea waste as biosorbents for the selected heavy metal ions was investigated in terms of the percentage heavy metal ions lost from the model solution over a period of 90 minutes. The results showing the change in concentration and percentage change in concentration are recorded in appendix 6. Factors that affected the rate of biosorption were also investigated together with the absorption isotherms and order of reaction that best fitted the biosorption process.

4.2 Effect of contact time on potential and rate of biosorption

Contact time is the time it takes the biosorbent (tea waste) to absorb a certain quantity of metal ions. The amount removed from solution at a particular time can be represented by the percentage of the fraction removed over the initial concentration. When the percentage uptake was plotted against time the graphs in figures 4.1 to 4.4 were obtained. The biosorption process in all cases exhibited a two stage process; the first involved rapid biosorption onto the surface of the tea waste while the second was gradual biosorption during which the metal ions diffused into the cells of the tea waste by a process of ion exchange. In Appendix 4 the approximate time taken for a quantitative amount of the metal ions to be absorbed by the tea waste was summarized and shows the percentage uptake after 20 minutes and after 90 minutes for; single ions at low initial concentration (SL), single ions at high initial concentration (SH), mixed ions at low initial concentration (ML) and mixed ions at high initial concentration

(MH). Tea waste is a porous material. The solution containing the heavy metal ions permeate into the available spaces in the tea waste leading to a high percentage of removal within the first few minutes. This first part of removal is mainly a physical process which is then followed by a slow chemical process composed of ion exchange and complexation. This behaviour has been reported by Cay and co-workers (2004). This information is presented in the following graphs (figures 4.1 - 4.4). Figure 4.1 presents the absorption for single ions at low initial concentration.
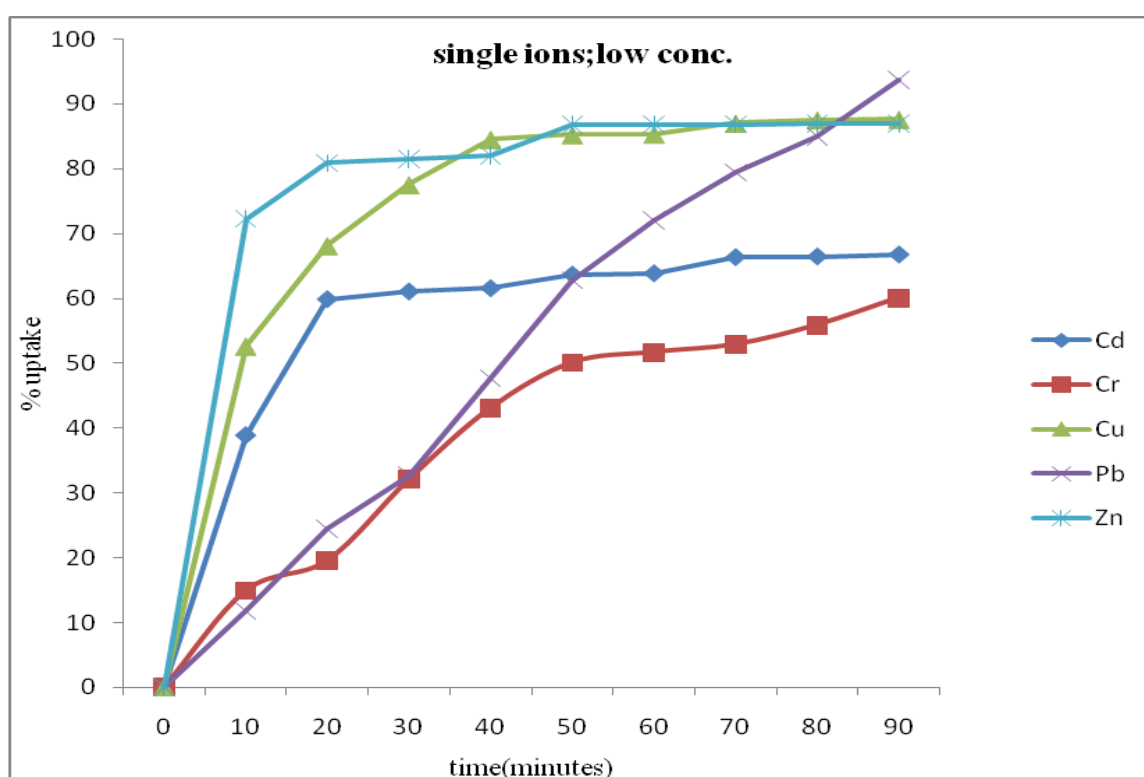
Figure 4.1: % absorption for single ions; low concentration

Zn (II) ions were adsorbed more than the rest of the ions for the first 30 minutes but by the end of 90 minutes Pb (II) ions ended up being the most absorbed. Cr ions were, however, the least absorbed because at pH 5, Cr ions form a precipitate $Cr(OH)_3$. The order of biosorption was $Pb^{2+} > Cu^{2+} > Zn^{2+} > Cd^{2+} > Cr^{3+.}$ In aqueous solution, metal

ions are surrounded by water molecules which offer a negative centre of attraction to the positive metal ions a process called hydration. The larger the ion the less the surrounding water molecules hence Pb (82) has the least hydration radius followed by Cd (48), Cr (24) Cu (29) and Zn (30), in that order. This explains why Pb with the greatest ionic radius of the five ions was the most biosorbed while Cu and Zn were among the least. Zn being the smallest ion penetrated the pores in tea waste very fast and was the most biosorbed within the first twenty minutes. This supports the speculation that the biosorption process starts with a physical uptake of the ions followed by the chemical ones. The percentages and order of biosorption in figures 4.1-4.4 agreed with Mahvi and co-workers (2005). Figure 4.2 presents the absorption for single ions at high initial concentration.
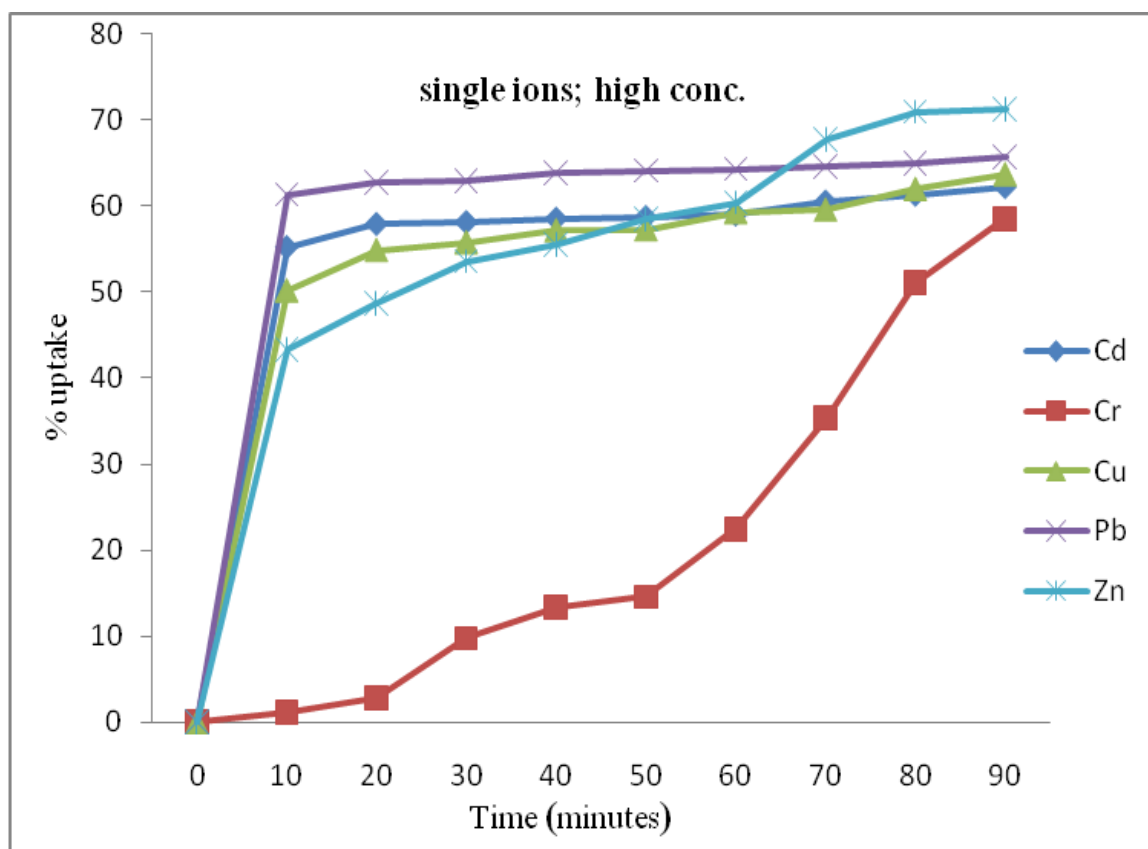
Figure 4.1: % absorption for single ions; high concentration

