CAPACITY OF REGENERATED BONE CHAR IN REMOVAL OF FLUORIDE IN TREATMENT OF DRINKING WATER

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DECLARATION AND RECOMMENDATION

DECLARATION

This research is my original work and has not been presented for any award of a degree in any institution.

Kanyora Amos Kimemia

Signature _____ Date _____

SM11/2807/10

RECOMMENDATION

This thesis has been submitted for examination with our approval as supervisors according to Egerton University regulations.

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DEDICATION

This work is dedicated to my lovely parents Mr. and Mrs. Kanyora, My dear siblings Beth, Irene, Ruth, and Andrew for their support and encouragement during my studies.

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ABSTRACT

Reuse of fluoride saturated bone char as a way of reducing fluoride in domestic water was investigated. The study was aimed at determining the most effective solution (NaOH, Na_2CO_3, $NaHCO_3$ or Na_3PO_4) to use in the regeneration of fluoride saturated bone char. Effect of temperature on regeneration and removal capacity of regenerated bone char is also reported. Samples of 40 grams of bone char per 50 mL solution were used in determination of effectiveness of different sodium solutions. Samples were taken at contact times of 0.5-24 hours for fluoride analysis. Determination of temperature effect was carried out using 40 grams bone char/ 50 mL 1% NaOH solution, one-hour contact time and temperature of 20-60℃. Removal capacity was determined using 40 grams bone char/ 50 mL natural water containing 5.96-ppm fluoride at contact time of 20 minutes. Fluoride and pH of unknown samples was analysed using fluoride and pH meter respectively. The data was analysed using Two Way Analysis of Variance to compare the efficiency of different concentrations of different solutions. NaOH solution was the most effective with 130.55-186.14 ppm and lowest was $NaHCO_3$ with 4.35-6.03 ppm of fluoride released from fluoride saturated bone char. The efficiency of the four solutions was found to follow the order $NaHCO_3$ < Na_2CO_3<Na_3PO_4 < NaOH. Increasing regeneration temperature increased amount of fluoride released from bone char. Regenerated bone char was able to treat about 6.85 litres of water before breakthrough of 1.5 ppm as compared to fresh bone char that treated 7.56 litres. Removal capacities were found to be 0.880 and 0.988 mg/g for regenerated and fresh bone char respectively. The study confirmed that sodium hydroxide solution is the most effective solution for regeneration. Regenerated bone char was found to be effective as fresh bone char. In conclusion, regenerated bone char is a viable option for reducing excess fluoride in drinking water. However, further investigation will be required to determine the most effective and economical method for regeneration that is either bucket or continuous. The findings from this research have potential of increasing the levels of renewable defluoridation materials available to communities with challenges of excessive fluoride in drinking water.

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LIST OF ABBREVIATIONS AND ACROYNMS

AWWA	American Water Works Association
CCEFW	Consultative Committee on Excess Fluoride in Water
CDN	Catholic Diocese of Nakuru
CTDA	1, 2 Cyclohexylenediaminetetraacetic acid
EPA	Environmental Protection Agency
IPCS	International Programme on Chemical Safety
KEBS	Kenya Bureau of Standards
NFIS	National Fluoridation Information Service
pH_{zpc}	pH of Point Zero Charge
PPM	Parts Per Million
TISAB	Total Ionic Strength Adjustment Buffer
UNEP	United Nations Environmental Programme
WQ	Water Quality

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Fluoride has certain physiological properties of great importance in human health. At low concentrations, fluoride stabilizes the skeletal system by increasing the size of apatite crystals and reduces their solubility thus minimizing tooth decay (Moges *et al*., 1996; Notcutt and Davis, 1999; Li *et al*., 2001, CCEFW, 2010). However, in excessive exposure in drinking water or combination from other sources such as tea (brick tea), vegetable juices, high fluoride toothpastes, and agricultural activities particularly the use of phosphate fertilizers can result in a number of adverse effects. These ranges from mild dental fluorosis to crippling skeletal fluorosis as the level and period of exposure increases and long-term damage to the brain, liver, thyroid and kidney (Fawell *et al.,* 2006; Barbier *et al*., 2010; Gazzano *et al*., 2010; Shepherd *et al*., 2012). The Kenya Society for Fluoride Research (KSFR) shows that 19 million Kenyans suffer from fluorosis.

In any attempt to mitigate fluoride contamination for an affected community, the first option is the provision of safe low fluoride water from alternative sources. However, the major problem in delivery of water from low fluoride sources and defluorinated water is the scarcity of piped distribution systems and the reliance of household in boreholes, wells, springs, and or surface water. There is thus, a dire need to improve some economical technology applicable at domestic levels to reduce the fluoride concentration from very high concentrations to acceptable concentrations in drinking water (Argaw and Kebede, 1999; Bhargava, 1997).

To date, several studies on removal of fluoride from drinking water have been carried out over the years using a wide variety of materials giving various efficiencies. The use of poly aluminium salts, magnesite, bone char and activated carbon demonstrated 95%, 81%, ~99% and 90% efficiency respectively (Mavura and Tiffany, 2002; Mjengera and Mkongo, 2003; Feenstra *et al.,* 2007; Rezaee *et al*., 2009;). Magnesium compounds, activated alumina, serpentine, bagasse, chitosan, clays, Nalgonda technique, and ion exchange have also been applied (Bulusu *et al.,* 1979; Bregnhøj *et al.,* 1990; Shirivastava and Sharma; 2012; Ardekani *et al*., 2013). Kenyan soil derived from igneous rock and volcanic ash showed adsorption capacity of 5.5mg/g (Zevenberrgen *et al.,* 1997; Dahi, 2000). Other methods include electro

dialysis, distillation, reverse osmosis, crystalactor, and memstill technology, which are more effective and can, remove fluoride to a suitable level. However, demand high cost, skilled labor, and frequent regeneration of ion exchange beds or cleaning of the scaling and fouling on the membrane further are becoming prohibitive in developing countries (Fawell *et al.,* 2006; Feenstra *et al.,* 2007; Bhatnagar *et al*., 2011).

Bone char are porous grains produced by heating animal bones in kiln to temperatures of 400 to 500^0C in oxygen-depleted atmosphere to control its quality. It has a specific ability to remove fluoride from water because of its chemical composition mainly hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. The colour of the bone char is used as a simple indicator of its defluoridation ability (Jacobsen, 1997; Jacobsen and Muller, 2007a). Grey-brown coloured bone char has the highest fluoride removal capacity, followed by white and black bone char is of lowest quality due to presence of organic impurities. With time, if the fluoride concentration in the treated water exceeds WHO recommended value of 1.5 mg/L, the bone char is exhausted and needs replacement with fresh bone char or regenerated to restore the fluoride removal capacity (WHO, 2004).

Upon saturation of bone char with fluoride, it is possible to regenerate it using different methods. These include regeneration using sodium hydroxide, surface coating, and contact precipitation. Present study was to determine best sodium solution for regeneration based on previous studies, which have shown carbonate, hydroxyl, hydrogen carbonate, and phosphate ions from hydroxyapatite of bone char exchange with fluoride ions from fluoride-contaminated water during defluoridation (Bailey, 1972; Wang *et al*., 2001; Abe *et al*., 2004; Kawasaki *et al*., 2009). Evaluate removal capacity of regenerated bone char, with a view to trying to find a solution to the problem of excessive ingestion of fluoride that is economical.

1.2 Statement of the Problem

Use of bone char has been extensively investigated for removal of fluoride from contaminated water, however, little is known on the efficacy on reuse of fluoride exhausted bone char that has been regenerated as a way of reducing fluoride. This study aimed at determining the effectiveness of regenerated bone char in removing fluoride from fluoride-contaminated water with a view of trying to find a solution to the problem of excessive ingestion of fluoride. Excessive fluoride concentration in drinking water causes; skeletal fluorosis, bone cancer, reduced IQ, and increased bone fractures. Kenya is among the Nations

around the globe where health problems occur due to the Great Rift Valley in Africa that has naturally high concentrations of fluoride in ground water caused by past volcanic activity in the area, interactions between volcanic sediments and water and lack of calcium in the area. Various treatment technologies have been investigated. Some of these methods are not sustainable due to inadequate technology and shortage of financial resources. Thus, there is a need to examine the use of low cost, locally available materials for defluoridation of water in these areas.

1.3 Objectives

1.3.1 General Objective

To assess the potential of regenerated bone char in removing fluoride ions from fluoride contaminated water.

1.3.2 Specific Objectives

1. To determine the best sodium solution among NaOH, $NaHCO_3$, Na_3PO_4, and Na_2CO_3 for regenerating fluoride-saturated bone char.
2. To determine the effect of temperature on regeneration process.
3. To compare the abilities regenerated and fresh bone char remove fluoride ions from fluoride contaminated water.
4. To determine the ratio of regenerated to fresh bone char for optimum fluoride removal.

1.4 Hypotheses

1. There is no significant difference in fluoride removal by use of different regenerants.
2. Temperature has no effect on regeneration efficacy process.
3. There is no significant difference of fluoride removal capacity and efficiency of regenerated bone char to that of fresh bone char.
4. There is no significant difference in varying ratios of regenerated bone char to fresh bone char for optimum fluoride removal.

1.5 Justification

Fluorosis is one of the most common health problems in areas containing high fluoride concentration in drinking water. It is not curable and therefore treatment is

sophisticated and expensive. Thus, efforts should be directed towards attempting to alleviate some of the symptoms (Fawell *et al*., 2006; CCFEW, 2010). The available ways for solving this issue is controlling and eliminating the pollution of fluoride in drinking water, researching into economic and practical methods of fluoride-removal agents and fluoride-removal methods. The use of bone char is a cheaper alternative that has high removal power and people centred approach that requires no daily dosage of chemicals. The materials used are widely available and affordable to majority of Kenyans. Hence the need for evaluation of the quality of regenerated bone char in fluoride removal because it can be cheaper in terms of cost compared to replacement with fresh bone char. It also reduces the problem of emptying, transportation and refilling of community and institution filters since regeneration is carried on site.

CHAPTER TWO

LITERATURE REVIEW

2.1 Occurrence of Fluoride in Environment

Fluorine (F_2) is a greenish diatomic gas, lightest member of halogens, and one of the most reactive of all chemical elements. It is so reactive and never encountered in its elemental gaseous state except in some industrial processes. It is the seventeenth in the order of frequency of occurrence of the elements and represents about 0.06 to 0.09% of the earth's crust. Fluorides are found at significant levels in a wide variety of minerals including sellaite (MgF_2), fluorspar (CaF_2) cryolite (Na_3AlF_6), fluorapatite ($Ca_3(PO_4)_3F$), bastnaesite and villanmite (NaF) (Bulusu *et al.,* 1979; Murray, 1986). Cryolite is used for production of aluminium and as a pesticide (USEPA, 1966) while fluorapatite is converted into phosphate fertilizers.

The dominant controls on fluoride buildup in water are mainly geology, contact times with fluoride minerals, groundwater chemical composition, and climate. Groundwater flow is slow and reaction times between water and rocks are therefore enhanced (Redda *et al.,* 2005). Fluoride build up is less pronounced in the less humid tropics because of high rainfall inputs and their diluting effects on groundwater chemical composition. Waters with high fluoride concentrations occur in large and extensive geographical belts associated with a) sediments of marine origin in mountainous areas b) volcanic rocks and c) granitic and gneissic rocks. A typical example of the first extends from Iraq and Iran through Syria and Turkey to the Mediterranean region, and then from Algeria to Morocco. Other important examples come from the southern parts of the USA, southern Europe and the southern parts of the former USSR (Fawell *et al.,* 2006).

