Public Health and Groundwater Quality in Sri Lanka and Defluoridation of Drinking Water

In relation to Chronic Kidney Disease of unknown etiology

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Doctoral Dissertation

Public Health and Groundwater Quality in Sri Lanka and Defluoridation of Drinking Water

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CONTENT

CHAPTER 1

Potential Risk of Drinking Water to Human Health in Sri Lanka in Relation to Public Health, and Chronic Kidney Disease of unknown etiology (CKDu)

3. Results and Discussion

3.5. Metals

CHAPTER 2

Arsenic, Cadmium, Lead, and Chromium in Rice, Human Urine, and Pesticides in Groundwater, and Rice in Sri Lanka in Relation to Chronic Kidney Disease of unknown etiology (CKDu)

3. Results and Discussion

CHAPTER 3

The Preparation of Chicken Bone Char (CBC), and Fluoride Removal from Sri Lankan Drinking Water by Larger Sized CBC – A Community Based CBC Filter

CHAPTER 4

Fluoride Removal by Finer Sized Chicken Bone Char (CBC), and Regeneration of CBC

GENERAL INTRODUCTION

Sri Lanka is an island located in the Indian Ocean just above the equator with a latitude, and longitude of 6^0-10^0 N, and 79^0-82^0 E, respectively. It covers an area of 65,610 km^2. Sri Lanka is divided into two main climatic zones: The Dry Zone, and the Wet Zone, based on the amount of precipitation. Sri Lanka has a tropical climate: year-round warm weather, moderated by ocean winds, and a considerable amount of moisture, with an average annual temperature for the country as a whole range from 28ºC to 30ºC.

Groundwater is the main drinking water source of most areas in Sri Lanka. it was reported that the Sri Lankan people suffer from numerous health defects due to inadequate clean drinking water such as dental fluorosis, skeleton fluorosis, cholera, typhoid, hepatitis, and dysentery. Further, Chronic kidney disease of unknown etiology (CKDu) is an epidemic, one of the most serious health issues, recently been increasing in Sri Lanka. It is a multifactorial disease which affects severely for human health, and causes death for many people. It is believed that the hydrogeochemistry of the drinking water is the most severe factor adversely affects CKDu, as the disease is highly endemic. The water quality of Sri Lanka was investigated by analyzing pH, fluoride, nitrate, hardness, and metal concentrations in groundwater samples collected from all districts of Sri Lanka, in order to access the potential risk of groundwater against the public health, and the CKDu in Sri Lanka. Further metal contents in rice, and urine from people living in both CKDu-endemic, and non-endemic areas in Sri Lanka were analyzed to investigate the relationship between the prevalence of CKDu, and metals. In addition, pesticides in groundwater from CKDu-endemic areas, and pesticides in rice from both CKDu-endemic, and non-endemic areas were analyzed.

Dental fluorosis, and skeleton fluorosis are directly affect by the high concentration of fluoride. Further, fluoride supposed to be one of the suspicious causes for CKDu. Therefore, profound attention should be given to minimize the contamination of drinking water by fluoride in Sri Lankan groundwater. It is worth noting that the high concentration of fluoride in groundwater is not confined to Sri Lanka. Excess fluoride concentrations in groundwater have been reported in more than 20 developing, and developed countries including Sri Lanka. Groundwater is a main source of fluoride ingestion into the human body where people use groundwater for drinking, and cooking purposes. Several techniques are used all over the world in order to remove fluoride from contaminated water. Adsorption is a widely used technique to remove fluoride from contaminated water due to cost-effectiveness, and easy operation. Among the adsorption techniques, adsorption of fluoride onto bone char is a highly effective, cost-effective, and enduring technology. Chicken bone char was investigated for fluoride removal from drinking water in this study.

The carbonizing temperature is the most important factor, when preparing chicken bone char, since it affects the efficacy of chicken bone char in removing fluoride from contaminated water. Different particle sizes of chicken bone char were investigated in relation to its adsorption capacity. The performance of larger sized chicken bone char was investigated using a community based chicken bone char filter, and the performance of finer sized chicken bone char was investigated using a laboratory scale chicken bone char filter, for defluoridation of drinking water.

The continuous usage of bone char in defluoridation causes the exhaustion of bone char media. The exhausted bone char media can either be replaced by newly processed bone char media or regenerated bone char media. Regeneration of exhausted bone char by heating is a new approach in fluoride removal from contaminated water. Heat treatment of chicken bone char was investigated as the regeneration technique because of ease operation, and cost effectiveness.

Defluoridation of drinking water is essential to avoid potential human health risks from water contaminated with fluoride. It is essential and one of the paramount environmental concerns in the world not only in Sri Lanka.

CHAPTER 1

Potential Risk of Drinking Water to Human Health in Sri Lanka in Relation to Public Health, and Chronic Kidney Disease of unknown etiology (CKDu)

1. Introduction

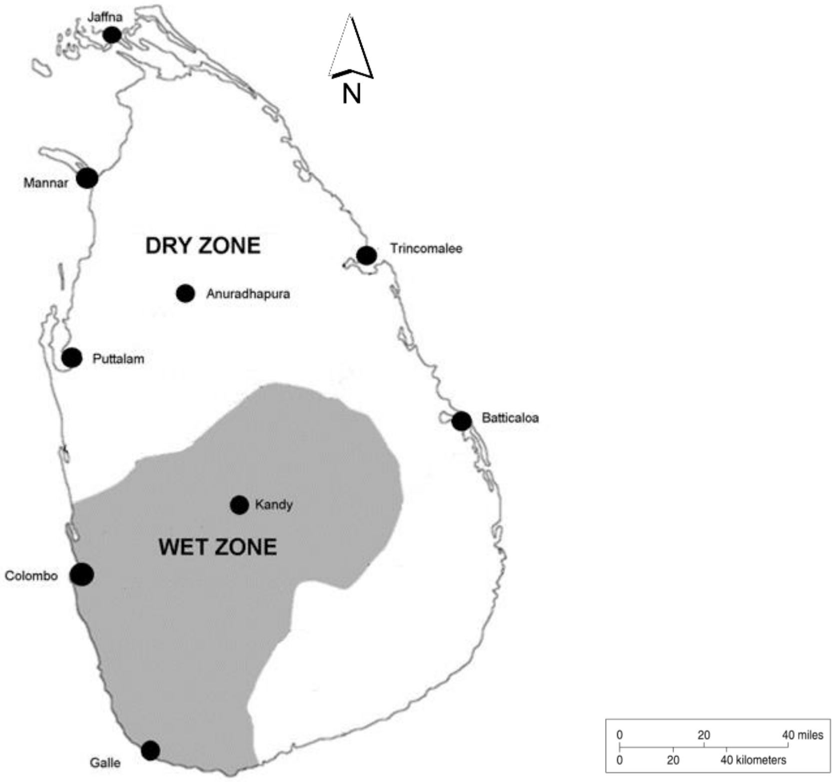


Fig. 1.1: Climatic zones of Sri Lanka

Sri Lanka is divided into two main climatic zones: The Dry Zone, and the Wet Zone, based on the amount of precipitation. Figure 1.1 shows the climatic zones of Sri Lanka. Groundwater is the main drinking water source of most areas in Sri Lanka (Dissanayake 2005), especially in the Dry Zone. Most residents obtain water for drinking, and cooking purposes through dug wells, and tube wells (IIeperuma 1995). Numerous wells supply people with water, since surface water is difficult to obtain in most of the dry zone, even though artificial canals have been constructed by successive dynasties since ancient times. However, the water quality had not been investigated due to the civil war that lasted for more than 30 years, from 1983 to 2009. An atlas of groundwater quality published in

1985 is the latest report showing the water quality in the whole area of Sri Lanka (Dissanayake and Weerasooriya 1985). Many residents suffer from health defects such as dental fluorosis, skeleton fluorosis, cholera, typhoid, hepatitis, and dysentery due to the insufficient availability of clean drinking water (IIeperuma 1995; Lunge et al. 2011). Chronic kidney disease of unknown etiology (CKDu) has recently been increasing mainly in the Dry Zone of Sri Lanka (Jayasumana et al. 2015). It is a multi-factorial disease which affects severely for human health, and causes death for many people.

CKDu is one of the most serious health issues in Sri Lanka, as it has recently been increasing regularly in the North Central Province of the Dry Zone, which covers 16% (10472 km^2) of the Sri Lankan landmass (Jayasumana et al. 2015). The North Central Province is inhabited by 6.2% of the Sri Lankan population; however, it has been reported that 73% of CKDu patients live in the North Central Province (Jayasumana et al. 2015). CKDu is an endemic disease that cannot be attributed to hypertension or diabetes (Jayasumana et al. 2013). The disease was first recorded in the early 1990s. It is more prevalent among males than females, with a ratio of 3:1, especially among males engaged in agriculture around the ages of 40–60 years (Nobel et al. 2014). Figure 1.2 shows the number of CKDu admissions as a percentage of total admissions at Anuradhapura hospital in the North Central Province. It is worth noting that the disease is not confined to Sri Lanka. Similar cases have been recorded in India, Nicaragua, Costa Rica, Central American states (Nobel et al. 2014), Uddanam, China, Serbia, Bulgaria, Romania, Croatia, and Bosnia.

Many hypotheses to explain CKDu have arisen, as follows: (1) high fluoride exposure through drinking water (Nanayakkara et al. 2012); (2) the use of aluminum containers for cooking (Jayasekara et al. 2013); (3) aflatoxins (Nobel et al. 2014); (4) ground water hardness (Jayasumana et al. 2014); (5) heavy metals contamination in rice, and/or drinking water (Redmon et al. 2014); (6) vanadium in drinking water; (7) lead, chromium, and vanadium in the soil (Jayawardana et al. 2014); (8) arsenic in drinking water (Jayasumana et al. 2013); (9) toxic levels of cadmium in food, and drinking water (Jayasumana et al. 2014); (10) various nucleotides; (11) uranium (Nobel et al. 2014); (12) pesticides (WHO 2012); fertilizers (Jayasumana et al. 2015); (13) low water intake, and exposure to high temperatures, resulting in significant dehydration (Jayasumana et al. 2014); (14) climate, and hydrogeochemistry (Redmon et al. 2014); (15) soil geochemistry (Jayawardana et al. 2014); (16) the usage of ayurvedic medicine; (17) selenium deficiency (Nobel et al. 2014); and (18) genetic susceptibility (Redmon et al. 2014). Unfortunately, conclusive evidence has not yet been obtained, as detailed investigation was hindered by the civil war that lasted 30 years, and the unavailability of analytical instruments in Sri Lanka.

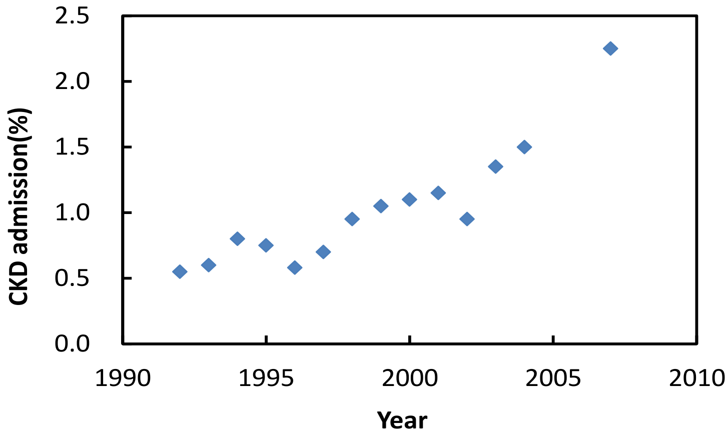


Fig. 1.2: Number of CKDu admissions as a percentage of total admissions at Anuradhapura hospital in the North Central Province (Chandrajith et al. 2011)

It is believed that the hydrogeochemistry of drinking water is the most severe factor adversely affect CKDu in Sri Lanka. Further, it was reported that the Sri Lankan people suffer from numerous health defects due to inadequate clean drinking water. Therefore, in this research, our attention was focused on the groundwater quality of Sri Lanka in order to evaluate potential risk against the public health, and CKDu. Accordingly, this research focused on pH, fluoride, nitrate, hardness, and metal concentrations of well water in Sri Lanka.

2. Materials and Methods

Water samples were collected from both dug wells, and tube wells in all districts of Sri Lanka from 2010 to 2015 to determine the water quality. Figure 1.3 shows the districts of Sri Lanka, and Figure 1.4 shows the locations from which samples were collected for analysis. 1304 samples were collected for the analysis of pH, fluoride, nitrate, hardness, aluminum, and manganese. 1435 samples were collected for the analysis of arsenic, cadmium, lead, and chromium. Table 1.1, and Table 1.2 show the number of well water samples collected in each Sri Lankan district. The wells including dug wells, and tube wells were selected randomly, and the locations were identified by GPS. The depth of wells was typically 5 m -10 m for dug wells, and sometimes more than 20 m for tube wells. Water samples were filtrated on site, using a membrane filter with a pore size of 0.45 µm to terminate bacterial activities which alter the water quality in samples. With this filtration, water quality was confirmed to be stabilized for more than 1 year at ambient temperature. The transportation of water samples from Sri Lanka to Japan took about 1-4 days after collection.

The pH value of well water was measured using a glass electrode method with a pH meter (Beckman Coulter, Inc., Model Φ260 and the STAR electrode 511070) after the samples were taken to Japan. The pH values indicated are the values after establishing the equilibrium between the solution and the atmosphere. Anions and cations were analyzed using ion chromatography (for anions: Dionex ICS-2000, separation column IonPac AS18, eluent KOH 23-40 mmol/l (gradient), suppressor ASRS 300 4mm; for cations: Dionex ICS-1500, separation column IonPac CS12, eluent methanesulfonic acid 30 mmol/l (isocratic), suppressor CSRS 500 4mm). The metal concentration of well water was analyzed by ICP-OES (Perkin Elmer Optima 5300) or ICP-MS (Agilent 7700). The data analysis, and graphing software, ORIGIN 9.1, was used to create the spatial distribution maps to represent the analyzed data. The MANDARA, and the QGIS software programs were used to create the location maps.

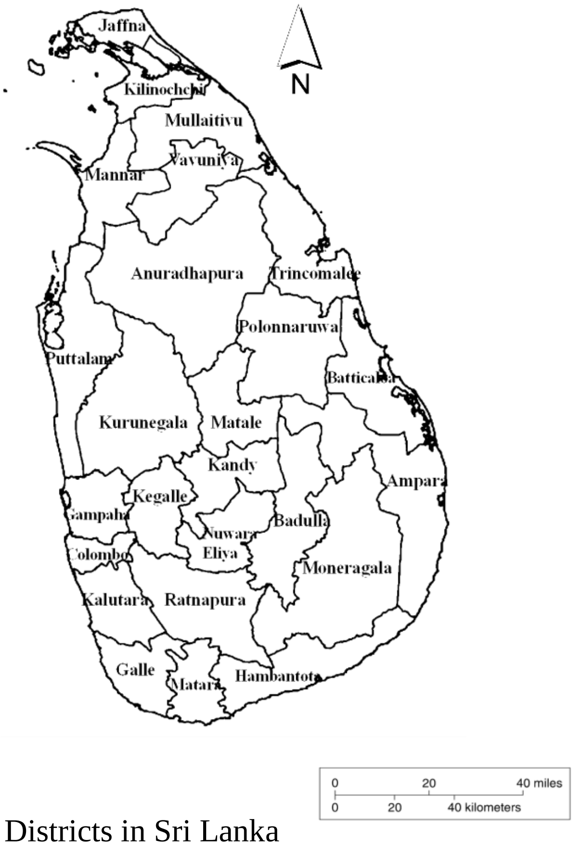


Fig. 1.3: Districts in Sri Lanka

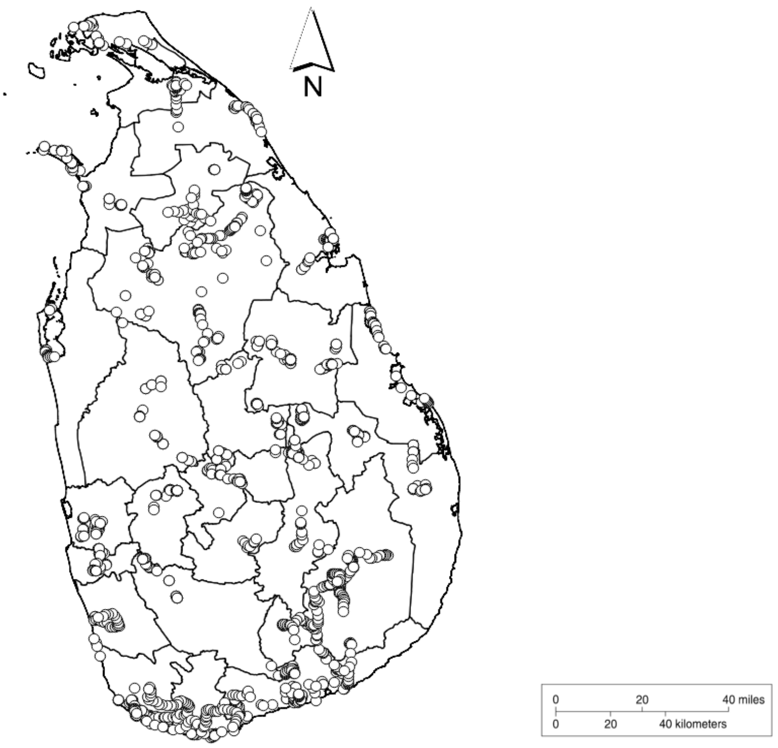


Fig. 1.4: Sampling locations in Sri Lanka

Table 1.1: Number of well water samples collected in each Sri Lankan district for the analysis of pH, fluoride, nitrate, hardness, aluminum, and manganese

District	**No. of samples**	**District**	**No. of samples**
Ampara	66	Kurunegala	32
Anuradhapura	187	Mannar	32
Badulla	65	Matale	61
Batticaloa	30	Matara	75
Colombo	25	Moneragala	141
Galle	86	Mullaitivu	23
Gampaha	25	NuwaraEliya	8
Hambantota	142	Polonnaruwa	56
Jaffna	23	Puttalam	28
Kalutara	31	Ratnapura	30
Kandy	25	Trincomalee	28
Kegalle	30	Vavuniya	30
Kilinochchi	25	**Total**	**1304**

Table 1.2: Number of well water samples collected in each Sri Lankan district for the analysis of arsenic, cadmium, lead, and chromium

District	**No. of samples**	**District**	**No. of samples**
Ampara	66	Kurunegala	32
Anuradhapura	228	Mannar	32
Badulla	65	Matale	151
Batticaloa	30	Matara	75
Colombo	25	Moneragala	141
Galle	86	Mullaitivu	23
Gampaha	25	Nuwara Eliya	8
Hambantota	142	Polonnaruwa	56
Jaffna	23	Puttalam	28
Kalutara	31	Ratnapura	30
Kandy	25	Trincomalee	28
Kegalle	30	Vavuniya	30
Kilinochchi	25	**Total**	**1435**

3. Results and Discussion

3.1. pH

The pH of land water usually ranges from 6.5 to 8.5, while the pH value in Sri Lankan well water ranged from 3.7 to 10.6. Figure 1.5 shows the frequency distribution of pH values in Sri Lankan well water. We found that 21.7% of well water contained a pH value lower than 7, and 78.2% of well water contained a pH value higher than 7. Figure 1.6 shows the spatial distribution of the pH of well water in Sri Lanka. It is obvious that most Sri Lankan well water has a basic condition, except for well water located in the Wet Zone. This is due to the dissolution of basic ions from the surface soil or underground soil to underground water, the source of well water. The number of well water with a pH value lower than 5 was 14, out of which 10 wells had a higher nitrate concentration than 10 mg/l. The lower pH could be attributed to the agricultural activity.

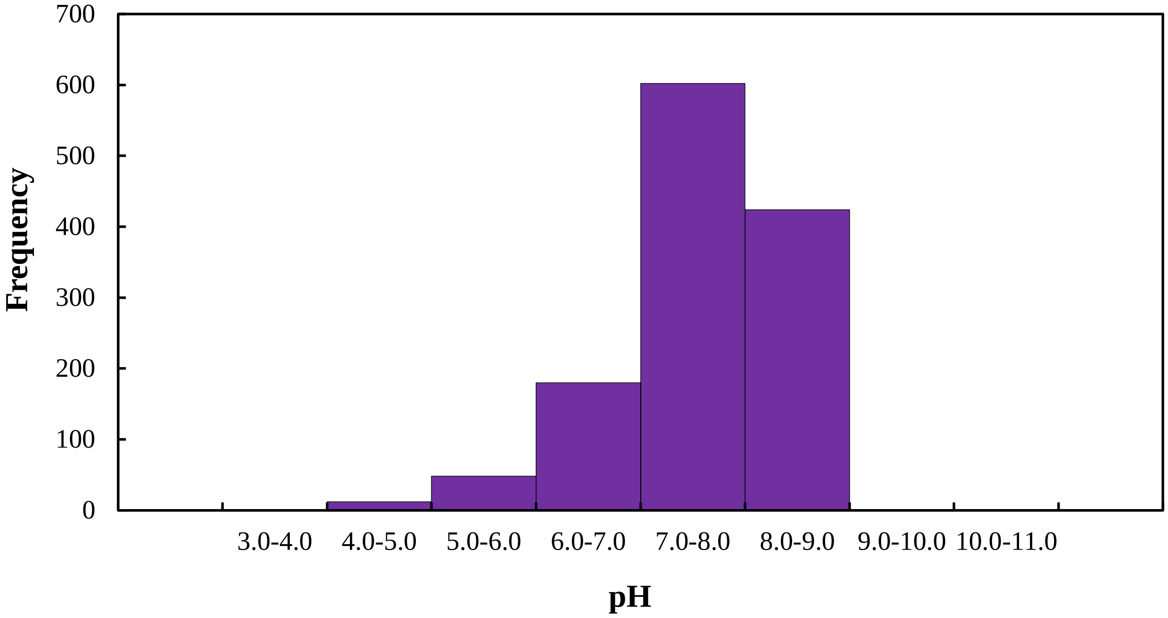


Fig. 1.5: Frequency distribution of pH values in Sri Lankan well water

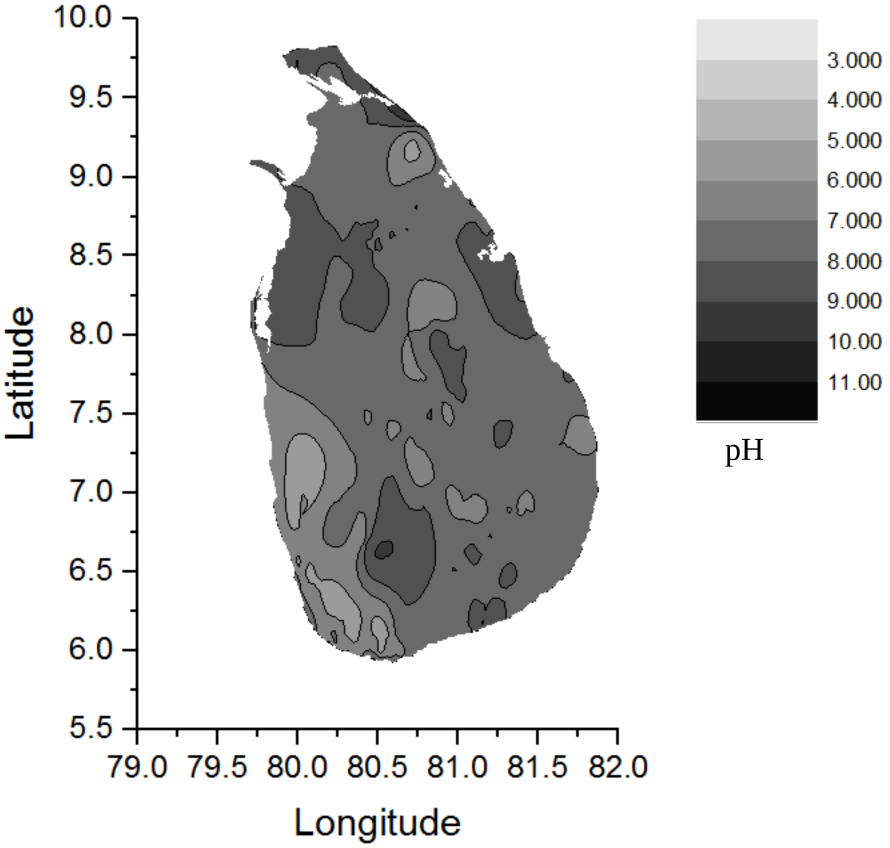


Fig. 1.6: Spatial distribution of pH values in Sri Lankan well water

3.2. Fluoride

Fluoride is an essential element for human life. This element causes the mineralization processes of bones, and teeth, cementing crystals to provide more strength, and hardness (Singh et al. 2001). An excess amount of fluoride intake causes human health hazards such as dental fluorosis, skeleton fluorosis (Yadav et al. 2006), increased rates of urolithiasis (kidney stones), impaired thyroid function, and lower intelligence in children (Loganathan et al. 2013). Fluoride is mainly ingested into the human body through drinking water (Loganathan et al. 2013). The WHO guideline for fluoride in drinking water is less than 1.5 mg/l (WHO 2011), while the Sri Lankan standard for fluoride in drinking water is less than 1.0 mg/l (Sri Lankan Standard Institute 2013). This is because the amount of intake water is larger in Sri Lanka than that in the moderate countries due to the tropical climate.

The maximum, and average fluoride concentrations in the well water of each district, and the standard deviation are summarized in Table 1.3. The highest concentration of fluoride, 7.0 mg/l, was recorded in the Anuradhapura District, followed by 6.8 mg/l in the Moneragala District. The samples from Anuradhapura, and Moneragala exceeded the average fluoride concentration of 1.0 mg/l. High values in the standard deviation represent the high variation of fluoride concentrations of each well, even among wells within the same district. The fluoride concentration of well water in Sri Lanka ranged from 0 to 7.0 mg/l. Eighty percent of wells contained a fluoride concentration of 0–1.0 mg/l, 10.1% of wells contained 1.0–1.5 mg/l of fluoride, and 9.9% contained a fluoride concentration of more than 1.5 mg/l. Figure 1.7 shows the frequency distribution of fluoride concentrations in Sri Lankan well water. Figure 1.8 shows the spatial distribution of fluoride in well water. High concentration of fluoride was found in the Dry Zone of Sri Lanka. Dental fluorosis, skeleton fluorosis, and CKDu are highly prevalent in the Dry Zone of Sri Lanka. Dental fluorosis, and skeleton fluorosis are directly affected by the high concentration of fluoride. 90% of 15 years old school children was reported to suffer from dental fluorosis in the Anuradhapura District belongs to the Dry Zone of Sri Lanka (Tennakoon 2004). Further, fluoride was supposed to be a suspicious cause for CKDu (Nanayakkara et al. 2012).

Table 1.3: Fluoride concentrations in the well water of each Sri Lankan district

District	Maximum Value (mg/l)	Average Value (mg/l)	Standard Deviation (mg/l)	District	Maximum Value (mg/l)	Average Value (mg/l)	Standard Deviation (mg/l)
Ampara	5.8	0.80	1.00	Kurunegala	5.0	0.70	1.20
Anuradhapura	7.0	1.10	0.90	Mannar	1.2	0.40	0.40
Badulla	2.3	0.60	0.50	Matale	2.6	0.40	0.60
Batticaloa	0.7	0.20	0.20	Matara	0.6	0.10	0.10
Colombo	0.1	0.00	0.00	Moneragala	6.8	1.40	1.30
Galle	5.8	0.10	0.60	Mullaitivu	0.4	0.10	0.10
Gampaha	0.1	0.00	0.00	Nuwara Eliya	0.1	0.04	0.03
Hambantota	2.3	0.60	0.40	Polonnaruwa	3.4	0.80	0.70
Jaffna	1.3	0.20	0.20	Puttalam	2.2	0.30	0.40
Kalutara	0.7	0.10	0.10	Ratnapura	0.2	0.10	0.10
Kandy	0.4	0.20	0.10	Trincomalee	2.4	0.80	0.70
Kegalle	0.3	0.10	0.10	Vavuniya	3.1	0.80	0.60
Kilinochchi	1.3	0.20	0.30				

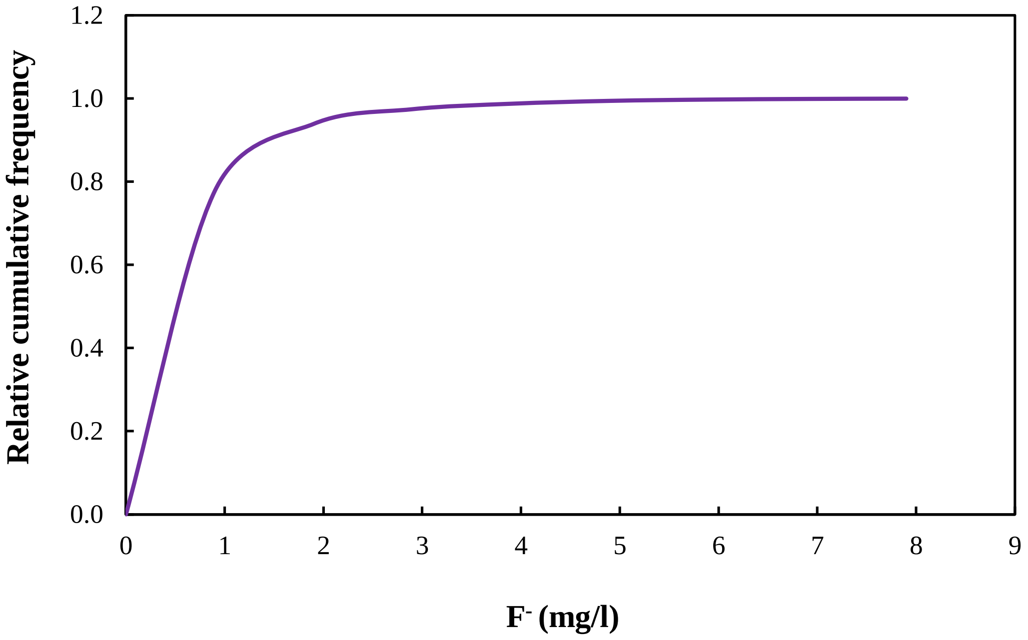


Fig. 1.7: Frequency distribution of fluoride concentrations in Sri Lankan well water

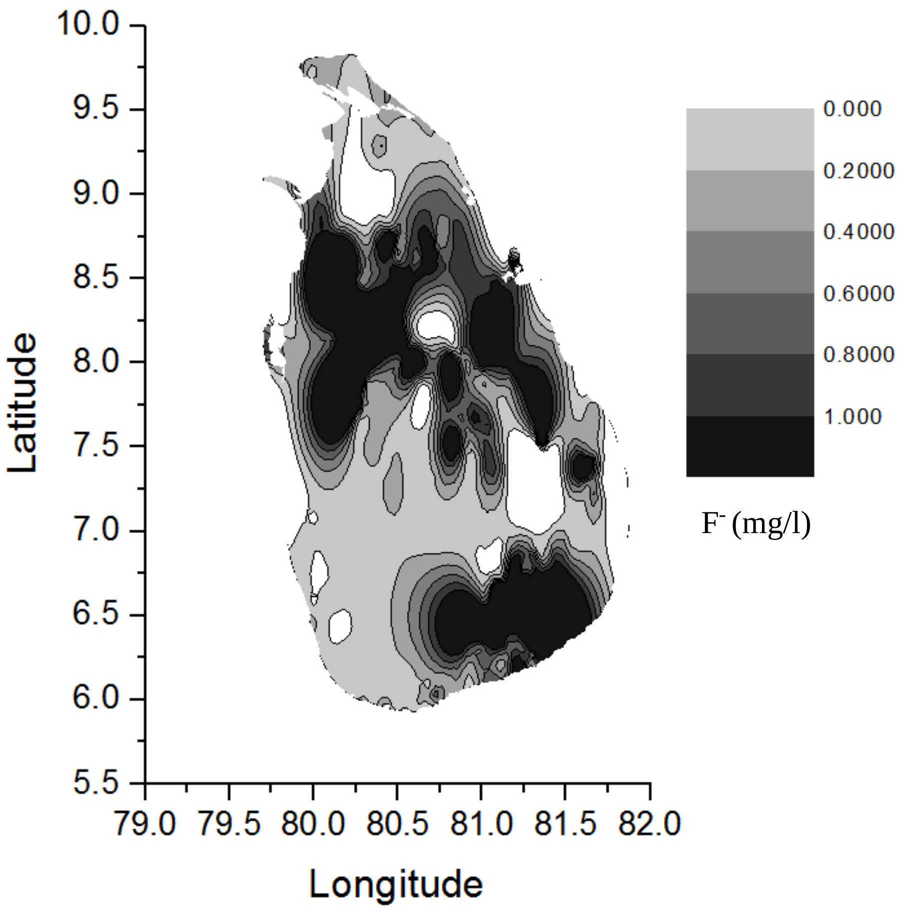


Fig. 1.8: Spatial distribution of fluoride concentrations in Sri Lankan well water

3.3. Nitrate

The WHO guideline for nitrate in drinking water is 50 mg/l (WHO 2011). An excess amount of nitrate, more than 50 mg/l, causes blue baby syndrome in infants (Gutierrez et al. 2014). Nitrate is reduced into nitrite in the stomach by nitrate-reducing bacteria. Nitrite binds with hemoglobin to produce methemoglobin, which obstructs oxygen transportation; hence, the baby falls into cyanosis (Mary et al. 1951). A nitrate concentration of more than 50 mg/l in groundwater also causes health issues such as cancer (Smith and Kellman 2011), cardiac defects (Cedergren et al. 2002), acute and chronic neurotoxicity, lung damage, chemical burns, immunological abnormalities, adverse reproductive, and developmental effects (Weisenburger 1993), adverse pregnancy outcomes, diabetes, thyroid disorders (Ward 2011). Nitrate is usually detected in water sources when groundwater is contaminated by nitrogenous fertilizer (Fan 2011) or by septic tank effluents (Rios et al. 2013).

The Sri Lankan standard for nitrate in drinking water is less than 50 mg/l (Sri Lankan Standard Institute 2013), and the nitrate concentrations in Sri Lankan well water ranged from 0 to 366 mg/l. Figure 1.9 shows the frequency distribution of nitrate concentrations in Sri Lankan well water. Nitrate concentrations higher than 50 mg/l were found in 2.2% of the sampled wells. The maximum value, the average value, and the standard deviation for nitrate concentrations in the well water of each district are shown in Table 1.4. Water in 13 out of 25 districts had a maximum nitrate concentration of more than 50 mg/l. The highest concentration of nitrate, 366 mg/l, was found in the Puttalam District. High concentrations of nitrate were also found in Mannar, Anuradhapura, Hambantota, Jaffna, Vavuniya. Figure 1.10 shows the spatial distribution of nitrate in Sri Lanka. The Puttalam, Jaffna, and Mannar Districts belong to the coastal zone of Sri Lanka, which consists of sandy soil (Survey Department 1967). Wells that showed a high concentration of nitrate in these areas were located on the soil type “sandy regosols on recent beach, and dune sands” (Survey Department 1967). Figure 1.11 shows the soil map of Sri Lanka (Based upon the maps of the Survey Department of Sri Lanka with the sanction of the Survey General). Agricultural activities on sandy soil may be a reason for the high contamination of nitrate in well water in the above districts, while the high concentration of nitrate found in Vavuniya could be from septic tank, since there are no dense agricultural activities in this area.

In a similar study done by C.B. Dissanayake, and S.V.R. Weerasooriya in 1985, the highest nitrate concentration in groundwater was recorded in Jaffna. This was due to the abundance of nitrogenous waste matter in the form of human excreta, synthetic and animal fertilizer reaching the shallow groundwater table (Dissanayake and Weerasooriya 1985).

In another detailed study about the groundwater quality in Jaffna performed by Gunasekeram in 1983 mentioned that 80 % of well waters were contaminated with fecal coliform, and he highlighted human excreta as the major factors responsible for the nitrate contamination in Jaffna. He also pointed out that excessive amount of urea was used on crop cultivations such as chili, onion, and further cattle manure was commonly used in cultivation purposes (Gunasekeram 1983). Sutharsiny et al. pointed out that there was a high risk of blue baby syndrome in Jaffna peninsula (Sutharsiny et al. 2014).