Cd, Cu, Pb and Zn ions were absorbed to over 50 % within the first 30 minutes. Cr ions showed very low absorption until after about 50 minutes when the percentage started rising. As the concentration of Cr ions increased the amount of precipitate also increased hence fewer ions could access the biosorbent binding sites. As time progressed, Cr ions replaced the hydrogen ions which in turn were released into solution. The increased hydrogen ions in solution could have caused the pH of the experimental solution to drop which favoured the rise in biosorption percentage after 50 minutes. The order of biosorption was therefore $Zn^{2+} > Pb^{2+} > Cu^{2+} > Cd^{2+} > Cr^{2+}$ which was different from that for single ions at low concentration. Figure 4.3 shows the absorption for mixed ions at low initial concentration.

Figure 4.2: % absorption for mixed ions; low concentration

When the ions were mixed the percent removal dropped (as shown in the result appendix 6) compared to those of single ion systems except for Cr. The decrease in percentage removal of ions could be attributed to the increase in the number of ions to be attached to the binding sites which remained constant as well as the competition for binding sites by the five ions in the mixture. Percentage uptake for Cr ions increased possibly as a result of both biosorption onto tea waste and reaction with the other ions present like Pb to give the respective solid chromate salts which remained as residues in the filter paper before the filtrate was nebulized in the AAS. The order of biosorption was $Pb^{2+} > Cu^{2+} > Zn^{2+} > Cd^{2+} > Cr^{3+}$. This order was the same as that for single ions at low initial concentration. The large Pb^{2+} ions have the largest naked radius, higher ionic mobility and approach sites of biosorbents more closely, while Cr^{3+} ions although large in size are precipitated before reaching the sites. Figure 4.4 reveals the absorption for mixed ions at high initial concentration.

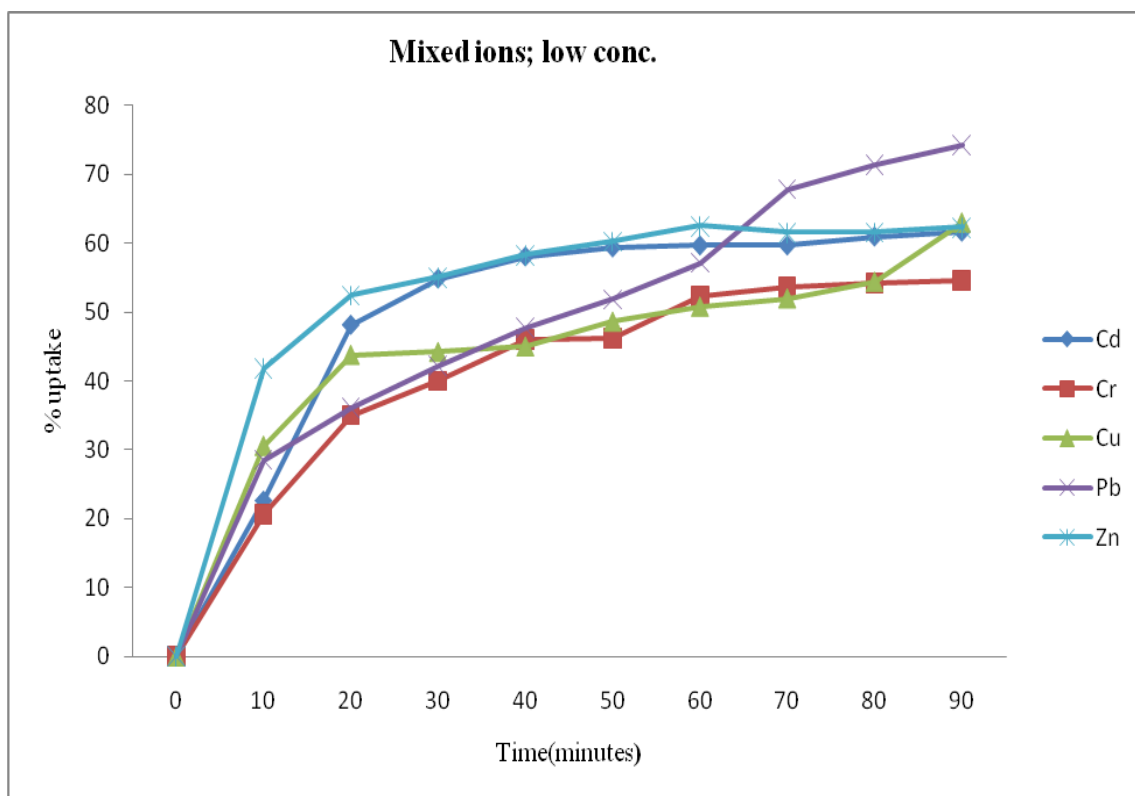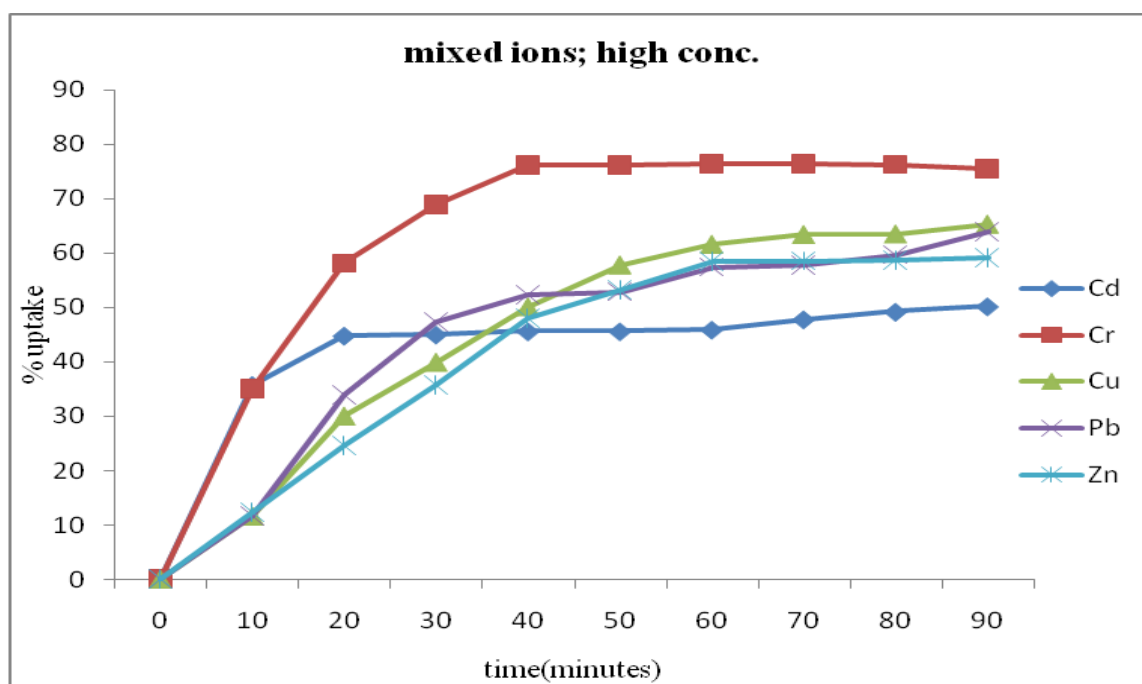**Figure 4.3: % absorption for mixed ions; high concentration**