One of the regions of the world most affected by fluorosis is East Africa, specifically, the East African Rift Valley possibly because fluorotic minerals are often carried by water. It is more common to find fluoride rich soils in lowlands and valleys than in nearby highlands. This phenomenon coupled with the high fluoride volcanic rocks in the East African Rift result in significant amounts of fluoride in the Rift Valley (Bårdsen, 1997; Jagtap *et al*., 2012).

Many of the lakes of the Rift Valley system, especially the soda lakes, have extremely high fluoride concentrations: 1,640, 2,800, and 690 ppm for Elmentaita, Nakuru and Momella respectively. In Kenya, Nair *et al.* (1984) undertook a survey of fluoride in groundwater. Of over 1,000 groundwater samples taken nationally, 61 per cent exceeded 1 ppm, almost 20 per cent exceeded 5 ppm and 12 per cent exceeded 8 ppm. The volcanic areas of the Nairobi, Rift Valley, and Central Provinces had the highest concentrations, with maximum groundwater fluoride concentrations reaching 30–50 ppm. In Nakuru district, 195 borehole water were analysed using GIS mapping, 43% was below 1.5 ppm, 24.1% was between 1.5-3.0 ppm, 26.7% was between 3-10 ppm and 6.2% exceeded 10 ppm (Jorgen, 2005).

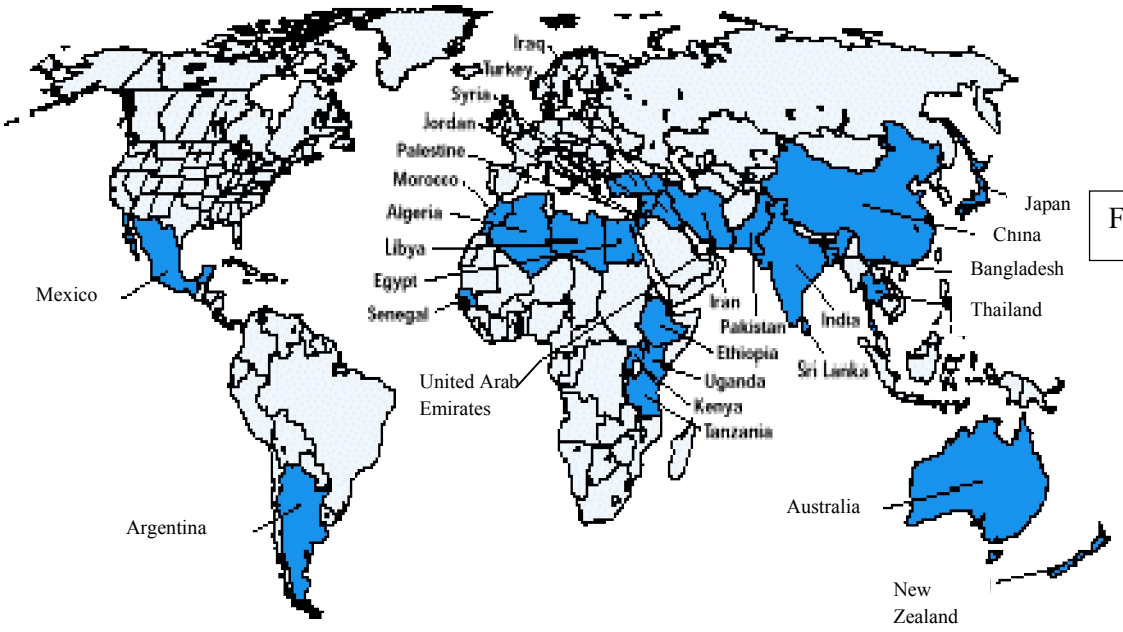

Figure 2.1: Location of Countries with Endemic Fluorosis

2.2 Effects of Prolonged Ingestion of High Fluoride Concentrations

It has been proven that with 1.0 mg of F/l the overall instances of dental health in a community can be greatly improved by the added protection fluoride gives to tooth enamel (Fawell *et al.*, 2006). Fluoride in water is not considered toxic until it reaches concentrations of 250-450 ppm (Crittenden *et al.*, 2005; Márquez-Mendoza *et al.*, 2012). However, because the body retains much of the fluoride consumed, it still has a cumulative effect when consumed in far smaller concentrations resulting in fluorosis. Fluoride is necessary for the body to function and proven helpful in smaller doses, the WHO recommends the ingestion of no more than 4.0 mg of fluoride per person per day (Dahi, 1997). Though much intake of

fluoride comes from food, it has been shown that the majority of occurrences of fluorosis come from the consumption of water with excessive amounts of fluoride (Karthikeyan, 1997). Thus, the WHO limits fluoride concentrations in drinking and cooking water to 1.5 ppm (WHO, 1996). Many consider the limit incomplete; this suggested that the optimum amount of fluoride in drinking water is approximately 0.5-1.0 ppm (Tekle *et al*., 1995). Because it is not so much the concentration of the fluoride that is of concern, but rather the total fluoride consumed, it has been suggested that a "sliding scale" of acceptable levels of fluoride should be used based on the average maximum temperature (Bårdsen, 1997).

Table 2.1: Recommended limits for fluoride concentration as a function of temperature

Maximum mean temperature of region (^{o}C)	**Maximum recommended concentration of fluoride (ppm)**
0	2.1
10	1.3
20	0.9
30	0.7
40	0.6
50	0.5
60	0.4

As a result, it has been noted that those who live in hotter/humid climates and/or labor outdoors are far more likely to develop symptoms of fluorosis than those who do not (Chikte, 1997). This is because they consume far more water than those in other regions and lifestyles. Studies have also shown that children are typically the most affected by fluoride as their developing bones and teeth are more susceptible to the effects of fluorosis. Because the amount of fluoride consumed in one's first year of life has more impact than any other phase of life (Bjorrvatn, 1997). Other factors that affect the severity of fluorosis in individuals are altitude of residence, nutritional status, and use of dentifrice (Rwenyonyi *et al*., 1997). It is estimated that about 60% (80-90% for infants) of fluoride ingested in person's body is retained while the rest is primarily expelled through urine (Fawell *et al*., 2006).

2.2.1 Dental Fluorosis

Dental fluorosis is the most common manifestation of over-consumption of fluoride. It is visible by white, yellow, and brown streaks on the teeth, characteristic of the hypoplasia and hypo calcification (WHO, 1984; Mcharo, 1986; Malik *et al.,* 2010).This damage is more than cosmetic, as it tends to be associated with painful "cavity-like" feelings. Additionally, there are social stigmas against those suffering from fluorosis. While all teeth are affected, the incisors (especially the maxillary incisors) and permanent molars are often the teeth most affected by fluorosis. It is speculated that this is because these are the first teeth to develop. According to Moturi *et al.,* (2001) results of examination of children's teeth in Njoro Division showed that 48.3% of the children suffer from moderate to severe dental fluorosis. In another study on incidence of dental fluorosis in Kenya, it was found that fluorosis incidences among populations was 60% in Central, 30% in Rift Valley, 45% in Nairobi and Eastern while in Coast and Western had 5% (Chibole, 1987).

2.2.2 Skeletal Fluorosis

Though it generally takes far more time, and higher concentrations (typically over 10 ppm) to develop, skeletal fluorosis is far more severe than its dental fluorosis. Skeletal fluorosis can be detected early using radiological techniques. Skeletal fluorosis is characterized by deformation of bone structure. Movement of the spine, pelvis, and joints become increasing difficult as fluoride deposits collect on ligaments and tendons and within the bones themselves. Fluoride levels beyond 10 mg/L result in crippling fluorosis, which is a serious bone disorder resembling osteoporosis and characterized by extreme density and hardness and abnormal fragility of the bones sometimes called "marble bones." (Water quality association, 2005)

2.2.3 Neurological Complications

There are increasing accounts of the neurological effects that fluoride can have in the body. It is suspected that fluorides effects on the spine and compression on the spinal cord (Meklan *et al*., 1997) cause neurological complications. Studies have shown that high levels of fluoride can cause headaches and insomnia (CCEFW, 2010; Sharma and Parul, 2009; WHO/IPCS, 2002).

2.2.4 Effect of Fluoride in Brain

A number of studies have attempted to quantify cognitive capacities in children in terms of a measure of IQ as a function of the fluoride concentrations in their drinking water. Most of these studies indicates that in endemic fluorosis areas, drinking water with high fluoride levels adversely affect the development of children's intelligence (Seraj *et al.,* 2012; Xiang *et al.,* 2003; Lu *et al.,* 2000). The ability of fluoride to enter in brain is enhanced by its ability to form a liquid-soluble complex with aluminium. Aluminium-fluoride complexes stimulate the guanine nucleotide binding proteins and can produce pharmacological and toxicological effects in human cells (Lu *et al.,* 2000). Most of these studies however provide little information on a range of important factors including the validity of test instruments and presence of potential confounding factors such as levels of parental education and income (NFIS, 2011)

2.3 Chemistry of Fluorosis

The structural inorganic part of bones and teeth consist mainly of apatite, a mixture of more hydroxyapatite (HAP), ($Ca_{10}(PO_4)_6(OH)_2$) and less fluorapatite (FAP), ($Ca_{10}(PO_4)_6F_2$). In this structure, F^- and OH^- are interchangeable. The parts of the apatite molecules, which are FAP, determine the properties of this hard tissue. At very low FAP ratios, teeth are easily soluble under acidic conditions, meaning a higher risk of dental carries. At higher FAP ratios, the solubility reduces. However, too high a ratio causes dental Fluorosis (Kaseva, 2006).

2.4 Sources of Fluoride

Drinking water is the largest single contributor to daily fluoride intake (Murray, 1986; Vijaya *et al*., 2010). For a given individual, fluoride exposure ($mgKg^{-1}$ of body weight per day) via drinking water is determined by the fluoride level in the water and daily water consumption (Fawell *et al.,* 2006). Since some fluoride compounds in the earth's upper crust are soluble in water, fluoride is found in both surface and ground water. In streaming surface fresh water, fluoride concentrations are usually lower than in groundwater because of the shorter contact time between water and rock. The natural concentration of fluoride depends on the geological, chemical, and physical characteristics of the aquifer, the porosity and acidity of the soil and rocks, the temperature and the action of other chemical elements (UNICEF, 2005). Another reason for high fluoride concentration in groundwater can be absorption of uprising, subterranean gas containing high levels of fluoride.

Due to dust, industrial production of phosphate fertilizers, coal ash from the burning of coal and volcanic activity, fluorides are widely distributed in the atmosphere. However, air is only responsible for only a small fraction of total fluoride exposure (USNRC, 1993). In non-industrial areas, the fluoride concentration in air are typically, low (Murray, 1986). However, in areas where fluoride-containing coal is burned or phosphate fertilizers are produced and used, the fluoride concentration is elevated leading to increased exposure by the inhalation route. This has resulted to serious complications of osteo-dental fluorosis (Ando *et al.,* 2001).