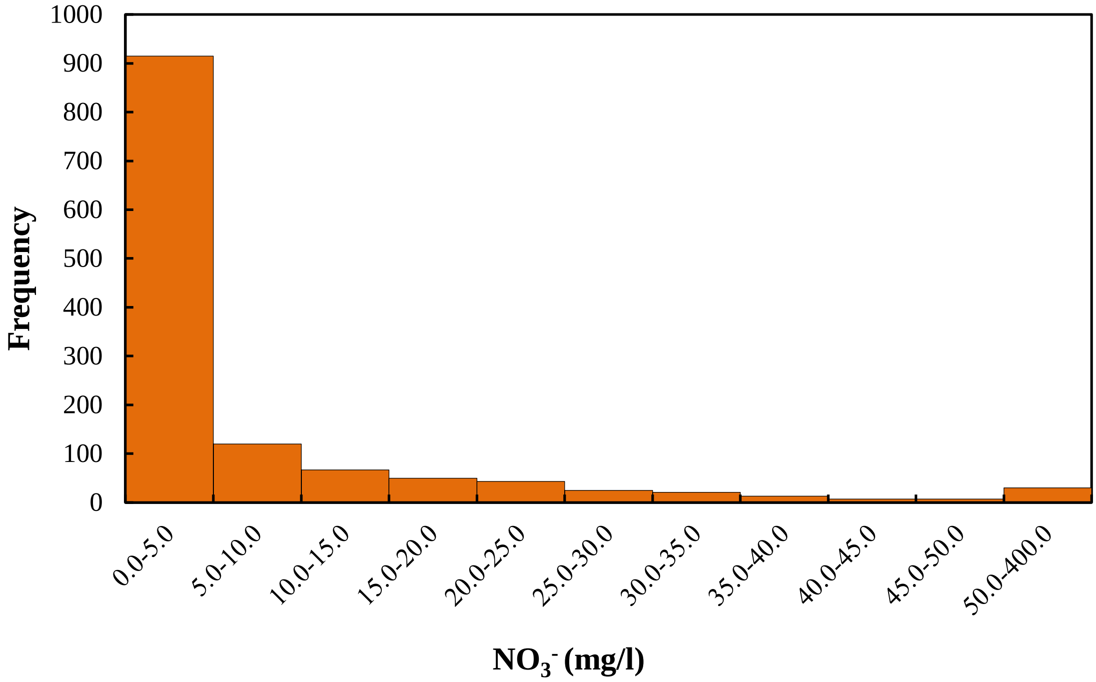


Fig. 1.9: Frequency distribution of nitrate concentrations in Sri Lankan well water

Table 1.4: Nitrate concentrations in the well water of each Sri Lankan district

District	Maximum Value (mg/l)	Average Value (mg/l)	Standard Deviation (mg/l)	District	Maximum Value (mg/l)	Average Value (mg/l)	Standard Deviation (mg/l)
Ampara	33	3	7	Kurunegala	22	2	5
Anuradhapura	131	6	15	Mannar	135	15	30
Badulla	47	4	8	Matale	53	8	13
Batticaloa	44	8	13	Matara	53	9	12
Colombo	37	13	10	Moneragala	82	4	10
Galle	37	8	8	Mullaitivu	51	12	15
Gampaha	58	18	14	Nuwara Eliya	10	5	4
Hambantota	128	2	13	Polonnaruwa	19	1	3
Jaffna	110	19	26	Puttalam	366	42	90
Kalutara	19	7	5	Ratnapura	28	11	9
Kandy	18	5	5	Trincomalee	79	7	16
Kegalle	35	5	8	Vavuniya	102	27	29
Kilinochchi	55	13	15				

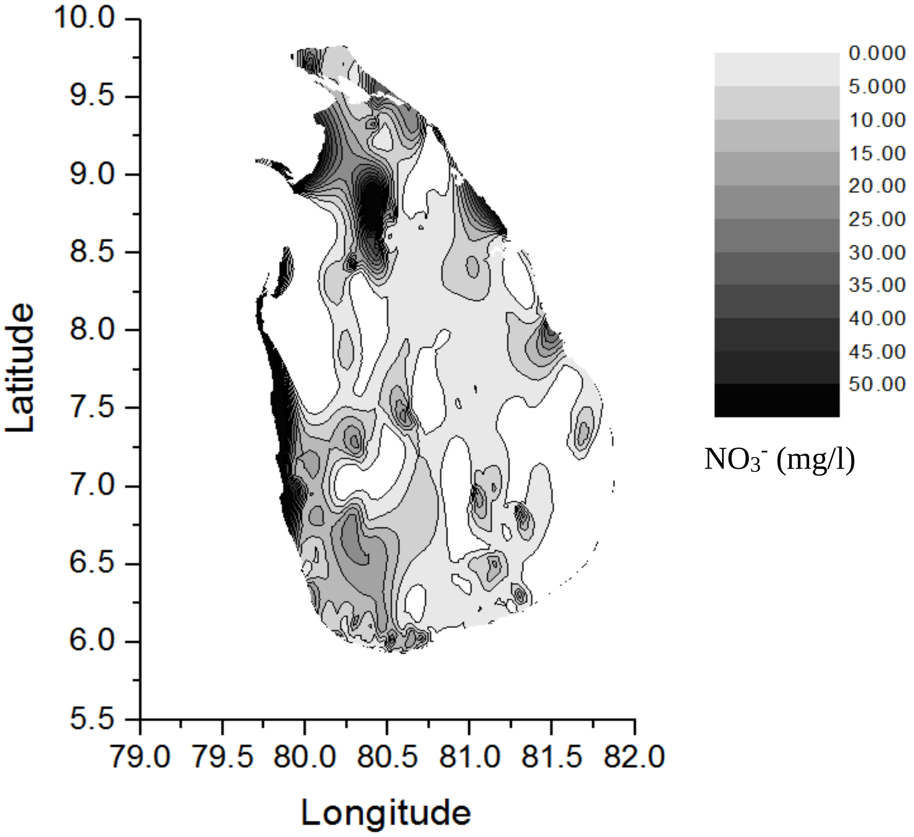
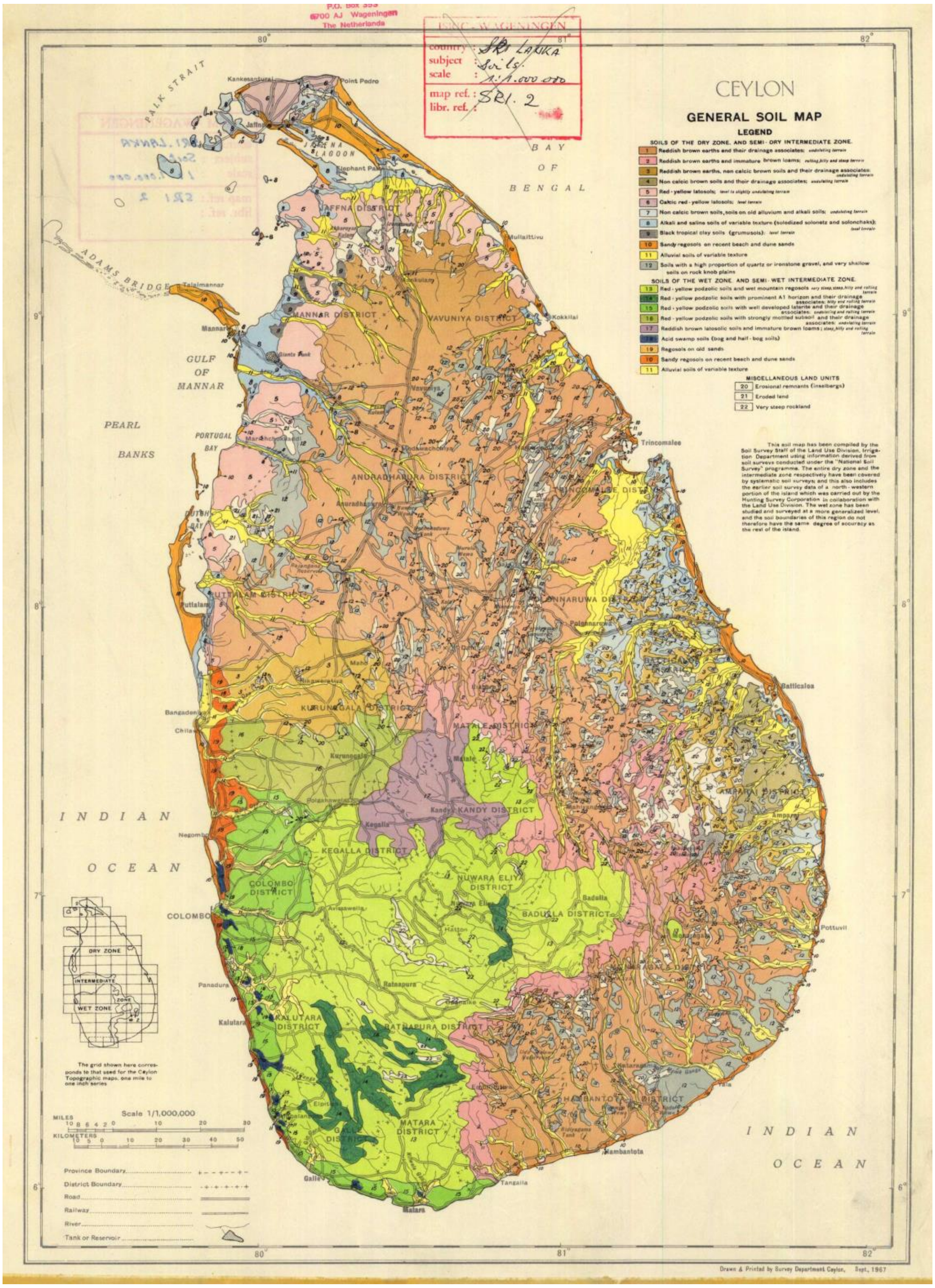


Fig. 1.10: Spatial distribution of nitrate concentrations in Sri Lankan well water



This soil map has been compiled by the Soil Survey Staff of the Land Use Division, Irrigation Department using information derived from soil surveys conducted under the "National Soil Survey" programme. The entire dry zone and the intermediate zone respectively have been covered by systematic soil surveys; and this also includes the earlier soil survey data of a north - western portion of the island which was carried out by the Hunting Survey Corporation in collaboration with the Land Use Division. The wet zone has been studied and surveyed at a more generalized level, and the soil boundaries of this region do not therefore have the same degree of accuracy as the rest of the island.

The grid shown here corresponds to that used for the Ceylon Topographic maps, one mile to one inch series

Drawn & Printed by Survey Department Ceylon, Sept. 1967

Fig. 1.11: Soil map of Sri Lanka

*Based upon the maps of the Survey Department of Sri Lanka with the sanction of the Survey General

3.4. Hardness

Permanent hardness occurs due to the dissolution of magnesium, and calcium salts in water. Hardness causes scale on cooking utensils, which decreases the efficiency of heat transfer (Ras and Ghizellaoui 2012). It was reported that no relationship was found between water hardness, and occurrence of cardiovascular diseases, and cancers (Lou et al. 2007), while another report mentioned that magnesium, and calcium ions, which cause water hardness, were found to cause cardiovascular diseases and health defects in humans (Lerga and O'Sullivan 2008). A study conducted in Osaka Prefecture, Japan, with 44 municipalities, concluded that water hardness may increase the risk of atopic dermatitis among elementary school children in Japan (Miyake et al. 2004). At the same time, hardness causes bad taste due to the bitterness that comes from magnesium (Hashimoto et al. 1987). It has been believed that hardness does not directly affect human health (Verissimo et al. 2007), however suspected the potential risk of CKDu by hardness. The degree of hardness is categorized as shown in Table 1.5 (Verissimo et al. 2007). Ion concentrations of Ca, and Mg in mg/l are converted to express hardness as an equivalent of $CaCO_3$ mg/l by using the following equation.

$$\mathbf{CaCO_3 = (Ca/40.1+Mg/24.3) * 100\ (mg/l)}$$

Table 1.5: Categories of hardness

Category of Hardness	**Range of Value ($CaCO_3$ mg/l)**
Soft	$CaCO_3$< 60 mg/l
Moderately hard	60 mg/l<= $CaCO_3$ < 120 mg/l
Hard	120 mg/l<= $CaCO_3$ < 180 mg/l
Very hard	180 mg/l<= $CaCO_3$

Figure 1.12 shows the frequency distribution of hardness in Sri Lankan well water. According to the analysis, 26.9% of well water was soft, 15.0% was moderately hard, 15.8% was hard, and 42.2% was very hard. Generally, we can conclude that Sri Lankan well water is very hard. The water hardness in each Sri Lankan district is represented in Table 1.6. The highest recorded value of hardness in Sri Lanka was in the Hambantota District. High values were also recorded in Anuradhapura, and Jaffna. When we consider the maximum value of hardness, the well water was very hard in all but except 5 out of

25 districts. When we consider the average values of hardness, a hardness concentration below 60 mg/l was shown only in well water in Colombo, Galle, Gampaha, Kalutara, and Nuwara Eliya, which belong to the Wet Zone of Sri Lanka.

Figure 1.13 shows the spatial distribution of hardness of Sri Lankan well water. It is clear that districts in the Dry Zone had high concentrations of hardness, and districts in the Wet Zone had considerably lower hardness concentrations. In the Dry Zone, due to limited rainfall and high temperatures, excessive evaporation is taking place, and the accumulation of salts in the soil is occurring, leading to high hardness concentrations in groundwater. Since CKDu is highly prevalence in the Dry Zone of Sri Lanka, our result coincided with the research by Jayasumana et al. indicating that hardness was supposed to be one of the suspicious causes for CKDu (Jayasumana et al. 2014).

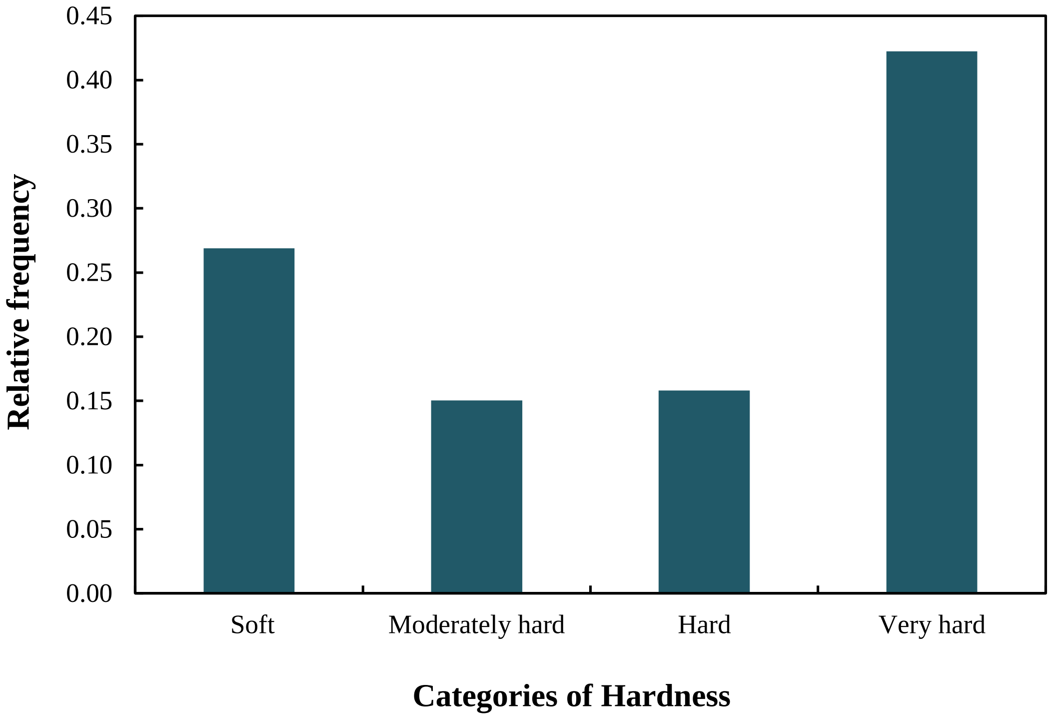


Fig. 1.12: Frequency distribution of hardness in Sri Lankan well water

Table 1.6: Hardness concentrations in the well water of each Sri Lankan district

District	Maximum ($CaCO_3$ mg/l)	Average ($CaCO_3$ mg/l)	Standard Deviation ($CaCO_3$ mg/l)	District	Maximum ($CaCO_3$ mg/l)	Average ($CaCO_3$mg/l)	Standard Deviation ($CaCO_3$ mg/l)
Ampara	362	135	78	Kurunegala	423	169	101
Anuradhapura	1373	283	158	Mannar	647	280	141
Badulla	495	140	107	Matale	494	120	96
Batticaloa	319	163	77	Matara	341	80	87
Colombo	132	31	31	Moneragala	891	244	159
Galle	232	34	37	Mullaitivu	619	172	140
Gampaha	110	20	24	Nuwara Eliya	149	36	50
Hambantota	1734	293	246	Polonnaruwa	980	231	192
Jaffna	1080	354	190	Puttalam	749	329	174
Kalutara	317	38	65	Ratnapura	174	63	52
Kandy	400	144	88	Trincomalee	581	254	137
Kegalle	166	69	51	Vavuniya	665	333	160
Kilinochchi	615	236	137				

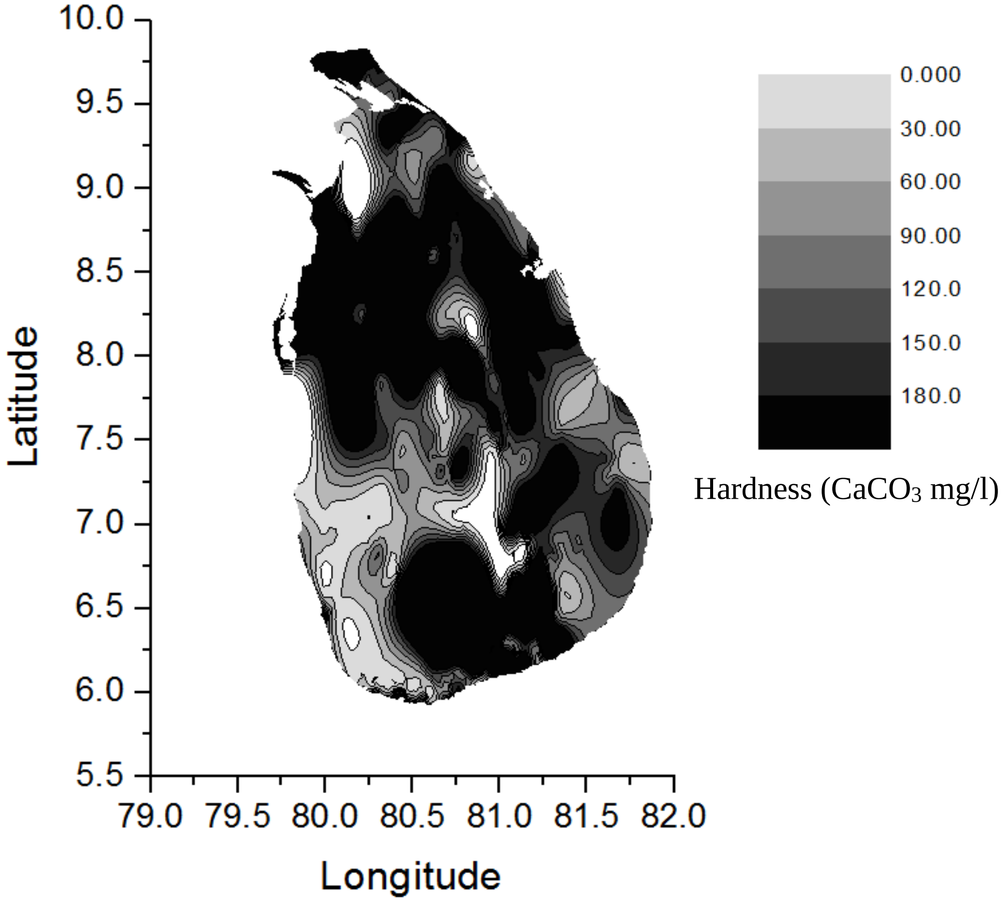


Fig. 1.13: Spatial distribution of hardness in Sri Lankan well water

3.5. Metals

3.5.1. Aluminum

Aluminum is the third most abundant element in the earth's crust (Zawilla et al. 2014; Gupta 2007). It is a light weight metal, and could be toxic to humans (Kumar and Gill 2014), causing various health defects or death, because it crosses the blood–brain barrier, and the placental barrier and produces neurotoxicity, and development toxicity (Gupta 2007). Water contaminated with a high concentration of aluminum may cause cognitive decline, and dementia. It also affects normal bone metabolism (Aslam et al. 2009), impairs mitochondrial bioenergetics, and may lead to the generation of oxidative stress (Kumar and Gill 2014) as well as several brain diseases, including dialysis encephalopathy, amyotrophic lateral sclerosis, Parkinson's disease dementia, Alzheimer's disease, and multiple sclerosis. It can also lead to cancers such as those of the bladder, lung, kidney, pancreas, and brain, and it increases the risk of leukemia (Crisponi et al. 2012). Other health defects resulting from exposure to aluminum include microcytic anemia, metastatic extra skeletal calcification (Savory et al. 1985), and effects on the immune function of humans (Zhu et al. 2014).

The WHO guideline for aluminum in drinking water is 200 µg/l which sets considering the taste, odour, and appearance (WHO 2011). The Sri Lankan standard for aluminum is the same as the WHO guideline, and also sets considering the taste, odour, and appearance (Sri Lankan Standard Institute 2013). Aluminum concentration in Sri Lankan well water ranged from 0 to 1457 µg/l. We found that 2.3% of well water samples had an aluminum concentration of more than 200 µg/l. The maximum value, and the average value of aluminum concentration obtained from each district are summarized in Table 1.7. The maximum aluminum concentration value was found in Moneragala; Mannar, Matara, and Kilinochchi also had high values of aluminum. In all but 11 districts, the maximum aluminum concentration exceeded the Sri Lankan standard as well as WHO guideline of 200 µg/l. No district reported an average aluminum concentration of more than 200 µg/l.

Figure 1.14 shows the spatial distribution of aluminum in well water. According to the graphical software although the spatial distribution map shows dense areas of high concentration of aluminum in the northern, and southern part of the Sri Lankan map, only few sampling locations are there with high concentration of aluminum. When comparing the pH values of well water having pH value of less than 6.0, and the aluminum concentrations of well water as indicated in Figure 1.15, it is clear that the aluminum concentration sharply decreased with increased pH. Further, the relationship between aluminum concentrations, and the nitrate concentrations in well water were studied since low pH accounted to high nitrate concentrations in well water. Figure 1.16 shows the relationship between aluminum concentrations, and the nitrate concentrations in Sri Lankan well water. There was no relationship between aluminum concentrations, and the nitrate concentrations in well water.

Table 1.7: Aluminum concentrations in the well water of each Sri Lankan district

District	Maximum Value (μg/l)	Average Value (μg/l)	District	Maximum Value (μg/l)	Average Value (μg/l)
Ampara	765	48	Kurunegala	559	19
Anuradhapura	135	5	Mannar	1364	46
Badulla	117	7	Matale	89	5
Batticaloa	252	19	Matara	1233	43
Colombo	567	47	Moneragala	1457	25
Galle	815	43	Mullaitivu	284	39
Gampaha	151	34	Nuwara Eliya	275	58
Hambantota	604	10	Polonnaruwa	57	3
Jaffna	10	0	Puttalam	86	11
Kalutara	434	33	Ratnapura	297	17
Kandy	18	3	Trincomalee	25	3
Kegalle	84	7	Vavuniya	40	4
Kilinochchi	966	43			

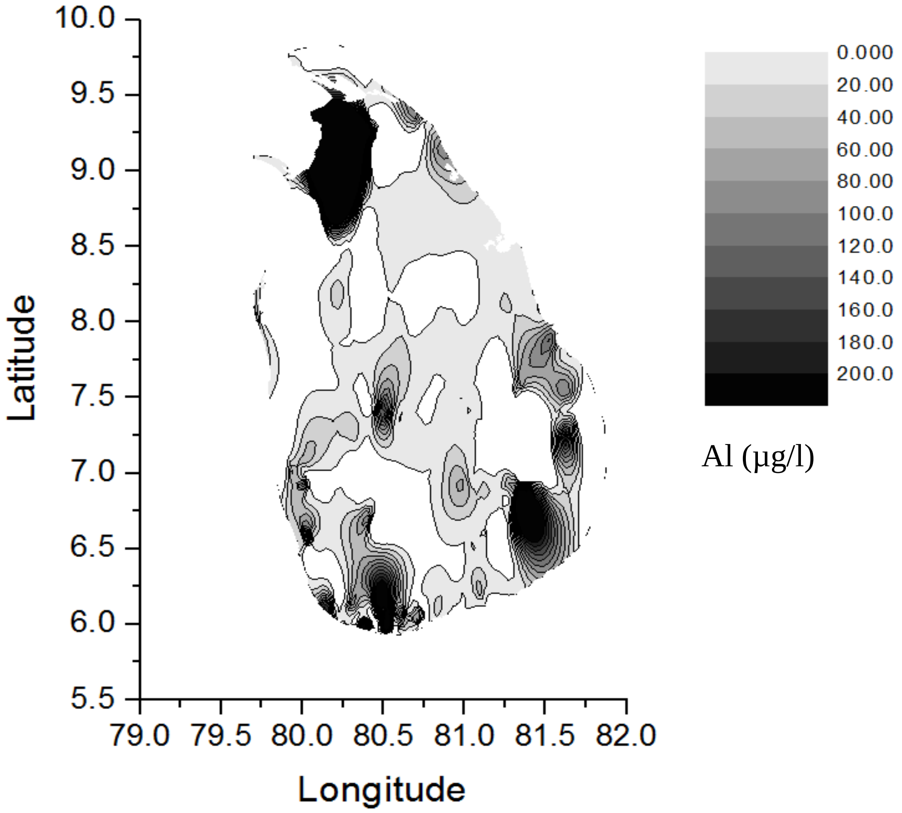


Fig. 1.14: Spatial distribution of aluminum concentrations in Sri Lankan well water

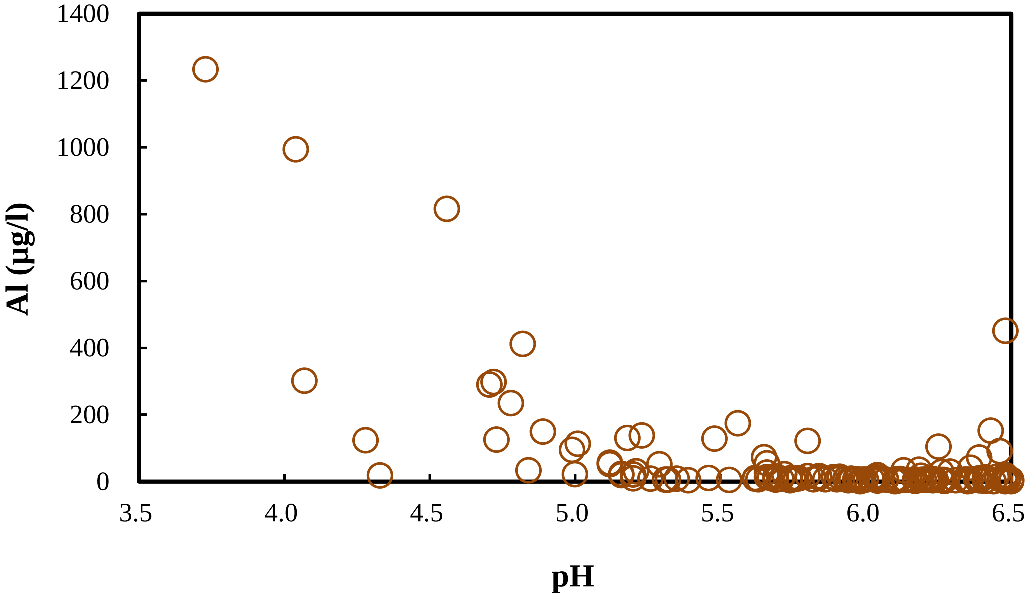


Fig. 1.15: Relationship between Al concentrations and pH in Sri Lankan well water

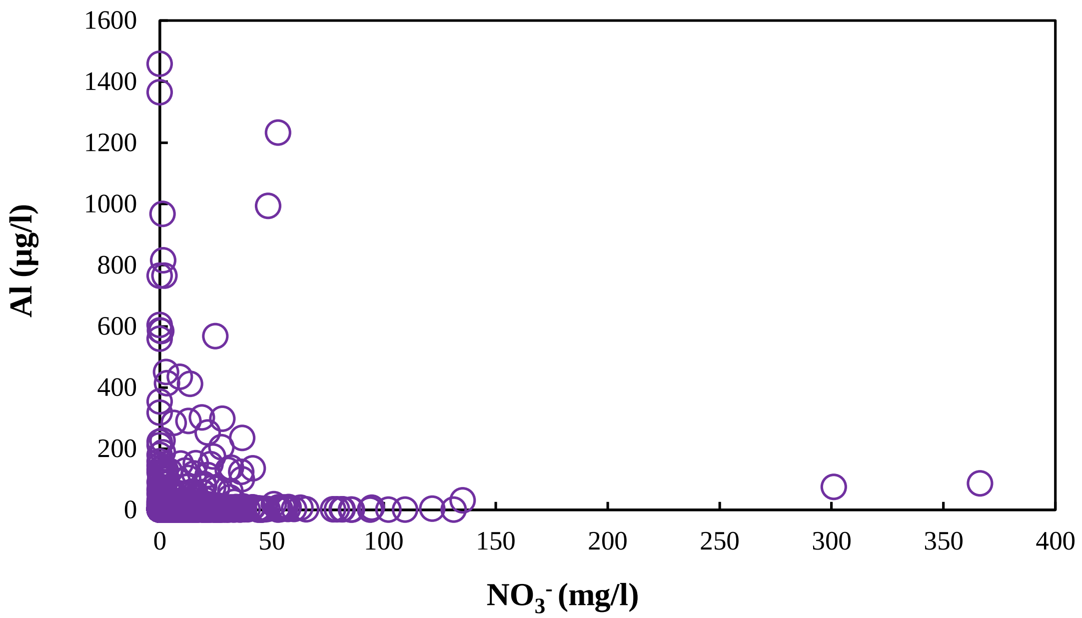


Fig. 1.16: Relationship between Al concentrations and NO_3^- concentrations in Sri Lankan well water

3.5.2. Manganese

Manganese is an element essential to humans. Manganese is naturally ubiquitous in the environment, and can be found in many types of rocks, soil, water, air, and food (Rollin 2011). However, excessive exposure to manganese cause toxicity (Yazbeck 2011), and health defects. Toxic levels of manganese are experienced through occupational exposure, and through exposure to contaminated groundwater. Such exposure causes human health defects at the acute level, chronic level, and sub-clinical level (Hess et al. 2015). One of the harmful effects of manganese is neurotoxic damage (Viana et al. 2014), because the half-life of manganese in the human brain is longer than that in other organs; hence, manganese is selectively retained in the brain, and causes central nervous system disorders, myasthenia, and manganese-induced Parkinsonism. Manganese also causes breathing discomfort, coughing, psychiatric symptoms (Yoon and Ahn 2014), and neuropsychiatric symptoms associated with the risk of alcohol-use disorders (Sassine et al. 2002). It affects reproductive, cardiovascular, hematological, endocrine, and immunological systems (Lucchini et al. 2015), as well as neonatal birth weight, head, and chest circumferences (Tsai et al. 2015), and night blindness in exposed children (Hess et al. 2015).

The Sri Lankan standard for manganese in drinking water is 100 µg/l (Sri Lankan Standard Institute 2013). The WHO guideline for manganese in drinking water is 400 µg/l which was set by considering taste, odour, and appearance (WHO 2011). The manganese concentration in Sri Lankan well water ranged from 0 to 9772 µg/l. Figure 1.17 shows the frequency distribution of manganese concentrations in Sri Lankan well water. We found that 14.1% of well water samples had manganese concentrations of more than 100 µg/l, and 3.6% of well water samples had manganese concentrations of more than 400 µg/l. The maximum, and average values obtained for manganese concentrations in each district are summarized in Table 1.8. A maximum manganese concentration value of 9772 µg/l was found in Polonnaruwa; manganese concentration values exceeding 1000 µg/l were recorded in Ampara, Kurunegala, Hambantota, Kandy, Matara, Anuradhapura, and Matale. The average manganese concentration values in samples from 5 districts exceeded the Sri Lankan standard of 100 µg/l, and those from 1 district exceeded the WHO standard of 400 µg/l.

Figure 1.18 shows the spatial distribution of manganese concentrations in well water. When we compared the analyzed manganese concentrations of well water with the Sri Lankan soil map (Figure 1.11), we could not identify any relationship between the manganese concentration, and the soil type.

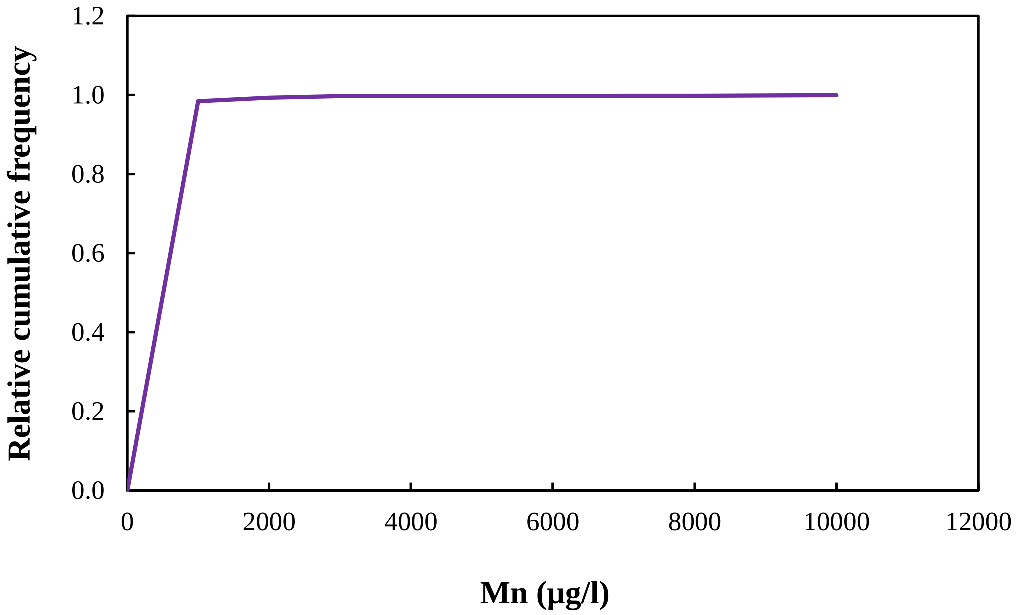


Fig. 1.17: Frequency distribution of manganese concentrations in Sri Lankan well water

Table 1.8: Manganese concentrations in the well water of each Sri Lankan district

District	**Maximum Value (μg/l)**	**Average Value (μg/l)**	**District**	**Maximum Value (μg/l)**	**Average Value (μg/l)**
Ampara	6068	192	Kurunegala	2479	219
Anuradhapura	1532	37	Mannar	353	56
Badulla	858	68	Matale	1058	58
Batticaloa	94	8	Matara	1796	80
Colombo	394	61	Moneragala	803	46
Galle	366	39	Mullaitivu	484	69
Gampaha	285	45	Nuwara Eliya	601	96
Hambantota	2346	144	Polonnaruwa	9772	440
Jaffna	216	20	Puttalam	79	19
Kalutara	148	31	Ratnapura	385	69
Kandy	2018	170	Trincomalee	219	15
Kegalle	349	38	Vavuniya	599	34
Kilinochchi	489	58			

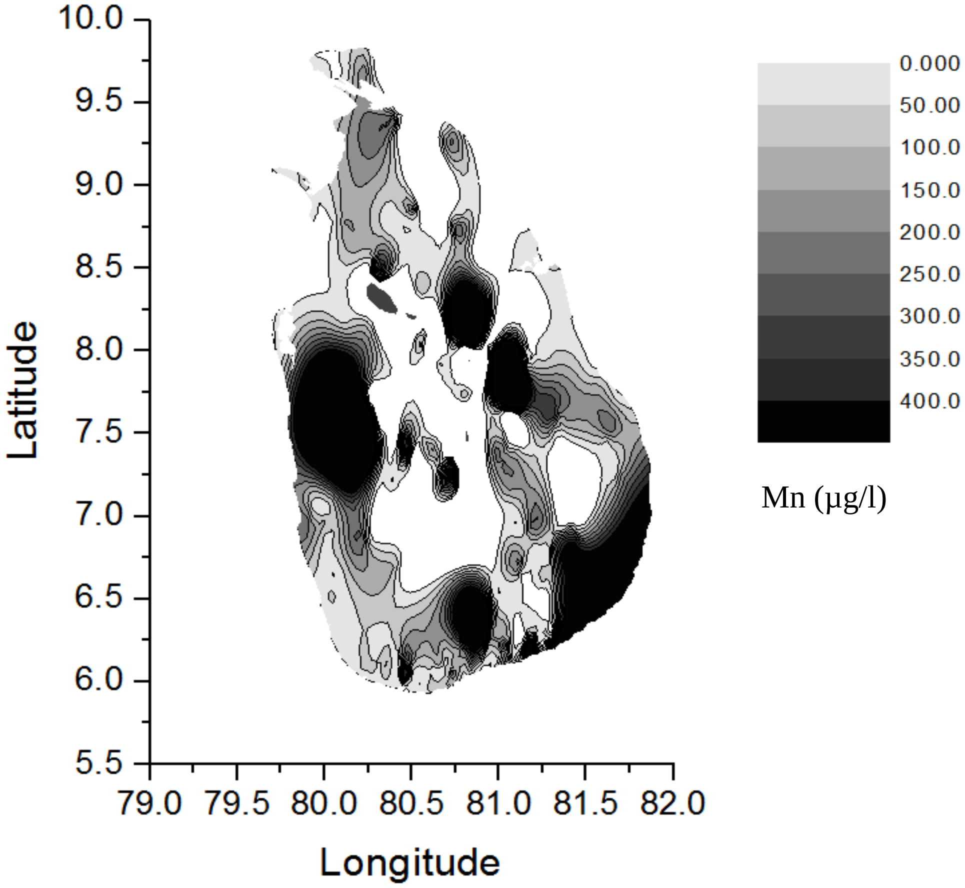


Fig. 1.18: Spatial distribution of manganese concentrations in Sri Lankan well water

3.5.3. Arsenic

Arsenic ranks as the 20th most abundant trace element which presents only in minute amounts in the earth's crust, 14th in sea water, and 12th in the human body (Singh et al. 2015). The primary source of arsenic in the environment is arsenic-enriched minerals (Singh et al. 2015), and a frequent source of human exposure to inorganic arsenic is through contaminated groundwater (Finley 2014). It has been reported that contamination of drinking water by arsenic has threaten to more than 150 million people all over the world (Singh et al. 2015).