When the concentration of the ions increased, the binding sites of the tea waste became too few to absorb all the metal ions. This was compounded by the fact that all the five ions were present at the same time. This is evidenced by the low percentage removal. Percentage removal of Cr ions was, however, abnormally high. This could be attributed to the high number of hydrogen ions released as the many ions displaced them from the biosorbent leading to reduced pH. A low pH favours the biosorption of Cr ions. Cr ions at pH 5, formed $Cr(OH)_3$ precipitates which were eliminated at the filtering stage. The order of biosorption was $Cr^{3+} > Cu^{2+} > Pb^{2+} > Zn^{2+} > Cd^{2+}$ and this was different from single ionic system. The order of biosorption, however, had no correlation with the sizes of the ions and by extension the hydration radii.

From these graphs it is shown that tea waste has a potential in absorbing heavy metal ions which was further investigated in terms of effect of other ions, nature of absorbing surface and the reaction kinetics of the absorption process.

4.3 The effect of initial concentration on rate of biosorption

Investigation was carried out on solutions with two levels of concentration, high and low for the two categories of singly occupied ions as well as for mixed ions. One way analysis of variance (ANOVA) at 95% confidence level was used to determine the variation in biosorption between the two levels of concentration. Tables 4.1 and 4.2 show the data for single and mixed ions, respectively.

Table 4.1: Effect of initial concentration on biosorption for single metal ions

parameter	p-value	F-calculated	F critical
Cd^{2+}	5.32×10^{-7}	57.3230	4.4139
Cr^{3+}	9.08×10^{-10}	133.8344	4.4139
Cu^{2+}	2.6×10^{-07}	63.4668	4.4139
Pb^{2+}	5.28×10^{-06}	40.6337	4.4139
Zn^{2+}	1.86×10^{-07}	66.5202	4.4139

Table 4.2: Effect of initial concentration on biosorption for mixed metal ions

parameter	p-value	F-calculated	F critical
Cd^{2+}	3.92×10^{-10}	148.5870	4.4139
Cr^{3+}	3.06×10^{-04}	19.8404	4.4139
Cu^{2+}	1.07×10^{-06}	51.7944	4.4139
Pb^{2+}	2.97×10^{-07}	62.2942	4.4139
Zn^{2+}	1.57×10^{-07}	68.1007	4.4139

In both cases $F_{\text{-calculated}}$ was greater than $F_{\text{-critical}}$ which implies that biosorption for ions that exist at low concentration differs significantly from that of ions that exist at high initial concentration. It is therefore clear that initial concentration determines the way metal ions are biosorbed whether they exist as the only individual contaminants or together with others. This is in agreement with the findings of Aksu and Gonen (2004), who postulated that a high concentration difference provides a driving force for the process hence a high adsorption capacity for higher concentration of metal ions since the concentration difference is high. Malkoc and Nuhoglu (2007) also had similar findings that a higher initial concentration provides the driving force to

overcome all mass transfer resistances of the pollutant between the aqueous and solid phases.

4.4 Effect of the presence of other ions (mixed ions) on the rate of biosorption

Industrial effluent could contain one or more metal ions as contaminants. It was thus considered prudent to compare the variation between biosorption when metal ions are present singly and when in combination with others. Table 4.3 records the analysis of variance at 95% confidence level for each ion between absorption for single and that of mixed.

Table 4.3: Effect of other ions on biosorption, at low initial concentration

parameter	p-value	$F_{\text{-calculated}}$	$F_{\text{-critical}}$
Cd^{2+}	0.2628	1.3364	4.4139
Cr^{3+}	0.6719	0.1853	4.4139
Cu^{2+}	0.0002	2.8719	4.4139
Pb^{2+}	0.5027	0.4678	4.4139
Zn^{2+}	0.0203	6.4820	4.4139

At low initial concentration there was no significant difference in the way specific metal ions were biosorbed whether present singly or mixed with other heavy metal ion contaminants since $F_{\text{-calculated}}$ was less than $F_{\text{-critical}}$ except for Zn as shown in table 8. Biosorption of Zn ions reached over 70 % within the first 20 minutes for single ions. However, when there were other ions at the same concentration the percentage uptake was less than 50 % hence the mode was significantly different. The small Zn ions were

the right size to occupy the empty spaces in tea waste without any hindrance but when the ions were mixed, this was reduced by collision and competition from other ions. (Kuyucak and Volesky 1988) hypothesized that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomasses takes place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells becomes the most adsorbed. The ion whose size matches with the spaces in the adsorbent

Table 4.4: Effect of other ions on biosorption, at high initial concentration

parameter	p-value	$F_{-calculated}$	$F_{-critical}$
Cd^{2+}	0.1101	2.8252	4.4139
Cr^{3+}	0.0445	4.6655	4.4139
Cu^{2+}	0.1368	2.4258	4.4139
Pb^{2+}	0.0897	3.2180	4.4139
Zn^{2+}	0.9209	0.0101	4.4139

At high initial concentration there was no significant difference in the way specific metal ions were biosorbed whether one ion was present alone or mixed with other metal ion, for four out of the five ions. In all cases $F_{-critical}$ was greater than $F_{-calculated}$. However, for Cr ions, the $F_{-calculated}$ value was higher than $F_{-critical}$ implying that there was significant difference in the biosorption of Cr ions. This exceptional behaviour for chromium is as a result of its ability to form precipitates of chromium hydroxide at pH values as low as 3. For the other ions which do not form precipitates at pH values below 5, there was no significant difference in the way the ions were biosorbed with or

without other heavy metal ion contaminants. These findings agree with those of Malkoc & Nuhoglu (2007).