Vegetables and fruits usually have low levels of fluoride and thus contribute little to exposure, but higher levels have been found in barley, rice, yams, and cassava (Murray, 1986). Tea leaves contain high levels of fluoride (up to 400 mg Kg^{-1} dry weight) a view supported by Cao *et al.,* (2004) that tea has natural fluoride levels since the tea plants easily absorb fluoride from the soil. Tibetans are known to ingest large amounts of fluoride about 14mg per day due to consumption of brick tea (tea made from older leaves that contains high levels of fluoride than standard tea) as a beverage (Cao *et al.,* 1997). On one hand, in the study of Malde *et al.,* (2006), the possible effect of original fluoride concentration in the water on the fluoride release from tea was tested and the possible capacity of commercial tea leaves to absorb fluoride from high-fluoride water. In low-fluoride water, fluoride is easily released from tealeaves. Depending upon the fluoride content of the water, dried tealeaves were also able to absorb fluoride. Thus, if a cup of tea is made from high-fluoride water, the fluoride concentration of the infusion may actually be lower than the original fluoride concentration of the water (Malde *et al.,* 2006).

Fluoride contaminated trona has significantly contributed to the prevalence and severity of dental fluorosis (Mabelya, 1997). Magadi soda, trona, contain fluoride in form of villiaumite, NaF, kogarkoite, $Na_2SO_4 \cdot NaF_{12,13}$, and the concentration varies considerably. The use of Magadi heavily contaminated with fluoride contributes to the high fluoride intake in fluorosis areas of East Africa. In some regions in China, significant dietary fluoride exposure occurs due to the consumption of maize polluted by fly ash generated by the burning of high fluoride coal (Chen, 1991).

2.5 Fluoride Removal Techniques

A wide range of defluoridation methods have been investigated and analysed, mainly in laboratory. However many have been found to be inefficient in fluoride removal, complicated maintenance and unaffordable costs especially in developing countries. According to CCEFW (2010), in choosing a technology suitable for the Kenya context, the following should be considered; Possible negative impacts, such as the consequences of wrong dosing of chemicals, possible chemical residuals in treated water, cost of defluoridation methods (both plant investment, and running costs) and scale of defluoridation / scale of service. The common methods used for the removal of fluoride from drinking water can be divided into the following four categories. They include precipitation, adsorption and ion exchange, membrane filtration process and electrochemical technique (Feenstra *et al.,* 2007; Malik *et al.,* 2010):

2.5.1 Precipitation

Precipitation processes involve addition of chemicals and formation of fluoride precipitates. Precipitation can be divided into two categories, those based on co precipitation of adsorbed fluoride and those based on the precipitation of insoluble fluoride compounds. Among these are precipitations with calcium and aluminium salts. Precipitation chemicals must be added daily in batches and precipitation techniques produce a certain amount of sludge every day. Examples include Nalgonda, poly aluminium chloride (PAC) and contact precipitation (Fawell *et al.,* 2006). Nalgonda technique is process by which aluminium salts (aluminium chloride and aluminium sulphate) is added to fluoride contaminated drinking water (Equations 2.1-2.3).

$$(Al_2SO_4)_3.18H_2O \rightarrow 2Al^{3+} + 3SO_4^{2-} + 18.H_2O \quad (2.1)$$

$$2Al^{3+} + 6H_2O \rightarrow 2Al(OH)_3 + 6H^+ \quad (2.2)$$

$$F^- + Al(OH)_3 \rightarrow \text{Al-F Complex + Undefined product} \quad (2.3)$$

The reaction results in an excess of H^+ ions, lime ($Ca(OH)_2$) is added to the water during the process to help maintain a neutral pH and hasten the settling of the sediment as in Equation 2.4 (Dahi, 2000).

$$6Ca(OH)_2 \rightarrow 12H^+ + 12.H_2O \quad (2.4)$$

Additionally, some of the fluoride is able to form precipitate with calcium (Equation 2.5).

$$Ca(OH)_2 + 2F^- \rightarrow CaF_2 + 2OH^- \quad (2.5)$$

2.5.2 Adsorption and Ion-Exchange

Adsorption processes involve the passage of water through a contact bed where fluoride is removed by ion exchange or surface chemical reaction with the solid bed matrix. After a period of operation, a saturated column must be refilled or regenerated. The different adsorbents used for fluoride removal include activated alumina, carbon, bone charcoal, and synthetic ion exchange resins. Examples include; activated alumina (Al_2O_3), clay and soils, and ion exchange resins.

2.5.3 Membrane Filtration Process

Reverse osmosis, Nano filtration and electro dialysis are membrane filtration processes that can be used for removal of fluoride. Large-scale electro dialysis plants are already used for making drinking water out of brackish water with high fluoride concentrations (Zakia *et al.,* 2001; Diawara *et al*, 2003). In many parts of North Africa, water is brackish and contains over 1.5 mg/L fluoride. All elements in water can be reduced by membrane filtration. Thus, this method is proposed to be the best water purification process available. Nevertheless, 30 % of raw water is lost in the process.

2.5.4 Electrochemical Technique/ Electro coagulation

It is a simple and efficient method for the treatment of potable water. Electro coagulation (EC) uses aluminium anodes, in EC cell, the aluminiun electrodes sacrifice themselves to form aluminiun ions first (Mameri *et al.*, 1998; Yang and Dlutty, 2002). Afterwards the aluminiun ions are transformed to $Al(OH)_3$ before being polymerized to $Al(OH)_3$ floc, which is believed to adsorb fluoride strongly as illustrated by the equation (2.6):

$$Al(OH)_3 + xF^- \rightarrow Al(OH)_{3-x}F_x + xOH^- \qquad (2.6)$$

The EC operation is completed by an electroflottation in order to separate the formed floc from water by floating them to the surface cell.

2.5.5 Other Technologies

They include crystalactor, memstill technology, and solar dew collector system (Fawell *et al.,* 2006).

The Solar Dew Collector System

Solar Dew developed a new porous membrane to purify water using solar energy (Solar, 2007). Water sweats through the membrane, evaporates on the membrane's surface and increases the air humidity in the evaporation chamber. Based on a temperature difference, pure water condenses on the cooler surfaces of the system. The product water quality is very constant and similar to that of distilled water. The quantity depends on the intensity of the solar radiation. To avoid crystallization, the brine has to be drained periodically. The system is able to process: sea brackish, contaminated wastewater with heavy metals, oil residue, boron, fluoride with an allowable pH range of 5-11 (Feenstra *et al.*, 2007).

Memstill Technology

The Netherlands Organization of Applied Scientific Research has developed a membrane based distillation concept, which radically improves the economy and ecology of existing desalination technology for seawater and brackish water. This "Memstill technology" combines multistage flash and multi-effect distillation modes into one membrane module (Hanemaaijer *et al.*, 2007). Cold feed water takes up heat in the condenser channel through condensation of water vapour, after which a small amount of (waste) heat is added, and flows counter currently back via the membrane channel. Driven by the small-added heat, water evaporates through the membrane, and discharged as cold condensate. The cooled brine is disposed, or extra concentrated in a next module. With the Memstill technology, anions like fluoride and arsenic are also removed.

Crystalactor

The Crystalactor is a type of contact precipitator that was developed in the Netherlands (Giessen, 1998). The Crystalactor is a fluidized-bed type crystallizer also called a pellet reactor. In the reactor, fluoride is removed from the water while calcium fluoride pellets with a diameter of 1 mm are produced. Cost comparisons show that the total treatment costs are typically in the range of 25% of the costs for conventional precipitation. However, the Crystalactor is more suitable for wastewaters with high fluoride concentrations > 10 ppm (Feenstra *et al.*, 2007).

2.6 Bone char

The use of bone char as a defluoridator started in USA in the 1940s through 1960s where it was commercially available because of its large-scale use in the sugar industry to absorb colour and inorganic ash impurities from sugar (AWWA, 1971). Today synthetic ion exchange resins have replaced bone char. Later research carried out by the Intercountry Centre for Oral Health (ICOH) in 1981 to 1983 in Thailand came up with ICOH filter (Phantumvanit *et al.,* 1988). Since then bone char use has been tested both in and outside Thailand. In Kenya, bone char defluoridation was first tested in laboratory in batch and column in late 1998 (Korir *et al.,* 2009). The technique as developed and marketed by the Catholic Diocese of Nakuru Water Quality offers four different types of defluoridation filters ranging from household filters, institutional, community to waterworks filters.

Bone char contains about 10% carbon (C) by weight with the remainder comprising mainly of hydroxyapatite, ($Ca_{10}(PO_4)_6(OH)_2$) but also a significant percentage of calcium carbonate ($CaCO_3$). The carbon, however, accounts for half the total surface area of the product and $CaCO_3$ gives bone char its alkaline properties (Lewis, 1995; Guedes *et al*., 2007).

Bone char ability to take up fluoride is complicated and involves more than one reaction. These reactions vary with fluoride concentration, pH and available surface area (Bregnhøj, 1995). Reactions involved are direct adsorption of fluoride on the empty sites on the bone char surface. Ion exchange where fluoride ion exchange position with OH^- (equation 2.2) or it exchanges with hydrogen carbonate/carbonate ion. Recrystallization, processes where the hydroxyapatite and bone minerals dissolve and precipitate with fluoride as fluorapatite (Bregnhøj and Dahi, 1995; Jorgen, 2005). The principal reactions are equations 2.7-2.8:

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^- \rightarrow Ca_{10}(PO_4)_6F_2 + 2OH^- \quad (2.7)$$

$$(Ca_3(PO_4)_2)_nCaCO_3 + 2F^- \rightarrow (Ca_3(PO_4)_2)_nCaF_2 + CO_3^{2-} \quad (2.8)$$

Studies have also shown that fluoride ion exchange is not only by hydroxyl ion but also by phosphate ion eluted from bone char (Kawasaki *et al*., 2009).

The main benefits of bone char as a fluoride removal technique compared to other methods such as activated alumina, magnesite, activated carbon, and clay include its high ability to remove fluoride and other pollutants in water such as heavy metals, colour, odour,

and taste (Sudaratn and Thares, 2010). Bone char can be re-used upon saturation by discarding it as fertilizer and soil conditioner, which is environmentally acceptable (Fawell *et al.,* 2006). Bone char defluoridation is based on materials that are readily available locally and affordable (Mavura *et al.,* 2002).

2.6.1 Production of Bone Char

Bones delivered from local butcheries are heated in a kiln to high temperatures of 400-500 ^{0}C in an oxygen-depleted atmosphere to control the quality (Jacobsen and Muller, 2007a). The required temperature and duration of heating depend largely on the batch size and the packing rather than the type or the nature of the bone (Dahi *et al.,* 1997).

The bone char separated manually from the metal pieces and separated according to its colour, where black ones are stored and added to the next charring batch. Grey-brownish and white bone char are then separately crushed using crushing machine and sieved to produce three different particle sizes. Powder and fine fraction (< 0.63 mm) used for the production of calcium phosphate pellets for contact precipitation. Medium (0.63-2 mm) is used in community and household filters and coarse (2-4 mm) is used in community filter. Homogenizing the size of the particles is to optimize both flow rate and removal capacity. Dust and other impurities from the charring and crushing process are removed by washing and then the bone char is dried for safe storage and use (Jacobsen and Muller 2007a).