Long-term exposure to arsenic causes various health effects on humans. Arsenic is a class 1 human carcinogen, which is identified as the second most important global health concern in groundwater contamination after the contamination by pathogenic organisms

(Chappells et al. 2015). In addition to cancer, arsenic causes birth-related risks such as lower birth weight, earlier delivery, smaller neonatal size in exposed mothers, and their fetuses (Bloom et al. 2014), and neural tube defects (Jin et al. 2013); it also causes defects in cardiovascular, integumentary, digestive, endocrine, and pulmonary systems in humans (Finley 2014). The WHO deemed that the contamination of groundwater by arsenic in Bangladesh is “the largest mass poisoning of a population in history” where the arsenic concentration in groundwater was reported to be 10–1000 µg/l (Singh et al. 2015).

According to Sri Lankan standards (Sri Lankan Standard Institute 2013), and World Health Organization (WHO) guidelines (WHO 2011), the maximum permissible level of arsenic in drinking water is 10 µg/l. Table 1.9 shows the arsenic concentration in the well water of each Sri Lankan district. The maximum, and average values, and the standard deviation of each district are given. Figure 1.19 shows the distribution of arsenic concentrations in Sri Lankan well water. The Mannar, Puttalam, Batticaloa, and Mullaitivu Districts reported maximum arsenic concentrations that are higher than the Sri Lankan standard, and the WHO guideline. Figures 1.20, 1.21, 1.22, and 1.23 respectively show the distribution of arsenic concentrations in Sri Lankan well water in Mullaitivu, Mannar, Puttalam, and Batticaloa Districts. Mannar District recorded the highest concentration, reaching 66 µg/l. These districts also exhibit high standard deviation values that show high variations in arsenic concentrations, even among wells within the same district. However, the average arsenic concentrations in all other districts were less than 10 µg/l.

As the incidence of CKDu has risen, it has been thought that arsenic is one cause of CKDu. Based on their analysis of 226 fertilizer samples collected from Padaviya, Medawachchiya, Mahawilachchiya, and Anuradhapura in the North Central Province of Sri Lanka, Jayasumana et al. (2015) found that phosphate fertilizers were a major source of inorganic arsenic in CKDu-endemic areas. Additionally, they reported that triple super phosphate contained the highest amounts of arsenic, and rock phosphate produced at Eppawala, a mining area in the North Central Province, contained the second highest levels of arsenic.

Jayasumana et al. (2013) reported that abnormal spotty pigmentation observed in the palms, and soles of CKDu patients in the Padavi Sri Pura Divisional Secretariat area of the Trincomalee District in the Eastern Province of Sri Lanka was due to chronic arsenic toxicity; thus, arsenic could be a cause of CKDu. Furthermore, the authors have reported that pesticides, and fertilizers used excessively in paddy farming are the most likely sources of arsenic in the study area. On the contrary, we have detected in our study that there was no high concentration of arsenic in the well water of CKDu-prevalent districts. High concentrations of arsenic that exceeded the Sri Lankan standard, and WHO guideline were detected in well water in areas other than CKDu-endemic areas; Mannar, Puttalam, Batticaloa, and Mullaitivu. In addition, Wasana et al. (2012) assessed the water quality of 60 samples from areas with high, and low prevalences of CKDu in Sri Lanka, and reported that the arsenic level in the water was well below the WHO guideline of 10 µg/l. Water quality analysis of 234 different sources of water from CKDu-endemic, and non-endemic areas of Sri Lanka conducted by the World Health Organization demonstrated that arsenic concentrations were borderline in just four samples; 9.9 µg/l, 10.2 µg/l, 10.5 µg/l, and 13.4 µg/l, in the endemic area (WHO 2012). These results coincide with our research, which shows low concentrations of arsenic in well water.

When we consider the soil map of Sri Lanka that categorizes soil into 21 types (Figure 1.11), it is clear that all of the wells with arsenic concentrations higher than 10 µg/l were located on the soil type of “sandy regosols on recent beach, and dune sands” (Survey Department 1967), indicating that the arsenic had a geological origin. In Sri Lanka, farmers use a lot of amount of fertilizer on the sandy soil due to low retention, causing nitrate contamination in well waters. Figure 1.24 shows the relationship between arsenic concentration, and nitrate concentration in Sri Lankan well water. It indicates that there is no relationship between them; hence, high concentration of arsenic did not come from nitrogenous fertilizer but from a geological reason.

Fortunately, no chronic diseases caused by arsenic have been reported in Sri Lanka so far, however; in several districts there are risks to occur adverse effects by arsenic in near future. Accordingly, we could conclude that arsenic in well water is not a cause of CKDu.

Table 1.9: Arsenic concentrations in the well water of each Sri Lankan district

District	Maximum Conc. (µg/l)	Average Conc. (µg/l)	Standard Deviation (µg/l)	District	Maximum Conc. (µg/l)	Average Conc. (µg/l)	Standard Deviation (µg/l)
Ampara	2	1	0.5	Kurunegala	1	0	0.1
Anuradhapura	3	0	0.4	Mannar	66	7	11.7
Badulla	1	0	0.3	Matale	2	0	0.3
Batticaloa	14	3	3.2	Matara	4	0	0.6
Colombo	0	0	0.1	Moneragala	2	0	0.4
Galle	0	0	0.1	Mullaitivu	13	3	3.7
Gampaha	0	0	0.1	Nuwara Eliya	0	0	0.0
Hambantota	3	1	0.6	Polonnaruwa	3	0	0.5
Jaffna	6	2	1.7	Puttalam	15	4	4.1
Kalutara	3	0	0.6	Ratnapura	0	0	0.1
Kandy	0	0	0.1	Trincomalee	9	1	1.7
Kegalle	0	0	0.1	Vavuniya	2	1	0.6
Kilinochchi	2	1	0.6				

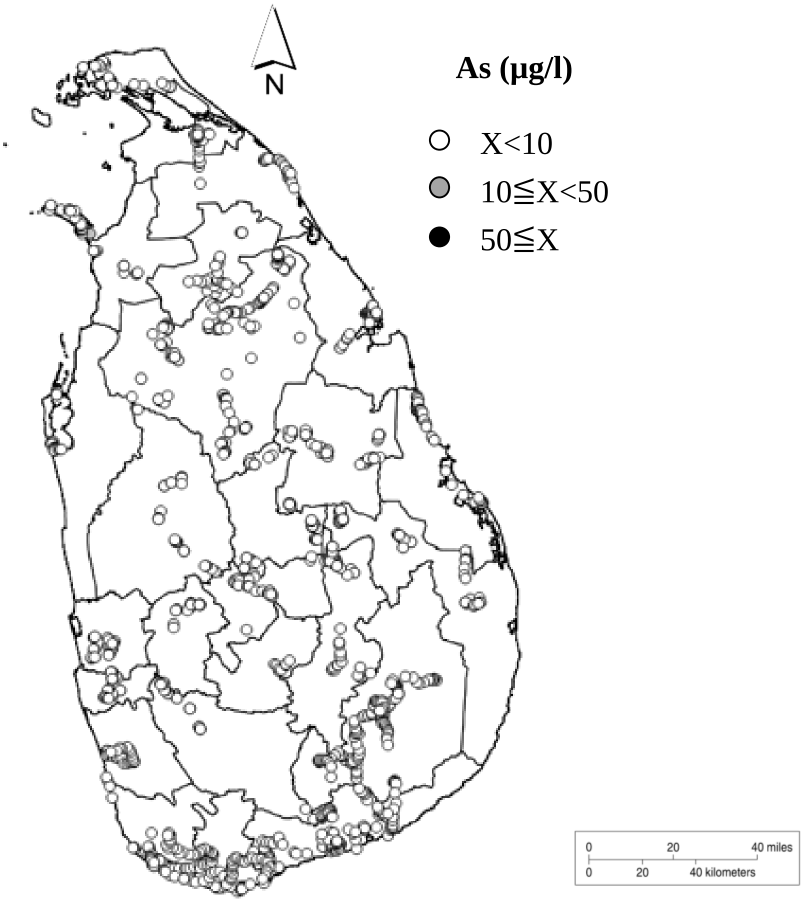


Fig. 1.19: Distribution of arsenic concentrations in Sri Lankan well water

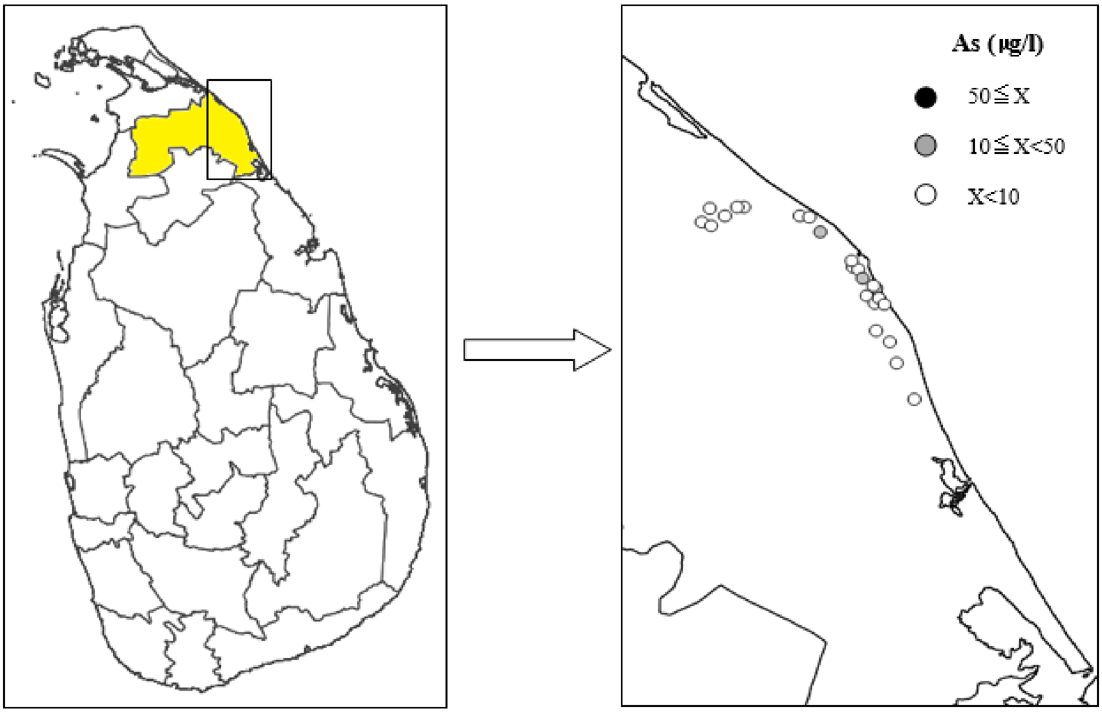


Fig.1.20: Distribution of arsenic concentrations in well water in Mullaitivu District in Sri Lanka

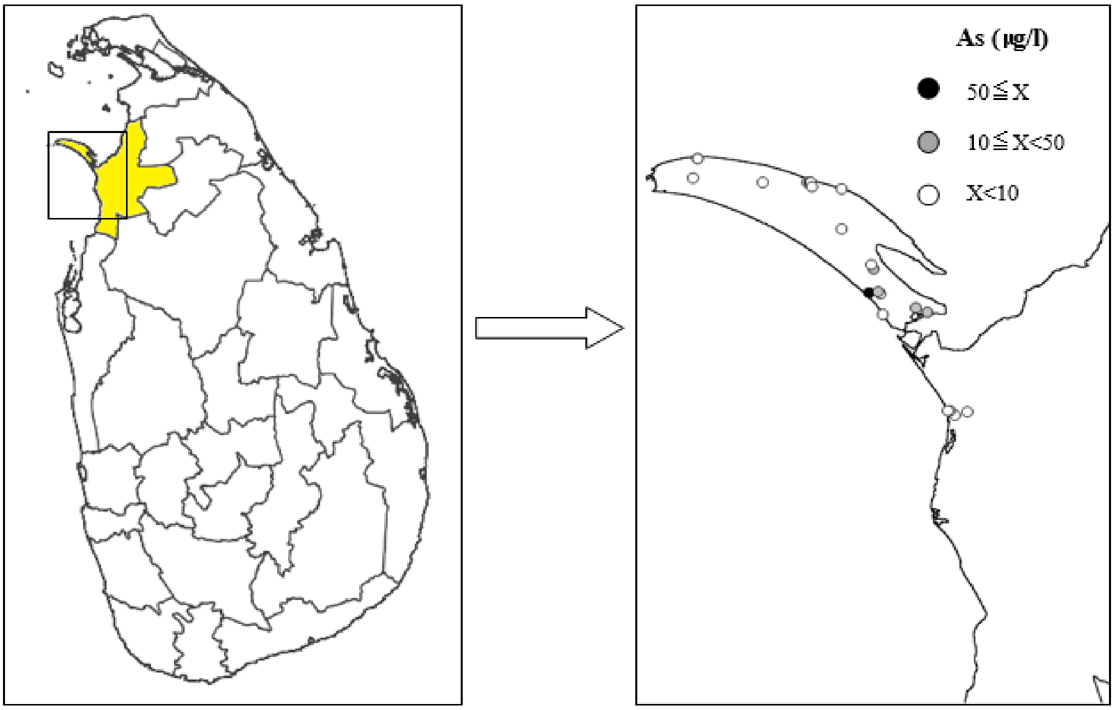


Fig.1.21: Distribution of arsenic concentrations in well water in Mannar district in Sri Lanka

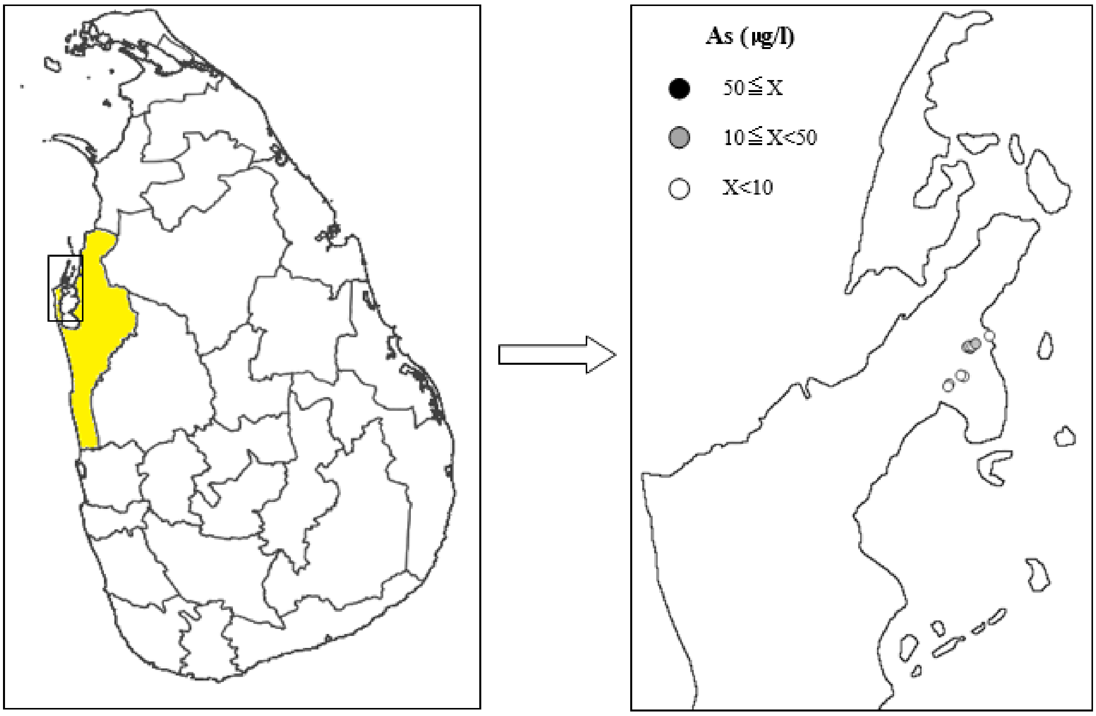


Fig.1.22: Distribution of arsenic concentrations in well water in Puttalam district in Sri Lanka

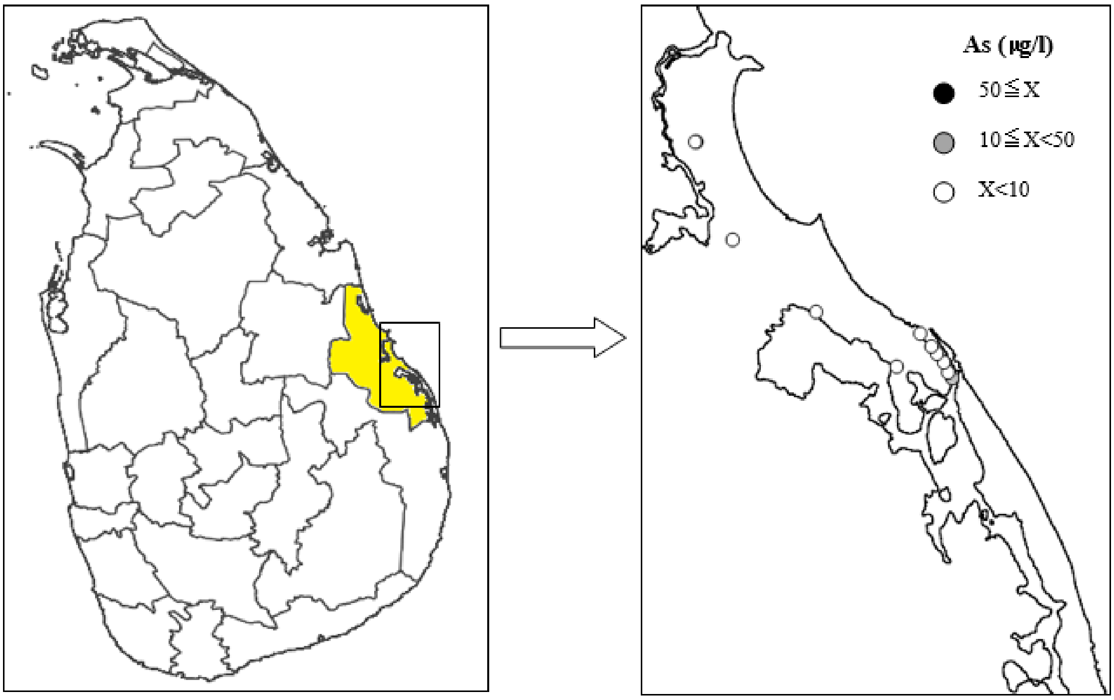


Fig.1.23: Distribution of arsenic concentrations in well water in Batticaloa district in Sri Lanka

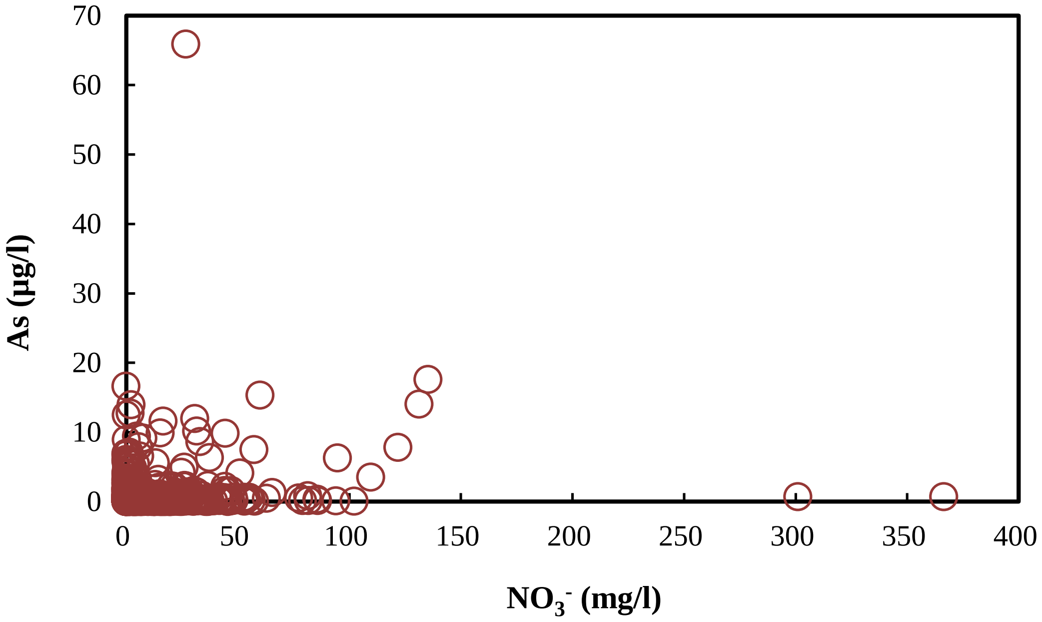


Fig. 1.24: Relationship between arsenic concentration and nitrate concentration in Sri Lankan well water

3.5.4. Cadmium

Cadmium is a non-nutritive (Roberts 2014), toxic, heavy metal (Averbeck and Bertin 2006) which occurs naturally in earth's crust and oceans. Collectively it is added to soil through natural sources as well as anthropogenic sources. As natural sources we can define forest fire, volcanic eruption, weathering of cadmium-containing phosphate rocks, soil erosion, and air pollution. As anthropogenic sources mining, and smelting of zinc-bearing ores, fossil fuel combustion, fertilizers derived from phosphate rocks, waste incineration, sewage sludge, irrigation waters can be defined (Roberts 2014). Cadmium can be easily contaminated groundwater (Rehman et al. 2015), and air (Averbeck and Bertin 2006). Smoking is also a possible source of cadmium exposure since tobacco leaves naturally accumulate high levels of cadmium (Roberts 2014). Toman, and his researchers reported that some dairy, and meat products are also acting as sources of cadmium (Toman et al. 2005). International Agency for Research on Cancer has listed cadmium as a Group 1 human carcinogen, and World Health Organization for smoke delivery reporting and recommended for regulatory policy in a subsequent report has selected cadmium as a priority toxicant (Piade et al. 2015). In the aquatic system it is one of the most abundant toxins (Tawwab and Wafeek 2014).

The Sri Lankan standard for cadmium in drinking water is 3 µg/l (Sri Lankan Standard Institute 2013), which is similar to the WHO guideline for cadmium in drinking water (WHO 2011). Cadmium causes adverse health effects in humans due to its long half-life of 15–20 years in the human body (Averbeck and Bertin 2006), and its rapid uptake and accumulation through food chains. Cadmium poisoning's adverse effect on human health, itai-itai disease, was first reported in Japan (Roberts 2014) in 1912 (Liu et al. 2015) in subsistence farmers who had grown rice with water contaminated by mining waste. The disease resulted in the softening of bones, and kidney failure (Roberts 2014). Mainly cadmium causes negative health effects on kidneys, and bones. It also responsible for human cancers in lungs, pancreas, breast, prostate, endometrium, urinary bladder (Piade et al. 2015), testes (Averbeck and Bertin 2006), adverse effects on hemoglobin (Chen et al. 2015), obstructive airway disease, emphysema, irreversible renal failure, immune-suppression in chronic exposure, diabetic, deregulated blood pressure, DNA damage (Averbeck and Bertin 2006), pulmonary, and cardiovascular defects (Roberts 2014). Researches up to date investigated that cadmium is one of the causes for CKDu (WHO 2012).

Table 1.10 shows the concentrations of cadmium in the well water of each district in Sri Lanka as the maximum value, the average value, and the standard deviation. No district exceeded the 3 µg/l cadmium concentration in well water; even the highest concentration recorded from the Moneragala District was 0.5 µg/l, well below the Sri Lankan standard, and the WHO guideline. Therefore, according to the analytical results of 1435 well water samples, there is no relationship between cadmium contamination in water, and CKDu.

Table 1.10: Cadmium concentrations in the well water of each Sri Lankan district

District	**Maximum Conc. (µg/l)**	**Average Conc. (µg/l)**	**Standard Deviation (µg/l)**	**District**	**Maximum Conc. (µg/l)**	**Average Conc. (µg/l)**	**Standard Deviation (µg/l)**
Ampara	0.1	0.01	0.02	Kurunegala	0.0	0.00	0.00
Anuradhapura	0.2	0.01	0.03	Mannar	0.1	0.02	0.07
Badulla	0.1	0.01	0.02	Matale	0.3	0.02	0.04
Batticaloa	0.2	0.01	0.03	Matara	0.1	0.01	0.01
Colombo	0.0	0.00	0.00	Moneragala	0.5	0.01	0.04
Galle	0.2	0.01	0.02	Mullaitivu	0.3	0.02	0.07
Gampaha	0.0	0.01	0.00	Nuwara Eliya	0.0	0.00	0.00
Hambantota	0.1	0.01	0.01	Polonnaruwa	0.0	0.01	0.01
Jaffna	0.1	0.02	0.04	Puttalam	0.0	0.00	0.00
Kalutara	0.0	0.00	0.00	Ratnapura	0.1	0.00	0.02
Kandy	0.0	0.00	0.00	Trincomalee	0.0	0.00	0.01
Kegalle	0.0	0.00	0.00	Vavuniya	0.1	0.01	0.01
Kilinochchi	0.1	0.01	0.02				

A study conducted by Jayatilake et al. (2013), based on water samples collected from CKDu-endemic, and non-endemic areas in Sri Lanka, concluded that drinking water has not been contaminated by cadmium that causes CKDu. Wasana et al. (2012) reported that the cadmium level in drinking water was well below the WHO guideline of 3 µg/l, based on the water quality assessment of areas of high, and low prevalence of CKDu in Sri Lanka (Wasana et al. 2012). In a study carried out by Chandrajith et al. 2011, drinking water samples collected from both CKDu-endemic, and non-endemic regions of the north central province of Sri Lanka had very low levels of cadmium, well below the WHO guideline of 3 µg/l (Chandrajith et al. 2011). The WHO final report stated that cadmium concentrations in drinking water sources used by CKDu patients in Sri Lanka were within the normal range (WHO 2012). All findings support our conclusion that cadmium in drinking water should not be a cause of CKDu.

3.5.5. Lead

The Sri Lankan standard for lead in drinking water is 10 µg/l (Sri Lankan Standard Institute 2013), which is similar to the WHO guideline for lead in drinking water (WHO 2011). The maximum, and average concentrations of lead in the well water of each district in Sri Lanka are shown in Table 1.11. The Galle District showed an extremely high maximum concentration of lead in well water of 288 µg/l. All other districts reported maximum concentrations of lead below the Sri Lankan standard of 10 µg/l. No district exceeded the average lead concentration in well water than the Sri Lankan standard.

Lead is suspected to be one cause of CKDu, since lead damages the human kidney, immune system, circulatory system, and neurons, and causes joint diseases (Siraj and Kitte 2013), and also adversely effect on hemoglobin (Chen et al. 2015). It is a commutative poison, and human carcinogen. Low level exposure to lead leads to increase the blood pressure, reduce intelligence quotient in children and cause for attention disorders. High level exposure to lead leads to irreversible brain damage. (Siraj and Kitte 2013). However, our results indicate that lead is not a cause of CKDu. The analysis of the WHO final report, which stated that lead concentrations in drinking water sources used by CKDu patients in Sri Lanka were within the normal range (WHO 2012), coincides with our analysis.

Table 1.11: Lead concentrations in the well water of each Sri Lankan district

District	Maximum Conc. (µg/l)	Average Conc. (µg/l)	District	Maximum Conc. (µg/l)	Average Conc. (µg/l)
Ampara	1.2	0.1	Kurunegala	0.1	0.0
Anuradhapura	4.6	0.1	Mannar	0.2	0.0
Badulla	0.9	0.1	Matale	2.8	0.1
Batticaloa	0.1	0.0	Matara	2.2	0.1
Colombo	3.3	0.3	Moneragala	4.3	0.1
Galle	288.0	3.8(0.5)*	Mullaitivu	3.9	0.3
Gampaha	0.7	0.2	Nuwara Eliya	3.0	0.5
Hambantota	0.3	0.0	Polonnaruwa	0.1	0.0
Jaffna	0.0	0.0	Puttalam	3.6	0.3
Kalutara	1.0	0.1	Ratnapura	2.6	0.2
Kandy	0.0	0.0	Trincomalee	0.1	0.0
Kegalle	0.0	0.0	Vavuniya	1.3	0.3
Kilinochchi	0.6	0.1			

*The average concentration for lead in the Galle District decreases from 3.8 µg/l to 0.5 µg/l when a single data with an extremely high concentration of 288 µg/l is eliminated.

3.5.6. Chromium

Chromium is listed as one of the 129 priority pollutants by the US Environmental Protection Agency (USEPA), and also it has listed among the 25 hazardous substances. In industrialized areas chromium is a common groundwater, and soil contaminant and in hazardous waste sites it is the second most abundant inorganic groundwater contaminant. Chromium is hard (Sueker 2005), highly toxic (Mohan and Pittman Jr 2006), steel-grey metallic element (Sueker 2005) persists for long period of time (Choppala et al. 2013). Chromium can be found in air, water, and soil (Shanker and Venkateswarlu 2011). Chromium release in to water by electroplating industries, leather tanning industries, steel works, refractories, pigments, timber processing companies, and oxidative dyeing (Matos et al. 2009).

The Sri Lankan standard for chromium in drinking water is 50 µg/l (Sri Lankan Standard Institute 2013). The WHO guideline for chromium in drinking water is the same as the Sri Lankan standard of 50 µg/l (WHO 2011). Chromium affects human health in many ways, such as kidney, and liver damage, skin rashes, respiratory defects, weakened immune systems, alteration of genetic materials (Shanker and Venkateswarlu 2011) lung cancer (Rafael et al. 2007), and death (Shanker and Venkateswarlu 2011). Table 1.12 shows the maximum, and average chromium concentrations in well water of each Sri Lankan district. It is obvious that no district has recorded maximum or average chromium concentrations above the Sri Lankan standard, and the WHO guideline. When we consider the average chromium concentration of each district, all districts showed average concentrations of 0 µg/l or 1 µg/l. Therefore, it is clear that Sri Lankan well water has not been contaminated with chromium, and that chromium is unlikely to be a cause of CKDu.

Table 1.12: Chromium concentrations in the well water of each Sri Lankan district

District	Maximum Conc. (µg/l)	Average Conc. (µg/l)	District	Maximum Conc. (µg/l)	Average Conc. (µg/l)
Ampara	14	1	Kurunegala	3	0
Anuradhapura	3	1	Mannar	3	1
Badulla	2	0	Matale	3	0
Batticaloa	1	0	Matara	1	0
Colombo	0	0	Moneragala	6	0
Galle	6	0	Mullaitivu	2	1
Gampaha	0	0	Nuwara Eliya	0	0
Hambantota	2	0	Polonnaruwa	1	0
Jaffna	2	0	Puttalam	2	1
Kalutara	1	0	Ratnapura	4	1
Kandy	0	0	Trincomalee	0	0
Kegalle	3	0	Vavuniya	6	1
Kilinochchi	2	1			

Table 1.13 summarized the percentage of wells with fluoride, nitrate, arsenic, aluminum, manganese concentrations relating to WHO guidelines for each district in Sri Lanka. It represented the percentages of parameters which did not fulfill the WHO guidelines in each district. Kegalle, and Kilinochchi Districts recorded that all the well waters in the area have fulfilled the WHO guidelines for fluoride, nitrate, arsenic, aluminum, and manganese.

Table 1.13: Percentage of wells with fluoride, nitrate, arsenic, aluminum, and manganese concentrations relating to WHO guidelines for each district in Sri Lanka

District	**Percentage of wells did not fulfill the WHO guidelines**					**NO. of parameters did not fulfill the WHO guideline**
	Fluoride	**Nitrate**	**Arsenic**	**Aluminum**	**Manganese**	
Ampara	6.1	0.0	0.0	9.1	10.6	3
Anuradhapura	23.5	1.7	0.0	0.0	1.3	3
Badulla	6.2	0.0	0.0	0.0	1.5	2
Batticalo	0.0	0.0	3.3	3.3	0.0	3
Colombo	0.0	0.0	0.0	4.0	0.0	1
Galle	0.0	0.0	0.0	5.8	0.0	1
Gampaha	0.0	8.0	0.0	0.0	0.0	1
Hambantota	2.8	1.4	0.0	1.4	9.2	4
Jaffna	0.0	8.7	0.0	0.0	0.0	1
Kalutara	0.0	0.0	0.0	6.5	0.0	1
Kandy	0.0	0.0	0.0	0.0	8.0	1
Kegalle	0.0	0.0	0.0	0.0	0.0	0
Kilinochchi	0.0	0.0	0.0	0.0	0.0	0
Kurunegala	9.4	0.0	0.0	3.1	9.4	3
Mannar	0.0	9.4	18.8	3.1	0.0	3
Matale	8.2	1.6	0.0	0.0	3.3	3
Matara	0.0	1.3	0.0	4.0	2.7	3
Moneragala	31.9	1.4	0.0	2.8	2.1	4
Mullaitivu	0.0	4.4	8.7	4.4	4.4	4
NuwaraEliya	0.0	0.0	0.0	14.3	14.3	2
Polonnaruwa	17.9	0.0	0.0	0.0	14.3	2
Puttalam	3.6	17.9	7.4	0.0	0.0	3
Ratnapura	0.0	0.0	0.0	3.3	0.0	1
Trincomalee	14.3	3.6	0.0	0.0	0.0	2
Vavuniya	10.0	17.9	0.0	0.0	4.0	3

From this study we have identified that most of the areas contained higher concentration of fluoride, hardness and few areas contained higher concentration of arsenic in Sri Lanka.