For the four categories namely; single metal ions at low initial concentration, single metal ions at high initial concentration, mixed metal ions at low initial concentration and mixed metal ions at high initial concentration, each category was subjected to one way ANOVA to find out whether tea waste biosorbed the ions in the same manner or in a significantly different manner. The findings are recorded in Table 4.5.

Table 4.5: Comparison of adsorption by tea waste, within categories

Parameter	P-value	$F_{calculated}$	$F_{critical}$
Single ions at low initial concentration	1.7×10^{-6}	7.7393	2.1240
Single ions at high initial concentration	3.54×10^{-8}	10.6119	2.1240
Mixed ions at low initial concentration	8.0×10^{-15}	28.9800	2.1240
Mixed ions at high initial concentration	5.11×10^{-10}	14.4150	2.1240

From the results of analysis of variance at 95% confidence level in the table above, the biosorption differed significantly since $F_{-calculated}$ is greater than $F_{-critical}$. This implies that the affinity of the ions at different concentration levels (low or high) to the functional groups in the tea waste was significantly different. The presence of other ions (single or mixed), made the biosorption for the two levels proceed in a significantly different speed and to different magnitudes of percentage removal. The difference in the rate of biosorption to the biosorbent could also be attributed to the

variation in the ionic size. Pb has a bigger size followed by Cd, Cr, Cu and Zn, in that order.

4.5 Adsorption isotherms

An adsorption isotherm is an equation that describes how the amount of a substance adsorbed onto a surface depends on its concentration (if in a solution), or its pressure (if a gas) at a constant temperature. Adsorption isotherms focus mainly on systems where the adsorbate particles are mostly concentrated on the surface of an adsorbent. The Langmuir isotherm describes the dependence of the surface coverage of an adsorbed species on the pressure/concentration of the species at a fixed temperature. The Freundlich describes physical adsorption in solution while the BET isotherm applies to multi-layer adsorption. The Dubinin-Radushkevich isotherm is commonly used to check whether an adsorption process is physical or chemical

To deduce the nature of surface that absorbs heavy metal ions, the experimental data were fitted to Freundlich and Langmuir equilibrium models. The amount of metal ion bound by the tea waste was calculated by the expression;

$$q = \frac{v(c_i - c_f)}{m}$$ ....Eqn. 4.1

Where q is the metal uptake (mg metal per g biosorbent), v is the liquid sample volume (ml), C_i is the initial concentration of the metal in the solution (mg/L), C_f is the final concentration (at equilibrium) of the metal in the supernatant (mg/L) and m is the amount of the added biosorbent on the dry mass basis (mg).

4.6 Langmuir isotherm

The Langmuir isotherm has been widely used to describe and determine the adsorption capacity q_{max} of metal ions during biosorption processes. The Langmuir adsorption isotherm is useful in quantifying and contrasting the performance of different biosorbents. In its formulation, binding to the surface is primarily by physical forces mainly electrostatic and implicit in its derivation is the assumption that all sites possess equal affinity for the adsorbate. It has been used to empirically describe equilibrium relationships between a bulk liquid phase and a solid phase. One of the simplest representations of the adsorption phenomenon calls for the migration to and the occupation of a surface site of adsorbent by an adsorbate.

The Langmuir model is given by the equation;

$$q_e = \frac{q_{max}k_L c_{eq}}{1 + k_L c_{eq}}$$ ....Eqn. 4.2

Where;

q_e is milligrams of metal accumulated per gram of the biosorbent material;

c_e is the metal residual concentration in solution;

q_{max} is the maximum specific uptake corresponding to the site saturation

k_L, is the ratio of adsorption and desorption rates. Langmuir equation can also be re-

written as: $\dfrac{c_e}{q_e} = \dfrac{1}{k_L q_m} + \dfrac{c_e}{q_m}$ .... Eqn. 4.3

A plot of $\dfrac{c_e}{q_e}$ versus c_e gave a straight line with y-intercept equal to $\dfrac{1}{k_L q_{max}}$ and the

slope given by the expression $\dfrac{1}{q_{max}}$. Figures 4.5 and 4.6 are examples of linearized

plot for Cd^{2+} single ions at high concentration and Pb^{2+} mixed ions at low concentration, respectively.
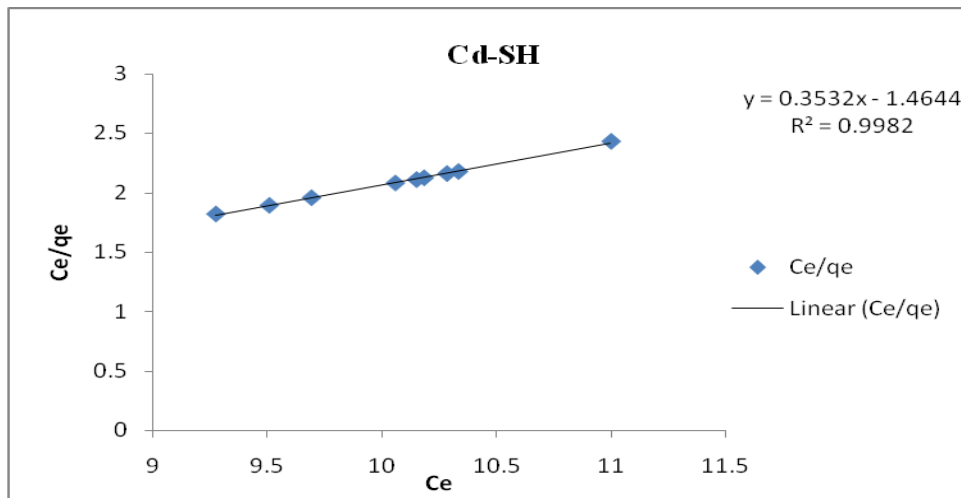
Figure 4.5: Langmuir plot for Cd^{2+} ions; high concentration
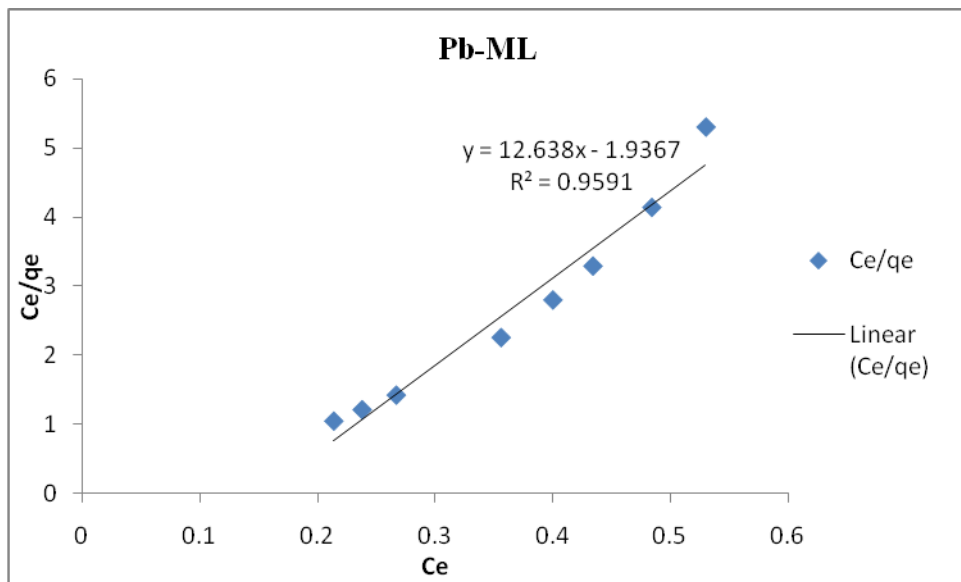
Figure 4.6: Langmuir plot for Pb^{2+} ions; low concentration