According to Albertus *et al*., (2000), charring can be carried out in two ways: As calcinations where bones are heated in the presence of continuous supply of oxygen from atmospheric air or as pyrolysis where no oxygen is present during heating. In calcinations, the organic carbon converted to CO_2 that is stripped off while in pyrolysis the organic carbon is converted to inorganic carbon that remains in the bone char. Calcined bones are brown-grey–white depending on the accessibility of the oxygen while pyrolyzed bones are black.

2.6.2 Regeneration of Bone Char

Upon saturation with fluoride, bone char can be regenerated through; surface coating, contact precipitation, and use of sodium hydroxide. Christoffersen *et al.,* (1991) studied surface coating in the laboratory. According to this process, fluoride-saturated bone char is immersed in an acidic solution of calcium and phosphate or of bone char powder and it takes up a fresh layer of hydroxyapatite, ($Ca_{10}(PO_4)_6(OH)_2$) on its surface. The surface coated bone char behaves as fresh bone char and can absorb a new amount of fluorides.

Contact precipitation method involves the addition of calcium and phosphate compounds to the raw water prior to its flow through the fluoride saturated bone char filter (Dahi, 1996; Jacobsen and Muller, 2007b; Korir *et al.,* 2009). In a mixed solution of calcium, phosphate and fluoride, the precipitation of calcium fluoride and/or fluorapatite is theoretically possible, but practically impossible due to reaction inertness (Fawell *et al.,* 2006). The precipitation is easily catalysed in a contact bed that acts as the filter for the precipitate, using calcium chloride and sodium dihydrogen phosphate or "monosodium phosphate." Equations 2.9 and 2.10 illustrate that the removal of fluoride; by contact precipitation involves dissolution and equations 2.11 and 2.12 show precipitations of calcium fluoride and fluorapatite respectively.

$$CaCl_2.2H_20_{(s)} \rightarrow Ca^{2+} + Cl^- + 2H_20 \quad (2.9)$$

$$NaH_2PO_4.H_20_{(s)} \rightarrow PO_4^{3-} + Na^{2+} + H^+ + H_20 \quad (2.10)$$

Calcium fluoride: $Ca^{2+} + 2F^- \rightarrow CaF_{2(s)}$ (2.11)

Fluorapatite: $10Ca^{2+} + 10\ PO_4^{3-} + 2F^- \rightarrow Ca_{10}(PO_4)_6F_{2(s)}$ (2.12)

Studies on the use of a 1-8% solution of sodium hydroxide for bone char regeneration before it is used have been reported and the mechanism is assumed to be ion exchange between fluoride and the hydroxyl ions (equations 2.13 & 2.14) (Horowitz *et al.,* 1972 ; Mcharo, 1986; Christoffersen *et al.,* 1991; Jacobsen and Muller 2007 a).:

$$Ca_{10}(PO_4)_6F^- + OH^- \rightarrow Ca_{10}(PO_4)_6OH^- + F^- \quad (2.13)$$

These reactions can be represented by the following equations:

$$Ca_{10}(PO_4)_6F_2 + OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 2F^- \quad (2.14)$$

Caustic soda of the calcium phosphate medium is usually followed by an acid rinse to remove residual caustic. The carbonic acid (dissolved CO_2) method for neutralizing excess residual caustic prolongs the activity of both tricalcium phosphate and hydroxyl apatite.

The exhausted bone char media can also be reactivated by heating (Wang *et al*., 2001; Kaseva, 2006).The bone char is then exposed to solution of sodium hydroxide. The mechanism that takes place is as shown in the following equation 2.15.

$$Ca_{10}(PO_4)_6F_2 + 2OH^- \xrightarrow{\text{Heat}} Ca_{10}(PO_4)_6 + 2HF + O_2 \qquad (2.15)$$

Conditions necessary for the above regeneration of bone char are temperature and contact time. The efficiency of bone char was found to improve as the temperature and contact time were increased.

2.6.3 Challenges of Use of Bone Char as a Defluoridator

Some of the challenges facing defluoridation are; religious beliefs, for instance use of bone char originating from cows among Hindus, pigs among Muslims. Nevertheless, according to Fawell *et al.,* (2006), from scientific point of view, all types of bones are equally good as raw materials, but in such cases, the problem can be solved through information and production of bone char in accordance with local acceptability. In Kenya, the CDN is trying to address the problem through education where they target students, teachers, and community at large; they network with stakeholders in health and water sectors. Other challenges may be poor quality mainly due to incomplete charring, overheating of bones especially if oxygen is admitted to the heated bone char, and inhomogeneous heating. All these can change taste of defluoridated water (Fawell *et al.,* 2006).

The bone char has other uses than fluoride removal, such as in sugar industries, due to active carbon, which has high surface area and the unique ability to absorb colour and inorganic impurities from, sugar, used in artistic painting, making pottery (Mameri *et al.,* 1998; Ghaneian *et al*., 2012). It has also been used in removal of metals like Mn (II), Pb (II), Cr (III), As (V) Cu and Zn from wastewater (Wilson *et al.,* 2000; Chen *et al.,* 2008; Brum *et al.,* 2010). It can also be used as fertilizer (Warren *et al*., 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection and Preparation of Regenerated Bone Char

Fresh brown-grey bone char of 0.63 to 2 mm diameter used for community and household filters was obtained from the Catholic Diocese of Nakuru Defluoridation Company. Borehole water samples with 5.96-ppm fluoride was collected within the Egerton University (borehole no. 4) and used as representative of fluoride-contaminated water.

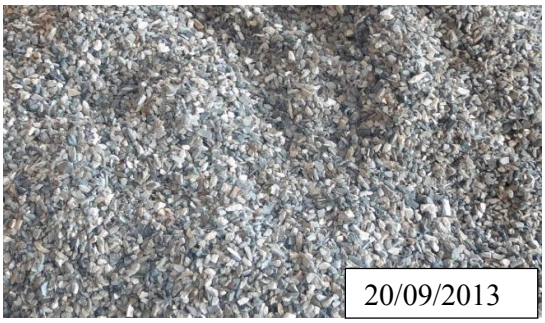

Figure 3.1: Sample of bone char

3.1.1 1000 ppm Fluoride Solution

A thousand ppm solution was prepared by weighing 2.21 grams NaF and putting it into a 1 litre volumetric flask. Distilled water was added to dissolve and then water was added to mark. This was labeled stock solution. Two-hundred ppm, 100 ppm and 10 ppm were prepared by serial dilution of 1000 ppm of stock solution.

3.1.2 Four Percent Sodium Solutions

All solutions used were of analytical grade. Four percent NaOH solution was prepared by dissolving 40 g of NaOH pellets in 1000 mL distilled water. One percent and 2% were prepared by appropriate dilution of 4% stock solution. The same procedure was used for preparation of Na_2CO_3, $NaHCO_3$ and Na_3PO_4 solutions.

3.1.3 Preparation of fluoride TISAB Solution

TISAB was prepared by weighing 29.2 g NaCl, 2.5 g CTDA, 28.5 mL acetic acid and placing them into 500 mL beakers. Four hundred mL distilled water was added to dissolve.

pH was then adjusted to pH 5.5 with 6 M NaOH. It was then transferred into 500 mL volumetric flask and diluted to mark with distilled water.

3.2 Experimental

3.2.1 Normal Saturation of Bone Char

Saturation of bone char was carried out using five different columns of the 5 cm diameter and 30 cm long. A piece of scrubber with 5 cm diameter as column was placed in the bottom to prevent the bone char particles passing through the outlet. The columns were propped up with wooden construction and each packed with 400 grams fresh bone char of 0.63 to 2 mm diameter. The columns were made from a glass of 30 cm long and a diameter of 5 cm that was fitted with inlet and outlet devices. Four columns were saturated using upward flow with 200-ppm solution fed by gravity from feed water tank that was elevated and one with 100 ppm. Samples of 5 mL regularly taken from each column and fluoride concentration determined to verify the saturation of bone char. Tap water, which contains 5.61-ppm fluorides was then passed through the bone char in a filter column to remove free F^- ions and to verify the saturation of bone char. Verification was done using batch process where tap water with 5.61 ppm fluoride added in each column and allowed 30 minutes contact time. Bone char used in the experiment was saturated to breakthrough point of fluoride effluent concentration of 1.5 ppm.

3.2.2 Determining the most Effective Solution for Regeneration

Regeneration was carried out by exposing the fluoride saturated bone char to NaOH solution in batch through the column. Samples of 40 grams each were packed in several columns and 50 mL of 1% NaOH was added and effluents taken at different duration of 0.5, 1, 2, 4 and 24 hours. The process was repeated with 2% and 4% NaOH solutions. The objective was to determine the effect of varying NaOH concentration on regeneration process. The same procedure was repeated for the other solutions ($NaHCO_3$, Na_3PO_4 and Na_2CO_3). All the effluents were collected in plastic bottles before the analysed for fluoride concentrations using fluoride ion selective electrode Metrohm 6.0502.150 and reference electrode (Ag/AgCl) Metrohm 6.0733.100. All the experiments were conducted in triplicate. From this experiment, 2% was NaOH was found to be most effective for regeneration as indicated in table 4.4.

3.2.3 Regeneration

Regeneration of fluoride bone char to be used in determination of removal capacity was carried out using 2% sodium hydroxide solution. Samples of the effluent were taken at different times and analyzed for F^- concentration to ensure complete fluoride removal. Fresh bone char was used as control that is to determine whether all fluoride in bone char has been completely removed from fluoride saturated bone char. After fluoride removal, bone char was washed with distilled water to reduce pH and to remove all the free fluoride ions. Further, pH was reduced by use of 0.02M HCl enriched water in place of the carbonic acid. pH was determined using a pH meter (Orion Combination pH 91-06).Finally; excess acid was rinsed using distilled water. Effluent should have a pH of 6.5 to 8.5 according to WHO recommended values. Bone char was dried for safe storage and use. Regenerated bone char was obtained and used to study fluoride removal capacity and efficiency.

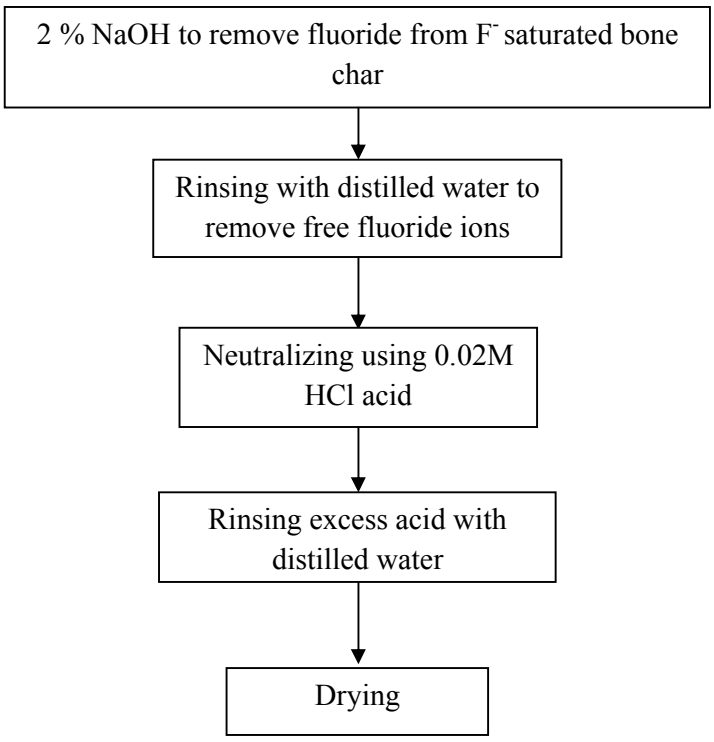

Figure 3.2: Summary of regeneration procedure

3.3 Effect of Temperature on Regeneration

Most regeneration studies are conducted at room temperature in laboratory settings. However, in real life situations temperatures above or below 25 °C depending on

environment are often met. Therefore it is important to understand what impact temperature has on the regeneration.