4. Conclusions

The well water quality of Sri Lanka was analyzed using 1304, and 1435 water samples collected from wells in all districts of Sri Lanka from 2010 to 2015. Spatial distribution maps were created for the pH, fluoride, nitrate, hardness, aluminum, and manganese concentrations to interpret the analyzed results. According to the analysis, it is clear that Sri Lankan well water was contaminated with fluoride, nitrate, aluminum, arsenic and manganese, and also it contained extremely high hardness. 78.2% of wells contained a pH value higher than 7, indicating that most of the Sri Lankan well water has a basic condition. 20% of the wells were recorded to have fluoride concentrations of more than 1.0 mg/l, although the Sri Lankan standard for fluoride in drinking water is 1.0 mg/l. The Moneragala, and Anuradhapura Districts had the highest recorded average concentrations of fluoride. We found that 2.2% of wells had nitrate concentrations of more than 50 mg/l, exceeding the Sri Lankan standard for nitrate in drinking water. Sri Lankan well water had high hardness. We found that 42.2% of well water was very hard which showed the hardness concentration more than 180 $CaCO_3$ mg/l.

We found that 2.3% of well water samples had aluminum concentrations of more than 200 µg/l exceeding the Sri Lankan standard. Five districts were exceeded the average concentration of manganese than Sri Lankan standard of 100 µg/l with the highest value as 440 µg/l in Polonnaruwa. No district recorded maximum, and average values of cadmium, and chromium concentrations above the Sri Lankan standard or the WHO guideline. Lead concentrations in well water were also recorded as being below the Sri Lankan standard, and the WHO guideline, with the exception of one sample. The well water arsenic in the Mannar, Puttalam, Batticaloa, and Mullaitivu Districts had concentrations that were above the Sri Lankan standard, and the WHO guideline of 10 µg/l.

Thus it is obvious that Sri Lankan well water needs treatment for fluoride, nitrate, aluminum, manganese, arsenic, and hardness for drinking purpose. Profound attention should be given to minimize the contamination of drinking water by fluoride because dental fluorosis, and skeleton fluorosis have been prevalent in Sri Lanka. In addition, it is supposed to be a suspicious cause for CKDu which was highly prevalent in the Dry Zone of Sri Lanka. Although four districts recorded higher arsenic concentrations, these areas have not yet been identified as CKDu-prevalent areas. So far prominent health hazardous were not reported due to the excess amount of nitrate, aluminum, manganese, and arsenic in Sri Lanka. It is obvious that there is a potential risk to human health since some of the well water exceeded the WHO guidelines.

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CHAPTER 2

Arsenic, Cadmium, Lead, and Chromium in Rice, Human Urine, and Pesticides in Groundwater, and Rice in Sri Lanka in Relation to Chronic Kidney Disease of unknown etiology (CKDu)

1. Introduction

The Chronic kidney disease of unknown etiology (CKDu) has recently been increasing in the Dry Zone of Sri Lanka especially in Anuradhapura, and Polonnaruwa Districts and spreading to some other areas causing number of deaths to mankind. In chapter 1, we have discussed the groundwater quality of Sri Lanka in relation to CKDu. The results revealed that there was no relationship between arsenic, cadmium, lead, and chromium in Sri Lankan groundwater, and CKDu. Literature reported that heavy metals cause CKDu; heavy metals in rice (Redmon et al. 2014), arsenic toxicity (Jayasumana et al. 2013), arsenic, and heavy metals in urine (WHO 2012), pesticides (WHO 2012). As we know cadmium poisoning's caused itai-itai disease which resulted chronic kidney disease (Roberts 2014) in humans. Therefore, further investigations are needed other than the groundwater quality, since the disease is highly endemic, and a multi-factorial disease.

In this chapter, arsenic, cadmium, lead, and chromium content in rice, and urine from people living in both CKDu-endemic, and non-endemic areas in Sri Lanka were analyzed to investigate the relationship between the prevalence of CKDu, and arsenic, cadmium, lead, and chromium. In addition, pesticides in groundwater from CKDu-endemic areas were analyzed as a pilot study. Pesticides in rice collected from both CKDu-endemic, and non-endemic areas were also analyzed.

2. Materials and Methods

Ninety-one rice samples were collected from Sri Lanka for the analysis of arsenic, cadmium, lead, and chromium contents. Samples were collected from the market, and households in both CKDu-endemic, and non-endemic areas in the Anuradhapura, Hambantota, Kandy, Matale, Ampara, Kegalle, Gampaha, Matara, and Colombo Districts in Sri Lanka. In total, 67 rice samples were from CKDu-endemic areas, and 24 rice samples were from CKDu non-endemic areas. Sixty-seven rice samples were polished, and 24 rice samples were unpolished. The rice samples were degraded with nitric acid by microwave (Analytik Jena TOPwave microwave system for pressure digestion) to determine the arsenic, cadmium, lead, and chromium contents of each sample by ICP-MS (Agilent 7700). Arsenic, cadmium, lead, and chromium in rice were statistically analyzed using SPSS software.

Fourty rice samples were collected from Sri Lanka for the analysis of pesticides in rice. Samples were collected from both CKDu-endemic, and non-endemic areas. The pesticides in rice samples were determined by Shokukanken Inc. Gunma, Japan. Each sample was analyzed for 9 types of neonicotinoid pesticides; Acetamiprid, Imidacloprid, Clothianidin, Dinotefuran, Thiacloprid, Thiamethoxam, Nitenpyram, Fipronil, and Flonicamid. Neonicotinoid pesticides in rice were extracted by Acetonitrile and purified by solid phase extraction with C-18-PSA, and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS).

Eighty-four human urine samples were collected from the Anuradhapura, Polonnaruwa, Matale, and Kandy Districts in Sri Lanka for the analysis of arsenic, cadmium, lead, and chromium concentrations, liver-type fatty acid binding proteins (L-FABPs), and creatinine concentrations. L-FABP is found in the cytoplasm of human proximal tubular cells of the kidney (Matsui et al. 2011). Human L-FABP binds to fatty acids and transports them into mitochondria or peroxisomes, where the fatty acids are β-oxidized, and contributes to intracellular fatty acid homeostasis. L-FABP has been detected as an early diagnostic of acute kidney injury, and a biological indicator for the monitoring of chronic kidney disease (Yokoyama et al. 2009; Susantitaphong et al. 2013). Deterioration of renal function increases the L-FABP in the proximal tubules of the kidney, and accelerates the excretion of L-FABP into the urine (Kamijo et al. 2006).

Creatinine is a chemical waste product produced by muscle metabolism (Pietrangelo 2017), and it is produced by the cells in the pancreas, kidneys, and liver from the methionine, arginine, and glycine amino acids (Coleman 2016). Creatinine is filter out of the blood by the kidney when the kidneys are functioning normally, and remove from the body through urination. the creatinine urine concentration can measure by a creatinine urine test (Pietrangelo 2017). high creatinine concentration in urine symbolize the malfunction of muscles or kidney (Coleman 2016).

The safe limit of L-FABP in urine is 8.4 µg/g cr (µg/g creatinine). In our study, people with L-FABP concentrations of more than 8.4 µg/g cr were categorized as CKDu affected, while people with L-FABP concentration of 8.4 µg/g cr or less were categorized as CKDu non-affected. Since 1.5 µg/l is the minimum determination limit of L-FABP in urine, for the people with L-FABP concentration less than the limit, 1.5 µg/l was used as the L-FABP concentration for the categorization. Accordingly, people with L-FABP concentrations of less than 1.5 µg/l and more than 8.4 µg/g cr were categorized as "unknown." Ten percent of people in CKDu-endemic areas were categorized as CKDu affected, while all of the people in the non-endemic area were categorized as CKDu non-affected, based on their L-FABP values.

Free and informed consent of the participants, and their legal representatives were obtained, and the study protocol was approved by Toyama Prefectural University Committee for Ethical Research Screening Involving Human Subjects.

Sixty human urine samples were collected from CKDu-endemic areas, and 24 human urine samples were collected from non-endemic areas. The samples were collected in sterilized plastic cups. Collected human urine samples were stored at 0 ^{0}C in an ice-cooled condition in plastic vials in a styrene foam box, and brought to Japan. The transportation of human urine samples from Sri Lanka to Japan took 1-4 days after collection.

Arsenic, cadmium, lead, and chromium concentrations were analyzed by ICP-MS, after acidifying the samples with 20% HNO_3. L-FABP and creatinine concentrations were determined by BML Inc., Tokyo, Japan. Arsenic, cadmium, lead, and chromium in human urine were statistically analyzed using SPSS software after correcting the concentrations by using the creatinine concentration.

Fifteen groundwater samples were collected from both dug wells, and tube wells in CKDu-endemic areas in Wilgamuwa, Anuradhapura, and Polonnaruwa Districts in Sri Lanka for the analysis of pesticides in groundwater. The depth of selected wells was 5 m - 10 m for dug wells, and 20 m or more for tube wells. Samples were collected in to glass bottles without filtrated on site since certain amount of pesticide residues are adsorbed onto the membrane filter. An air portion was kept on the surface of the collected water sample to maintain the samples in an aerobic condition. Collected samples were stored at 0 ^{0}C in an ice-cooled condition in a styrene foam box, and brought to Japan. The transportation from Sri Lanka to Japan took 1-4 days after collection. The pesticides in water samples were determined by Hokuriku Environmental Science Research

Laboratory, Japan and analyzing was done using liquid chromatography-mass spectrometry (LC-MS/MS). Each sample was analyzed for 245 pesticides.

3. Results and Discussion

3.1. Arsenic, Cadmium, Lead, and Chromium in Rice in Sri Lanka

The arsenic, cadmium, lead, and chromium content in 91 rice samples (both polished, and unpolished) collected from both CKDu-endemic, and non-endemic areas in the Anuradhapura, Hambantota, Kandy, Matale, Ampara, Kegalle, Gampaha, Matara, and Colombo Districts in Sri Lanka, are shown in Tables 2.1 and 2.2.

Table 2.1: Arsenic, cadmium, lead, and chromium in rice samples from CKDu-endemic areas (n=67)

	Maximum Value (mg/kg)		Average Value (mg/kg)		Standard Deviation (mg/kg)		CODEX* (mg/kg)
	Polished Rice	Unpolished Rice	Polished Rice	Unpolished Rice	Polished Rice	Unpolished Rice	
As	0.20	0.08	0.03	0.03	0.04	0.04	0.2
Cd	0.87	0.52	0.12	0.16	0.19	0.17	0.4
Pb	0.08	0.02	0.01	0.00	0.02	0.01	0.2
Cr	2.03	1.50	0.06	0.27	0.27	0.54	_

*Maximum level of the Codex standard for polished rice

Table 2.2: Arsenic, cadmium, lead, and chromium in rice samples from CKDu non-endemic areas (n=24)

	Maximum Value (mg/kg)		Average Value (mg/kg)		Standard Deviation (mg/kg)		CODEX* (mg/kg)
	Polished Rice	Unpolished Rice	Polished Rice	Unpolished Rice	Polished Rice	Unpolished Rice	
As	0.07	0.09	0.03	0.04	0.03	0.03	0.2
Cd	0.65	1.43	0.21	0.18	0.24	0.36	0.4
Pb	0.00	0.02	0.00	0.00	0.00	0.00	0.2
Cr	0.40	0.58	0.07	0.10	0.14	0.14	_

*Maximum level of the Codex standard for polished rice

The specific standards for the maximum level of arsenic, cadmium, lead, and chromium in polished rice (Codex 2014) according to the Codex Alimentarius Commission, as set by the UN Food, and Agricultural Organization (FAO), and WHO, are shown for comparison.

It was observed that the average arsenic contents in polished, and unpolished rice collected from both CKDu-endemic, and non-endemic areas were well below the maximum level of the Codex standard for polished rice. Chandrajith et al. (2011) analyzed rice samples collected from the CKDu-endemic regions of Girandurukotte, and Nikawewa in the North Central Province of the Dry Zone of Sri Lanka and found that the rice samples contained relatively high levels of arsenic, ranging between 0.09 and 0.26 mg/kg (Chandrajith et al. 2011). The highest value was relatively higher than the maximum level of the Codex standard, 0.2 mg/kg.

The average cadmium content in polished, and unpolished rice collected from both CKDu-endemic, and non-endemic areas were also well below the maximum level of the Codex standard for polished rice. The study conducted by Bandara et al. (2008) to determine cadmium levels in rice from households of individuals with CKDu in the North Central Province of Sri Lanka where CKDu was prevalent showed the following results. All collected rice samples contained cadmium. Rice grains collected from Medawachchiya contained cadmium ranging from 0.001–0.093 mg/kg, with a mean value of 0.0444 mg/kg ± 0.0165, while those from the Anuradhapura-Thuruwila area contained cadmium ranging from 0.001–0.194 mg/kg, with a mean value of 0.0404 ± 0.0196 mg/kg. The background value for cadmium in rice grains in Sri Lanka was found to be 0.001 mg/kg. Based on the analyzed results, the authors concluded that long-term exposure to dietary cadmium in rice over the years would adversely affect human kidneys (Bandara et al. 2008). On the contrary, Chandrajith et al. analyzed the cadmium content in rice collected from CKDu-endemic regions of Girandurukotte, and Nikawewa in the Dry Zone of Sri Lanka, and found substantially lower values; rice samples from the Girandurukotte area ranged from 0.009–0.018 mg/kg, while those in the Nikawewa area ranged from 0.003–0.013mg/kg. The authors concluded that cadmium in rice is not a possible risk factor for the occurrence of CKDu in Sri Lanka (Chandrajith et al. 2011).

The lead levels in polished, and unpolished rice collected from both CKDu-endemic, and non-endemic areas were well below the maximum level of the Codex standard for polished rice. The chromium content in rice from both CKDu-endemic, and non-endemic areas had lower recorded concentrations for both polished, and unpolished rice, although there is no standard value set in the Codex standard for comparison.

The two-sample t-test, assuming unequal variances ($p<0.05$), was performed to investigate whether there is a significant difference between arsenic, cadmium, lead, and chromium levels in rice collected from CKDu-endemic, and non-endemic areas. Results indicated that there was no significant difference between the arsenic, cadmium, lead, and chromium levels in CKDu-endemic, and non-endemic areas. Therefore, it was concluded that arsenic, cadmium, lead, and chromium in rice are not causing CKDu.

3.2. L-FABP, Arsenic, Cadmium, Lead, and Chromium in Human Urine in Sri Lanka

Urine liver-type fatty acid binding protein (L-FABP) concentrations were analyzed to reveal the renal function of people in both CKDu-endemic, and non-endemic areas. Figures 2.1, 2.2, 2.3, and 2.4, respectively, show the relationships between L-FABP concentrations, and arsenic, cadmium, lead, and chromium concentrations in urine corrected by the creatinine concentration of both CKDu affected, and non-affected people. Only the data showing L-FABP concentrations of more than 1.5 µg/l were used for the figures. It is obvious that there was no positive correlation between L-FABP concentrations, and the arsenic, cadmium, lead, and chromium concentrations in urine. The arsenic, cadmium, lead, and chromium concentrations in urine of both CKDu affected, and non-affected people are independent of L-FABP concentrations.

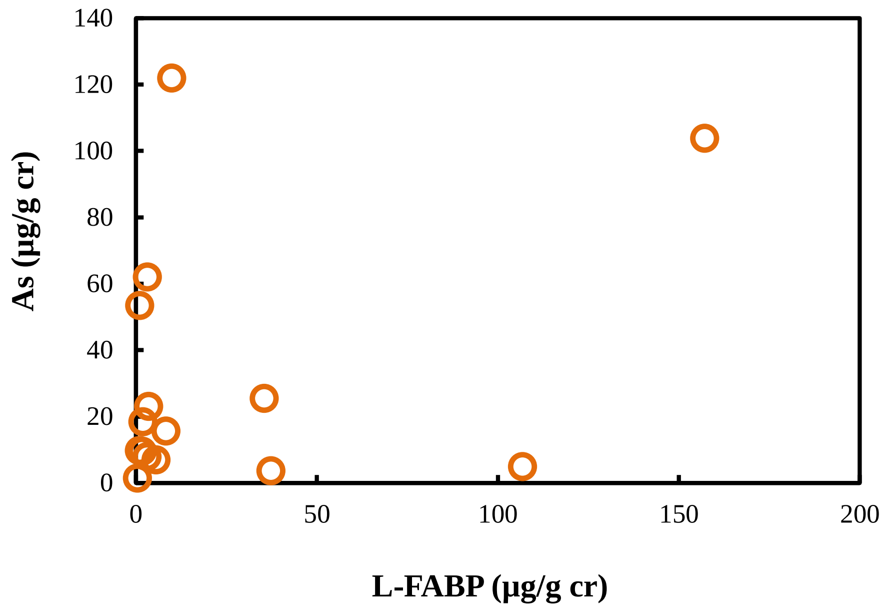

Fig. 2.1: Relationship between L-FABP and arsenic concentrations in human urine

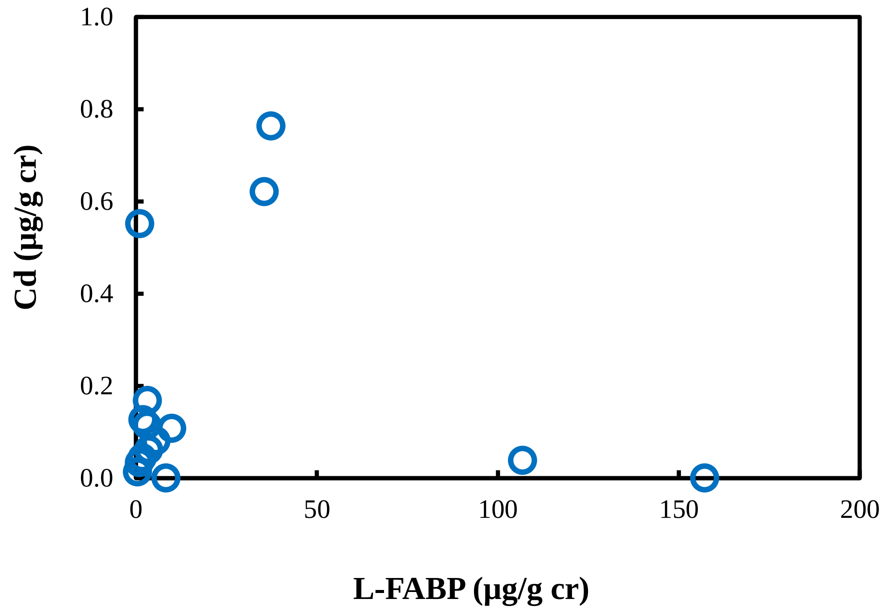

Fig. 2.2: Relationship between L-FABP and cadmium concentrations in human urine

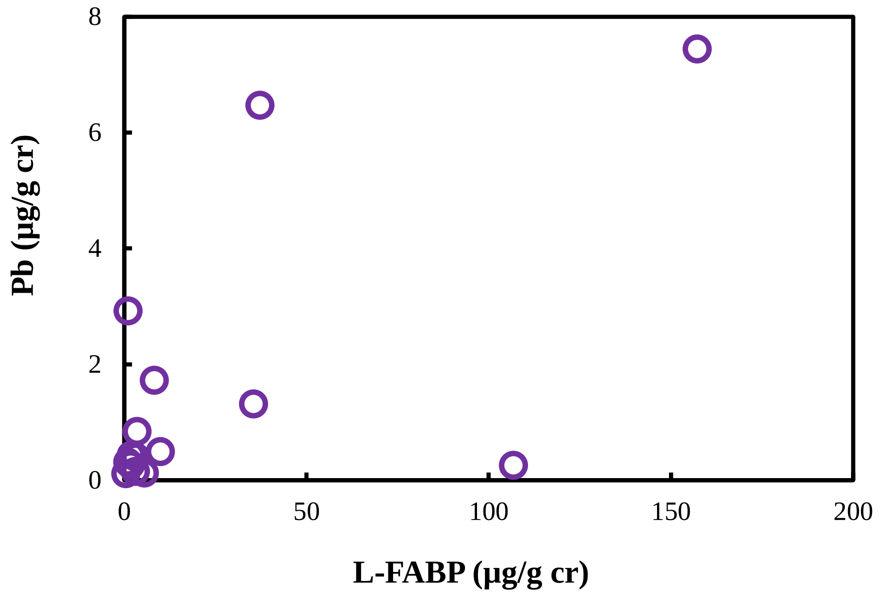

Fig. 2.3: Relationship between L-FABP and lead concentrations in human urine

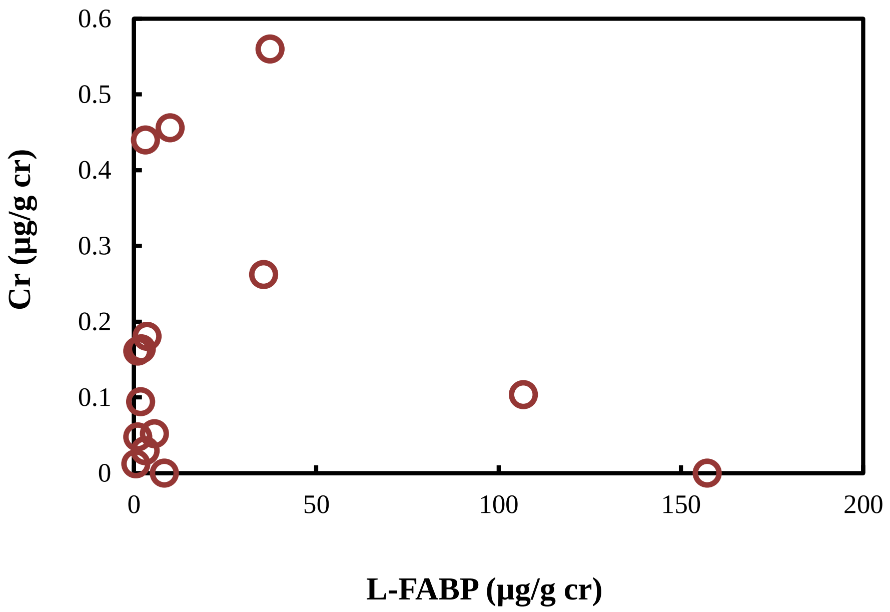

Fig. 2.4: Relationship between L-FABP and chromium concentrations in human urine

Table 2.3: Arsenic, cadmium, lead, and chromium concentrations in human urine samples

	Maximum (µg/g cr)	Average (µg/g cr)	Standard Deviation (µg/g cr)
CKDu-endemic			
As	326.0	33.0	47.1
Cd	0.8	0.2	0.2
Pb	10.8	1.8	2.3
Cr	3.9	0.4	0.7
CKDu non-endemic			
As	82.3	44.2	19.2
Cd	6.5	0.6	1.3
Pb	47.5	2.8	9.6
Cr	2.2	0.5	0.5

Table 2.3 shows the arsenic, cadmium, lead, and chromium concentrations in human urine samples as the maximum, average, and standard deviation for both CKDu-endemic, and non-endemic areas.

The two-sample t-test, assuming unequal variances ($p<0.05$), was performed to identify whether there is a significant difference between arsenic, cadmium, lead, and chromium concentrations in the urine of people in CKDu-endemic areas, and non-endemic areas. Analyzed results revealed that there were no significant differences between the groups.

After analyzing 745 samples, Jayatilake et al. reported that no significant differences were found in arsenic concentrations in the urine samples of CKDu affected people as compared to controls in Sri Lanka (Jayatilake et al. 2013). This coincided well with our analysis of arsenic. The WHO final report of 2012 (WHO 2012) revealed that there was significantly lower urine arsenic excretion in CKDu affected people (mean 45.4, max 617 µg/g cr) than in the urine arsenic excretion of non-affected people (mean 92.4, max 966 µg/g cr) ($p<0.01$) in endemic areas after analyzing 495 urine samples from CKDu affected people, and 250 urine samples from non-affected people. However, our study showed no significant difference between the arsenic concentrations in CKDu affected people (mean 45.8, max 122 µg/g cr), and non-affected people (mean 25.1, max 100 µg/g cr) ($p<0.05$) in endemic areas. Further, there was no significant difference between the arsenic concentrations in CKDu affected people (mean 45.8, max 122 µg/g cr), and CKDu non-affected people (mean 30.3, max 100 µg/g cr) ($p<0.05$) when considering both CKDu-endemic, and non-endemic areas. The fact that there is no relationship between L-FABP, and arsenic concentrations, as shown in Figure 2.1, indicates that arsenic excretion is not a possible indicator of CKDu.

Jayatilake et al. reported that the urine cadmium concentration in CKDu affected people was significantly higher as compared to that of controls in both endemic, and non-endemic areas of Sri Lanka (Jayatilake et al. 2013). The WHO reported that considerably higher cadmium concentrations in urine were found in healthy people in endemic areas as compared to those in people in non-endemic areas (mean 0.35, max 2.08 µg/g cr) ($p<0.05$) (WHO 2012). The WHO also reported that significantly higher urine cadmium concentrations were detected in CKDu affected people (mean 1.04, max 8.93 µg/g cr) as compared to those of healthy people (mean 0.65, max 5.13 µg/g cr) ($p<0.05$) in endemic areas (WHO 2012). On the contrary, we have detected that the urine cadmium concentrations in CKDu affected people (mean 0.25, max 0.76 µg/g cr) were not significantly different from those in non-affected people (mean 0.13, max 0.55 µg/g cr) ($p<0.05$) in CKDu-endemic areas. Urine cadmium concentrations in non-affected people in CKDu-endemic areas (mean 0.13, max 0.55 µg/g cr) were significantly lower than those in non-endemic areas (mean 0.28, max 0.96 µg/g cr) ($p<0.05$). Further, there was no significant difference between the cadmium concentrations in CKDu affected people (mean 0.25, max 0.76 µg/g cr), and CKDu non-affected people (mean 0.18, max 0.96 µg/g cr) ($p<0.05$) when considering both CKDu-endemic, and non-endemic areas.

The WHO revealed that there was no significant difference in the lead excreted in the urine of CKDu affected people (mean 1.15, max 8.53 µg/g cr), and healthy people (mean 1.02, max 2.25 µg/g cr) in CKDu-endemic areas (WHO 2012). In our study as well, the concentrations of lead in the urine of CKDu affected people (mean 2.94, max 7.44 µg/g cr) were not significantly different than those of non-affected people (mean 1.50, max 10.82 µg/g cr) ($p<0.05$) in CKDu-endemic areas. Further, there was no significant difference between the lead concentrations in CKDu affected people (mean 2.94, max 7.44 µg/g cr), and CKDu non-affected people (mean 1.28, max 10.82 µg/g cr) ($p<0.05$) when considering both CKDu-endemic, and non-endemic areas.

The chromium concentrations in the urine of CKDu affected people (mean 0.23, max 0.56 µg/g cr) were not significantly different from non-affected people (mean 0.21, max 0.54 µg/g cr) ($p<0.05$) in CKDu-endemic areas. Further, there was no significant difference between the chromium concentrations in CKDu affected people (mean 0.23, max 0.56 µg/g cr), and CKDu non-affected people (mean 0.25, max 0.96 µg/g cr) ($p<0.05$) when considering both CKDu-endemic, and non-endemic areas.

3.3. Pesticides in Groundwater in Sri Lanka

Each well water sample collected from CKDu-endemic area was analyzed for 245 types of pesticides. No any type of pesticides was detected in the collected samples. The lowest detection limit of each pesticide was 10 µg/l. A similar study was reported in literature. Jayasumana and his fellow researchers have analyzed 60 water samples for glyphosate, which represents 52% of total pesticides used in Sri Lanka. The water samples were collected from wells, reservoirs, taps, and reverse osmosis machines, from both CKDu-endemic, and non-endemic areas. Further, samples were collected from both serving wells, and abandoned wells. They have found that 94% of abandoned wells contained glyphosate more than 1 µg/l, and 31% of serving wells contained glyphosate more than 1 µg/l in CKDu-endemic area. The glyphosate was not detected in water samples collected from non-endemic area. Trace amount of glyphosate was detected in the surface water collected from reservoirs indicating that glyphosate may have been washed from the agricultural or paddy lands to reservoirs (Jayasumana et al. 2015).

According to results obtained from our study, pesticides in groundwater was not a suspicious cause for CKDu. Anyhow, further investigations are needed with more number of samples from both CKDu-endemic, and non-endemic areas for a firm conclusion, since we have collected only fifteen water samples for the analysis as a pilot study.

20 groundwater samples from CKDu-endemic areas in Anuradhapura, Polonnaruwa, and Wilgamawa, and 10 groundwater samples from CKDu non-endemic areas in Kegalle, Kandy, Gampaha will collect for the analysis of glyphosate in water as further investigations of the current study. All the samples will collect from dug wells, and tube wells near to agricultural areas. Glyphosate will analyze using enzyme-linked immunosorbent assay (ELISA). ELISA has a lower detection limit of glyphosate of 0.6 µg/l (Jayasumana et al. 2015).

3.4. Pesticides in Rice in Sri Lanka

Each rice sample collected from both CKDu-endemic, and non-endemic areas was analyzed for 9 types of neonicotinoid pesticides. No any type of pesticides was detected in the collected rice samples. The lowest detection limit of each pesticide was 0.01 mg/g.

4. Conclusions

Rice samples collected from CKDu-endemic areas, and non-endemic areas were analyzed for arsenic, cadmium, lead, and chromium. The rice samples showed arsenic, cadmium, and lead contents below the Codex standards, and the chromium content in rice also had a lower value. Furthermore, we found that there was no significant difference in the arsenic, cadmium, lead, and chromium contents in rice between CKDu-endemic, and non-endemic areas. Thus, it is clear that arsenic, cadmium, lead, and chromium in rice are not possible causes of CKDu.

Furthermore, the L-FABP concentration in human urine was compared with arsenic, cadmium, lead, and chromium concentrations in human urine. No relationships were found between them. In addition, arsenic, cadmium, lead, and chromium concentrations in human urine samples from CKDu-endemic areas were not significantly different from those non-endemic areas, indicating that cadmium, lead, chromium, and arsenic are not causes of CKDu.

Pesticides in groundwater were analyzed, and the pesticides were not detected in groundwater samples although the samples were from CKDu-endemic areas. A broad investigation is needed for a firm conclusion.

Rice samples collected from both CKDu-endemic, and non-endemic areas were analyzed for neonicotinoid pesticides, and neonicotinoid pesticides were not detected in rice. Therefore, it was concluded that neonicotinoid pesticides are not cause of CKDu.

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CHAPTER 3

The Preparation of Chicken Bone Char (CBC), and Fluoride Removal from Sri Lankan Drinking Water by Larger Sized CBC – A Community Based CBC Filter

1. Introduction

Most of the groundwater sources in Sri Lanka were contaminated with high concentration of fluoride as we discussed in chapter 1. Groundwater is the main drinking water source of most areas in Sri Lanka (Dissanayake 2005). Profound attention should be given to minimize the groundwater contamination by fluoride, since it causes dental fluorosis, and skeleton fluorosis in humans (Yadav et al. 2006). As well it is one of the suspicious causes for chronic kidney disease of unknown etiology (CKDu) (Nanayakkara et al. 2012) spreading most of the areas in Sri Lanka by causing deaths to many people. An efficiency fluoride removal method is needed in Sri Lanka for a low cost, since it is a developing country.

Several defluoridation techniques are used all over the world in order to remove fluoride from contaminated water, such as precipitation (Sakhare et al. 2012), coagulation (Essadki et al. 2009), adsorption (Sundaram et al. 2009), ion exchange (Feng et al. 2012), reverse osmosis (Nicolas et al. 2010), nanofiltration (Cui et al. 2011), electrolysis (Sivasankar et al. 2012), electrodialysis (Loganathan et al. 2013), membrane separation process (Meenakshi and Maheshwari 2006), Donnan dialysis (Tomar et al. 2014), and Nalgonda technique (Yadav et al. 2006). Access to most of these techniques is limited due to the high operational cost, and the formation of sludge. Among the techniques mentioned above, adsorption is a widely used method of defluoridation due to its cost effectiveness, and ease of operation (Sivasankar et al. 2012). When considering adsorption techniques, the adsorption of fluoride onto bone char is a highly effective, and cost-effective technology that can be used in defluoridation (Phantumvanit and LeGeros 1997). Defluoridation of drinking water by bone char is an old, enduring technology (Larsen et al. 1994).

Fluoride removal by bone char was selected as a low cost, and efficiency raw material to remove fluoride from Sri Lankan drinking water. Majority of Sri Lankans are Buddhists, and minority are Muslims, and Hindus. Muslims don’t prefer pig bones and

Hindus don’t prefer cow bones based on their religious beliefs. Therefore, pig bone char, and cow bone char cannot be used in Sri Lanka for fluoride removal. Chicken bones char was selected to remove fluoride from Sri Lankan drinking water based on the cultural background in Sri Lanka.

In this study our attention was focused on finding the best carbonizing temperature for the preparation of chicken bone char (CBC), and the fluoride removal from drinking water in Sri Lanka by larger sized CBC using a community based CBC filter.

2. Materials and Methods

2.1. Preparation of CBC, and Selecting the Best Carbonizing Temperature for the Preparation of CBC

Three temperatures were selected to determine the best carbonizing temperature for preparing CBC. Figure 3.1 shows the preparation process of CBC. The raw chicken bones were boiled in hot water for 100 minutes in a steel container. Boiled chicken bones were cleaned by removing meat residues. Then chicken bones were carbonized under an anaerobic condition in closed metal containers by an electrical muffle furnace with temperatures of 673 K, 873 K, and 1073 K for 1.5 hours in order to prepare the CBC. Carbonized chicken bones were allowed to cool down to room temperature, and washed with clean water several times to remove remaining meat residues, and dried at 378 K in an electrical oven for one night.

Fig. 3.1: Preparation of CBC

Isotherm studies were conducted for each set of carbonized CBC at 673 K, 873 K, and 1073 K to investigate the adsorption capacities of CBC at different temperatures. Synthesized drinking water with a fluoride (F^-) concentration of 10 mg/l was prepared by using sodium fluoride (NaF), and deionized water for the isotherm studies. Each set of CBC with a diameter of less than 45 µm was shaken with weights of 0.01 g, 0.02 g, 0.03 g, 0.04 g, 0.05 g, 0.1 g, 0.15 g, and 0.2 g, and 50 ml of prepared F^- solution in 50 ml vials in an electrical shaker with a 180 reciprocation. After 24 hours of shaking, samples were taken from each vial, and filtered through a membrane filter with a pore size of 0.45 µm to remove any suspended CBC. The fluoride concentration of each sample was measured with an ion selective electrode (ORION STAR A324 pH/ISE Meter and ORION 9609BNWP Ionplus Sure-Flow Fluoride Electrode). Total Ionic Strength Adjustment Buffer (TISAB III) was added to each sample before the analysis to avoid the interference during the measurements.

One of the 3 sets of carbonized CBC at 3 different temperatures with the highest fluoride adsorption capacity was selected for further experiments.

2.2. Fluoride Removal from Sri Lankan Drinking Water by Larger Sized CBC

A community based CBC filter was established to investigate the performance of fluoride removal by larger sized CBC, at Wilgamuwa village locate in the eastern boundary of the central province of Matale District in Sri Lanka with a latitude, and longitude of 7.5482^0 N, and 80.9491^0 E, respectively, on 24th July 2014. Wilgamuwa is locate in one of the driest regions in Sri Lanka belongs to Dry Zone, with an average annual precipitation of 1100 mm, and an average annual temperature with 28^0C - 30^0C.

Figure 3.2 shows the schematic diagram of the community based CBC filter, and Figure 3.3 shows the photograph of the community based CBC filter. Three polyvinyl chloride (PVC) pipes with a bed heigh of 1.5 m, and a diameter of 16 cm were connected in series to arrange the filter as shown in Figure 3.2, and Figure 3.3. The well water was allowed to pass upward through each column to provide a uniform flow across the cross-sectional area of each column. CBC with a diameter of 5-10 mm was placed in the filter and the total amount of CBC used for the filter was 23 kg. Operation capacity of the filter was 150 L/day.