From these plots, the parameters for each ion at the different conditions were obtained and tabulated in Table 4.6.

Table 4.6: Constants for linearized Langmuir model

Metal ion	SH			SL			MH			ML		
	$-k_L$	q_{max}	R^2	$-k_L$	q_{max}	R^2	$-k_L$	q_{max}	R^2	$-k_L$	q_{max}	R^2
Cd^{2+}	0.241	2.832	0.998	4.656	0.086	0.988	0.122	1.468	0.986	4.532	0.032	0.967
Cr^{3+}	0.142	0.359	0.827	3.649	0.023	0.908	0.288	2.049	0.967	4.401	0.051	0.984
Cu^{2+}	0.306	1.923	0.992	26.082	0.098	0.982	0.138	0.619	0.808	5.444	0.076	0.987
Pb^{2+}	0.354	2.816	0.999	12.365	0.119	0.959	0.169	1.616	0.964	6.526	0.079	0.959
Zn^{2+}	0.156	1.684	0.982	29.150	0.193	0.996	0.161	1.032	0.951	4.598	0.088	0.986

KEY

SL- single metal ions at low concentration

SH- single metal ions at high concentration

ML- mixed metal ions at low concentration

MH- mixed metal ions at high concentration

Theoretically the order of biosorption is $Pb^{2+} > Cd^{2+} > Zn^{2+} > Cu^{2+} > Cr^{3+}$, based on the varying sizes of ions. Ions with larger ionic radii have a higher likelihood of being biosorbed compared to those which have smaller ionic radii. Larger ions are least

hydrated while the smaller ions are highly hydrated in solution making them more difficult to biosorb compared to the bigger ions. Using the averages of the maximum specific uptake (q_{max}) in the Langmuir model recorded in table 11 for example, for Pb (2.816 for SH, 0.119 for SL, 1.616 for MH and 0.079 for ML), the values were added and the sum divided by four to give the value 1.158. The same was done for all the other ions and the order was found to be: Pb(1.158) > Cd(1.105) > Zn(0.749) > Cu(0.6790) > Cr(0.540). This represents the average general trend from the graphs drawn in figures 4.1-4.4. This order agrees with the findings of Mahvi and co workers (2005).

4.6.1 Freundlich isotherm

The Freundlich isotherm (Freundlich, 1907) was originally of an empirical nature, but was later interpreted as sorption to heterogeneous surfaces or surfaces supporting sites of varied affinities. It is assumed that the stronger binding sites are occupied first and that the binding strength decreases with increasing degree of site occupation. In this model, the energy of a metal ion binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. The Freundlich equation takes the form:

$$q_e = k_f \left(c_e \right)^{\frac{1}{n}}$$ ……………………………………………………………eqn. 4.4

This equation can be expressed in linear form as;

$$\log q_e = \log k_f + \frac{1}{n} \log c_e$$ …………………….......…….…………………eqn. 4.5

Where q_e and c_e are corresponding to the mass of solute absorbed per gram of biosorbent and the solute concentration at equilibrium, respectively. k_f and n are

constants relating to respective biosorption capacity and biosorption intensity. The corresponding values of k_f and n indicate high biosorption capacity and intensity. The equation; $\log q_e = \log k_f + \dfrac{1}{n} \log c_e$ was used to plot log q_e (y-axis) against log c_e and a straight line was obtained with n equal to the reciprocal of the gradient and k_f the anti-logarithm of the y-intercept. When these parameters were plotted, graphs similar to the one for Cu ions at high initial concentration in figure 4.7 were obtained. The constants n, k_f and R^2 were obtained and recorded in table 4.7.
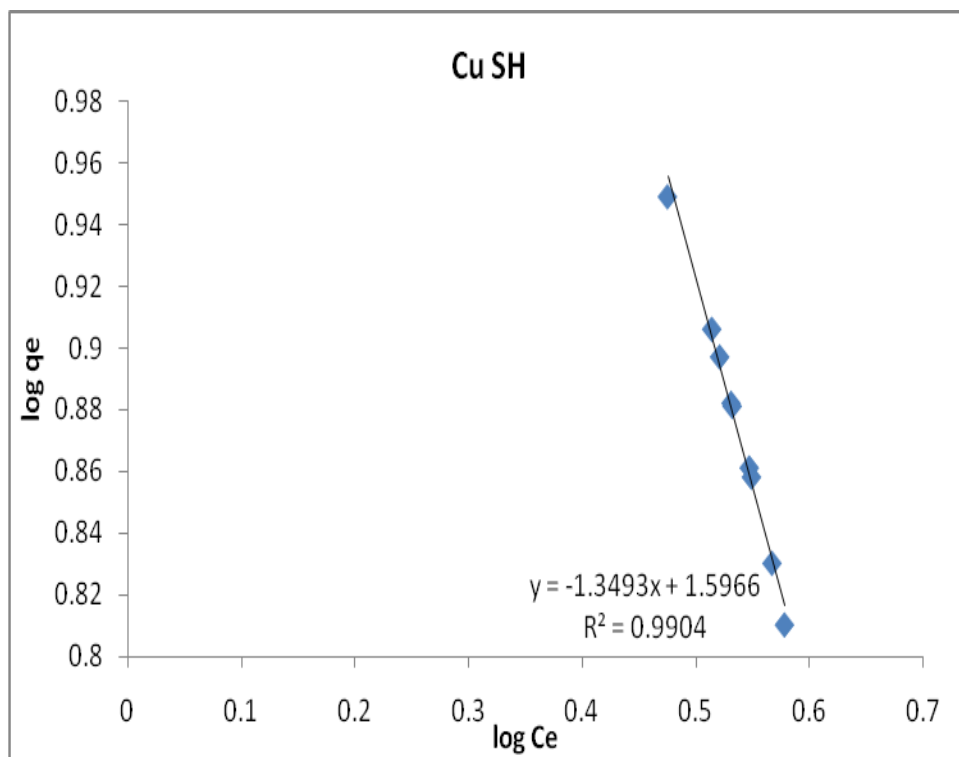
Figure 4.7: Freundlich plot for Cu single ion; high concentration.