Forty grams of fluoride saturated bone char was placed in 150- mL plastic bottle; to it, 50 mL of 1% NaOH was added. Shaken in water bath at 20°C for one hour. Thereafter, the medium was separated by filtration through Whatman No. 42 filter paper and the filtrate analysed for desorbed fluoride. The procedure was then repeated at different temperatures of 30, 40, 50 and 60 ^{o}C. The fluoride released was plotted against temperature.

3.4 Determination of Fluoride Removal Capacity and Efficiency

The experiment set up was carried out using identical columns packed with 40 grams of NaOH regenerated bone char. Borehole water containing 5.96-ppm initial fluoride concentration was then added in each column and allowed a contact time of 20 minutes as recommended by CDN WQs. The effluents were collected in plastic bottles before residual fluoride was analysed. This was repeated severally until concentration of the effluent was above 1.5 ppm. Experiments were carried out in duplicate. The removal capacity and efficiency of regenerated bone char was calculated using this data. This was then repeated using fresh bone char and ratios of 1:1 regenerated to fresh bone char. The various runs were terminated when the effluent fluoride concentration at the bottom of the column beds exceeded 1.5 mg/L (the permissible concentration, designated as the break-through concentration). The volume of the effluent treated prior to the breakthrough concentration was designated as the `useful (or effective) treated effluent volume.

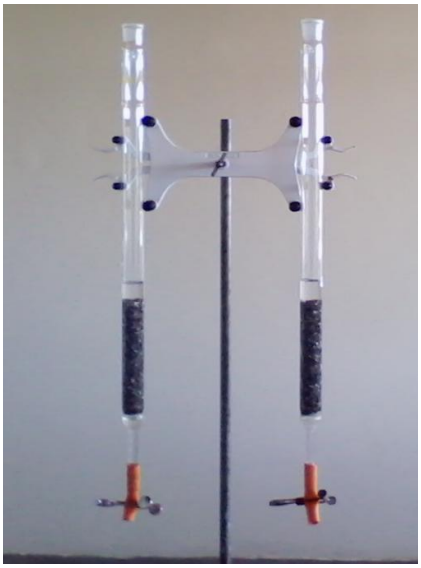
Figure 3.3: Experimental set up

3.4.1 Fluoride Removal Capacity

For this work, removal capacity was calculated using accumulated volume of treated water before break point of 1.5 ppm fluoride by dividing it with volume of bone char packed in the column.

3.4.2 Removal Efficiency

The quantities removed in a given period and removal efficiency was calculated based on the following equation 3.1:

$$Qt=\frac{S_0-S_t}{S_0}\times 100 \qquad 3.1$$

where

Q_t= percentage removal efficiency,

S_0 = initial fluoride concentration (mg/L) and

S_t =residual fluoride concentration (mg/L).

3.4.3 Fluoride Ion Measurements

Fluoride analysis was done using standard method (ALPHA, 1995). The concentration of fluoride ions in the solutions was determined using a selective electrode fluoride ion selective electrode Metrohm 6.0502.150 and reference electrode (Ag/AgCl) Metrohm 6.0733.100.

Reference standards were made from appropriate dilutions of the stock solution of sodium fluoride (NaF) 100 mg/L and Total Ion Strength Adjusting Buffer (TISAB) solution. A water sample of 5 mL was transferred into a 25 mL plastic beaker by means of a measuring pipette. After rinsing the pipette by distilled water, 5 mL of TISAB was measured and transferred into the beaker containing the water sample. The electrodes were immersed into the sample and stirred slowly for 30 s, and then the specific pH ion meter was switched on in order to read the voltage when a steady state was reached. A TISAB buffer was added a prior to measurement to attain constant pH and break up fluoride complexes.

3.5 Data Analysis

MSTAT-C, 1993 and Excel-2007 software were used for data analysis. Results were expressed as mean ± standard deviation. Two-way Analysis of Variance was carried out to determine significance difference of varying concentrations of various sodium solutions at selected conditions using a p value of 0.05 and to determine significant differences of the regenerated bone char compared to that of fresh bone char in terms of fluoride removal capacity and removal efficiency. The Bonferroni test was used for posttest's analyses.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1Normal Saturation of Bone Char

Normal saturation in this context is the concentration of fluoride in the effluent water and this is equal to 1.5-ppm fluoride (WHO recommended value).

Tables 4.1: Results of fluoride retained during saturation using water with initial concentration of 200 ppm

Contact time in hours	Fluoride in water (ppm)	Fluoride retained in bone char (ppm)	% Efficiency
15	26.32±4.32	173.68	86.84
30	31.97±5.68	168.03	84.02
45	55.35±3.79	144.65	72.33
60	73.05±4.15	126.95	63.48

Table 4.1 shows average results of the four columns during saturation. More samples were taken randomly during saturation process to test the saturation of bone char. Column saturated using 100 ppm fluoride had highest efficiency of 97.63% that is fluoride released from bone char of 2.37 ppm.

Table 4.2: Fluoride concentrations obtained after rinsing bone char with 5.61 ppm water at a contact time of 30 mins

Column number	Final fluoride concentration (ppm) (initial conc.=5.61 ppm)
III	1.33
IV	1.19
V	1.74
VI	1.80

Water containing 5.61ppm fluoride was used to rinse bone char from free fluoride ions. To test the saturation of the bone char, measurement of the effluent concentrations of fluoride was carried out. Table 4.2 shows the concentrations of the fluoride in water released after 30 minutes of contact time for the four columns. Bone char in the four columns was then thoroughly mixed for use in regeneration.

From the results, the highest removal efficiency was found to be 97.63%, which had initial fluoride concentration of 100 ppm and a contact time of four hours. Previous studies indicated that bone char had high efficiency of between 97.4-99.8 % (Mavura and Tiffany, 2002; Korir *et al*., 2009). The highest efficiency of 97.63% had 2.37 ppm fluoride in water. This is an indication that the concentration was above the WHO guideline value of 1.5 ppm. Therefore, even though bone char has very high efficiency, it cannot be used to remove fluoride from waters with high concentrations up to 100 ppm and above for human consumption.

During the verification of normal saturation of bone char (Table 4.2), it was found that columns V and VI had fluoride concentrations of 1.74 and 1.80 ppm respectively slightly above breakthrough point of 1.5 ppm that is WHO guideline value while that of columns III and IV were 1.33 and 1.19 ppm respectively. The bone char the four columns was mixed for use in regeneration step.

4.2 Reactivation of Fluoride Saturated Bone Char

Fluoride ion is known to exchange with hydroxyl, carbonate, hydrogen carbonate, and phosphate ions of bone char during fluoride removal from water using bone char (Bailey, 1972; Abe *et al*., 2004; Kawasaki, 2009). The purpose of using different sodium solutions was to determine whether regeneration is possible. During reactivation of fluoride saturated bone char, three different concentrations of NaOH were used to determine optimum fluoride removal: 1%, 2%, and 4% NaOH solution respectively. The same procedure was repeated using Na_2CO_3, $NaHCO_3$ and Na_3PO_4 solutions in order to determine the best solutions of fluoride regeneration. Tables 4.3 through 4.6 show the results obtained for fluoride released from fluoride saturated bone char for the four solutions at different concentrations.

Table 4.3: Fluoride concentration released from bone char using 1%, 2% and 4% of $NaHCO_3$ solution for various contact times

	1% $NaHCO_3$	**2% $NaHCO_3$**	**4% $NaHCO_3$**
Time in hours		Fluoride concentration in ppm	
0.5	5.12± 0.41	4.73± 0.38	4.35± 0.09
1	4.20± 0.11	4.65± 0.21	5.32± 0.25
2	4.45± 0.07	4.94± 0.43	5.43± 0.08
4	4.34± 0.20	4.75± 0.18	5.46± 0.20
24	4.73± 0.42	4.83± 0.06	6.03± 0.10

The amounts of fluoride released by bone char at 95% confidence interval for 1% $NaHCO_3$ vs. 2% $NaHCO_3$ concentrations were not statistically different while 2% vs. 4% were statistically different.

Table 4.3 shows that the highest fluoride concentration released was 6.03 ppm at 4% $NaHCO_3$, 5.83, and 5.12 ppm for 2% $NaHCO_3$ and 1% $NaHCO_3$ respectively. The minimum concentrations were 4.35, 4.65, and 4.20 ppm for 4%, 2%, and 1% respectively. The concentrations of fluoride removed increased with time. ANOVA study at $P<0.05$ in Table 4.3 show that the means for the 1% vs. 2% $NaHCO_3$ concentrations were not significantly different that is, increasing the concentration of sodium bicarbonate from 1% to 2% had no effect on the fluoride removed. For 2% vs. 4%, there is no significant difference at 0.5 and 2 hours respectively. These low concentrations of fluorides released may be attributed to the fact that hydrogen carbonate in both fluorapatite and hydroxyapatite is not a functional group in ion exchange, or the concentration of OH^- ions is very low.

Table 4.4: Fluoride concentration released from bone char using 1%, 2% and 4 % of NaOH solution for various contact times

	1% NaOH	2% NaOH	4% NaOH
Time in hours	Fluoride concentration in ppm		
0.5	130.55± 3.04	176.96± 9.79	175.89± 3.08
1	142.02± 4.24	186.14±12.55	182.18± 5.52
2	145.06± 1.99	179.14± 3.20	177.33± 4.38
4	138.96± 5.90	176.08± 0.70	177.78± 5.91
24	140.17± 1.79	172.18± 1.72	182.16± 4.75

Comparisons of 1% vs. 2% concentrations at 95% confidence interval were statistically different while 2% vs. 4% concentrations were not statistically different.

From Table 4.4, the higher the NaOH concentration, the higher the fluoride effluent released from the bone char. The fluoride concentration released from saturated bone char was found to be 186.1 ppm maximum and 172.0 minimum using 4% NaOH, 2% NaOH, 181.4 maximum and 175.9 ppm minimum and 1% NaOH, 145.1 maximum and 130.6 ppm respectively. Optimum contact time for regeneration was obtained at two hours for all the concentrations used. According to the ANOVA analysis in Table 4.4, at 95% confidence interval indicated that the mean concentrations are statistically significant at $P< 0.05$ for 1% vs. 2% NaOH concentrations. This suggests that increasing the concentration of sodium hydroxide, increases the fluoride concentration removed. However, no significant difference was obtained for 2% vs. 4% NaOH, suggesting that increasing the concentration of sodium hydroxide from 2% to 4% does not increase the concentration of fluoride ions removed from the saturated bone char. The reaction involved is ion exchange between fluoride ions from fluorapatite and hydroxyl ion from sodium hydroxide (Bailey, 1972; Dahi, 1997; Jacobsen and Muller, 2007 a).