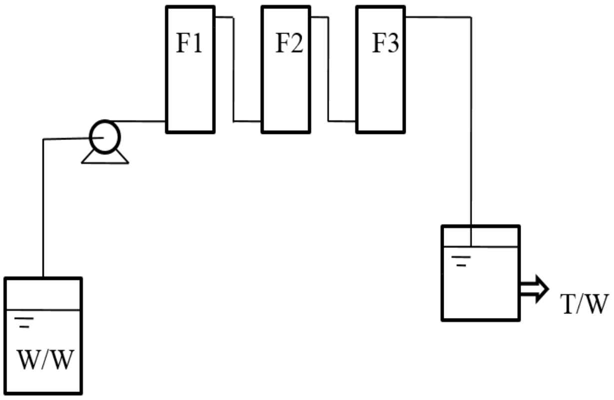

Fig. 3.2: Schematic diagram of the community based CBC filter

Fig. 3.3: Photograph of the community based CBC filter etablished at Wilgamuwa, Sri Lanka

Water from a selected well was allowed to pass through the CBC filter. Water was pumped upto 60 m distance to the filter from the selected well via a pump. Well water (W/W) was filtrated from filter 1 (F1), filter 2 (F2), filter 3 (F3), and treated water (T/W) was taken out from the CBC filter. Water samples from W/W, F1, F2, and T/W (F3) were collected and filtrated on site, using a membrane filter with a pore size of 0.45 µm to stabilize the water quality. Collected water samples were taken to Japan for the analysis of pH, anion and cation concentrations, and alkalinity.

The pH of the solutions was measured using a pH meter (ORION STAR A324 pH/ISE Meter and Beckman Electrode 511070). Anions and cations were analysed using ion chromatograpgy (for anions: Dionex ICS-2000, separation column IonPac AS18, eluent KOH 23-40 mmol/l (gradient), suppressor ASRS 300 4mm; for cations: Dionex ICS-1500, separation column IonPac CS12, eluent methanesulfonic acid 30 mmol/l (isocratic), suppressor CSRS 500 4mm). Alkalinity was measured using Gran's plot titration method (Network Center for EANET 2010), and ion balance calculation for the solutions.

Exhausted CBC in the filter was replaced with raw CBC for a better fluoride removal. First operation was conducted for 142 days, and the second operation was conducted for 220 days.

3. Results and Discussion

3.1. Selecting the Best Carbonizing Temperature for the Preparation of CBC

When preparing CBC, selecting the appropriate carbonizing temperature is the most important factor, since it affects the efficacy of CBC in removing fluoride from contaminated water. Figure 3.4 shows the Freundlich isotherm for CBC carbonized at 673 K, 873 K, and 1073 K. In the Figure, C is fluoride concentration in the solution expressed in a unit of mg/l. Q is maximum adsorption capacity expressed in a unit of mg/g.

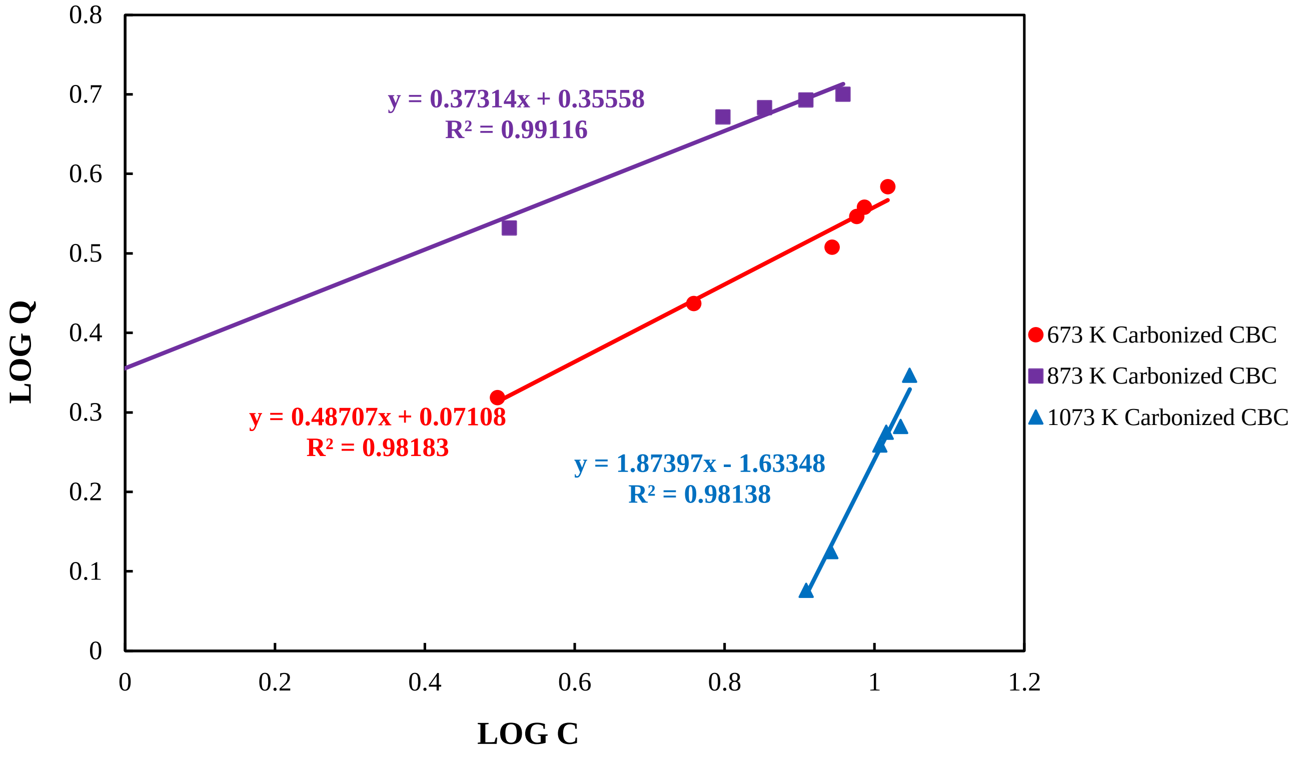

Fig. 3.4: Freundlich isotherm for CBC carbonized at different temperatures

The F^- adsorption by CBC carbonized at three temperatures was well matched with Freundlich isotherm. According to the Freundlich isotherm, CBC carbonized at 673 K, 873 K, and 1073 K, respectively, showed fluoride adsorption capacities of 3.62 mg/g, 5.35 mg/g, and 1.74 mg/g at the equilibrium fluoride concentration of 10 mg/l. It was obvious that chicken bones carbonized at 873 K showed the highest adsorption capacity. Further, we observed a yellowish colour, undesirable taste, and unpleasant odour in water treated by chicken bones carbonized at 673 K. Therefore, we selected 873 K as the best carbonizing temperature for further experiments.

The results obtained from our study were consistent with the literature. When the carbonizing temperature decreases to less than 773 K, odour, undesirable taste, and yellowish colour occur in the treated water due to less (or no) removal of organic matter such as fats, oil, and meat residuals (Mjengera and Mkongo 2003) from chicken bones. Leyva-Ramos and his fellows also reported that carbonizing temperatures below 773 K caused an unpleasant taste, and smell, and yellowish colour in the treated water due to the organic matter in bone char (Leyva-Ramos et al. 2010), as we found in our study. We detected that the weight-basis C content in chicken bones carbonized at 673 K (14%) was higher than the C content in CBC carbonized at 873 K (9%), and 1073 K (6%), showing that there was considerably more organic matter in CBC carbonized at 673 K. At lower carbonizing temperatures (573 K), the organic matter in bones was removed insufficiently, which made it unable to provide a large specific surface area, and enough pore space for the efficient removal of fluoride (Brunson and Sabatini 2009).

When the carbonizing temperature increased to more than 873 K, the adsorption capacity of CBC was reduced dramatically. Carbonizing temperatures higher than 873 K can alter the hydroxyapatite structure of bone (Brunson and Sabatini 2009), leading to reduction in fluoride removal capacity (Kaseva 2006), as we found in our study. Kawasaki et al. investigated the fact that the adsorption capacity of fluoride onto bone char carbonized at 1073 K for 2 hours was higher than that carbonized for 2 hours at 1273 K by studying four types of bone char: cow bone char, pig bone char, chicken bone char, and fish bone char (Kawasaki et al. 2009). Mayorga et al. reported that the best fluoride removal performance of bone char was observed at the carbonizing temperature of 973 K for 2 hours by studying cow bone char. Further, they mentioned that when the carbonizing temperature increased to more than 1073 K, the fluoride ion adsorption capacity of bone char decreased from 6.0 mg/g to 3.0 mg/g, and when increasing the carbonizing temperature to 1273 K, the fluoride adsorption capacity was reduced to 1.0 mg/g. Finally, they justified by their results that the carbonizing temperature of bone char plays a major role in water defluoridation (Rojas-Mayorga et al. 2013). We selected 873 K as the best carbonizing temperature considering the adsorption capacity, and the energy consumption.

3.2. Fluoride Removal from Sri Lankan Drinking Water by Larger Sized CBC

3.2.1. Operation 1

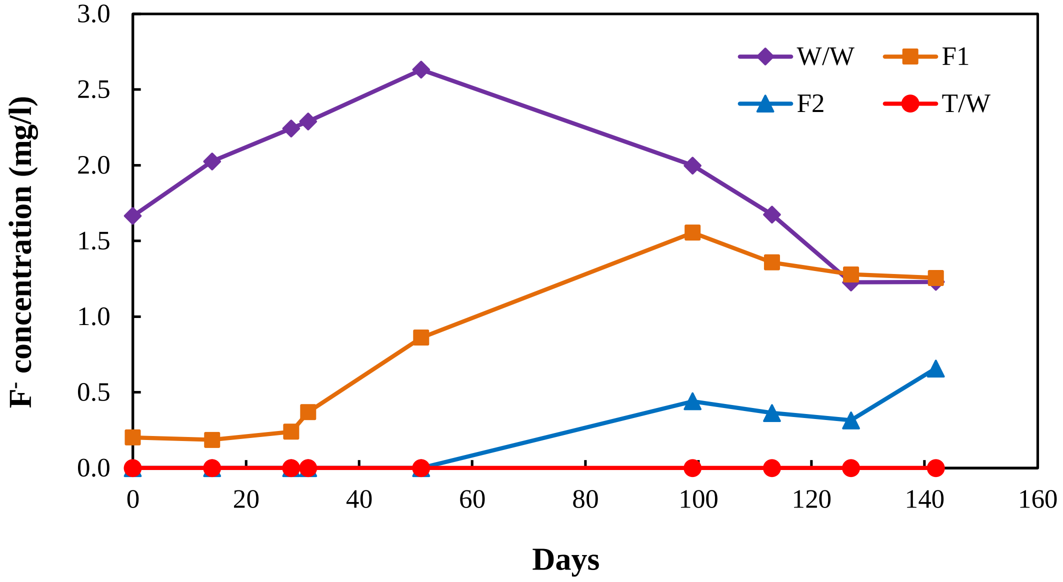

Fig. 3.5: Fluoride concentrations of the well water and the community based CBC filter (Operation 1)

Figure 3.5 shows the fluoride concentrations of the well water (W/W), the filter 1 (F1), the filter 2 (F2), and the treated water (T/W) for 142 days of operation (Operation 1). There was a considerably high concentration of fluoride in the well water. The concentration of fluoride increased in the former period due to evaporation by the dry climate, and then decreased in the later period due to the wet climate. After being filtrated by F1, and F2, the fluoride concentration in well water was reduced dramatically. Samples collected from T/W were totally free from fluoride up to 142 days' operation equivalent to 23201 L. The Sri Lankan standard for fluoride in drinking water is 1.0 mg/l (Sri Lankan Standard Institute 2013). Fluoride was effectively removed by the CBC filter, to a level much lower than the Sri Lankan standard.

After 127 days of operation fluoride concentration of F1 reached to an equilibrium concentration (1.23 mg/l). The adsorption capacity of CBC was calculated based on the area of the graph between W/W, and F1 at the equilibrium represented in Figure 3.5. The area of the graph was equivalent to 23011 mg of F^- adsorption. Total amount of CBC used for F1 was 23000 g /3 = 7700 g. Therefore, the adsorption capacity of CBC was 3.00 mg F^-/g CBC.

According to the Freundlich isotherm (Figure 3.4), adsorption capacity of CBC was 2.45 mg F^-/g CBC at the equilibrium fluoride concentration of 1.23 mg/l. The adsorption capacity of CBC obtained from the CBC filter coincided well with the adsorption capacity of CBC obtained by the Freundlich isotherm.

It was expected to operate for 250 days, equivalent to 37500 L, without replacing CBC until the fluoride concentration reaches the Sri Lankan standard of 1.0 mg/l. This was calculated based on the area of the graph between F2, and F3 in Figure 3.5, assuming that F2, and F3 reached to the equilibrium concentration of 1.0 mg/l. According to the calculation, it was expected to operate additional 100 days. Therefore, the total operation period is 250 days (142+100) equivalent to 37500 L (250*150 L/day).

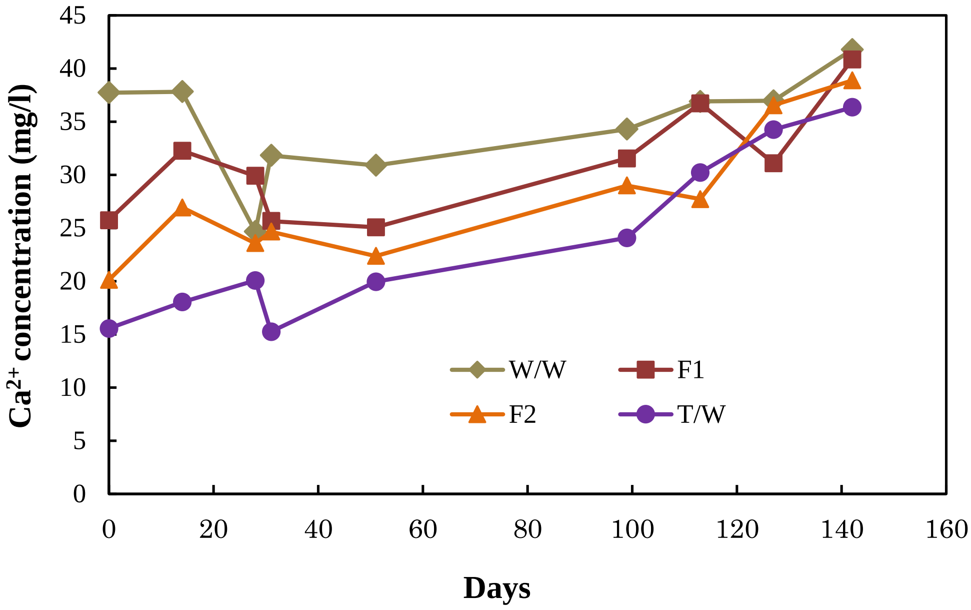

Fig. 3.6: Calcium concentrations of the well water and the community based CBC filter (Operation 1)

Figure 3.6 shows the calcium concentrations of W/W, F1, F2, and T/W for 142 days of operation (Operation 1). Calcium concentration in T/W was dramatically reduced after being filtrated by F1, F2, and F3. In the former period, it is obvious that CBC was capable of removing calcium to a considerable level although the well water calcium concentration was well below the Sri Lankan standard of 100 mg/l (Sri Lankan Standard Institute 2013).

Figure 3.7 shows the magnesium concentrations of W/W, F1, F2, and T/W for 142 days of operation (Operation 1). It is obvious that the CBC filter was not efficiency in removing magnesium although the well water magnesium concentration was well below the Sri Lankan standard of 30 mg/l (Sri Lankan Standard Institute 2013).

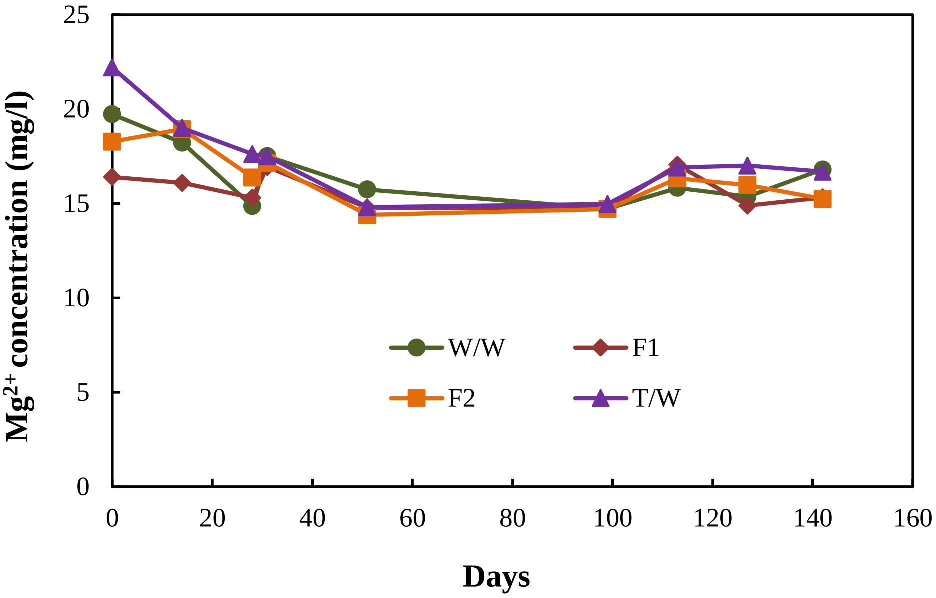

Fig. 3.7: Magnesium concentrations of the well water and the community based CBC filter (Operation 1)

Figure 3.8 shows the hardness of W/W, F1, F2, and T/W for 142 days of operation (Operation 1). Hardness in T/W was dramatically reduced after being filtrated by F1, F2, and F3. It is obvious that CBC was capable of removing hardness to a considerable level in the former period. The well water hardness was well below the Sri Lankan standard of 250 mg/l (Sri Lankan Standard Institute 2013).

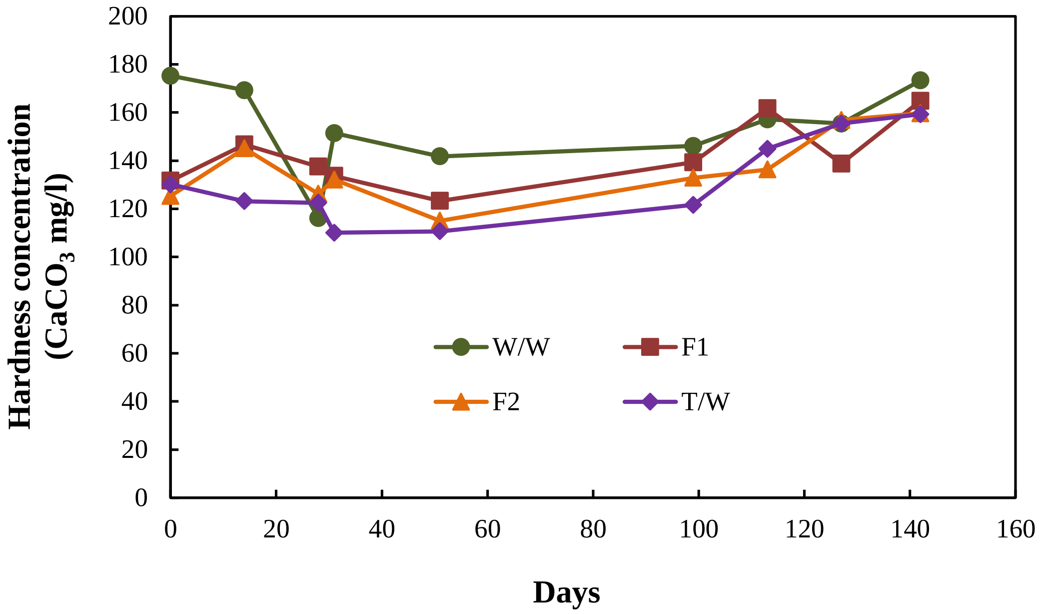

Fig. 3.8: Hardness of the well water and the community based CBC filter (Operation 1)

Table 3.1: Anion and cation concentrations of the well water and the community based CBC filter (Operation 1)

Days		Concentration (mg/l)					
		Cl^-	SO_4^{2-}	NO_3^-	PO_4^{3-}	Na^+	K^+
0	W/W	10	5	0	0	55	0
	F1	11	6	1	0	69	0
	F2	11	4	0	2	62	0
	T/W	10	5	0	5	61	3
99	W/W	12	7	1	0	66	0
	F1	12	7	0	0	69	0
	F2	12	7	1	0	69	0
	T/W	12	8	0	1	72	0
142	W/W	7	5	0	0	38	0
	F1	7	5	0	0	38	0
	F2	7	5	1	0	38	0
	T/W	7	5	0	0	38	0

Table 3.2: Alkalinity of the well water and the community based CBC filter (Operation 1)

Days	Alkalinity (µeq/l)			
	W/W	F1	F2	T/W
0	5418	5209	4723	4806
99	5677	5681	5558	5620
142	5178	4984	4988	4996

Table 3.1, and 3.2 respectively show the anion and cation concentrations, and the alkalinity of the well water, and the community based CBC filter (Operation 1). Anion and cation concentrations were measured throughout the operation period of 142 days. There was no dramatic change in anion and cation concentrations in W/W, F1, F2, and T/W with time. Concentration data of only three different time intervals were shown as representative samples. The alkalinity was measured relevant to three different time intervals, and alkalinity of W/W, F1, F2, and T/W were almost in a similar range. There was no dramatic change in the alkalinity with time.

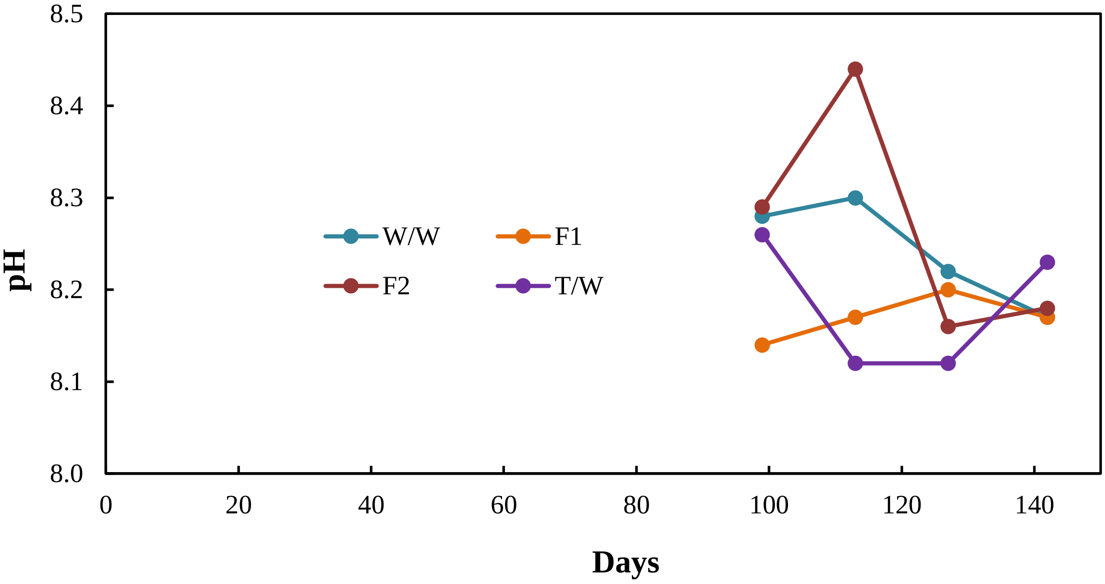

Fig. 3.9: pH of the well water and the community based CBC filter (Operation 1)

Figure 3.9 shows the pH of W/W, F1, F2, and T/W (Operation 1). There was no remarkable variation in pH in W/W, F1, F2, and T/W with time. The pH value of T/W ranges from 8.12-8.26. Sri Lankan standard for pH in drinking water is 6.5-8.5 (pH at 25^0C $\pm 2^0$C) (Sri Lankan Standard Institute 2013). The pH of T/W was within the pH range of Sri Lankan standard.

The first-order reaction was used to evaluate the model for the community based CBC filter. Figure 3.10 shows the evaluated model; the fluoride concentrations in W/W, and at the outlets of F1, F2, and F3 (T/W) with water volume (Operation 1). In Figure 3.10, lines have used to represent the W/W fluoride concentration, and the estimated fluoride concentrations at the outlets of F1, F2, and F3, and square marks have used to represent the W/W fluoride concentration, and the observed fluoride concentrations at the outlets of F1, F2, and F3.

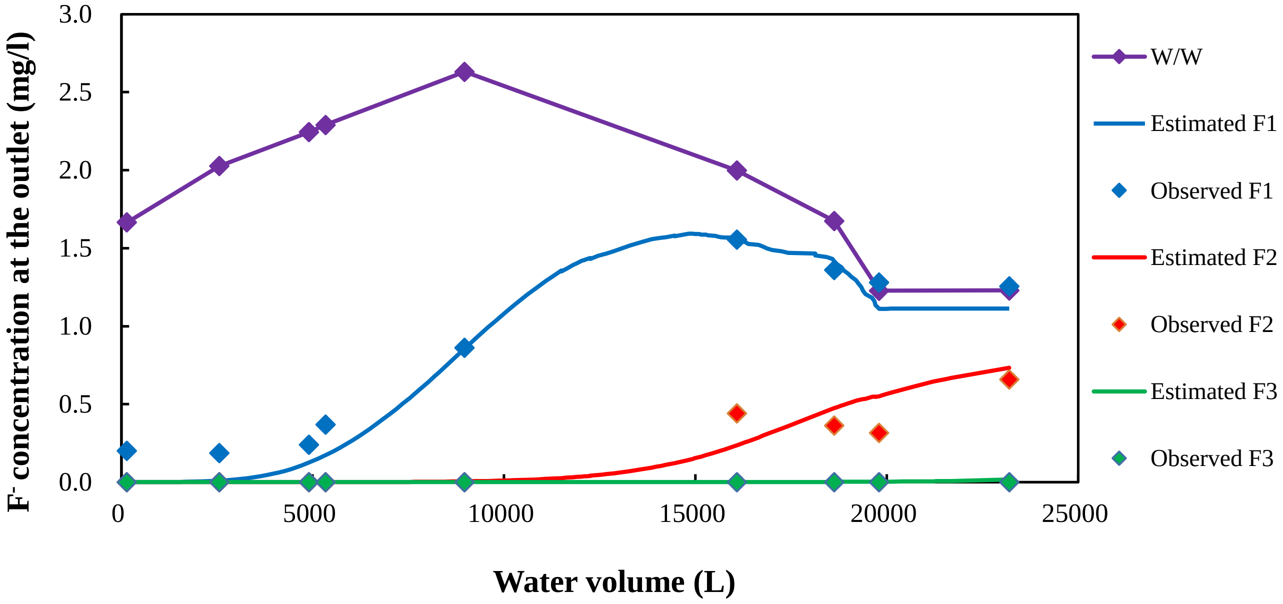

Fig. 3.10: Fluoride concentrations of the well water and at the outlets of the filter (Operation 1)

The first-order reaction is expressed as equation 1.

$$C_t = C_0 e^{-kt} \qquad (1)$$

Where, C_t is the fluoride concentration at time t, C_0 is the initial fluoride concentration, k is the rate constant, and t is the reaction time.

The equation 2, estimating k value, was obtained by several column experiments.

$k = 4.61e^{-3.34x}$ (2)

Where, x is fluoride saturation ratio. The fluoride saturation ratio is the ratio of the amount of adsorbed fluoride (cumulative fluoride) onto CBC to the maximum adsorption capacity of CBC. The cumulative amount of adsorbed fluoride was obtained from the amount of fluoride adsorbed onto CBC. The maximum adsorption capacity of CBC was calculated by the Freundlich isotherm equation.

The fluoride concentration at the outlet, C_{out} was calculated by the k value obtained from equation 2. C_{out} is expressed as equation 3.

$C_{out} = C_0e^{-kt}$ (3)

Where, C_0 is the fluoride concentration in the inlet (well water), and t is the retention time of the water in the CBC filter bed.

The flow rate of the filter was 240 ml/min (operation 1).

The estimated C_0 values for the fluoride concentrations of F1, F2, and F3 was obtained by a step-by-step calculation according to the model. The obtained estimated values for the fluoride concentrations of F1, F2, and F3 was compared with the measured values for the fluoride concentrations of F1, F2, and F3. It was obvious that the estimated values for F1, F2, and F3 were coincided well with observed values of F1, F2, and F3 (Figure 3.10).

3.2.2. Operation 2

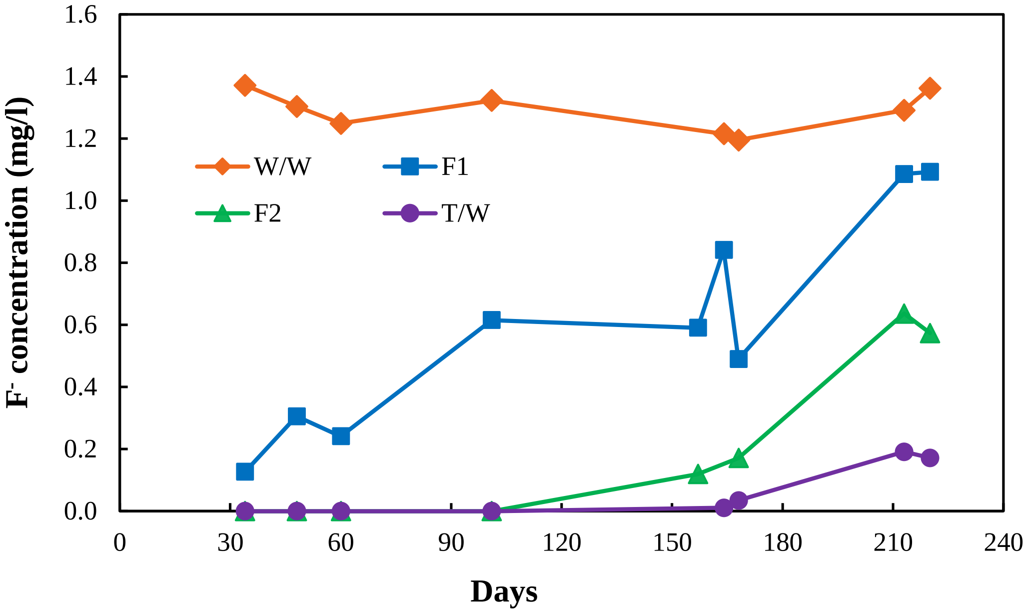

Fig. 3.11: Fluoride concentrations of the well water and the community based CBC filter (Operation 2)

Figure 3.11 shows the fluoride concentrations of W/W, F1, F2, and T/W for 220 days of operation (Operation 2). In operation 2, the CBC filter removed fluoride efficiently to a level much below the Sri Lankan standard as the same manner in operation 1, equivalent to 40366 L. In operation 2, the well water fluoride concentration is almost same throughout the operation period and lower than the operation 1. It was due to the wet climate presence throughout the operation period. The CBC filter has not reached to the equilibrium in operation 2, hence; it is possible to use the filter further without replacing CBC.

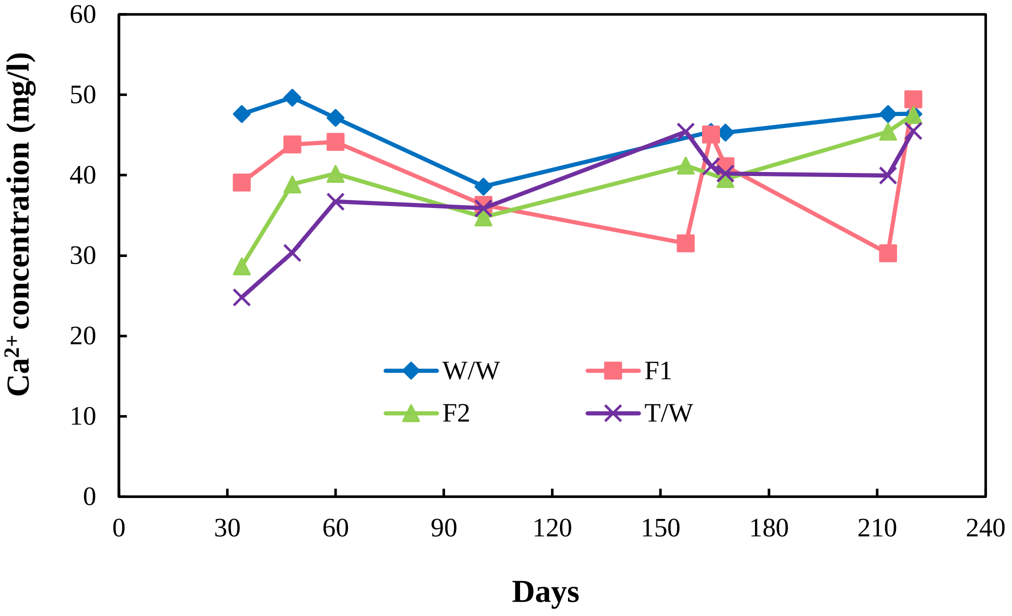

Fig. 3.12: Calcium concentrations of the well water and the community based CBC filter (Operation 2)

Figure 3.12 shows the calcium concentrations of W/W, F1, F2, and T/W for 220 days of operation (Operation 2). According to Figure 3.12, it is obvious that the CBC filter removed calcium efficiency in the former period. In the later period, the removal efficiency was not continuous as in operation 1; it fluctuated with time. The well water calcium concentration was well below the Sri Lankan standard as the same manner in operation 1.

Figure 3.13 shows the magnesium concentrations of W/W, F1, F2, and T/W for 220 days of operation (Operation 2). It is obvious that the CBC filter was not efficiency in removing magnesium as the same manner in operation 1 although the well water magnesium concentration was well below the Sri Lankan standard.

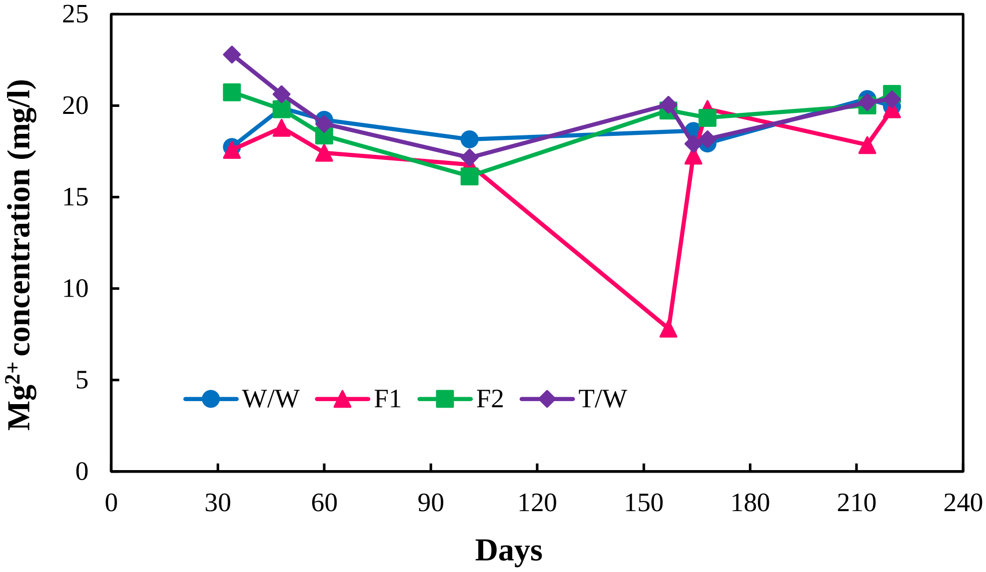

Fig. 3.13: Magnesium concentrations of the well water and the community based CBC filter (Operation 2)

Figure 3.14 shows the hardness of W/W, F1, F2, and T/W for 220 days of operation (Operation 2). The CBC filter removed hardness in the former period. In the later period, the removal was not continuous; it fluctuated with time. The well water hardness was well below the Sri Lankan standard.