Table 4.7: Constants for linearized Freundlich model

Metal ion	SH			SL			MH			ML		
	n	K_f	R^2	n	K_f	R^2	n	K_f	R^2	n	K_f	R^2
Cd^{2+}	0.694	97.05	0.996	4.065	6.761	0.671	1.359	35.645	0.988	1.475	13.428	0.966
Cr^{3+}	5.050	11.194	0.694	1.927	8.871	0.949	0.742	54.075	0.948	1.704	8.690	0.984
Cu^{2+}	0.741	39.446	0.990	0.373	1510.08	0.915	1.818	18.239	0.878	1.196	12.793	0.986
Pb^{2+}	20.0	8.222	0.942	1.049	28.576	0.843	2.096	19.907	0.913	0.927	22.542	0.959
Zn^{2+}	0.709	76.736	0.968	0.247	1776.03	0.983	1.942	17.179	0.918	0.887	15.922	0.986

The sequence of biosorption was evaluated by the ability of the ions to bind to tea waste (biosorbent) given by value (n) in the table above. All the values of n for each ion were added and divided by four. By using the average value of n, the order of biosorption was found to be: Pb (24.072) > Cd (1.898) > Cr (1.853) > Cu (1.032) > Zn (0.946).

R^2 values for Langmuir isotherms were, however, higher than those of Freundlich isotherms and this implies that the biosorption takes place at specific homogenous sites of the tea waste biomass. This also indicates that ion exchange mechanism took

place as opposed to adsorption-complexation reaction. Competitive biosorption and displacement of metals are complicated processes that are influenced by a multitude of factors. The occurrence of more than one possible adsorption mechanism onto tea waste contributes to the multi-faceted nature of these interactions. The binding of metal ions is thought to occur through a combination of physical, electrostatic, biosorption, ion-exchange, complexation, and chelation reactions. Biosorption is the predominant mechanism. The extent of biosorption and the mechanisms involved are also influenced by pH, initial metal concentration, the presence and properties of competing metals in solution, and contact time with tea waste. The associated properties of ion charge, size and solubility could determine how effectively a particular metal is absorbed and how likely it will be displaced by a more preferred competitor. This was investigated by Holberg (2006).

4.7 Order of reaction

The order of reaction explains the functional relationship between the concentration of reactants and the rate of reaction. It determines how the amount of compound speeds up or retards the reaction. It is given by the sum of the exponents of the concentration terms of the rate law: $Rate = k[A]^x[B]^y$ ....Eqn. 4.6. The reaction order for this equation is given by the sum of x and y. Example;

$$Rate = k[A]^1[B]^0 = k[A]$$. The reaction is first order in $[A]$ and zeroth order in $[B]$. The data was subjected to reaction kinetics to deduce whether the process followed pseudo first order or pseudo second order. The rate equation gives an

expression of the variation in concentration of metal ions with time. The order of reaction was deduced from the integrated rate equation given as;

$$\frac{\partial q_t}{\partial t} = k_1(q_e - q_t)$$ …………………...Eqn. 4.7

The symbols q_t and q_e are the masses of metal which are biosorbed by the tea waste in (mg/g) at any time t and at equilibrium, respectively. The value k_1 is the rate constant for the biosorption. On integration, equation 4.6 gives;

$$k_1 t = \ln q_e - \ln(q_e - q_t)$$ …………………….....................................Eqn. 4.8

A plot of time t against $\ln(q_e - q_t)$ gives a straight line with a slope of $-1/k_1$

If a graph of t against $\ln(q_e - q_t)$ is plotted, it gives a straight line with a correlation coefficient (R^2) value) tending to unity, then the experimental data agrees with first order kinetics and the reaction is first order. The second order rate equation can be represented as;

$$\frac{\partial q_t}{\partial t} = k_2(q_e - q_t)^2$$ …………………….....................................Eqn. 4.9,

where k_2 is the second order rate constant, q_e and q_t have the same meaning as defined above. Upon integration, equation 4.8 gives the equation;

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2}$$ …...Eqn. 4.10

Equation 4.9 predicts that for a second order process, plotting t/q_t against t should give a straight line from which the rate constant k_2 and the metal uptake at equilibrium q_e, can be calculated. From the R^2 values for the first and second order plots, it is possible to deduce the order of reaction. The curve with the higher value of linear

correlation coefficient corresponds to the order of the reaction. The plot of t/q_t against t

gave a straight line from which the rate constants k_1, k_2 and q_e were calculated and

tabulated in the following tables 4.8-4.11.

Table 4.8: Data for first and second order kinetics for single ions at low initial concentration

Metal	Pseudo first order			Pseudo second order		
	k_1	q_e	R^2	k_2	q_e	R^2
Cd^{2+}	0.008	17.270	0.949	2.412	0.108	0.995
Cr^{3+}	0.097	93901.34	0.873	1.405	0.094	0.976
Cu^{2+}	0.022	33.049	0.500	5.163	0.023	0.980
Pb^{2+}	0.029	54.707	0.941	3.272	0.141	0.976
Zn^{2+}	0.005	6.404	0.681	4.650	0.0376	0.986

Table 4.9 Data for first and second order kinetics for single ions at high initial concentration

Metal	Pseudo first order			Pseudo second order		
	k_1	q_e	R^2	k_2	q_e	R^2
Cd^{2+}	0.052	1	0.856	0.1554	3.125	0.997
Cr^{3+}	0.014	6.385	0.787	0.0355	2.463	0.882
Cu^{2+}	0.036	9.478	0.541	0.126	2.193	0.991
Pb^{2+}	0.003	1.627	0.917	0.309	2.421	0.999
Zn^{2+}	0.020	2.638	0.878	0.081	2.817	0.993

Table 4.10: Data for first and second order kinetics for mixed ions at low initial concentration

Metal	Pseudo first order			Pseudo second order		
	k_1	q_e	R^2	k_2	q_e	R^2
Cd^{2+}	0.011	49.402	0.753	2.978	0.087	0.996
Cr^{3+}	0.022	305.82	0.881	1.723	0.113	0.991
Cu^{2+}	0.092	59874.14	0.996	1.741	0.127	0.997
Pb^{2+}	0.062	1426.53	0.836	1.197	0.073	0.918
Zn^{2+}	0.020	56.149	0.703	2.547	0.120	0.997

Table 4.11: Data for first and second order kinetics for mixed ions at high initial concentration