In regeneration, using NaOH the fluoride in the molecule of fluorapatite is displaced by OH^- as follows (Equations 4.1-4.3)

$$Ca_{10}(PO_4)F_2 + 2OH \rightarrow Ca_{10}(PO_4)(OH)_2 + 2F \quad (4.1)$$

$$Ca_{10}(PO_4)_6.2F + 2OH^- \rightarrow Ca_{10}(PO_4)(OH)_2 + 2F^- \quad (4.2)$$

$$(Ca_3(PO_4)_2)_nCaF_2 \rightarrow Ca_3(PO_4)_2)_nCa(OH)_2 + 2NaF \quad (4.3)$$

Table 4.5: Fluoride concentration released from bone char using 1%, 2% and 4 % of Na_3PO_4 solution for various contact times

	1% Na_3PO_4	**2% Na_3PO_4**	**4% Na_3PO_4**
Time in hours	Fluoride concentration in ppm		
0.5	28.64± 1.16	42.33± 1.27	52.97± 6.97
1	29.10± 0.51	45.87± 1.63	62.98± 0.54
2	30.64± 0.96	49.60± 0.62	74.43± 1.90
4	31.51± 0.40	47.60± 0.46	73.50± 0.53
24	28.82± 0.53	46.06± 0.84	63.85± 0.55

Comparison of 1% vs. 2% and 2% and 4% concentrations at 95% confidence interval, were significantly different.

For sodium phosphate, maximum fluoride removed was 74.43 ppm and minimum was 28.64 ppm in Table 4.5. All the p values were less than 0.001. There was significant difference for 1% vs. 2% and 2% vs. 4% thus increasing the concentration from 1% to 2% or from 2% to 4%, the concentration of the fluoride removed from bone char increases. The concentrations of phosphate ions eluted during de-fluoridation were compared to fluoride ions removed by bone char, and phosphate ions were found to be very low depending on the animal bone char used (Kawasaki *et al*., 2009). Equation 4.4 shows how phosphate ion exchanges with fluoride ion in water (Brunson and Sabatini, 2009).

$$Ca_{10}(PO_4)_6(OH)_2 + 20F^- + 2H^+ \rightarrow 10CaF_2 + 6PO_4^{3-} + 2H_20 \quad (4.4)$$

Table 4.6: Fluoride concentration released from bone char using 1%, 2% and 4 % of Na_2CO_3 solution for various contact times

	1% Na_2CO_3	**2% Na_2CO_3**	**4% Na_2CO_3**
Time in hours	Concentration in ppm	Concentration in ppm	Concentration in ppm
0.5	31.58± 1.31	36.63± 0.67	38.69± 1.73
1	32.56± 0.59	39.75± 1.89	42.51± 0.86
2	32.77± 1.96	42.68± 0.96	46.02± 0.22
4	34.85± 1.08	43.79± 0.21	47.46± 0.39
24	33.92± 0.91	40.77± 1.45	46.31± 1.67

Comparing 1% vs. 2% and 2% vs. 4% Na_2CO_3 concentrations at 95% confidence interval, were found to be significantly different.

The removal mechanism was via ion exchange in which the carbonate ion of apatite was replaced by fluoride ion to form fluorapatite (Ayoob *et al.*, 2008; Shrikant and Nitin, 2012). The carbonate ion is thought to be the active part of the apatite (equation 4.5).

$$Ca_9(PO_4)_6.CaCO_3 + 2F^- + Ca_9(PO_4)_6.CaF_2 + CO_3^{2-} \quad (4.5)$$

Regeneration using sodium carbonate solution is the reverse of the equation 4.6. From Table 4.6, maximum fluoride removed was 47.46 ppm while minimum was 31.58 ppm. At 95% confidence interval in Table 4.6, the means for 1% vs. 2% concentrations were statistically significant. This suggests that increasing concentration of sodium carbonate from 1% to 2%, increases the amount of fluoride concentration removed from bone char. Increasing concentration of sodium carbonate had no significant effect for 2% vs. 4% between 0.5 and 1 hour. Between 2 to 4 hours, there is a slight significant difference and at 24 hours.

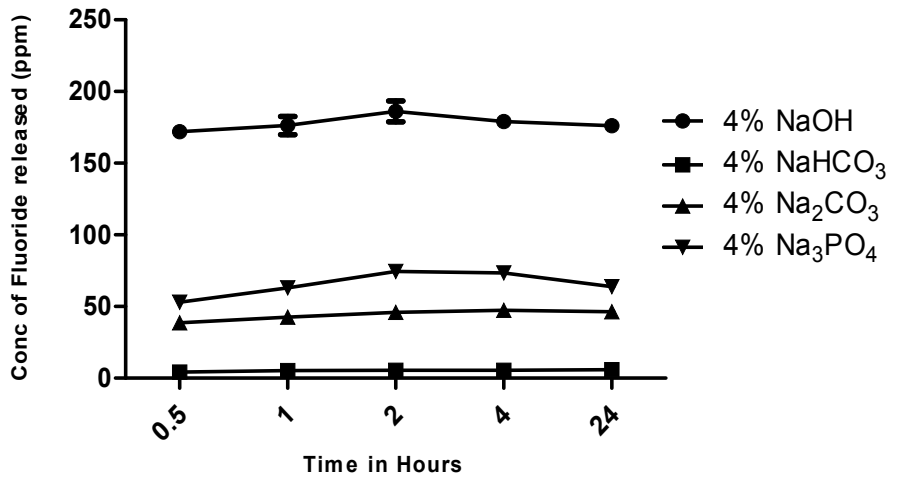

Figure 4.1: Concentration of fluoride released by different sodium solutions of 4% concentration.

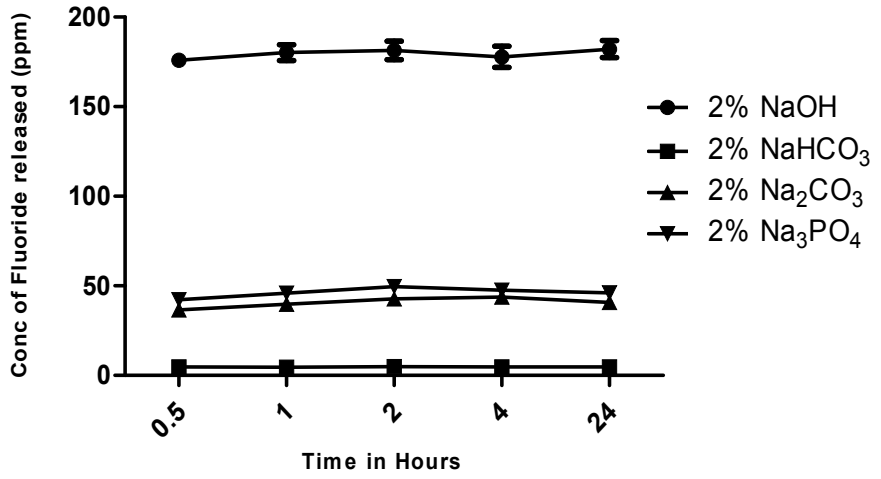

Figure 4.2: Concentration of fluoride released by different sodium solutions of 2% concentration

All the four solutions were found to be statistically different except 2% for carbonate vs. phosphate solutions.

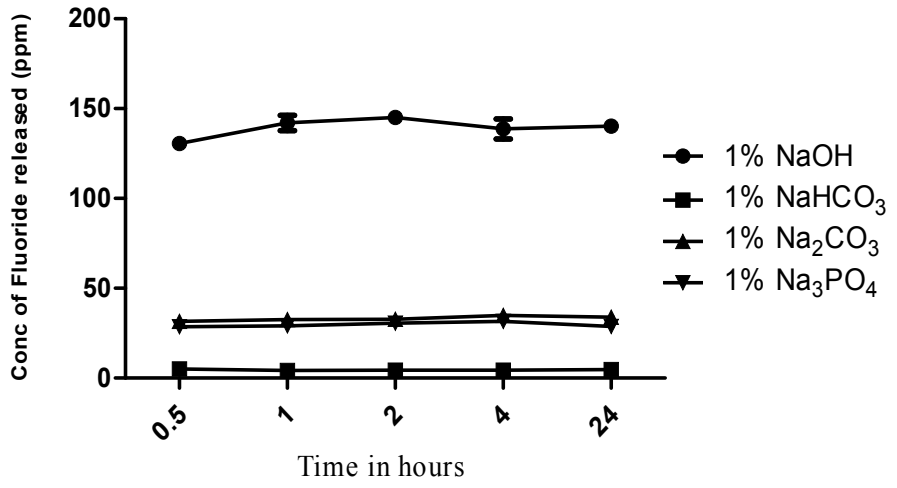

Figure 4.3: Concentration of fluoride released by different sodium solutions of 1% concentration

All solutions were statistically different for fluoride removal except that of carbonate and phosphate solutions.

The pH values of the solutions were measured to determine whether pH has any effect on the amount of fluoride released from the bone char. The pH values of the four solutions were observed to follow the following order $NaHCO_3 < Na_2CO_3 \approx Na_3PO_4 <$NaOH. The pH of the solution was found to be the most important factor affecting the amount of fluoride removed. Sodium Hydrogen carbonate provided the lowest concentration of fluoride (4.12-6.03 ppm) and it has the lowest pH and sodium hydroxide the highest (130.55-182.18 ppm). Sodium phosphate had a concentration of between 22.60-74.43 ppm and carbonates a concentration of between 31.58-47.46 ppm of fluoride removed. The results obtained indicate that the best desorption was achieved in the solution with highest pH.

One of the reasons for better desorption at high pH values may be attributed to a large number of OH^- ions present at these pH values, which in turn increases diffusion and mobility of fluoride ions. At low pH values, the reduction in desorption may be possible due to the abundance of H^+ ions thus fluoride ions are immobile and this hinders diffusion. The surface desorbed anions favorably in high pH range due to the presence of OH^- ions, whereas the surface is active for the adsorption of anions at low pH values due to the accumulation of H^+ ions (Tembhurkar and Shilpa, 2006). Thus, greater pH gives maximum fluoride removal. The

other contribution could be the contents of these ions in bone char or their arrangement - determine the contents) carbonate composition is less, phosphate bonds are stronger and hydrogen carbonate. Previous studies indicated that lower pH values, bone char will be positively charged, and thus it should have a higher affinity for fluoride making it a viable adsorbent for fluoride removal (Abe *et al.,* 2004).

The major regeneration process therefore, involves ion exchange between hydroxyl ions in solution and fluoride ions from the fluorapatite according to equation 4.6.

$$BC - F + OH^- \rightarrow BC - OH + F^- \quad (4.6)$$

This reaction readily occurs because fluoride ion and hydroxide ion have the same charge and radius (Bregnhøj, 1995; Chidambaram *et al*., 2003). Regeneration process also involves other reactions such as diffusion, precipitation and desorption.

Comparing statistically similar concentrations among the four solutions, it was found that all solutions differ significantly that is $p<0.001$ except for 1% Na_2CO_3 vs. 1% Na_3PO_4 and 2% Na_2CO_3 vs. Two% Na_3PO_4 where p-value was $p>0.05$. This could be attributed to the pH of 1% Na_2CO_3 vs. 1% Na_3PO_4 and 2% Na_2CO_3 vs. 2% Na_3PO_4 are almost equal.