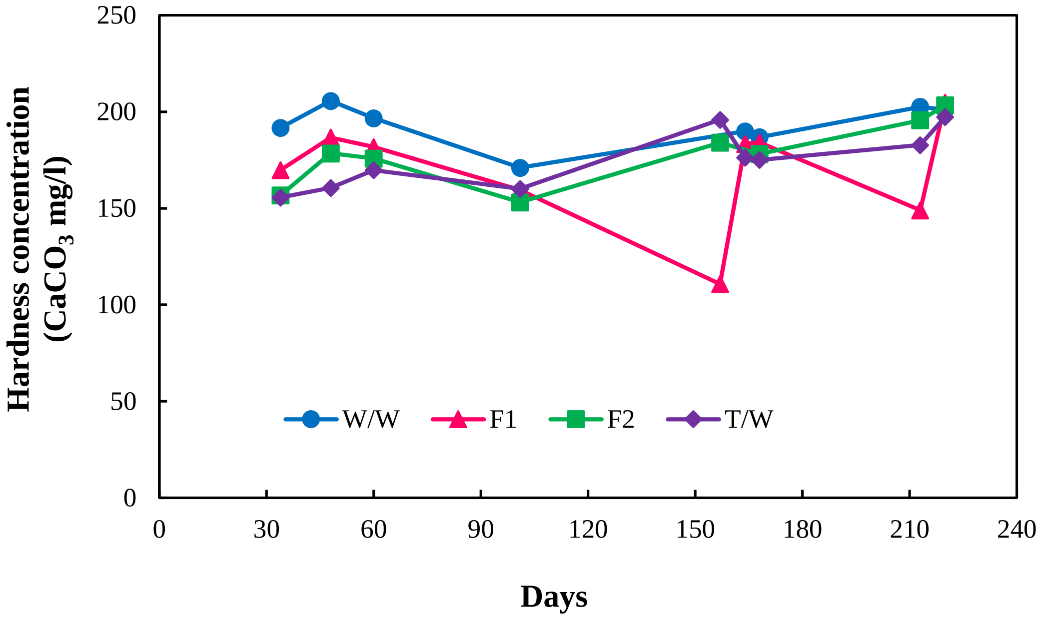

Fig. 3.14: Hardness of the well water and the community based CBC filter (Operation 2)

Table 3.3: Anion and cation concentrations of the well water and the community based CBC filter (Operation 2)

Days		Concentration (mg/l)					
		Cl^-	SO_4^{2-}	NO_3^-	PO_4^{3-}	Na^+	K^+
34	W/W	7	4	1	0	39	0
	F1	8	4	0	0	38	1
	F2	7	4	1	0	38	1
	T/W	7	4	1	0	38	1
101	W/W	7	3	0	0	33	0
	F1	7	3	1	0	34	0
	F2	7	3	0	0	34	0
	T/W	7	3	1	0	36	0
220	W/W	5	2	0	0	37	0
	F1	5	2	0	0	39	0
	F2	5	2	0	0	38	0
	T/W	5	2	0	0	37	0

Table 3.4: Alkalinity of the well water and the community based CBC filter (Operation 2)

Days	Alkalinity (μeq/l)			
	W/W	F1	F2	T/W
34	5152	4732	4483	4493
168	4921	4773	4679	4676
220	5386	5544	5480	5363

Table 3.3, and 3.4 respectively show the anion and cation concentrations, and the alkalinity of the well water, and the community based CBC filter (Operation 2). Anion and cation concentrations were measured throughout the operation period of 220 days. There was no dramatic change in anion and cation concentrations in W/W, F1, F2, and T/W with time as the same manner in operation 1. Concentration data of only three different time intervals were shown as representative samples. The alkalinity was calculated by ion balance calculation for the solutions, relevant to three different time

intervals, and alkalinity of W/W, F1, F2, and T/W were almost in a similar range. There was no dramatic change in the alkalinity with time.

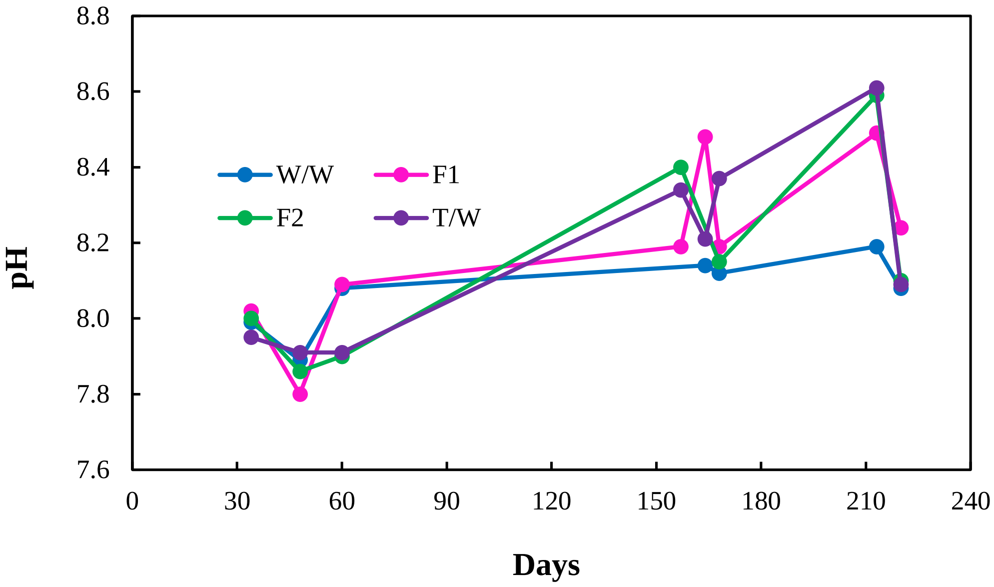

Fig. 3.15: pH of the well water and the community based CBC filter (Operation 2)

Figure 3.15 shows the pH of W/W, F1, F2, and T/W (operation 2). There is no remarkable variation in the pH in W/W, F1, F2, and T/W with time, as the same manner in operation 1. The pH value of T/W ranges from 7.91-8.61. The pH of T/W was slightly higher than the pH range of Sri Lankan standard.

The first-order reaction was used to evaluate the model for operation 2 as the same way in operation 1. Figure 3.16 shows the evaluated model; the fluoride concentrations in W/W, and at the outlets of F1, F2, and F3 (T/W) with water volume (Operation 2). The flow rate of the filter was 308 ml/min (Operation 2). In the evaluated model shown in Figure 3.16, lines have used to represent the W/W fluoride concentration, and the estimated fluoride concentrations at the outlets of F1, F2, and F3, and square marks have used to represent the W/W fluoride concentration, and the observed fluoride concentrations at the outlets of F1, F2, and F3. The estimated values for F1, F2, and F3 were accorded with observed values of F1, F2, and F3.

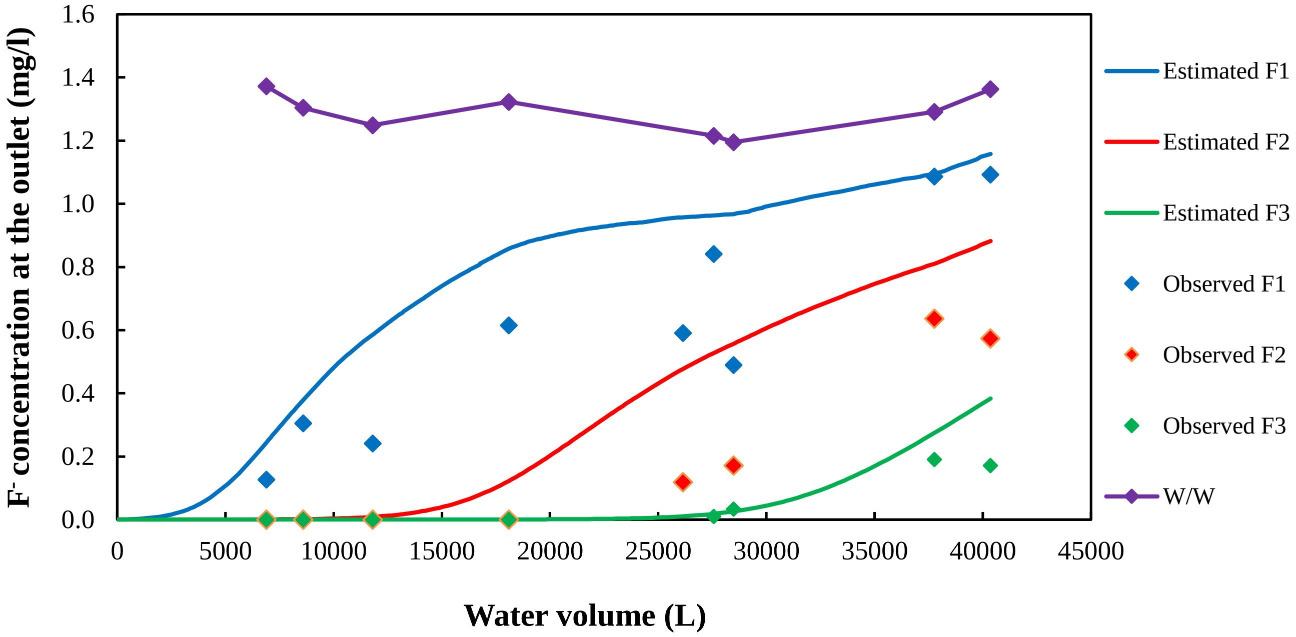

Fig. 3.16: Fluoride concentration of well water and at the outlets of the filter (Operation 2)

4. Conclusions

Most of the groundwater sources in Sri Lanka were contaminated with high concentration of fluoride. Groundwater is the main drinking water source of most areas in Sri Lanka. Fluoride removal from Sri Lankan drinking water is essential to avoid potential risk to human health. CBC was selected as a low cost, and efficiency raw material in removing fluoride from Sri Lankan drinking water. The best carbonizing temperature for the preparation of CBC was investigated, and it was found to be 873 K.

The performance of larger sized CBC was investigated using a community based CBC filter. The particles with a diameter of 5-10 mm was selected as the larger particle size, and it showed an adsorption capacity of 2.45 mg F^-/g CBC. The fluoride concentration of treated water was well below the Sri Lankan standard for fluoride in drinking water. The CBC filter removed fluoride efficiency from drinking water. Further, the CBC filter was not efficiency in removing calcium, magnesium, and hardness in water. The first-order reaction was used to evaluate the model for the CBC filter. The estimated fluoride concentration for the filter was well coincided with the observed fluoride concentration.

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CHAPTER 4

Fluoride Removal by Finer Sized Chicken Bone Char (CBC), and Regeneration of CBC

1. Introduction

Fluoride is widespread in the geological environment (Bhatnagar et al. 2011), and fluoride commonly occurs as fluorspars (CaF_2), cryolite (Na_3AlF_6), and fluorapatite ($Ca_{10}(PO4)_6F_2$) (Nemade et al. 2002). The fluorspars mineral is found in sedimentary rocks, and the cryolite mineral is found in igneous rocks (Mohapatra et al. 2009). The dissolution of fluorine-containing rocks releases fluoride into groundwater (Bhatnagar et al. 2011). The industrial effluents which contain high amount of fluoride discharged into groundwater cause contamination of groundwater (Mohapatra et al. 2009). Groundwater is a main source of fluoride ingestion into the human body (Bhatnagar et al. 2011) where people use groundwater for drinking and cooking (Mjengera and Mkongo 2003).

The WHO guideline for fluoride in drinking water is less than 1.5 mg/l (WHO 2011). Excess fluoride concentrations in groundwaters have been reported in more than 20 developing and developed countries (Meenakshi and Mageshwari 2006). It was estimated that 260 million people in the world are consuming drinking water with a fluoride concentration of more than 1.0 mg/l (Rojas-Mayorga et al. 2013). The fluoride concentration in drinking water is an important factor when determining water quality for human consumption. The ingestion of fluoride into the human body will be either beneficial or detrimental based on its concentration (Medellin-Castillo et al. 2007). Fluoride is an important micro-element for keeping human bones, and teeth in a healthy condition (Feng et al. 2012). An excess amount of fluoride intake causes human health hazards such as dental fluorosis, and skeleton fluorosis (Yadav et al. 2006). Therefore, treatment of fluoride contaminated water is essential, and is one of the paramount environmental concerns in the world (Ma et al. 2008).

The adsorption of fluoride onto bone char is a highly effective, and cost-effective technology that can be used in defluoridation (Phantumvanit and LeGeros 1997). literature reported that finer sized bone char enhances the mechanism of fluoride removal by bone char (Ismail and Abdelkareem 2015). Therefore, in this study finer sized CBC was investigated in fluoride removal as an implementation work of the contents we have discussed in chapter 3.

The continuous usage of bone char in defluoridation causes the exhausted of the bone char media. The exhausted bone char media can either be replaced by newly processed bone char media or regenerated bone char media. Regeneration of exhausted bone char by heating is a new approach in defluoridation (Kaseva 2006).

In this study our attention was focused on the fluoride removal by finer sized CBC in relation to its adsorption capacity, the regeneration of CBC, and the fluoride removal by regenerated CBC.

2. Materials and Methods

2.1. Preparation of Different Particle Sizes of CBC

Eight particle sizes of CBC; with diameters between 5 µm-5 mm were selected for the investigation of best particle size. Prepared CBC was hand crushed in a dry condition by using a mortar, and pestle, and then hand sieved using 10 µm, 20 µm, 45 µm, 106 µm, 212 µm, 1.2 mm, and 5 mm sieves to prepare the particles with diameters of 10 µm, 20 µm, 45 µm, 106 µm, 212 µm, 1.2 mm (1200 µm), and 5 mm (5000 µm). Hand sieving is more reproducible, hence; it is usually recommended for particle preparation (Carpenter and Deitz 1950).

It was impossible to prepare particle size with a diameter of 5 µm by hand crushing in a dry condition, and hand sieving through the 5 µm sieve. Therefore, CBC was sent to Fritsch Co. LTD, Japan, for the preparation of particle size with a diameter of 5 µm. They had wet grinded CBC with 99.5% ethanol by using the Planetary Mono Mill PULVERISETTE 6 Classic Line (Zirconium Oxide 500 cc milling bowl, Zirconium Oxide 30 mm - 5 mm milling ball), and prepared a wet sample of CBC with a diameter of 5 µm. The wet sample was allowed to pass through the 5 µm sieve by adding distilled water, and by continues stirring using a glass rod, and the filtrate was collected to a beaker. Then the filtrate was poured into 50 ml centrifugal vials, and centrifuged in a centrifugal machine (himac compact centrifuges RX ii series) at 7000 rpm for two minutes. Separated particles from centrifugation were dried in an electrical oven at 338 K for 24 hours.

2.2. Investigating the Effect of Particle Size of CBC on Fluoride Removal

Isotherm studies were conducted to investigate the effect of particle size of CBC on fluoride removal. Synthesized drinking water with fluoride (F^-) concentration of 10 mg/l was prepared by using sodium fluoride (NaF), and deionized water for the isotherm studies. Each prepared particle size of CBC were shaken with weights of 0.01 g, 0.02 g, 0.03 g, 0.04 g, 0.1 g, and 0.2 g, and 50 ml of prepared F^- solution in 50 ml vials for 24 hours at different temperatures of 277 K, 283 K, 293 K, 303 K, and 313 K in an electrical shaker with a 30 reciprocation per minute. Low reciprocation was used in order to prevent CBC from crushing during the shaking process. At a time 48 vials were loaded into the shaker with eight particle sizes, and six different weights for each particle size at each temperature. Shaking was done inside a bio-chamber (TAITEC BCP-320 F) which could maintain a constant temperature. Temperature logger was used in the Bio-chamber to check the temperature throughout the shaking process. After 24 hours shaking, fluoride concentration was measured using an ion selective electrode (ORION STAR A324 pH/ISE Meter and ORION 9609BNWP Ionplus Sure-Flow Fluoride Electrode). Total Ionic Strength Adjustment Buffer (TISAB III) was added to each sample before the analysis to avoid the interference during the measurements.

2.3. Fluoride Removal by Finer Sized CBC

CBC with a diameter of 106-212 µm was selected to investigate the effectiveness of defluoridation by finer sized CBC. This was the minimum possible particle size that shows a high adsorption capacity in the defluoridation of drinking water.

Isotherm-analyzing studies were conducted to investigate the effect of Cl^- on the adsorption capacity of the CBC since high concentration of Cl^- was detected in the final solution after F^- adsorption. Synthesized drinking water with a fluoride concentration of 10 mg/l, and Cl^- concentrations of 0 mol/l, 0.01 mol/l, 0.1mol/l, and 1.0 mol/l were prepared by dissolving NaF, and NaCl into deionized water. The CBC with a diameter of 106-212 µm was shaken with weights of 0.03 g, 0.04 g, 0.1 g, 0.2 g, 0.3 g, and 0.5 g and 50 ml of the solution in 50 ml vials in an electrical shaker with a 150 reciprocation. After 48 hours of shaking, the fluoride concentration was measured using the ion selective electrode.

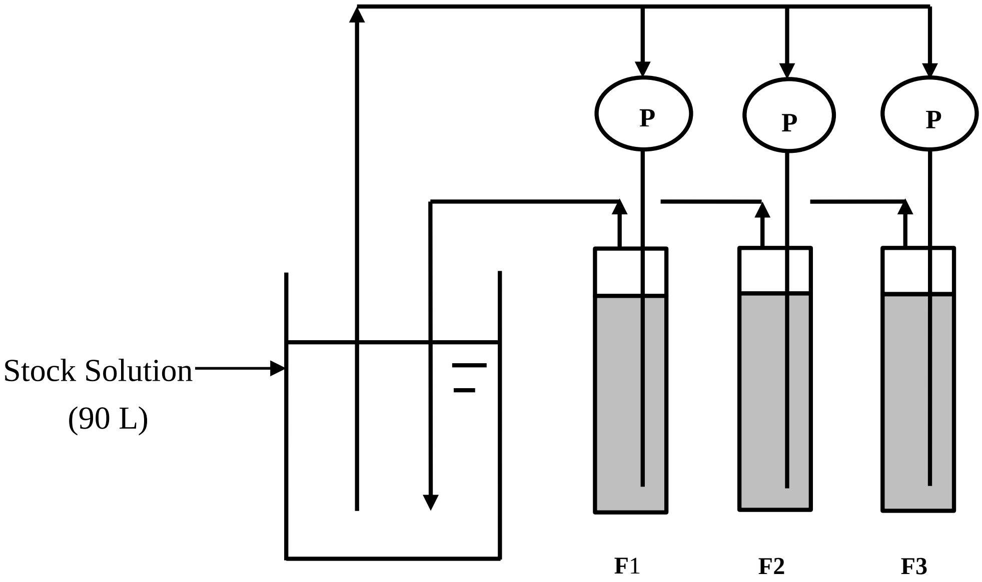

Fig. 4.1: Schematic diagram of the laboratory scale CBC column filter

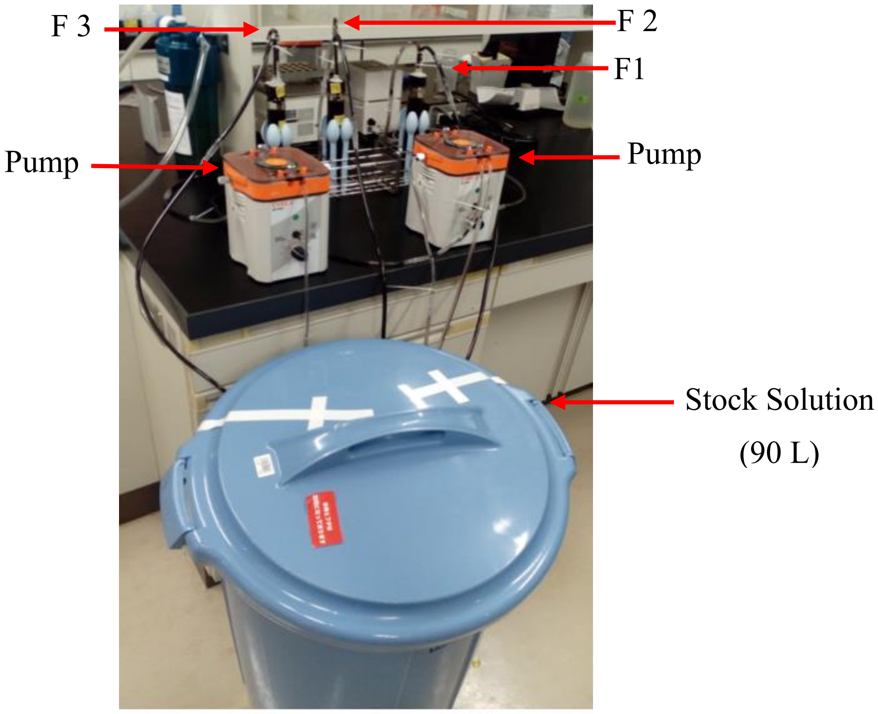

Fig. 4.2: Photograph of the laboratory scale CBC column filter

Figure 4.1 shows the schematic diagram of the laboratory scale CBC column filter, and Figure 4.2 shows the photograph of the laboratory scale CBC column filter. Three plastic vessels (F1, F2, and F3) with a bed height of 8 cm, and a diameter of 6.5 cm were used for the experiment. Fifty-five grams of CBC with a diameter of 106-212 µm was placed into each column. Synthesized drinking water with a fluoride concentration of 20 mg/l (stock solution) prepared by sodium fluoride (NaF), and deionized water was allowed to pass through each column with a flow rate of 2.4 l/day via a pump (P). The outlets of the F1, F2, and F3 columns were connected to the bucket for the stock solution. The three plastic vessels (F1, F2, and F3) with a bed height of 8 cm, and a diameter of 6.5 cm were changed into three glass columns (F1, F2, and F3) with a bed height of 21 cm, and diameter of 3 cm, after 20 days of operation for a uniform flow. The same amount of CBC was placed into the glass columns, and the same flow rate was maintained. The fluoride concentration of the stock solution, and the outlets were regularly measured using the ion selective electrode. The pH of the solution was measured using the pH meter (ORION STAR A324 pH/ISE Meter and Beckman Electrode 511070). EC of the solutions was measured using a conductivity meter (HORIBA Twin cond B-173). Since after the 48-day operation, the fluoride concentration was found to be decreased to a level below 10 mg/l, NaF equivalent to the concentration of 10 mg/l was added to the stock solution. F^- was added to the solution at days 58, 106, 130, 132, 139, and 143 by using NaF to keep the F^- concentration around 10 mg/l.

The synthesized fluoride solution was allowed to pass upward through each column to avoid overflowing in the case of clogging of the CBC media, and to provide a uniform flow across the cross-sectional area of each column. This strategy of upward flow was consistent with that in the study by Mjengera and Mkongo, who used cow bone char to remove fluoride from water. They also mentioned that very fine particles are not recommended for use in column experiments because of the tendency of the fine particle sizes to clog (Mjengera and Mkongo 2003). In our study, we used a fine particle size of CBC, 106-212 µm in diameter, in columns with regular ultra-sonication to successfully avoid the clogging.

Chemical composition analysis, X-ray diffraction (XRD) pattern analysis, scanning electron microscope (SEM) analysis, and Brunauer-Emmett-Teller (BET) surface area analysis were conducted for 106-212 µm CBC used for the study. CBC particles before the fluoride adsorption were analyzed as a control.

The chemical composition of CBC, and the solutions were analyzed by using ion chromatography (for anions: Dionex ICS-2000, separation column IonPac AS18, eluent KOH 23-40 mmol/l (gradient), suppressor ASRS 300 4mm; for cations: Dionex ICS-1500, separation column IonPac CS12, eluent methanesulfonic acid 30 mmol/l (isocratic), suppressor CSRS 500 4mm). Samples (0.1 g) were digested with 5% nitric acid to analyze the anions and cations in CBC other than F^-. The F^- in CBC was determined after the steam distillation (JIS K0102). The followings were kept at 418 K in a flask: 0.1 g of CBC, 40 ml of $HClO_4$, 1 g of SiO_2, 1 ml of H_3PO_4, and 10 ml of distilled water. Steam was introduced into the flask to vaporize F^-. The vaporized F^- was condensed with steam in a water-jacketed condenser for measurement by ion chromatography. The carbon (C), hydrogen (H), nitrogen (N), and carbonate (CO_3^{2-}) contents of CBC were analyzed using a CHN Corder (Yanaco, MT - 5). The CO_3^{2-} content of CBC was analyzed by the CHN Corder after heating chicken bones to 973 K under an aerobic condition in an electrical muffle furnace to remove organic carbon. The XRD of hydroxyapatite (HAP), and CBC were analyzed using MiniFlex (Rigaku Co.); X-ray 30kV/15mA, radiation CuK alpha line (Ni filter), scintillation detector. SEM images of the CBC were taken using a VE-8800 (Keyence Co.). The BET surface area of the CBC was analyzed using the NOVA 3200e Surface Area & Pore Size Analyzer (Quantachrome Instruments).

2.4. Regeneration of CBC, and Fluoride Removal

Regeneration of CBC, and fluoride removal by regenerated CBC was studied using two experimental setups. Heat treatment of CBC was selected as the regeneration method in the study because of ease operation, and cost effectiveness. Since the heat regeneration of CBC is not a chemical treatment method, the release of chemical components to treated water during a chemical regeneration may not occur. Therefore, the treated water may safe for human health than from a chemical treatment.

Exhausted CBC was prepared by dipping CBC for several days in a solution containing fluoride. In Experiment 1, F^- exhausted CBC was regenerated at 3 temperatures: 673 K, 773 K, and 873 K, and studied the fluoride adsorption capacity of regenerated CBC. The selected best regenerating temperature from Experiment 1 was further investigated in regeneration of CBC, and fluoride adsorption in Experiment 2.

2.4.1. Experiment 1

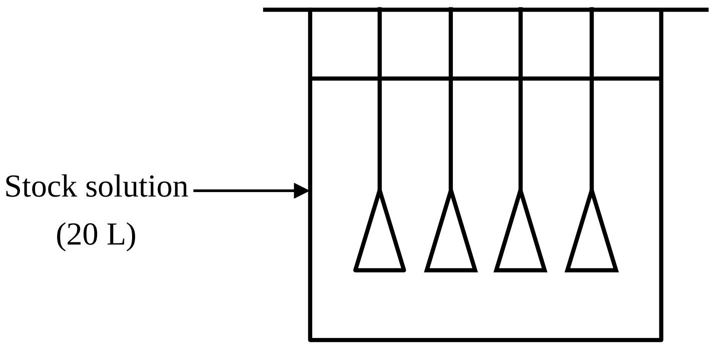

Fig. 4.3: Schematic diagram of the setup of preparation of fluoride exhausted CBC (Experiment 1)

CBC with a diameter of 5-10 mm was saturated with fluoride in order to obtain fluoride-exhausted CBC. Figure 4.3 shows the schematic diagram of the setup of preparation of fluoride-exhausted CBC (Experiment 1). Four tea bags filled with CBC were used for the experiment. 15 g of CBC was used in each tea bag. Four tea bags filled with CBC were dipped in the 20 L solution with a fluoride concentration of 20 mg/l (stock solution) prepared by dissolving sodium fluoride (NaF) into tap water. The experimental setup was allowed to adsorb fluoride for 5 days, and the fluoride concentration of the solution was regularly measured using the ion selective electrode.

Exhausted CBC was taken out from tea bags after 5 days, and all the four sets were dried at 338 K temperature in an electrical oven for 24 hours. Three samples of 15 g of exhausted CBC were reactivated by heat at 3 temperatures: 673 K, 773 K, and 873 K under an anaerobic condition in closed metal containers in an electrical muffle furnace for 2 hours. The remaining sample of 15 g of exhausted CBC was kept as the control setup without reactivated by heat.

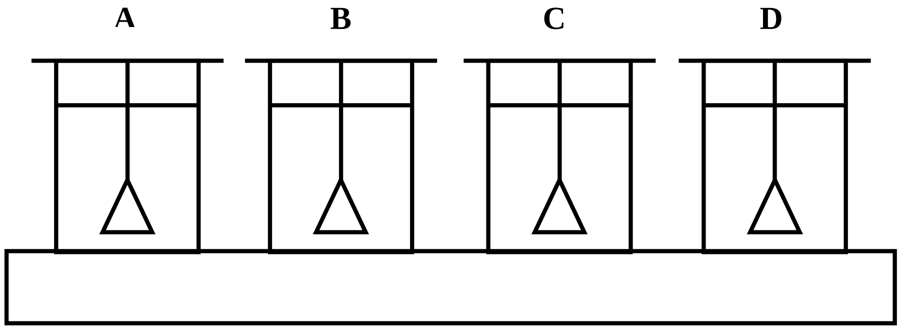

Fig. 4.4: Schematic diagram of the adsorption setup (Experiment 1)
*A: control setup, B:673 K regenerated setup, C:773 K regenerated setup, D:873 K regenerated setup

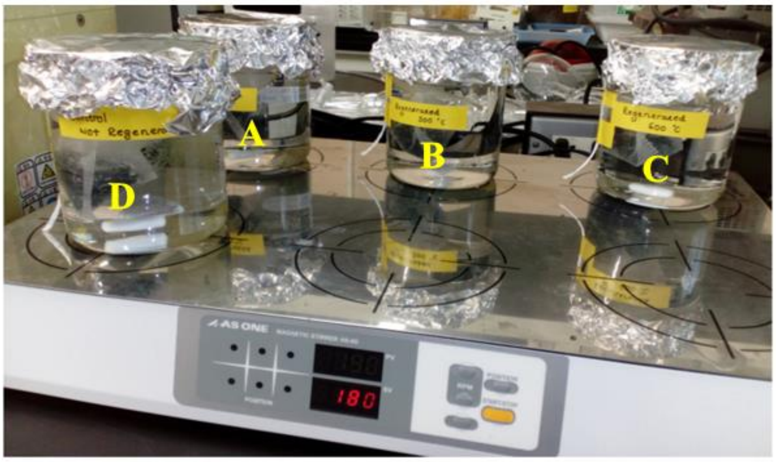

Fig. 4.5: Photograph of the adsorption setup (Experiment 1)
*A: control setup, B:673 K regenerated setup, C:773 K regenerated setup, D:873 K regenerated setup

Figure 4.4 shows the schematic diagram of the adsorption setup, and Figure 4.5 shows the photograph of the adsorption setup (Experiment 1). Four tea bags filled with CBC, and four 1 L beakers were used for the experiment. Ten grams of each regenerated CBC was used in three tea bags, and 10 g of CBC left without regenerated (control) was used in one tea bag. 800 ml of the same solution which used to prepare fluoride-exhausted CBC was used in each beaker. The four tea bags filled with CBC were dipped in solutions in four beakers as shown in Figures 4.4, and 4.5. Four setups were allowed to shake with a 270 reciprocation on a magnetic stirrer. A, B, C, and D, in Figure 4.4, and 4.5, respectively represent the control set up, 673 K regenerated setup, 773 K regenerated setup, and 873 K regenerated setup.

Fluoride concentrations of the solutions were regularly measured using the ion selective electrode. The pH of the solutions was measured using the pH meter. Anion and cation concentrations of the solutions were measured using ion chromatography.

Isotherm studies were conducted for each set of regenerated CBC at 673 K, 773 K, 873 K, and for the control setup to investigate the adsorption capacities of CBC at different temperatures. Synthesized drinking water with fluoride (F^-) concentration of 10 mg/l was prepared by using sodium fluoride (NaF), and deionized water for the isotherm studies. Each set of regenerated CBC with a diameter of less than 45 µm was shaken with weights of 0.03 g, 0.04 g, 0.1 g, 0.2 g, 0.3 g, and 0.5 g, and 50 ml of prepared F^- solution in 50 ml vials for 48 hours in an electrical shaker with a 90 reciprocation. After 48 hours shaking, fluoride concentration was measured using the ion selective electrode.

The chemical composition (anions and cations) of CBC was analyzed after digesting the samples (0.1 g) with 5% nitric acid. The X-ray diffraction (XRD) pattern analysis were conducted for regenerated CBC at 673 K, 773 K, 873 K, and for the control setup to investigate the structural difference of CBC after regeneration. The XRD pattern of initial CBC was analyzed as a control.

2.4.2. Experiment 2

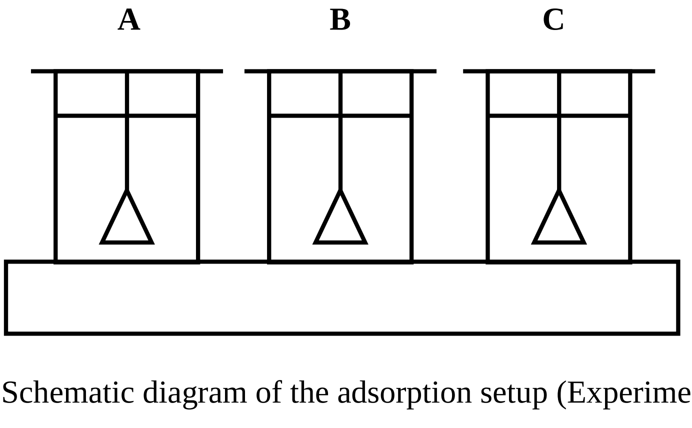

Fig. 4.6: Schematic diagram of the adsorption setup (Experiment 2)
*A: control setup, B:338 K regenerated setup, C:673 K regenerated setup

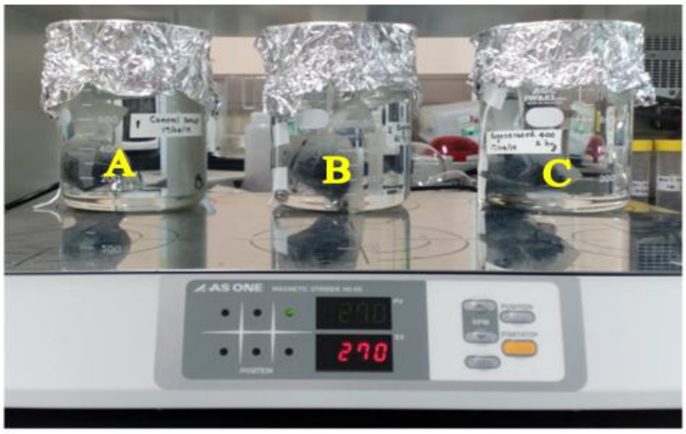

Fig. 4.7: Photograph of the adsorption setup (Experiment 2)
*A: control setup, B:338 K regenerated setup, C:673 K regenerated setup

Three setups (A, B, and C) of CBC with a diameter of 5-10 mm were used for the experiment. Three tea bags filled with 10 g of CBC were dipped in 1 L of solution with a fluoride concentration of 20 mg/l prepared by dissolving sodium fluoride (NaF) into tap water, in three 1 L beakers to obtain fluoride-exhausted CBC. The experimental setup was allowed to adsorb fluoride for 13 days. Three setups were allowed to shake with a 270 reciprocation on a magnetic stirrer. The fluoride concentrations of the solutions were regularly measured using the ion selective electrode.

Exhausted CBC in setups B, and C were taken out from tea bags after 13 days, and dried at 338 K temperature in an electrical oven for 24 hours. CBC in the setup C was reactivated by heat at 673 K for 2 hours as the same manner mentioned previously, after dried at 338 K in an electrical oven for 24 hours. Both sets of CBC (B, and C) were dipped in the same solutions in setups B, and C after heat treatment and continued the experiment. The setup A was continued as the control setup without regeneration. Figure 4.6 shows the schematic diagram of the adsorption setup, and Figure 4.7 shows the photograph of the adsorption setup (Experiment 2). A, B, and C, in Figure 4.6, and 4.7, respectively show the control setup, 338 K regenerated setup, and 673 K regenerated setup.

Fluoride concentrations of the solutions were regularly measured using the ion selective electrode. The pH of the solutions was measured using the pH meter. Anion and cation concentrations of the solutions were measured using ion chromatography. The F^- in CBC was determined by steam distillation as mentioned previously and also by acid digestion of 0.1 g of sample with concentrated nitric acid for measurement by the ion selective electrode. SEM images were analyzed for the control CBC, and 673 K regenerated CBC after the adsorption.

3. Results and Discussion

3.1. Investigating the Effect of Particle Size of CBC on Fluoride Removal

Freundlich isotherm was evaluated to investigate the adsorption capacities of the eight particle sizes of CBC at each temperature. Obtained results were summarized in Figures 4.8, and 4.9. Figure 4.8 shows the adsorption capacities of particle sizes of CBC against the temperature at an equilibrium fluoride concentration of 4 mg/l. It was obvious that adsorption capacity increased with decreasing particle size. The particles with a diameter of 10 µm showed the highest adsorption capacity, and particles with diameters of 1.2 mm and 5 mm showed the lowest adsorption capacity compared to other particle sizes, since the particles with a diameter of 10 µm was the best particle size of CBC.