Metal	Pseudo first order			Pseudo second order		
	k_1	q_e	R^2	k_2	q_e	R^2
Cd^{2+}	0.038	3.915	0.821	0.109	4.149	0.997
Cr^{3+}	0.011	1.649	0.489	0.123	1.618	0.991
Cu^{2+}	0.074	162.715	0.540	0.055	2.278	0.981
Pb^{2+}	0.036	11.393	0.916	0.053	2.740	0.980
Zn^{2+}	0.020	4.622	0.557	0.059	2.525	0.986

The q_e values did not show a consistent pattern between the first and the second order hence the values of k_1 and R^2 were used in determining the preference of the biosorption of the selected metal ions onto tea waste. A comparison of R^2 values between first and second order shows that the values for second order are higher than those of first order. Therefore the biosorption process for the ions by tea waste followed pseudo second order and these findings agree with those of Dhanakumar and co-workers (2007). The rate constants k_2 is also higher for the second order reactions (Wasewar, 2010).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

a). The rate of biosorption was high for the first thirty minutes which implies that the process taking place was physical adsorption where the ions enter the empty spaces in the porous tea waste biomass. Between thirty and ninety minutes the process was slow ion exchange as summarized in appendix 5. The rate of reaction followed pseudo second order kinetics which implies that the concentration of both the ions and the absorbing material significantly affect the process.

b).The longer the time of contact between the tea waste and the metal ions the higher the percentage uptake. When only one metal ion was present, the percentage uptake by the tea waste was higher than when other ions were also present in the simulated contaminated water. The capacity of tea waste to remove the metal ions uder study was higher when only one ion was present than when a mixture of ions were present in solution.

c). Initial concentration significantly affected the rate of biosorption. The difference in concentration between the ions and the absorbent provides the driving force for the process. The rate of uptake was higher when the initial concentration was high.

d).To deduce the nature of absorbing surface in tea waste, the data was fitted to Freundlich and Langmuir equilibrium models. The data fitted the Langmuir isotherm better than the Freundlich isotherm which implies that biosorption took place at specific homogenous sites of the tea waste. The averages of the maximum specific

uptake (q_{max}) in the Langmuir model gave the order; Pb^{2+} (1.158) > Cd^{2+} (1.105) > Zn^{2+} (0.749) > Cu^{2+} (0.6790 > Cr^{3+} (0.540),

Tea waste proved to be an efficient biosorbent reaching over 50 % for all the ions studied and Pb was the most biosorbed while Cr was the least. Tea waste is a cheap material and thus it would be convenient to use it in industrial waste water treatment plants.

5.2 Recommendations

a).Tea waste can be used to treat industrial and municipal wastes whose effluents contain; Cd^{2+}, Cr^{3+}, Cu^{2+}, Pb^{2+} and Zn^{2+} ions since it is efficient and cheap.

b). Efficiency of tea waste increases with increased contact time and increased amount of tea waste. Engineering prospects which utilize batch method and excess tea waste should be applied in removing these metal ions from effluents.

c) The contaminated tea waste is removed, dried and disposed of through incineration. The bottom ash obtained after its combustion can be blended with the cementitious mixtures. These cementitious materials could be used in building and construction works hence heavy metals remain permanently embedded in buildings.

5.3 Recommendation for further research

More research needs to be carried out on;

a). The effect of pre-treatment of the tea waste using some chemicals such as acids, bases and detergents. Instead of washing the tea waste with water only, the tea waste could be washed with a detergent and the percentage uptake of heavy metals compared with that of biosorbents washed with water only.

b). The ability of tea wastes to biosorb other metal ions like mercury (II), nickel (II) and chromium (VI) which were not covered by this research yet are very common pollutants of water from recycling of batteries and paint manufacturing industries.

c). Other biological materials which are cheap and easy to be disposed of and effective in removing heavy metals ions from contaminated water be studied with a view of making biosorption the technology of the future.

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APPENDICES

Appendix I: WHO, FAO and KEBS recommended maximum permissible limits of heavy metals in foodstuffs and drinking water

Element	WHO and FAO maximum permissible limits (ppm)	KEBS maximum permissible limits (ppm)
Pb	0.01	0.1
Cd	0.003	N/A
Cr	0.05	N/A
Cu	1.0	0.1
Zn	5.0	5.0

Appendix II: Dilution formula of stock solution into required standard solution

for calibration of the AAS instrument: $C_1V_1 = C_2V_2$

Where C_1 – Initial concentration of the stock solution

V_1 – Initial volume of the stock solution

C_2 – Final concentration (required) of analyte

V_2 – Final volume of analyte required to prepare the required

Standard solution

Appendix III: Preparatory procedures for the metal adsorption experiments on tea waste

Protocol for the preparation of reagents and materials used in the metal adsorption experiments. The procedures were based on protocol applied in the experiments by Kalmykova (2004; 2006).

A. Preparation of metal stock solutions

Metal stock solutions, each with a concentration of 1000 ppm (1 g/L) were prepared from metal nitrate salts. A calculated amount of salt was weighed, transferred into a volumetric flask, and then diluted to 100 mL. Preservation of the stock solutions was done by adding 1 mL HNO_3.

The formula used for the calculation of the weight of salt:

$$\frac{1\,gram\,of\,element\,dissolved\,in\,12\%\,Nitric\,acid}{R.A.M\,of\,element\,to\,be\,analysed} \times RFM\,of\,the\,salt\,used = Mass\,of\,salt.$$

This gives 1.000 µg/ml or 1000 ppm at density range of $1.01 - 1.02$ g/ml at 20° C

B) Preparation of acetate buffer

0.1 M acetate buffer was prepared by mixing equal volumes of 0.2 M sodium acetate and 0.2 M acetic acid solutions, prepared from fresh hydrated sodium acetate and glacial acetic acid, respectively from tablets of acetate buffer: sodium acetate(pH 5.6) and acetic acid(pH 4)

Appendix IV: Summary of % metal ion uptake

Metal ion	First stage(rapid adsorption)		Second stage(Gradual adsorption)	
	Time(minutes)	% uptake	Time(minutes)	% uptake
Cd^{2+} SL	20	59.5	90	66.8
Cd^{2+} SH	10	55.2	90	62.2
Cd^{2+} ML	20	48.1	90	61.6
Cd^{2+} MH	20	44.8	90	50.2
Cr^{3+} SL	30	32.1	90	60.1
Cr^{3+} SH	30	9.8	90	58.6
Cr^{3+} ML	30	35.0	90	54.6
Cr^{3+} MH	20	58.1	90	75.5
Cu^{2+} SL	10	52.6	90	87.6
Cu^{2+} SH	10	50.2	90	63.7
Cu^{2+} ML	10	30.6	90	63.0
Cu^{2+} MH	20	30.0	90	65.3
Pb^{2+} SL	30	32.7	90	93.7
Pb^{2+} SH	10	61.3	90	65.7
Pb^{2+} ML	30	42.2	90	74.2
Pb^{2+} MH	30	47.3	90	64.0
Zn^{2+} SL	10	72.2	90	86.9
Zn^{+2} SH	10	43.3	90	62.5
Zn^{2+} ML	10	41.8	90	74.2
Zn^{2+} MH	30	35.7	90	59.1