Regeneration can be carried out by heating fluoride saturated bone char in the presence of hydroxide (Kaseva, 2006). Wang *et al*., 2001 suggested that during fluoride removal from water, fluoride ion might combine with hydroxyapatite in two ways according to equations 4.7-4.8.

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^- + H^+ \rightarrow Ca_{10}(PO_4)_6(OH)_2.2F \text{ (Free calcium)} \quad (4.7)$$

$$Ca_{10}(PO_4)_6(OH)_2 + 2Ca^{2+} + 6F^- + H^+ \rightarrow Ca_{10}(PO_4)_6.2CaF_2 + 2H_20 \text{ (Need calcium)} \quad (4.8)$$

In regeneration using sodium hydroxide, the F^- in the $Ca_{10}(PO_4)_6(OH)_2.2F$ can be replaced by OH^- (Equations 4.9-4.10). The molecule $Ca_{10}(PO_4)_6.2CaF_2$ cannot react with OH^- but dissolves and releases fluoride from the molecule heating as shown in equations 4.11-12. The reaction may be as follows:

a) $$Ca_{10}(PO_4)_6(OH)_2.2F + 2OH^- \rightarrow Ca_{10}(PO_4)_5O + 2F_2 \quad (4.9)$$

$$Ca_{10}(PO_4)_6O + 2H_20 \rightarrow Ca_{10}(PO_4)_6(OH)_2 \quad (4.10)$$

b) $$Ca_{10}(PO_4)_6.2CaF_2 + 2OH^- \rightarrow 2Ca_{10}(PO_4)_6.2CaFO + 2F_2 \quad (4.11)$$

$$Ca_{10}(PO_4)_62CaFO + 2H_20 \rightarrow 2Ca_{10}(PO_4)_62F + 2Ca(OH)_2 \quad (4.12)$$

4.3 Effect of Temperature in Regeneration

Study on the effect of temperature was conducted by varying it from 20-60 ℃ keeping bone char of 40 g/50 mL and 1hour contact time.

Table 4.7: Effect of Temperature on Regeneration

Effect of temperature on regeneration using 1% NaOH	
Temperature (^{o}C)	Fluoride effluent (ppm)
60	155.92±3.43
50	159.87±2.62
40	151.14±3.04
30	128.80±2.44
20	113.67±2.25

The effect of temperature on the regeneration of fluoride saturated bone char was studied at temperatures of 20, 30, 40, 50 and 60°C. It was found that fluoride released was essentially dependent on temperature as seen in Table 4.7. At 50 °C, it was found to be the best temperature for regeneration since it provided the highest concentration of fluoride released 159.87 ppm.

López *et al.,* (2006) showed that temperature appears to play a slight role in the optimal pH for sorption in that by increasing temperature from 10 °C to 40 °C the pH_{pzc} decreased from 9.6 to 8.1. This change suggests that increased temperatures either favors desorption of protons from the surface or hydroxide sorption to the surface, shifting protonation constants to lower values as shown in the following surface equilibria. As temperature increased, sorption was shown to be less favored most likely due to increased deprotonation or hydroxylation of the surface causing more negatively charges sorbant surfaces.

At higher temperature, the hydroxyl ions move faster and more can penetrate into cavities of the porous bone char's structure. This result in more exchange of hydroxyl ions with the fluoride ions of the bone char is fluorapatite. The amount of fluoride ions that were desorbed increased at higher temperatures. This result indicated that desorption mechanism of

fluoride ion from bone char is an endothermic reaction; that is, the fluoride in bone char consumes heat in exchanging with a hydroxyl ion. Diffusion of hydroxyl ions seems also to increase with increased temperature (Meena *et al.*, 2005).

4.4 Determination of efficiency of regenerated bone char

Table 4.8: Efficiency of regenerated, fresh and mixture (1:1) bone char

	Fresh bone char		Regenerated bone char		Mixture	
Volume of treated water in litres	Final fluoride concentration in ppm	Efficiency in %	Final fluoride concentration in ppm	Efficiency in %	Final fluoride concentration in ppm	Efficiency in %
0.25	0.25±0.085	95.81	0.46±0.035	92.37	0.50±0.099	91.61
0.50	0.34±0.000	94.30	0.46±0.007	92.37	0.55±0.035	90.69
0.75	0.31±0.000	94.80	0.45±0.028	92.45	0.38±0.014	93.62
1.00	0.34±0.014	94.30	0.50±0.014	91.61	0.32±0.042	94.63
1.30	0.42±0.035	93.04	0.46±0.064	92.37	0.43±0.007	92.87
1.55	0.45±0.163	92.53	0.53±0.007	91.19	0.36±0.028	93.96
2.05	0.34±0.064	94.38	0.56±0.007	90.69	0.58±0.113	90.27
2.35	0.37±0.042	93.79	0.58±0.000	90.27	0.47±0.057	92.11
2.70	0.38±0.078	93.71	0.60±0.007	90.02	0.44±0.021	92.70
3.05	0.37±0.085	93.79	0.63±0.014	89.43	0.44±0.035	92.70
3.45	0.74±0.021	87.67	0.77±0.021	87.16	0.79±0.212	86.74
3.80	0.74±0.028	87.58	0.83±0.021	86.16	0.78±0.035	87.00
4.80	0.83±0.071	86.07	1.08±0.035	81.96	0.87±0.078	85.49
5.80	1.21±0.021	79.78	1.45±0.092	75.76	1.29±0.042	78.36
6.80	1.34±0.106	77.60	1.55±0.127	73.99	1.57±0.000	73.66
7.30	1.53±0.021	74.41	1.67±0.057	71.98	1.75±0.028	70.64
8.30	1.58±0.163	73.57	1.89±0.035	68.37	1.81±0.071	69.63
8.80	1.86±0.262	68.88	1.99±0.021	66.69	1.84±0.049	69.21

Untreated borehole water had initial fluoride concentration of 5.96 ppm. At 95% confidence interval, there was no significant different in efficiency between fresh and

regenerated bone chars. Fresh bone char versus mixture bone char there was no significant different that is p>0.05. From table 4.8, optimum percentages of fluoride removal were 95.81, 92.45 and 94.63% for the fresh, regenerated and mixture bone char respectively. Percentage of fluoride removed decreased with increase of treated water (L).

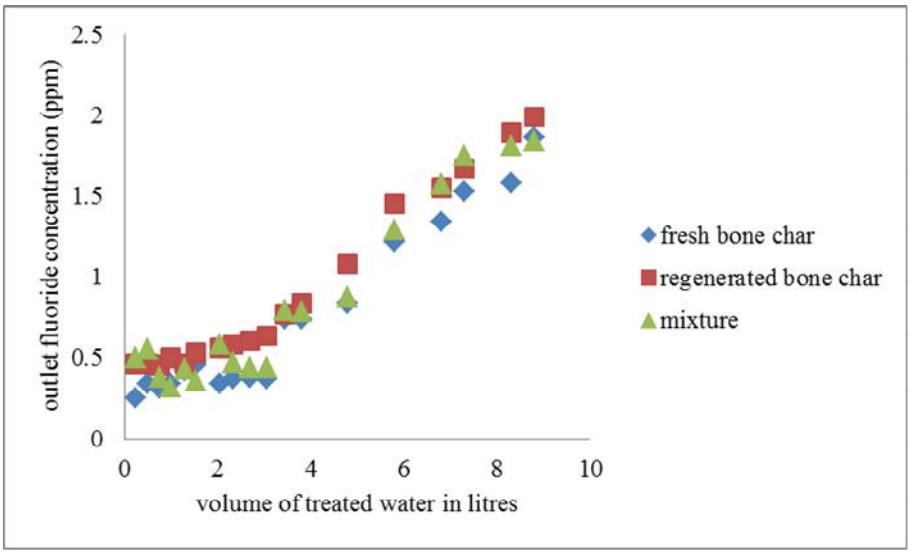

Figure 4.4: Fluoride concentration released as a function of the amount of treated water. Initial fluoride concentration of water 5.96 ppm.

Equations for the curves in figure 4.4:

$$y = 0.014x^2 + 0.060x + 0.246,\ R^2 = 0.968 \quad (4.13)$$

$$y = 0.013x^2 + 0.075x + 0.377,\ R^2 = 0.984 \quad (4.14)$$

$$y = 0.017x^2 + 0.036x + 0.363,\ R^2 = 0.948 \quad (4.15)$$

for fresh, regenerated and mixture bone char respectively.

Where; Y= fluoride effluent in mg/L (ppm) and x= the volume in litres of water treated during the experiment.

The equations 4.13-15 were used to determine the fluoride concentration in treated water. Amount of water treated before breakpoint of 1.5 ppm was 7.56, 6.85, and 7.18 litres for fresh, regenerated and mixture respectively:

$1.5 = 0.014x^2 + 0.060x + 0.246$ or $0.014x^2 + 0.060x - 1.254 = 0$, then calculate x.

Total amount of fluoride in water before treatment was; 45.06 (5.96 ppm× 7.56 l), 42.79 and 40.83 mg F^-. Amount of fluoride in water after treatment was; 5.53, 5.54, and 5.64 mg $F^-$for fresh, mixture, and regenerated bone char respectively obtained through integration of equations 4.13-4.15.

The results obtained were plotted as effluent fluoride concentration versus volume of treated water (Figure 4.8). The data in figure 4.8 indicates that the concentrations of fluoride removed was high in initial stages, and decreased up to about 6.85, 7.18 and 7.56 litres for regenerated, mixture of bone char and fresh bone char respectively. The removal rate was fast at the beginning of the process and then it decreased until equilibrium was reached. This trend may be due to; initially all the adsorbent sites were vacant and the solute gradient was high for ion exchange but with time the number of sites decreases. After this, the bone char is still active but effluent has a fluoride concentration above the WHO recommended value of 1.5 ppm.

From the results obtained, the highest removal efficiency of regenerated bone char, fresh bone char, and a mixture of bone char was 92.45, 95.81, and 94.63 % respectively. The efficiency of regenerated bone char was compared to fresh bone char and results have showed that there was no significant difference that is $p>0.05$ (Table 4.8). This is in agreement with previous experiments carried out (Kaseva, 2006). The first regenerated bone char was capable of removing fluoride from drinking water to meet the Kenyan and WHO recommended values of 1.5 ppm. This indicated that a large part of the hydroxyapatite structure was not damaged during the regeneration process. Mixing of regenerated and fresh bone char does not add any advantage in improving the efficiency of bone char, that is the efficiency of the mixture and that of regenerated bone char was not statistically different, $p>0.05$ (Table 4.8b). From the trend of the effluent fluoride level observed during the defluoridation process, the fluoride retained in the bone char can be calculated from the equations 4.13 -4.15.