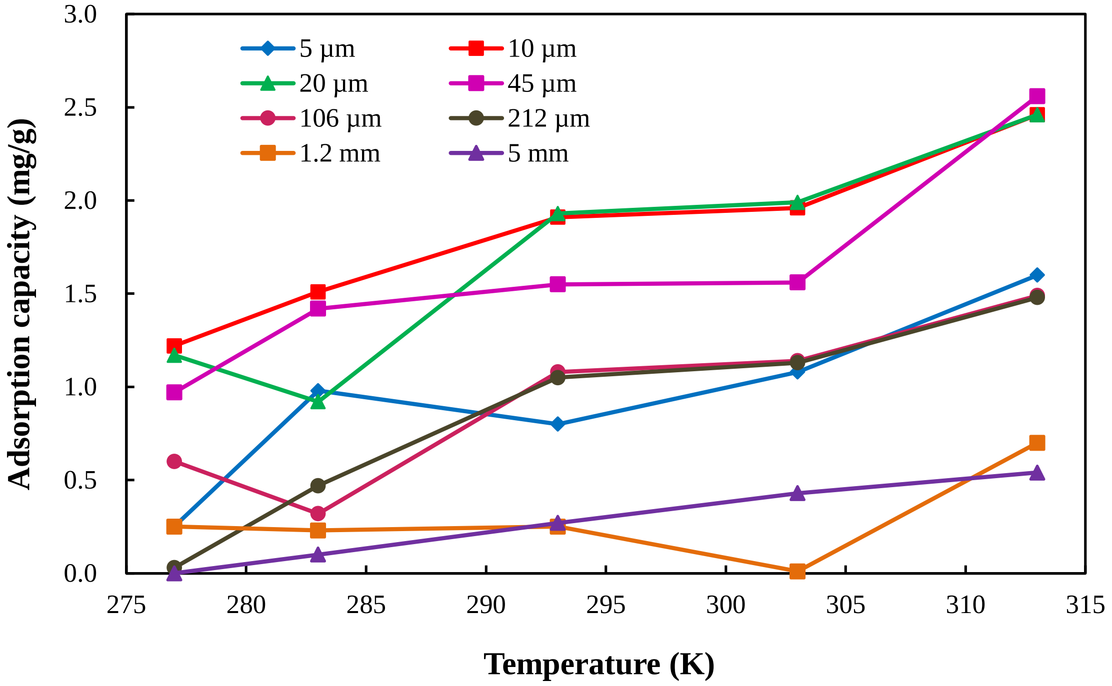

Fig 4.8: Adsorption capacities of particle sizes of CBC against the temperature

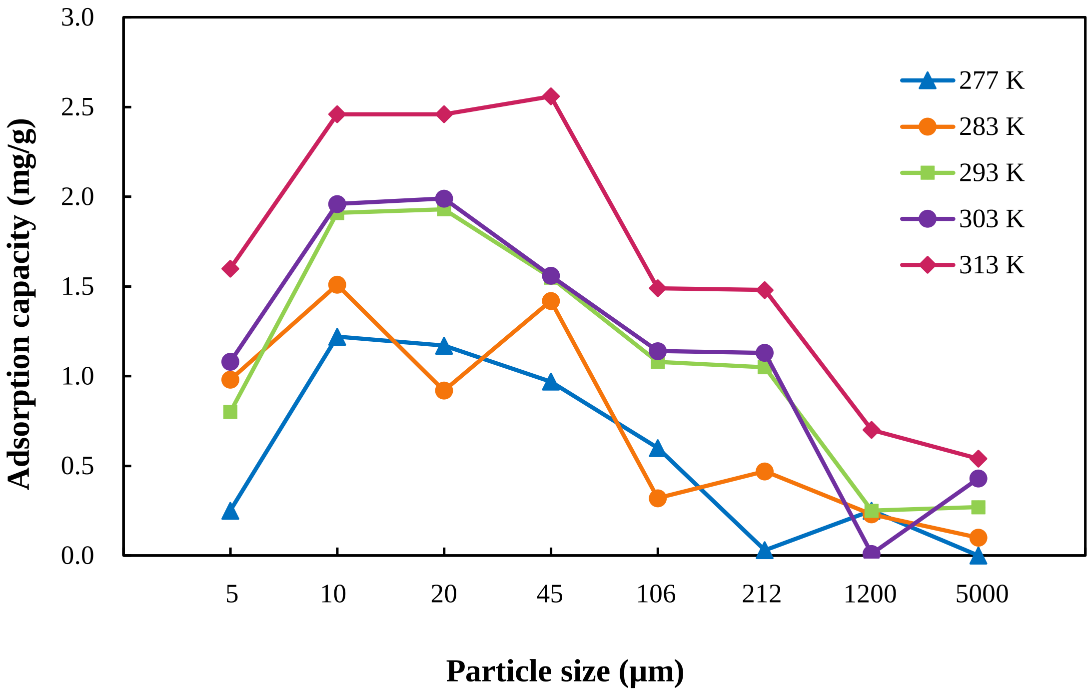

Fig 4.9: Adsorption capacities at different temperature against the particle sizes of CBC

Figure 4.9 represents the adsorption capacities at different temperature against the particle sizes of CBC at the equilibrium fluoride concentration of 4 mg/l. The graph shows that all the particle sizes showed the maximum adsorption capacities at the highest temperature of 313 K. This result indicates that the adsorption mechanism of fluoride ion onto CBC is an endothermic reaction.

Based on the obtained results minimum possible particle size (finer size) was selected to investigate the performance of CBC in fluoride removal in a laboratory scale constructed filter.

A laboratory scale CBC column filter was designed to evaluate the performance of finer sized CBC. The selected best particle size with a diameter of 10 µm which shows the highest adsorption capacity was impossible to produce in a larger scale. The particle sizes with diameters of 20 µm, and 45 µm, which shows the highest adsorption capacities next to 10 µm were also impossible in large scale production. When consider particle sizes with diameters of 106 µm, and 212 µm, those particle sizes showed almost a similar adsorption capacity in 313 K, 303 K, and 293 K (Figure 4.9). Therefore, the particle size with a diameter of 106–212 µm was selected as the minimum possible particle size for the laboratory scale CBC column filter.

3.2. Fluoride Removal by Finer Sized CBC

Freundlich, and Langmuir isotherm models were evaluated to investigate the adsorption capacity of CBC. Figure 4.10 shows the Freundlich isotherm for CBC. Figure 4.11 shows the Langmuir isotherm for the CBC. In the Figures, C is fluoride concentration in the solution expressed in the unit of mg/l. Q is maximum adsorption capacity expressed in the unit of mg/g.

The Langmuir isotherm model was found to be a better fitted than the Freundlich isotherm model. According to the Freundlich, and Langmuir isotherms, the adsorption capacity of CBC at the equilibrium fluoride concentration of 10 mg/l was 5.35 mg/g, and 5.88 mg/g, respectively.

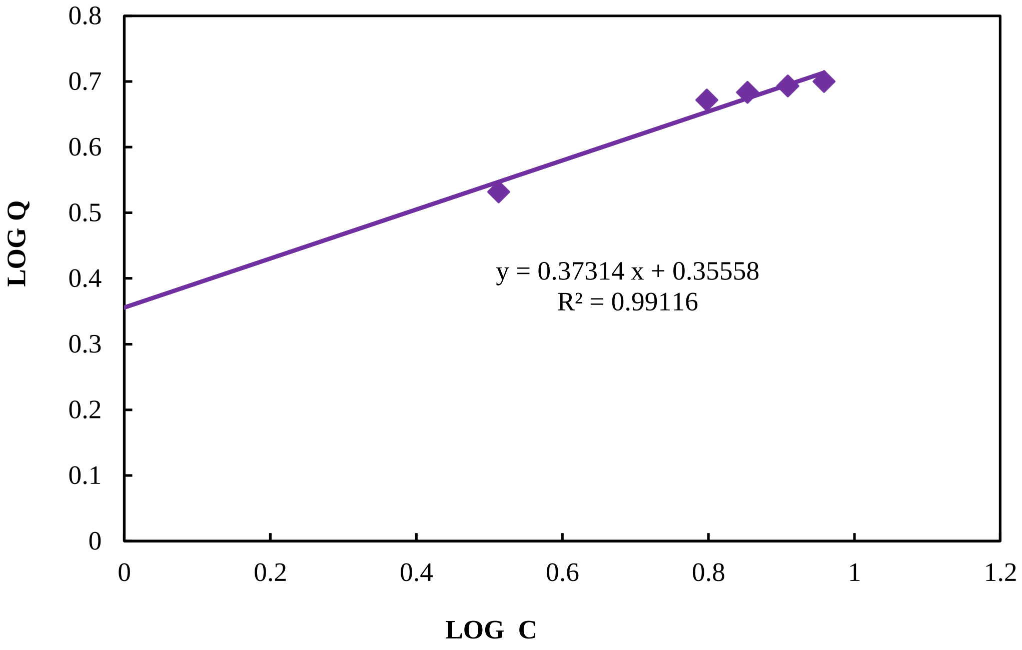

Fig. 4.10: Freundlich isotherm for CBC

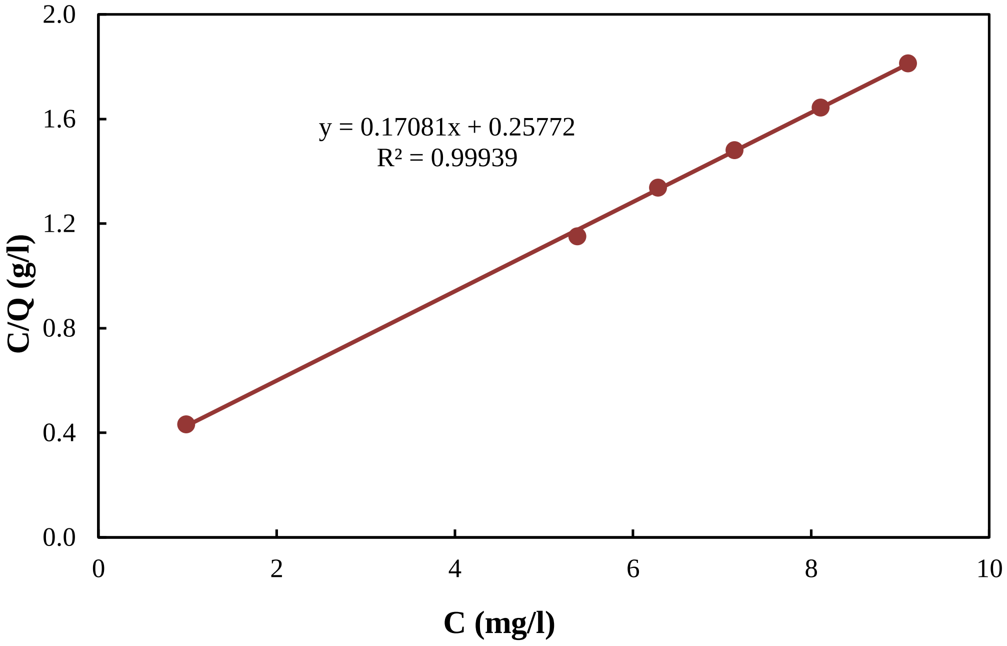

Fig. 4.11: Langmuir isotherm for CBC

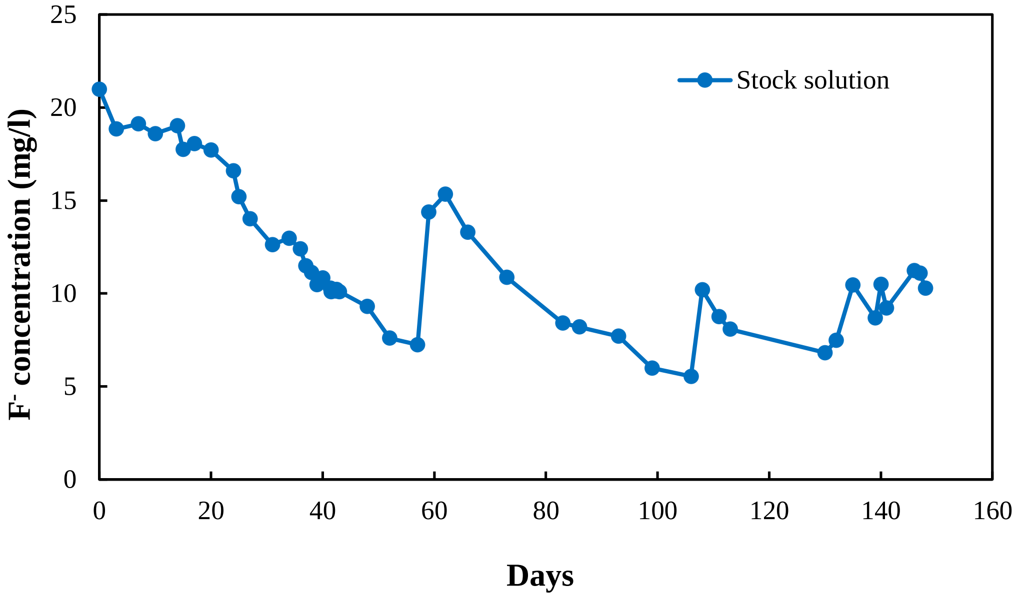

Fig. 4.12: Change in the fluoride concentration of the stock solution

Table 4.1: Amount of F^- in the solution

	mg
F^- added to the solution	2831
F^- lost from the solution by leakage	492
F^- remaining in the solution	483
F^- adsorbed by CBC (55 g*3)	1855

Figure 4.12 shows the change in the fluoride concentration of the stock solution throughout the operation period. Table 4.1 gives a detailed description of the amount of F^- in the solution throughout the operation period. A certain amount of F^- was lost from the stock solution due to leakages, and this was considered when calculating the adsorption capacity. The CBC particles with a diameter of 106-212 µm showed an adsorption capacity of 11.2 mg/g at a fluoride concentration of 10 mg/l after the operation period of 148 days. The adsorption capacity was calculated according to the data in Table 4.1 (1855 mg F^-/(55*3) g CBC). It is obvious that the CBC showed an unusually high adsorption capacity, nearly 2 times the adsorption capacity obtained by the Langmuir isotherm, indicating that the equilibrium was not established within 24 hours. To confirm the unusual fluoride adsorption capacity, the fluoride content in the CBC before, and after 148 days' operation was measured using the steam distillation method.

Table 4.2: Averaged value of adsorption capacities of F1, F2, and F3

F^- adsorption capacity (mg/g)				
F^- content in CBC before adsorption	F^- content in CBC after adsorption		Net adsorption	Averaged value
0.46	F1	12.13	11.67	11.1
	F2	12.05	11.59	
	F3	10.43	9.97	

Table 4.2 shows the averaged value of the adsorption capacities of F1, F2, and F3 after the adsorption. An adsorption capacity of 11.1 mg/g coincided well with the adsorption capacity obtained from the mass balance calculation for the solution, as shown in Table 4.1.

According to studies relating to the fluoride adsorption capacity of bone char, CBC with a size of >0.075 mm, 0.075-0.30 mm, 0.30-1.18 mm, and 1.18-2.34 mm respectively showed a fluoride adsorption capacity of 0.665 mg/g, 0.661 mg/g, 0.660 mg/g, and 0.643 mg/g at the equilibrium fluoride concentration of 10 mg/l (Ismail and Abdelkareem 2015). They also reported the fluoride adsorption capacity of lamb bone char with sizes of >0.075 mm, 0.075-0.30 mm, 0.30-1.18 mm, and 1.18-2.34 mm for which the fluoride adsorption capacity was 0.482 mg/g, 0.475 mg/g, 0.459 mg/g, and 0.414 mg/g respectively, at the equilibrium fluoride concentration of 10 mg/l (Ismail and Abdelkareem 2015).

In a study using 0.79 mm cattle bone char particles, 2.71 mg/g was recorded at the equilibrium fluoride concentration of 1 mg/l (Medellin-Castillo et al. 2007). Rojas-Mayorga and his fellow researchers showed a fluoride adsorption capacity of 7.32 mg/g by using ~1 mm cow bones at the equilibrium fluoride concentration of 60 mg/l (Rojas-Mayorga et al. 2013).

No studies have reported such an unusually high fluoride adsorption capacity of bone char.

Figure 4.13 shows the X-ray diffraction patterns of CBC before, and after the fluoride adsorption.

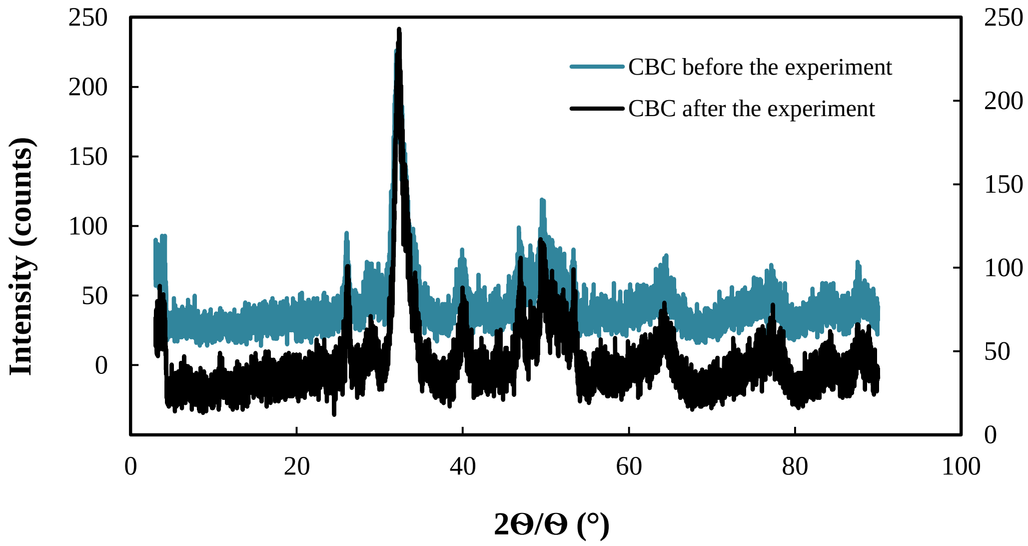

Fig. 4.13: X-ray diffraction patterns for the CBC before and after the fluoride adsorption

*CBC before the experiment (left Y axis), CBC after the experiment (right Y axis)

According to Figure 4.13, it is clear that the two X-ray diffraction patterns of CBC before, and after the fluoride adsorption are overlapping together showing that the structure of CBC before, and after the fluoride adsorption is similar.

It was reported that fluoride removal by bone char is a surface reaction process (Kaseva 2006). Table 4.3 shows the BET surface area of CBC before, and after the fluoride adsorption, with the surface area of 1mm CBC also represented for comparison.

Table 4.3: BET surface area of CBC before and after the fluoride adsorption

Particle size		BET surface area (m^2/g)
1 mm	CBC	145
106-212 µm	CBC before the fluoride adsorption	126
	CBC after the fluoride adsorption	136

According to Table 4.3, the finer particle size of CBC, 106-212 µm, before, and after the fluoride adsorption showed a similar surface area. The surface area of the larger CBC was also similar to the surface area of the smaller 106-212 µm CBC.

SEM images of 106-212 µm CBC used for the study were taken in two different stages to compare the surface, morphology, and size distribution. Figures 4.14, and 4.15 respectively show SEM images of the CBC before, and after fluoride adsorption.

Fig. 4.14: SEM image of the CBC before fluoride adsorption

Fig. 4.15: SEM image of the CBC after fluoride adsorption

The SEM images in Figures 4.14, and 4.15 show similar structures of CBC, as evidence from the similar X-ray diffraction patterns for the CBC before, and after the fluoride adsorption in Figure 4.13. This was further confirmed by the almost equal surface area of CBC before, and after the fluoride adsorption as shown in Table 4.3.

It was reported in the literature that fluoride removal by bone char (CBC) is associated with the two main mechanisms of ion exchange, and chemical precipitation. In the presence of fluoride ion, the hydroxyl ion in HAP is replaced by fluoride ion to form insoluble fluorapatite (FAP) (Ismail and Abdelkareem 2015), and release the hydroxyl ion into the solution. F^- and OH^- consist of the same charge, and a similar size of radius. Therefore, the fluoride ion can replace the hydroxyl ion in mineral structures (Brunson and Sabatini 2009).

The relevant chemical reaction can be represented in equation (1) (Fawell et al. 2006):

$$Ca_{10}(PO_4)_6(OH)_2 + 2\,F^- \longrightarrow Ca_{10}(PO_4)_6F_2\downarrow + 2\,OH^- \qquad (1)$$

In the presence of an excess fluoride ion, HAP precipitates into calcium fluoride (CaF_2), and the phosphate in HAP is released into the solution.

The relevant chemical reaction can be represented in equation (2) (Brunson and Sabatini 2009):

$$Ca_{10}(PO_4)_6(OH)_2 + 20\,F^- + 2\,H^+ \longrightarrow 10\,CaF_2\downarrow + 6PO_4^{3-} + 2\,H_2O \qquad (2)$$

According to the similar XRD patterns, SEM images, and BET surface area of CBC, there was no evidence indicating that the formation of CaF_2 took place.

Table 4.4 shows the anion and cation concentrations of the solution before, and after the adsorption. A detailed description of the fluoride concentration in the solution was given in Table 4.1. The increase of Na^+ in the final solution is mainly due to the addition of NaF to the solution to maintain the fluoride concentration. Cl^-, K^+, Mg^{2+}, and Ca^{2+} ions, which were not present in the initial solution, were detected in the final solution after 148 days' operation. This was due to the dissolution of those ions to the final solution from CBC as we detected them as the components in CBC. A certain amount of Na^+ may also be released into the solution by the dissolution from CBC, as we also detected Na^+ as a trace component in the CBC.

According to equation (2), the phosphate in HAP should be released into the solution with the formation of CaF_2. To the contrary, there was no evidence of phosphate in the solution.

Table 4.4: Anion and cation concentrations of solution used for the experiment

	Concentration (mg/l)							
	F^-	Cl^-	PO_4^{3-}	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
Initial solution	20	0	0	19	0	0	0	0
Final solution	10	87	0	61	0	3	5	10

According to the solubility product constant (Ksp) of CaF_2, and the molar concentrations of Ca^{2+}, and F^- in the final solution, there was a possibility that CaF_2 precipitated in the solution due to the reaction of F^- in the solution, and released Ca^{2+} from the CBC to the solution. The Ksp of CaF_2 ($3.4*10^{-11}$ mol^3/l^3) was calculated from the solubility of CaF_2 (0.016 g/l in water at 20 ^0C). The molar concentrations of Ca^{2+}, F^- in the final solution was calculated as $2.7*10^{-10}$ mol^3/l^3, which exceeded the Ksp value. However, there was no visible CaF_2 precipitation in the experimental setup.

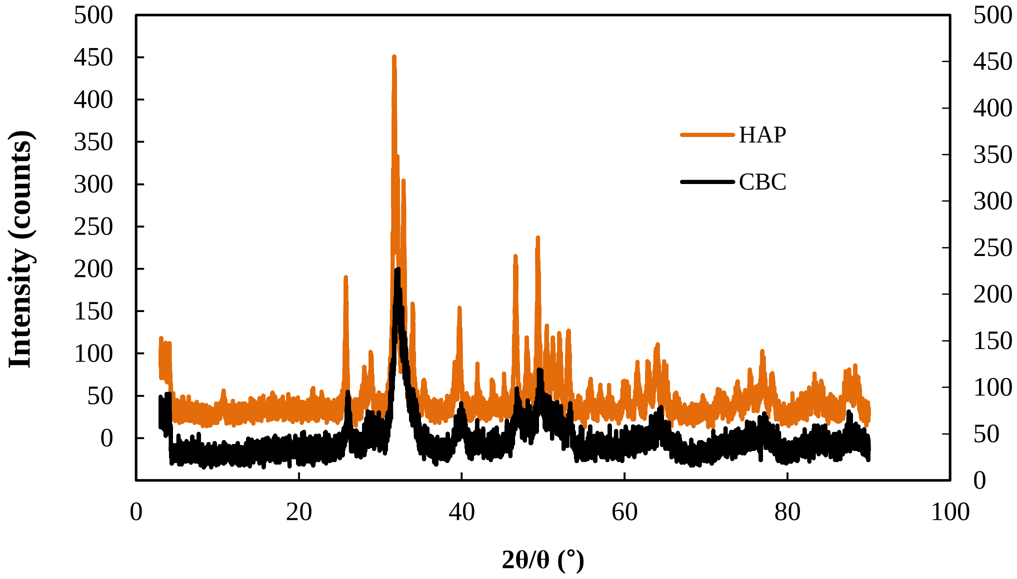

Fig. 4.16: XRD patterns of the HAP and CBC
*HAP (left Y axis), CBC (right Y axis)

Figure 4.16 shows the XRD patterns of the HAP, and CBC. Their similar patterns indicate that the major component of CBC was HAP. Table 4.5 shows the number of moles of PO_4^{3-}, Ca^{2+}, F^-, and OH^- in 100 g of CBC before, and after the fluoride adsorption based on the chemical analysis. The Ca^{2+}/PO_4^{3-} molar ratio of 1.86 for CBC (before the fluoride adsorption) was similar to that of 1.67 for hydroxyapatite: $[Ca_{10}(PO_4)_6(OH)_2]$ (HAP).

The number of moles of OH^- in CBC before the fluoride adsorption was calculated based on the molar ratio of $Ca^{2+}:OH^-$ (10:2) before the fluoride adsorption, assuming that the major component of CBC is hydroxyapatite. The number of moles of OH^- in CBC after the fluoride adsorption was calculated based on the molar ratio of $Ca^{2+}:OH^-$ (10:2) and by reducing the F^- moles.

Table 4.5: No. of moles of PO_4^{3-}, Ca^{2+}, F^-, and OH^- in CBC before and after the fluoride adsorption

	No. of moles in 100 g of CBC			
	PO_4^{3-}	Ca^{2+}	F^-	OH^-
CBC before the fluoride adsorption	0.371	0.691	0.002	0.138
CBC after the fluoride adsorption	0.351	0.684	0.061	0.075

According to the chemical composition, CBC contained 65.3% HAP, and 9% of C on a weight basis. The percentage of HAP in the CBC was calculated by the sum of the percentages of Ca^{2+} (27.7%), and PO_4^{3-} (35.2%) in the CBC digested with nitric acid, and OH^- (2.4%) which was calculated from the molar ratio of Ca^{2+}:OH^- (10:2). The result obtained in our study is consistent with the literature. Brunson and Sabatini, and Abe et al. have mentioned that bone char contains approximately 75% of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], 9-11% of calcite ($CaCO_3$) (Brunson and Sabatini 2009), and 8-10% C (Abe et al. 2004). Further, we could detect 0.6% Mg^{2+}, 0.5% Na^+, 0.1% K^+, 0.1% Cl^-, 1.3% N, and 0% CO_3^{2-} on a weight basis as trace components in the CBC. Ooi et al. also reported that Ca, and P are the major components in bone char, and that Na, Mg, O, and C are minor components in bone char based on their study of bovine bone char (Ooi et al. 2007).

When the reaction of equation (1) is taken into consideration, a certain amount of HAP was converted to FAP. Considering the number of OH^- moles in CBC before, and after the experiment in Table 4.5, 45.6% of HAP could be converted to FAP. According to equation (1), the same molar of OH^- should be released into the solution; however, a significant change in pH value was not observed. Table 4.6 shows the pH, number of OH^- moles, alkalinity, and electrical conductivity (EC) of the solutions.

Table 4.6: Solution pH, no. of OH^- moles, alkalinity, and electrical conductivity

Solution	pH	No. of OH^- moles (μ moles/l)	Alkalinity (μeq/l)	EC (μS/cm)
Initial solution	5.21	0.002	0	75
Final solution	7.82	0.661	762	330

The total amount of F^- removal was 97.63 mmol. This was calculated according to the data in Table 4.1 (1855 mg/19 g/mol). The released OH^- could be partly neutralized by CO_2 dissolved from the atmosphere to produce alkalinity as we detected 762 μeq/l of alkalinity in the final solution. The EC values of final solution was increased than the initial solution as mentioned in Table 4.6. It was due to the dissolution of mainly Na^+, and Cl^- ions, and other ions to the final solutions from CBC (Table 4.4).

In relation to the high adsorption by bone char, Mwaniki reported that Cl^- ions increased the rate of fluoride adsorption onto bone charcoal (Mwaniki 1992). Abe et al. also reported that fluoride adsorption by bone char increased in the presence of Cl^- ions in the solution. They discussed the "salting out" effect of NaCl relevant to the excess fluoride adsorption by bone char. NaCl dissociates in water by giving Na^+, and Cl^- ions to the solution. Na^+, and Cl^- ions in the solution are hydrated with water molecules by reducing the water molecules for the dissolution of fluoride. Therefore, the fluoride ion in the solution is enhanced to be adsorbed onto bone char (Abe et al. 2004).

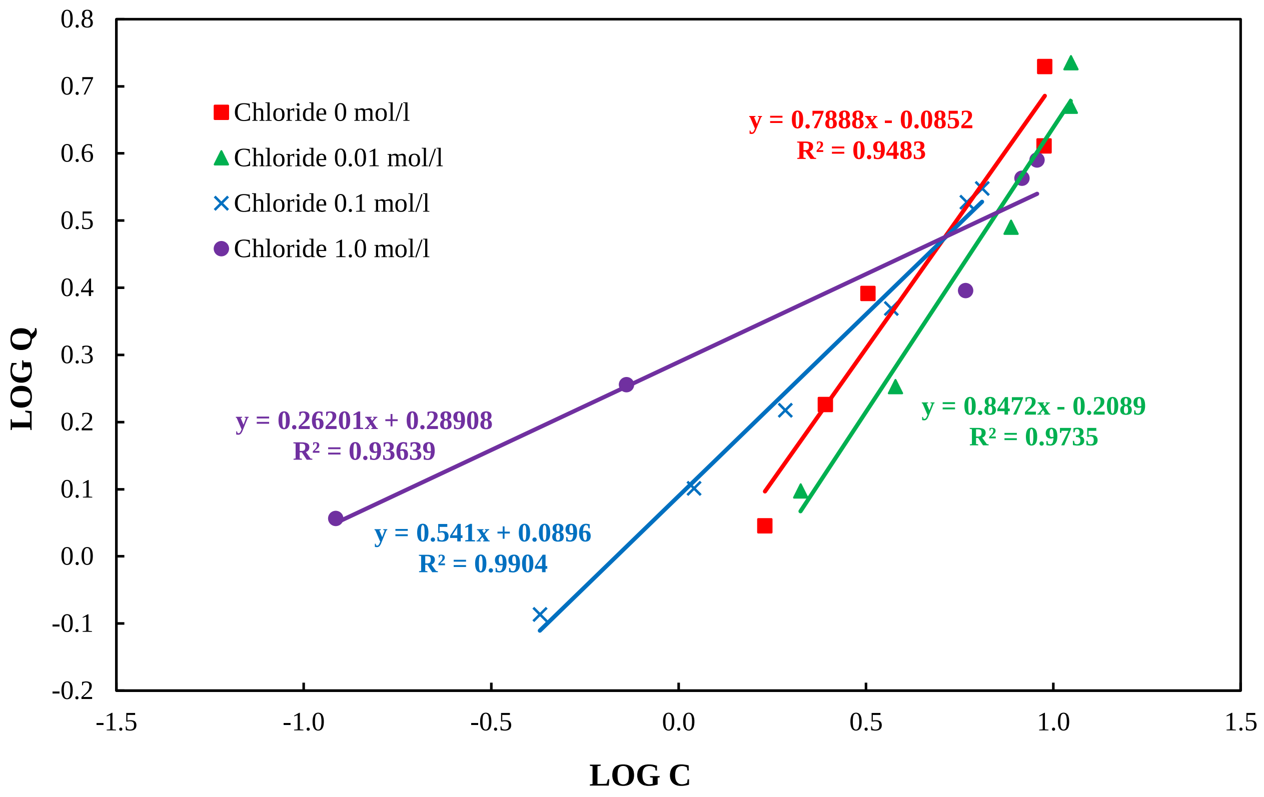

Fig. 4.17: Freundlich isotherm for CBC in the presence of chloride

In contrast, our experiment showed that higher Cl^- concentrations decreased the fluoride adsorption capacity of CBC. Figure 4.17 shows the Freundlich isotherm for CBC in the presence of chloride. According to the Freundlich isotherm, the adsorption capacities of CBC in the presence of Cl^- concentrations of 0 mol/l, 0.01 mol/l, 0.1mol/l, and 1.0 mol/l were respectively 5.1 mg/g, 4.4 mg/g, 4.3 mg/g, and 3.6 mg/g at a fluoride concentration of 10 mg/l. According to Table 4.4, Cl^- ions were slightly released into the solution from CBC as 0.002 mol/l was detected in the final solution. The release of Cl^- from CBC to the solution caused a decrease in the adsorption of fluoride onto the CBC. Consequently, “salting out” is not the reason for the excess adsorption of fluoride.

3.3. Regeneration of CBC and Fluoride Removal

3.3.1. Experiment 1

Figure 4.18 shows the fluoride concentration of the solution used for the preparation of fluoride-exhausted CBC throughout operation period of 5 days (Experiment 1).

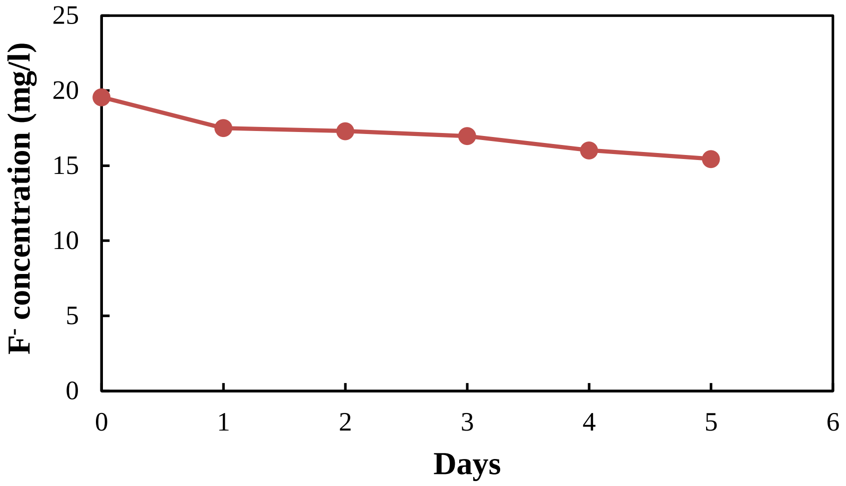

Fig. 4.18: Fluoride concentration of the solution used for the preparation of fluoride-exhausted CBC (Experiment 1)

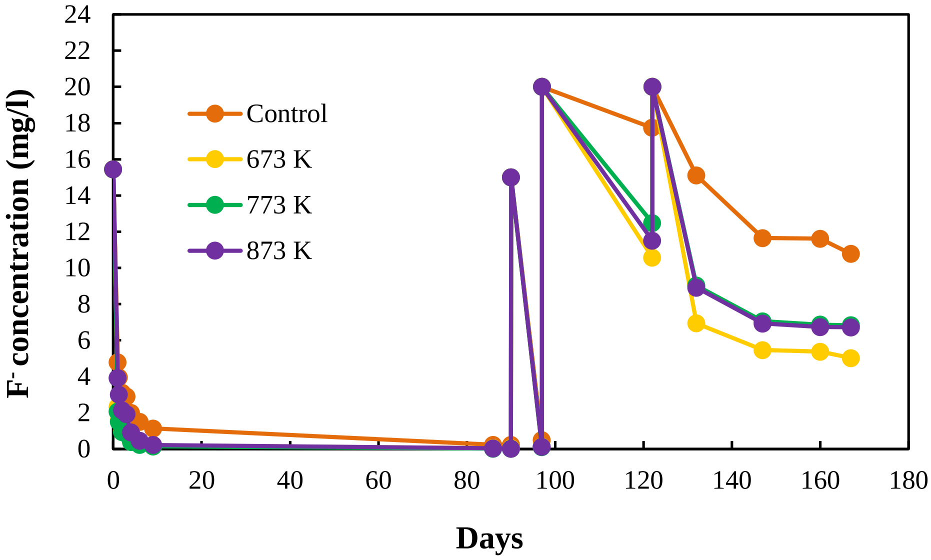

Fig. 4.19: Fluoride concentrations of the solutions after the regeneration of CBC (Experiment 1)

Figure 4.19 shows the fluoride concentrations of the solutions after the regeneration of CBC throughout the operation period of 167 days (Experiment 1). The fluoride concentrations of the solutions were increased to 15 mg/l, 20 mg/l, 20 mg/l respectively at days 90, 97, and 122 to investigate the fluoride adsorption capacity of regenerated CBC for more period of time at a higher fluoride concentration. NaF solution was used to increase the fluoride concentrations of the solutions. According to Figure 4.19, it was obvious that the control setup also adsorb fluoride similar to regenerated CBC, indicating that 5 days of operation period was not sufficient to prepare fluoride exhausted CBC.