Appendix V: Glossary of terms

Adsorbent	Solid material on which adsorption occurs
Adsorption	A surface phenomenon defined as the increase in concentration of a particular component at the surface or interface between two phases
Adsorptive	Adsorbable substance in the fluid phase. A molecule or an ion in the soil solution that potentially can be adsorbed.
Chelation	The reaction between a metal ion and a chelating reagent. Chelating reagents are substances with multiple sites available for bonding with metal ions; such bonding typically results in the formation of stable five- or six-membered rings.
Chemisorption	Adsorption involving chemical bonding
Physisorption	Adsorption without chemical bonding
Functional group	An atom or group of atoms in an organic compound that gives the compound some of its characteristic properties, such as the C=O functional group in aldehydes and ketones, or –COOH in carboxylic acids
Ion exchange	The interchange between an ion in solution and another ion on a charged surface

Appendix VI: Results

Changes in metal ion concentration with time, for single ions, at low initial

concentration (5 ppm).

	Time (min)									
	0	10	20	30	40	50	60	70	80	90
Cd^{2+}	1.00	0.61	0.40	0.39	0.38	0.36	0.36	0.34	0.34	0.33
Cr^{3+}	0.70	0.60	0.56	0.48	0.40	0.35	0.34	0.33	0.31	0.28
Cu^{2+}	0.57	0.27	0.18	0.13	0.09	0.09	0.08	0.08	0.07	0.07
Pb^{2+}	0.79	0.60	0.53	0.50	0.42	0.30	0.22	0.16	0.12	0.05
Zn^{2+}	0.91	0.25	0.17	0.17	0.17	0.12	0.12	0.12	0.12	0.12

Changes in percentage uptake of metal ions by tea waste with time for single ions
at low initial concentration (5 ppm)

	Time (min)									
	0	10	20	30	40	50	60	70	80	90
Cd^{2+}	0	38.9	59.9	61.1	61.6	63.7	63.9	66.4	66.5	66.8
Cr^{3+}	0	14.9	19.6	32.1	43.1	50.1	51.7	52.9	55.9	60.1
Cu^{2+}	0	52.6	68.1	77.5	84.5	85.2	85.3	87.0	87.5	87.6
Pb^{2+}	0	11.8	24.4	32.7	47.7	62.8	72.0	79.4	84.9	93.7
Zn^{2+}	0	72.2	80.8	81.4	81.9	86.7	86.8	86.8	86.9	86.9

Changes in concentration for single metal ions with time, at high initial concentration (50 ppm)

	Time (min)									
	0	10	20	30	40	50	60	70	80	90
Cd^{2+}	24.55	11.00	10.33	10.28	10.18	10.15	10.06	9.69	9.51	9.28
Cr^{3+}	16.16	15.97	15.72	14.58	14.01	13.82	12.53	10.45	7.91	6.69
Cu^{2+}	17.83	8.89	8.05	7.89	7.63	7.61	7.26	7.21	6.76	6.46
Pb^{2+}	21.06	8.14	7.86	7.81	7.62	7.57	7.51	7.45	7.40	7.23
Zn^{2+}	23.107	13.11	11.86	10.72	10.28	10.00	9.16	7.46	6.70	6.62

Changes in percentage uptake of metal ions for single metals at high initial concentration (50 ppm)

	Time (min)									
	0	10	20	30	40	50	60	70	80	90
Cd^{2+}	0	55.2	57.9	58.1	58.5	58.7	59.0	60.5	61.3	62.2
Cr^{3+}	0	1.2	2.8	9.8	13.4	14.6	22.5	35.4	51.1	58.6
Cu^{2+}	0	50.2	54.9	55.8	57.2	57.3	59.3	59.6	62.0	63.7
Pb^{2+}	0	61.3	62.7	62.9	63.8	64.1	64.3	64.6	64.9	65.7
Zn^{2+}	0	43.3	48.7	53.6	55.5	58.5	60.4	67.7	71.0	71.3

Changes in concentration with time for mixed metal ions at low initial concentration (5 ppm)

	Time (min)									
	0	10	20	30	40	50	60	70	80	90
Cd^{2+}	0.70	0.53	0.36	0.31	0.29	0.28	0.28	0.28	0.27	0.28
Cr^{3+}	0.77	0.61	0.50	0.46	0.42	0.42	0.37	0.36	0.35	0.35
Cu^{2+}	0.83	0.58	0.47	0.47	0.46	0.43	0.41	0.40	0.38	0.31
Pb^{2+}	0.83	0.59	0.53	0.48	0.43	0.40	0.36	0..27	0.24	0.21
Zn^{2+}	0.98	0.57	0.47	0.44	0.41	0.39	0.37	0.38	0.378	0.37

Changes in percentage uptake of metal ions for mixed metal ions at low initial concentration (5 ppm)

	Time (min)									
	0	10	20	30	40	50	60	70	80	90
Cd^{2+}	0	22.6	48.1	54.8	58.0	59.3	59.6	59.6	60.9	61.6
Cr^{3+}	0	20.6	35.0	40.0	46.0	46.2	52.3	53.6	54.2	54.6
Cu^{2+}	0	30.6	43.8	44.2	45.0	48.6	50.8	52.0	54.4	63.0
Pb^{2+}	0	28.5	36.1	42.2	47.7	51.8	57.1	67.8	71.3	74.2
Zn^{2+}	0	41.8	52.4	55.0	58.3	60.3	62.5	61.5	61.6	62.3

Changes in concentration for mixed metal ions with time, at high initial concentration (50 ppm)

	Time (min)									
	0	10	20	30	40	50	60	70	80	90
Cd^{2+}	24.82	15.96	13.70	13.64	13.52	13.50	13.42	12.96	12.60	12.36
Cr^{3+}	21.89	14.24	9.18	6.81	5.24	5.23	5.19	5.18	5.20	5.15
Cu^{2+}	20.55	18.18	14.40	12.35	10.25	8.68	7.90	7.53	7.50	7.13
Pb^{2+}	22.45	19.85	14.85	11.83	10.70	10.58	9.56	9.48	9.05	8.08
Zn^{2+}	19.68	17.26	14.83	12.66	10.24	9.20	8.18	8.16	8.15	8.05

Changes in percentage uptake of metal ions for mixed metal ions at high initial concentration (50 ppm)

	Time(min)									
	0	10	20	30	40	50	60	70	80	90
Cd^{2+}	0	35.7	44.8	45.0	45.6	45.6	45.9	47.8	49.2	50.2
Cr^{3+}	0	35.0	58.1	68.9	76.1	76.1	76.3	76.4	76.2	75.5
Cu^{2+}	0	11.6	30.0	39.9	50.1	57.8	61.6	63.4	63.5	65.3
Pb^{2+}	0	11.6	33.9	47.3	52.3	52.9	57.5	57.8	59.7	64.0
Zn^{2+}	0	12.3	24.6	35.7	48.0	53.2	58.4	58.5	58.6	59.1