The useful removal capacity of the bone char was defined as the volume of treated water before the breakthrough point at concentration of 1.5 ppm (Bhargava, 1997). The amount of fluoride retained in bone char at C_o of 5.96 ppm for regenerated bone char, fresh bone char, and mixture of bone char were 35.19 mg F^-, 39.53 mg F^-, and 37.25 mgF^- respectively. This corresponds to removal capacities of about 0.880 ($^{35.19\ mg}/_{40\ grams}$), 0.988 and 0.932mg/g respectively which is equivalent to 0.880, 0.988 and 0.932 g/kg. This

showed that the removal capacity has decreased by 10.93% only. The capacities found in this experiment were smaller but in the same order of magnitude. This suggests that results from different tests of bone char may not be directly comparable due to variations in the design of the experiments. The estimated removal capacities from small-scale experiments can therefore not be used as an exact determination of the capacities. They are however; very useful for comparing different types of bone chars and can rank them by quality for adsorption (Albertus *et al.*, 2000). This work was to compare the regenerated and fresh bone char with respect to their capacity to remove fluoride. The other factor that may influence the capacity of bone char is the presence of competitive ions present in the borehole water such as carbonate, hydrogen carbonate generate basicity that reduces the removal capacity. Presence of calcium, magnesium ions promote to some extent fluoride removal. pH of the water, the lower the pH, the higher the amount of fluoride removed. Flow rate of water during defluoridation, the higher the flow rate, the lower the capacity (Bailey, 1972; Abe *et al*., 2004; Mishra *et al*., 2010). pH_{zc}, production of bone char (Posner, 1987; Brunson and Sabatini, 2007; Razaee *et al*., 2009; Moreno *et al*., 2011).

Some adsorption of fluoride occurs onto the activated carbon although the primary uptake reaction is believed to be ion exchange between hydroxyapatite and fluoride resulting in formation of fluorapatite (Bregnhøj and Dahi, 1995; Crittenden *et al*., 2005).

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^- \rightarrow Ca_{10}(PO_4)_6F_2 + 2OH^- \quad (4.16)$$

The main ingredient in the bone char is hydroxyapatite and in water, it combines with fluoride. The reaction may include two ways involving free calcium and need calcium as in (Equations 4.7-4.8). The percentage loss could be because of $Ca_{10}(PO_4)_6.2CaF_2$ molecule that cannot react with OH^- ion during regeneration using sodium hydroxide. Bone char is soluble in an acid, due to this; some of its efficiency could have been lost during acid neutralization of sodium hydroxide used for regeneration.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Sodium hydroxide was the most effective among the four solutions tested for regeneration of fluoride saturated bone char that had the highest pH.
2. Temperature was found to influence the amount of fluoride released from bone char during regeneration process. Thus, increasing temperature increases the amount of fluoride removed from fluoride saturated bone char but its influence is low compared to pH.
3. From the results, obtained regenerated bone char was equally effective as fresh bone char. There was no significant in removal capacities of regenerated compared to fresh bone char. That is, for the first regeneration process, the bone char has not lost its fluoride binding capacity. This implies that the regenerated bone char is a potential material that can be used in communities where fluoride concentration in the water system is above the recommended level. Thus making it possible to prevent health complications like dental fluorosis, skeletal fluorosis, neurological problems, muscular problems, and allergic manifestations.
4. There was no significant difference between the ratios of regenerated bone char to unused bone char. Mixing of regenerated bone char with fresh bone char did not improve the efficiency.

5.2 Recommendations

1. In order to understand regeneration process well, data on thermodynamic should be obtained.
2. Further studies should be carried out to determine the efficiency of the process of regeneration in large scale, and the practicability and suitability of the process. A method of reusing sodium hydroxide that will reduce the amount of fluoride going back to environment and maximize the use of sodium hydroxide should be devised. There is also need to compare regeneration using continuous and batch methods to determine the most effective.

3. Since regenerated bone char has shown no significant difference as compared to fresh bone char for first regeneration, series of regeneration processes should be carried out to determine when regenerated bone char would no longer be effective that is it has lost fluoride-binding capacity.

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APPENDICES

Sample of data analysis

APPENDIX 1: Two ANOVA for NaOH solution

Two-way ANOVA for NaOH Solution

Bonferroni posttests

1 % NaOH vs. 2% NaOH

Row Factor	1 % NaOH	2% NaOH	Difference	95% CI of diff.
0.5	130.5	175.9	45.34	30.78 to 59.90
1	142.0	180.2	38.16	23.60 to 52.72
2	145.1	181.4	36.38	21.82 to 50.94
4	138.7	177.8	39.09	24.53 to 53.65
24	140.2	182.2	42.00	27.44 to 56.55

Row Factor	Difference	t	P value	Summary
0.5	45.34	9.932	P<0.001	***
1	38.16	8.359	P<0.001	***
2	36.38	7.969	P<0.001	***
4	39.09	8.563	P<0.001	***
24	42.00	9.199	P<0.001	***

1 % NaOH vs. 4%NaOH

Row Factor	1 % NaOH	4%NaOH	Difference	95% CI of diff.
0.5	130.5	172.0	41.46	26.90 to 56.02
1	142.0	176.3	34.27	19.72 to 48.83
2	145.1	186.1	41.08	26.52 to 55.63
4	138.7	179.1	40.46	25.90 to 55.01
24	140.2	176.1	35.91	21.36 to 50.47

Row Factor	Difference	t	P value	Summary
0.5	41.46	9.082	P<0.001	***
1	34.27	7.508	P<0.001	***
2	41.08	8.998	P<0.001	***
4	40.46	8.862	P<0.001	***
24	35.91	7.867	P<0.001	***

2% NaOH vs. 4%NaOH

Row Factor	2% NaOH	4%NaOH	Difference	95% CI of diff.
0.5	175.9	172.0	-3.880	-18.44 to 10.68
1	180.2	176.3	-3.887	-18.44 to 10.67
2	181.4	186.1	4.697	-9.861 to 19.25

4	177.8	179.1	1.367	-13.19 to 15.92
24	182.2	176.1	-6.083	-20.64 to 8.474

Row Factor	Difference	t	P value	Summary
0.5	-3.880	0.8499	P > 0.05	Ns
1	-3.887	0.8514	P > 0.05	Ns
2	4.697	1.029	P > 0.05	Ns
4	1.367	0.2994	P > 0.05	Ns
24	-6.083	1.333	P > 0.05	Ns

APPENDIX 2: Two-way ANOVA for efficiency determination

Parameter				
Table Analyzed	EFFICIENCY			
Two-way ANOVA				
Bonferroni posttests				
UNUSED vs. REGENERATED				
Row Factor	UNUSED	REGENERATED	Difference	95% CI of diff.
.25	0.2500	0.4550	0.2050	-0.04453 to 0.4545
.50	0.3400	0.4550	0.1150	-0.1345 to 0.3645
.75	0.3100	0.4500	0.1400	-0.1095 to 0.3895
1.00	0.3400	0.5000	0.1600	-0.08953 to 0.4095
1.30	0.4150	0.4550	0.04000	-0.2095 to 0.2895
1.55	0.4450	0.5250	0.08000	-0.1695 to 0.3295
2.05	0.3350	0.5550	0.2200	-0.02953 to 0.4695
2.35	0.3700	0.5800	0.2100	-0.03953 to 0.4595
2.70	0.3750	0.5950	0.2200	-0.02953 to 0.4695
3.05	0.3700	0.6300	0.2600	0.01047 to 0.5095
3.45	0.7350	0.7650	0.03000	-0.2195 to 0.2795
3.80	0.7400	0.8250	0.08500	-0.1645 to 0.3345
4.80	0.8300	1.075	0.2450	-0.004529 to 0.4945
5.80	1.205	1.445	0.2400	-0.009529 to 0.4895
6.80	1.335	1.550	0.2150	-0.03453 to

				0.4645
7.30	1.525	1.670	0.1450	-0.1045 to 0.3945
8.30	1.575	1.885	0.3100	0.06047 to 0.5595
8.80	1.855	1.985	0.1300	-0.1195 to 0.3795

Row Factor	Difference	t	P value	Summary
.25	0.2050	2.770	P > 0.05	ns
.50	0.1150	1.554	P > 0.05	ns
.75	0.1400	1.891	P > 0.05	ns
1.00	0.1600	2.162	P > 0.05	ns
1.30	0.04000	0.5404	P > 0.05	ns
1.55	0.08000	1.081	P > 0.05	ns
2.05	0.2200	2.972	P > 0.05	ns
2.35	0.2100	2.837	P > 0.05	ns
2.70	0.2200	2.972	P > 0.05	ns
3.05	0.2600	3.513	P < 0.05	*
3.45	0.03000	0.4053	P > 0.05	ns
3.80	0.08500	1.148	P > 0.05	ns
4.80	0.2450	3.310	P < 0.05	*
5.80	0.2400	3.242	P < 0.05	*
6.80	0.2150	2.905	P > 0.05	ns
7.30	0.1450	1.959	P > 0.05	ns
8.30	0.3100	4.188	P<0.01	**
8.80	0.1300	1.756	P > 0.05	ns

UNUSED vs. MIXTURE (1:1)

Row Factor	UNUSED	MIXTURE	Difference	95% CI of diff.
.25	0.2500	0.5000	0.2500	0.0004707 to 0.4995
.50	0.3400	0.5550	0.2150	-0.03453 to 0.4645
.75	0.3100	0.3800	0.07000	-0.1795 to 0.3195
1.00	0.3400	0.3200	-0.02000	-0.2695 to 0.2295
1.30	0.4150	0.4250	0.01000	-0.2395 to 0.2595
1.55	0.4450	0.3600	-0.08500	-0.3345 to 0.1645
2.05	0.3350	0.5800	0.2450	-0.004529 to 0.4945
2.35	0.3700	0.4700	0.1000	-0.1495 to 0.3495
2.70	0.3750	0.4350	0.0600	-0.1895 to 0.3095
3.05	0.3700	0.4350	0.0650	-0.1845 to 0.3145

3.45	0.7350	0.7900	0.05500	-0.1945 to 0.3045
3.80	0.7400	0.7750	0.03500	-0.2145 to 0.2845
4.80	0.8300	0.8650	0.03500	-0.2145 to 0.2845
5.80	1.205	1.290	0.08500	-0.1645 to 0.3345
6.80	1.335	1.570	0.2350	-0.01453 to 0.4845
7.30	1.525	1.750	0.2250	-0.02453 to 0.4745
8.30	1.575	1.810	0.2350	-0.01453 to 0.4845
8.80	1.855	1.835	-0.02000	-0.2695 to 0.2295

Row Factor	Difference	t	P value	Summary
.25	0.2500	3.378	P < 0.05	*
.50	0.2150	2.905	P > 0.05	ns
.75	0.07000	0.9457	P > 0.05	ns
1.00	-0.02000	0.2702	P > 0.05	ns
1.30	0.01000	0.1351	P > 0.05	ns
1.55	-0.08500	1.148	P > 0.05	ns
2.05	0.2450	3.310	P < 0.05	*
2.35	0.1000	1.351	P > 0.05	ns
2.70	0.0600	0.8106	P > 0.05	ns
3.05	0.0650	0.8782	P > 0.05	ns
3.45	0.05500	0.7431	P > 0.05	ns
3.80	0.03500	0.4729	P > 0.05	ns
4.80	0.03500	0.4729	P > 0.05	ns
5.80	0.08500	1.148	P > 0.05	ns
6.80	0.2350	3.175	P < 0.05	*
7.30	0.2250	3.040	P > 0.05	ns
8.30	0.2350	3.175	P < 0.05	*
8.80	-0.02000	0.2702	P > 0.05	ns