When we compare the rate of F^- adsorption capacities before (0 mg/g CBC/day), and after the regeneration, it was clear that the rate of adsorption capacities after the regeneration; regenerated CBC to 673 K (1.04 mg/g CBC/day), 773 K (1.06 mg/g CBC/day), and 873 K (0.92 mg/g CBC/day) were higher than the rate of adsorption capacity before regeneration. Further, the rate of adsorption capacities after the regeneration were higher than the rate of adsorption capacity of the control (0.84 mg/g CBC/day). Therefore, the regeneration of CBC was effective in fluoride removal. The rate of adsorption capacity was calculated by the slope of the Figures 4.18 and 4.19.

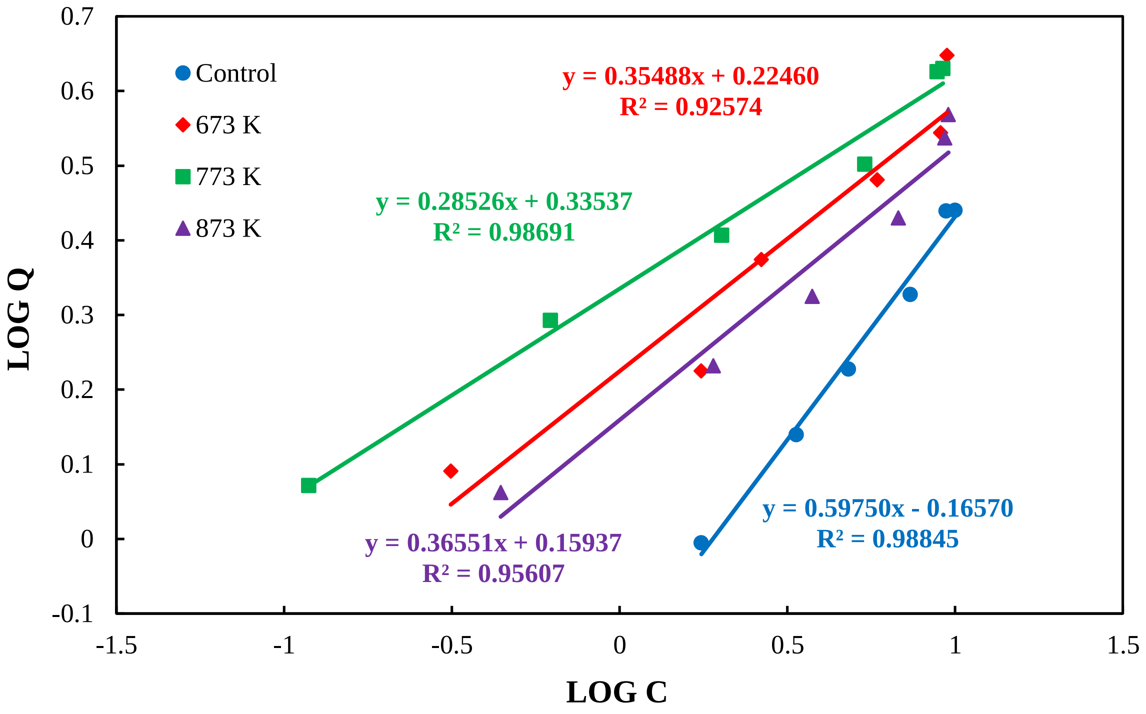

Fig. 4.20: Freundlich isotherm for CBC used for the regeneration study (Experiment 1)

Freundlich isotherm model was used to evaluate the adsorption capacity of regenerated CBC. Figure 4.20 shows the Freundlich isotherm for CBC used for the regeneration study (Experiment 1). Table 4.7 shows the adsorption capacities of CBC obtained by the Freundlich isotherm, at the equilibrium concentrations. The final fluoride concentrations of the solutions after 167 days were used as the equilibrium concentrations to calculate the adsorption capacity by Freundlich isotherm (Figure 4.20). The adsorption capacity of the control was 2.83 mg/g, indicating that it was not fully saturated, however; when comparing it with that from Figure 4.10, it is obvious that the adsorption capacity decreased from 5.35 mg/g to 2.83 mg/g.

Table 4.7: Adsorption capacities of CBC obtained by the Freundlich isotherm (Experiment 1)

	Equilibrium concentration (mg/l)	Adsorption capacity (mg/g)
Control	11	2.83
673 K regenerated	5	2.97
773 K regenerated	7	3.74
873 regenerated	7	2.90

Table 4.8 shows the adsorption capacities of CBC used for the regeneration study obtained from the mass balance calculation for the solutions, at the equilibrium concentrations (Experiment 1). Figure 4.21 shows the adsorption capacities of CBC used for the regeneration study obtained from the mass balance calculation for the solutions throughout the operation period (Experiment 1). The adsorption capacities obtained by the Freundlich isotherm were similar to the adsorption capacities obtained from the mass balance calculation for the solutions.

The CBC regenerated to 673 K showed the highest adsorption capacity, hence; 673 K was the best regenerating temperature. The adsorption capacity of CBC regenerated to 773 K, and 873 K was almost similar. When comparing the adsorption capacities of regenerated CBC, and the control setup, adsorption capacities of regenerated CBC were slightly higher than the control setup.

Adsorption of fluoride onto heat regenerated bone char was reported in literature. Kaseva has used cattle bones for defluoridation, and he showed that the cattle bones regenerated at 773 K for 2 hours showed the maximum fluoride ion adsorption capacity of 0.75 mg/g among cattle bones regenerated to 373 K, 473 K, 573 K, 673 K, 773 K, 873 K, 973 K, and 1073 K (Kaseva 2006).

Table 4.8: Adsorption capacities of CBC used for the regeneration study obtained from the mass balance calculation for the solutions (Experiment 1)

		Weight of CBC (g)	Adsorbed F^- (mg)	Adsorption capacity (mg/g)	Total Adsorption capacity (mg/g)
Control	Exhausted CBC	60.5	81.7	1.35	3.69
	After regeneration	10.1	23.6	2.34	
673 K regenerated	Exhausted CBC	60.5	81.7	1.35	4.40
	After regeneration	10.0	30.6	3.05	
773 K regenerated	Exhausted CBC	60.5	81.7	1.35	4.23
	After regeneration	10.1	28.9	2.88	
873 K regenerated	Exhausted CBC	60.5	81.7	1.35	4.26
	After regeneration	10.0	29.1	2.91	

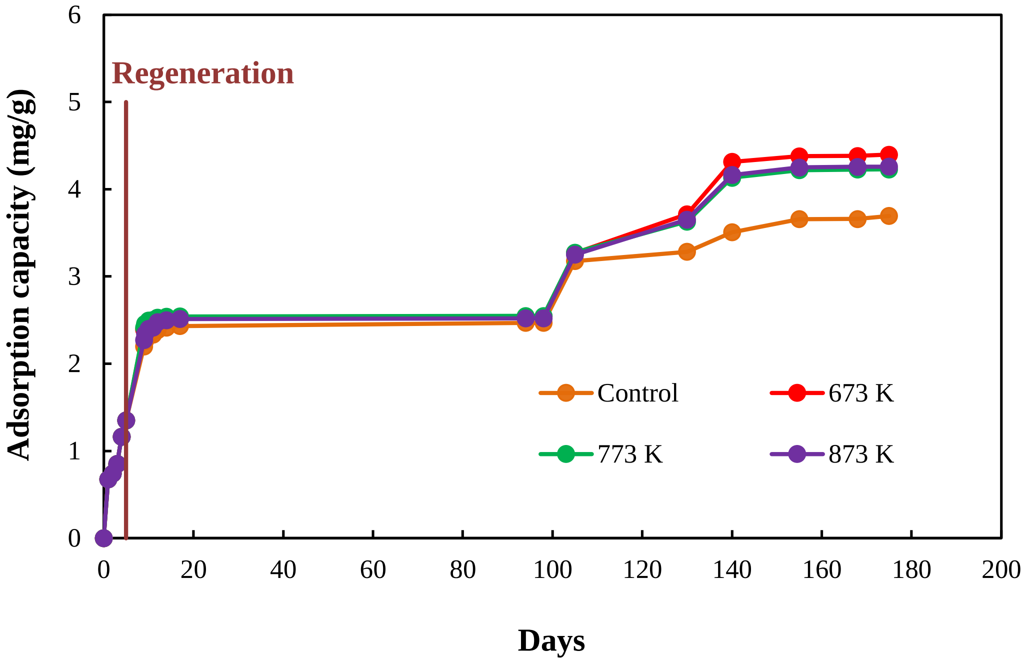

Fig. 4.21: Adsorption capacities of CBC used for the regeneration study obtained from the mass balance calculation for the solutions throughout the operation period (Experiment 1)

Figures 4.22, 4.23, 4.24, and 4.25 respectively show the XRD patterns of initial CBC, and control setup, regenerated CBC to 673 K, 773 K, and 873 K (Experiment 1). It is clear that the XRD patterns of initial CBC, and the regenerated CBC were overlapping together showing that the structure of CBC was not changed during the regeneration. The major component of CBC was found to be HAP (Figure 4.16). According to Figures 4.22, 4.23, 4.24, and 4.25, it was concluded that the HAP structure of CBC was not changed during the regeneration.

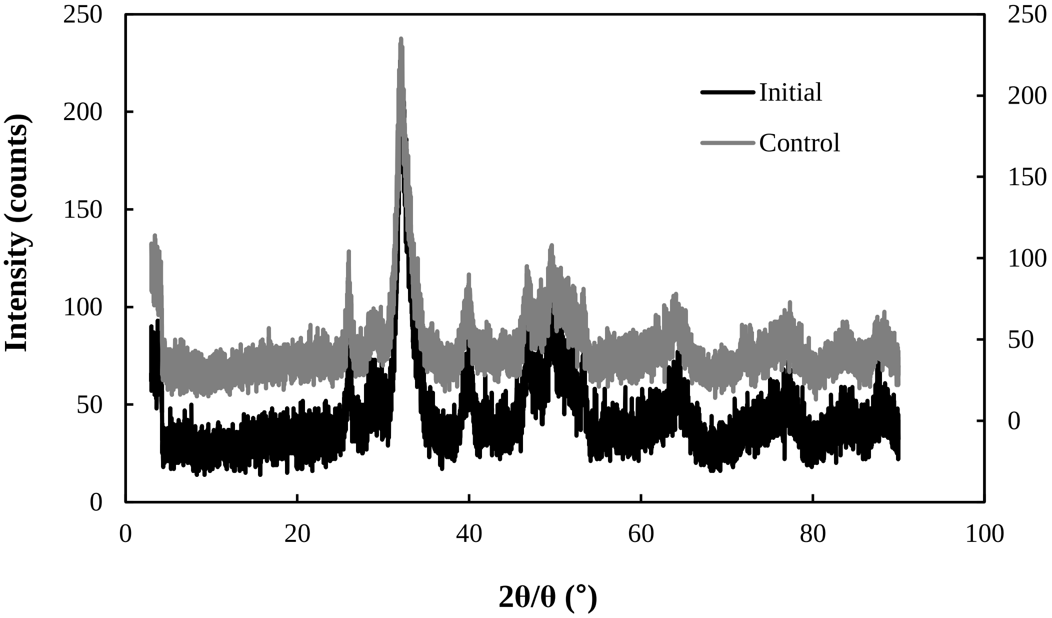

Fig. 4.22: XRD patterns for the initial CBC and control setup (Experiment 1)
*Initial (left Y axis), Control (right Y axis)

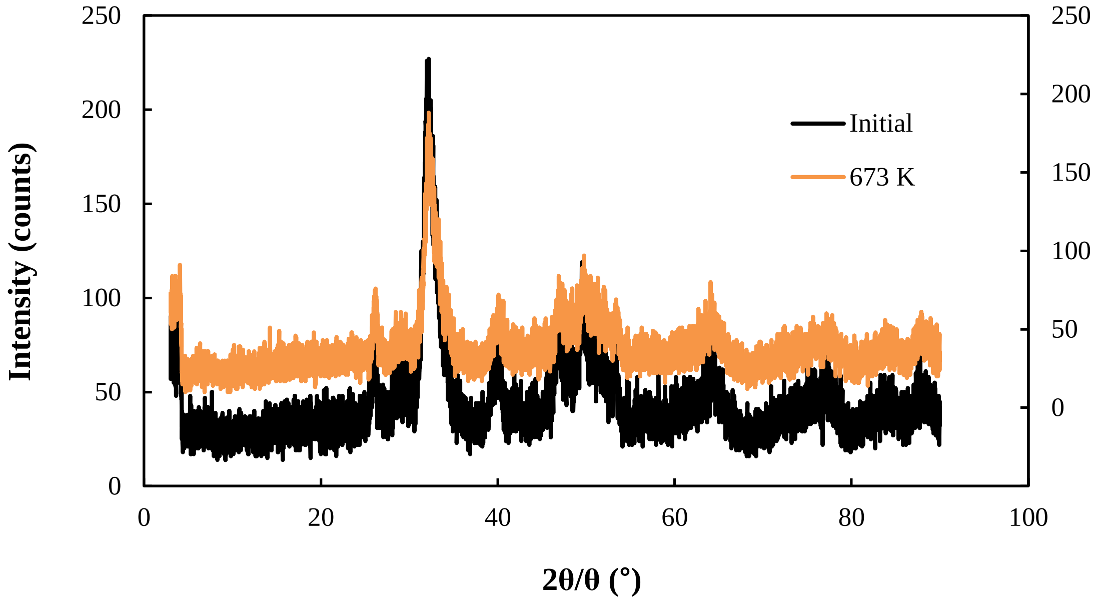

Fig. 4.23: XRD patterns for the initial CBC and 673 K regenerated CBC (Experiment 1)
*Initial (left Y axis), 673 K (right Y axis)

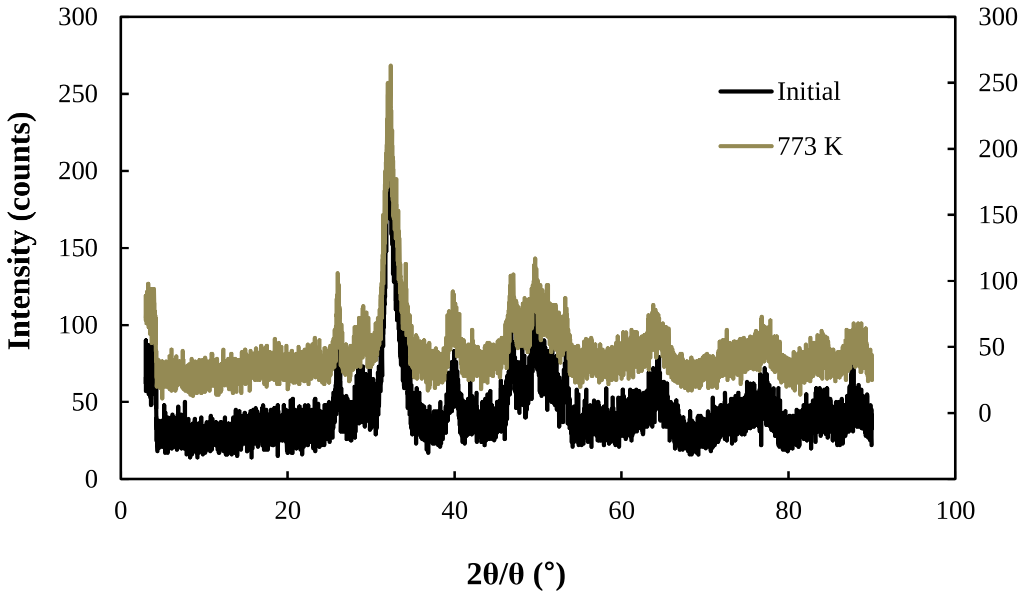

Fig. 4.24: XRD patterns for the initial CBC and 773 K regenerated CBC (Experiment 1)
*Initial (left Y axis), 773 K (right Y axis)

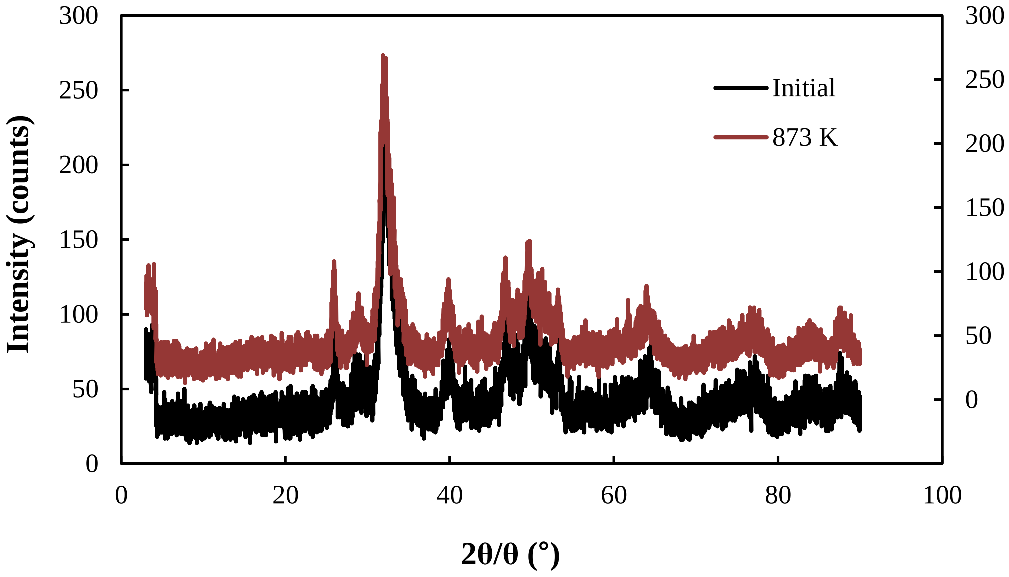

Fig. 4.25: XRD patterns for the initial CBC and 873 K regenerated CBC (Experiment 1) *Initial (left Y axis), 873 K (right Y axis)

Table 4.9: No. of moles of Ca^{2+}, PO_4^{3-} in CBC (Experiment 1)

	No. of moles in 100 g of CBC		
	Ca^{2+}	PO_4^{3-}	molar ratio (Ca^{2+}/PO_4^{3-})
Control	0.638	0.394	1.62
673 K regenerated	0.610	0.363	1.68
773 K regenerated	0.583	0.345	1.69
873 K regenerated	0.639	0.383	1.67

Table 4.9 shows the number of moles of Ca^{2+}, and PO_4^{3-} in 100 g of control setup, and regenerated CBC based on the chemical analysis (Experiment 1). The Ca^{2+}/PO_4^{3-} molar ratio of regenerated CBC was also similar to that of 1.67 for HAP, confirming that the HAP structure was not changed during the regeneration as evidence from the similar XRD patterns of the initial CBC, and the regenerated CBC (Figures 4.22, 4.23, 4.24, 4.25).

Table 4.10: Anion and cation concentrations of solutions (Experiment 1)

		Concentration (mg/l)									
		F^-	**Cl^-**	**SO_4^{2-}**	**NO_3^-**	**PO_4^{3-}**	**Na^+**	**NH_4^+**	**K^+**	**Mg^{2+}**	**Ca^{2+}**
	Initial solution	20	8	6	0	0	30	0	1	1	8
	Final solution before regeneration	15	8	7	1	5	34	0	3	1	6
Final solutions after regeneration	Control	11	6	6	1	36	93	1	3	0	0
	673 K regenerated	5	6	42	1	33	98	2	4	0	0
	773 K regenerated	7	7	47	1	36	109	2	4	0	0
	873 K regenerated	7	8	27	1	27	105	2	4	0	0

Table 4.10 shows the anion and cation concentrations of the initial solution, final solutions before, and after regeneration (Experiment 1). The increase of Na^+ in the final solutions was mainly due to the addition of NaF to the solutions. A certain amount of Na^+ may also be released into the solution by the dissolution from CBC.

SO_4^{2-} were detected in the final solutions, and PO_4^{3-} ions released to the final solutions due to the dissolution of those ions from CBC. We detected 35 mg/l, 40 mg/l, and 20 mg/l of SO_4^{2-} ions respectively in the final solutions after regeneration in the 673 K regenerated setup, 773 K regenerated setup, and 873 K regenerated setup than the final solutions before regeneration. 31 mg/l, 28 mg/l, 31 mg/l, and 22 mg/l of PO_4^{3-} ions were detected respectively in the final solutions after regeneration in the control setup, 673 K regenerated setup, 773 K regenerated setup, and 873 K regenerated setup than the final solutions before regeneration. Mg^{2+}, and Ca^{2+} ions were not detected in the final solutions due to adsorption of those ions by CBC from the solutions.

Table 4.11 shows the pH, alkalinity, and PO_4^{3-} concentrations of the solutions (Experiment 1). A slight increase of solution pH was detected, however, a significant change in pH value was not detected. Alkalinity of final solutions was increased due to releasing basic ions into the solutions.

Table 4.11: Solution pH, alkalinity, and PO_4^{3-} concentrations (Experiment 1)

		pH	Alkalinity (μeq/l)	PO_4^{3-} (mg/l)
	Initial Solution	7.26	317	0
	Final solution before regeneration	7.81	530	5
Final solutions after regeneration	Control	8.39	2166	36
	673 K regenerated	8.40	1976	33
	773 K regenerated	8.43	2153	36
	873 K regenerated	8.49	2673	27

According to equation (1), pH value of the solution should be increased due to release of OH^- into the solution with the formation of FAP. The pH value was not increased sharply in the final solutions after regeneration (Table 4.11). According to equation 2, phosphate in HAP should be released into the solutions with the formation of CaF_2. Phosphate was detected in the final solutions after regeneration, compared to the final solution before regeneration (Table 4.10). Therefore, the equation (2) took place during the adsorption.

3.3.2. Experiment 2

Experiment 2 was conducted for further investigation of the best regenerating temperature detected from Experiment 1, and to investigate the heat treatment of 338 K in the electrical oven for 24 hours. Figure 4.26 shows the fluoride concentrations of the solutions used for the preparation of fluoride-exhausted CBC throughout operation period of 13 days (Experiment 2). The fluoride concentrations of the solutions were increased to 20 mg/l at days 2, 5, 7, and 9 to allow to adsorbed more amount of fluoride than in Experiment 1, to obtain a better adsorption capacity than Experiment 1. NaF solution was used to increase the fluoride concentrations of the solutions.

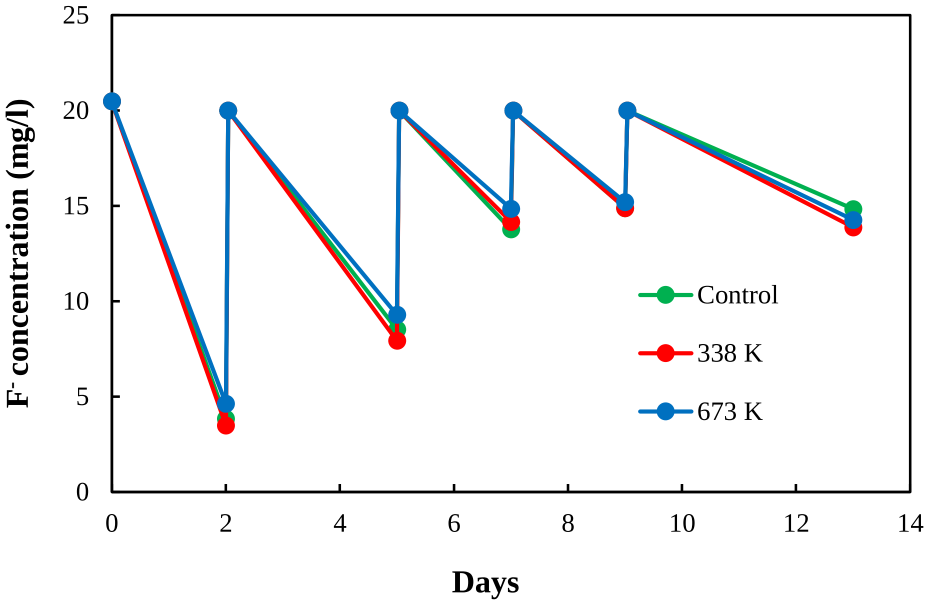

Fig. 4.26: Fluoride concentrations of the solutions used for the preparation of fluoride-exhausted CBC (Experiment 2)

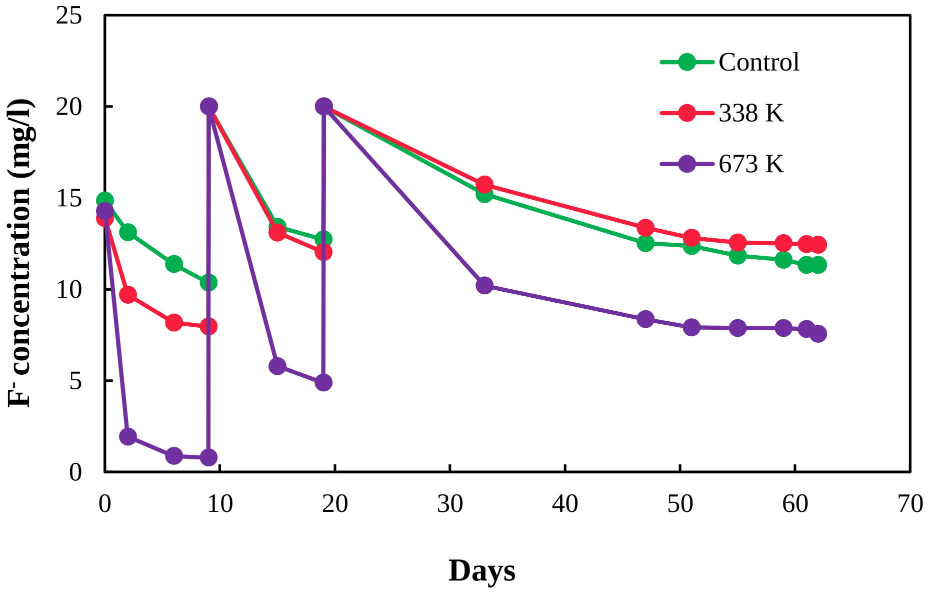

Fig. 4.27: Fluoride concentrations of the solutions after the regeneration of CBC (Experiment 2)

Figure 4.27 shows the fluoride concentrations of the solutions after the regeneration of CBC throughout operation period of 62 days (Experiment 2). The fluoride concentrations of the solutions were increased to 20 mg/l at days 9, and 19 to obtain a considerable adsorption capacity within a short period of time. NaF solution was used to increase the fluoride concentrations of the solutions. The equilibrium fluoride concentrations of the solutions were used to investigate the performance of fluoride adsorption onto regenerated CBC.

Table 4.12: Adsorption capacities of CBC used for the regeneration study obtained from the mass balance calculation for the solutions (Experiment 2)

		Weight of CBC (g)	Adsorbed F^- (mg)	Adsorption capacity (mg/g)	Total Adsorption capacity (mg/g)
Control	Exhausted CBC	10.3	44.1	4.27	6.20
	After regeneration	10.3	19.9	1.93	
338 K regenerated	Exhausted CBC	10.3	45.6	4.42	6.51
	After regeneration	10.0	21.0	2.10	
673 K regenerated	Exhausted CBC	10.4	41.7	4.02	8.17
	After regeneration	9.7	40.3	4.15	

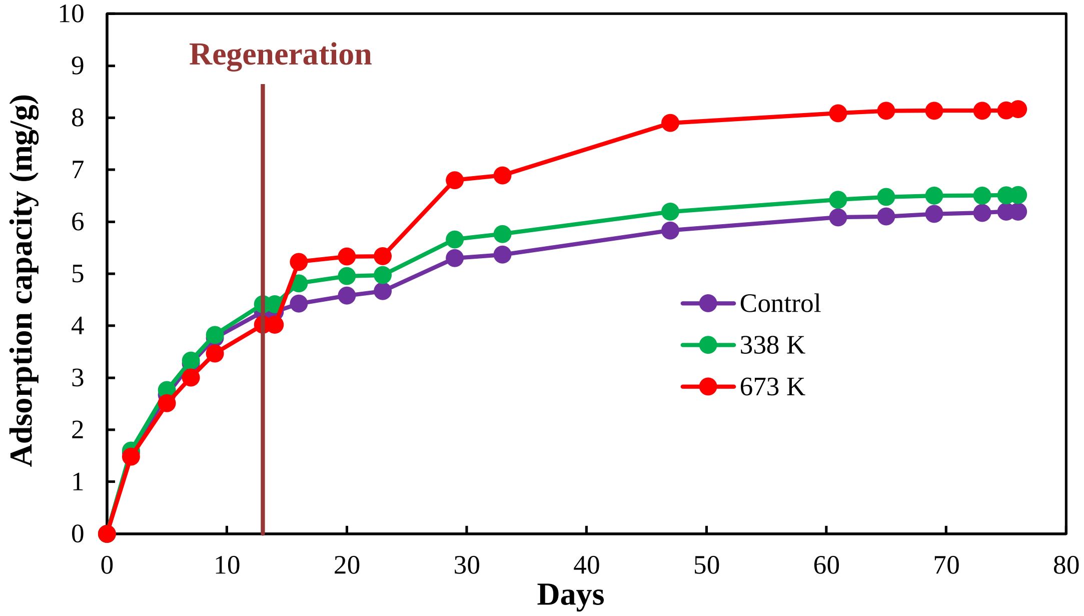

Fig. 4.28: Adsorption capacities of CBC used for the regeneration study obtained from the mass balance calculation for the solutions throughout the operation period (Experiment 2)

Table 4.13: Adsorption capacities of CBC according to the stem distillation, and HNO_3 digestion (Experiment 2)

	Adsorption capacity (mg/g)	
	Steam distillation	HNO_3 digestion
Control	5.60	5.66
338 K regenerated	5.82	5.95
673 K regenerated	7.95	8.09

Table 4.12 shows the adsorption capacities of CBC used for the regeneration study obtained from the mass balance calculation for the solutions (Experiment 2). Figure 4.28 shows the adsorption capacities of CBC used for the regeneration study obtained from the mass balance calculation for the solutions throughout the operation period (Experiment 2). Experiment 2 resulted a considerable higher amount of adsorption capacities compared to Experiment 1. Therefore, stem distillation, and acid digestion were conducted for the solid CBC used for Experiment 2. Table 4.13 shows the adsorption capacities of CBC used for the regeneration study according to the stem distillation, and acid digestion (Experiment 2). Adsorption capacities of CBC obtained from the mass balance calculation for the solutions were well coincided with the adsorption capacities obtained from the steam distillation, and acid digestion by solid CBC.

CBC regenerated at 673 K showed the highest adsorption capacity, hence; 673 K was the best regenerating temperature. The CBC regenerated to 338 K in the electrical oven for 24 hours showed an adsorption capacity slightly higher than the adsorption capacity of the control setup, indicating that there is a certain effect of heat treatment at 338 K in the electrical oven for 24 hours as a regeneration method.

According to equation (1), FAP forms from HAP during the adsorption of fluoride from CBC. Kaseva reported that the adsorbed fluoride on to CBC in the formation of exhausted CBC was evaporating as HF during the heat regeneration (Kaseva 2006).

The relevant chemical reaction can be represented in equation (3):

$$Ca_{10}(PO_4)_6F_2 + 2\ OH^- \xrightarrow{\text{Heat}} Ca_{10}(PO_4)_6 + 2\ HF\uparrow + O_2\uparrow \qquad (3)$$

According to the results obtained in our study, it was obvious that the adsorbed fluoride during the formation of exhausted CBC was not evaporated as HF during the heat treatment, since total amount of adsorbed fluoride onto CBC (Table 4.12) was detected by the steam distillation, and the acid digestion of CBC (Table 4.13). Therefore, it was concluded that the phenomenon mentioned in equation (3) (Kaseva 2006) did not take place during the heat treatment of exhausted CBC.

Table 4.14: Anion and cation concentrations of solutions (Experiment 2)

		Concentration (mg/l)									
		F^-	**Cl^-**	**SO_4^{2-}**	**NO_3^-**	**PO_4^{3-}**	**Na^+**	**NH_4^+**	**K^+**	**Mg^{2+}**	**Ca^{2+}**
	Initial solution	20	9	6	0	0	31	0	1	1	8
Final solutions before regeneration	Control	15	8	7	1	38	70	0	6	0	0
	338 K regenerated	14	8	7	1	39	73	0	6	0	0
	673 K regenerated	14	8	7	1	34	72	0	6	0	0
Final solutions after regeneration	Control	11	7	6	0	40	76	1	6	0	0
	338 K regenerated	12	7	6	0	45	83	1	6	0	0
	673 K regenerated	8	8	59	1	35	100	2	6	0	0

Table 4.14 shows the anion and cation concentrations of the initial solution, final solutions before, and after regeneration (Experiment 2). The Na^+ in the final solutions was increased mainly due to the addition of NaF to the solutions. A certain amount of Na^+ may also be released into the solution by the dissolution from CBC.

SO_4^{2-} concentration was increased only in the final solution after regenerated CBC at 673 K. We detected 52 mg/l of SO_4^{2-} in the final solution after regeneration in the 673 K regenerated setup than the final solution before regeneration. Figure 4.29 shows the sulfate concentrations of the solutions after the regeneration of CBC throughout operation period of 62 days (Experiment 2).

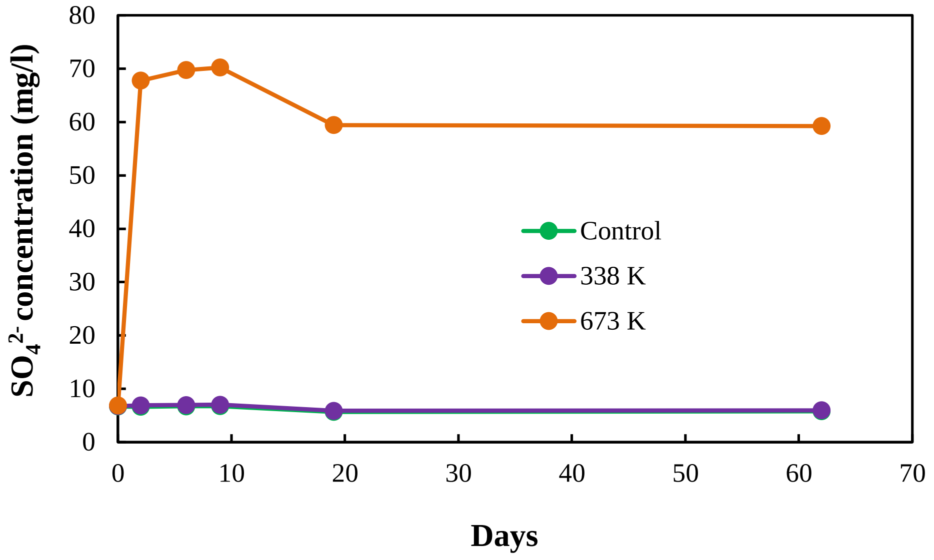

Fig. 4.29: Sulfate concentrations of the solutions after the regeneration of CBC (Experiment 2)

The PO_4^{3-} ions slightly released to the final solutions after regeneration, compared to the final solutions before regeneration. Respectively 2 mg/l, 6 mg/l, and 1 mg/l of PO_4^{3-} ions were detected in the final solutions after regeneration in the control setup, 338 K regenerated setup, and 673 K regenerated setup than the final solutions before regeneration. Figure 4.30 shows the phosphate concentrations of the solutions after the regeneration of CBC throughout operation period of 62 days (Experiment 2). Mg^{2+}, and Ca^{2+} ions were not detected in the final solutions due to adsorption of those ions by CBC from the solutions.

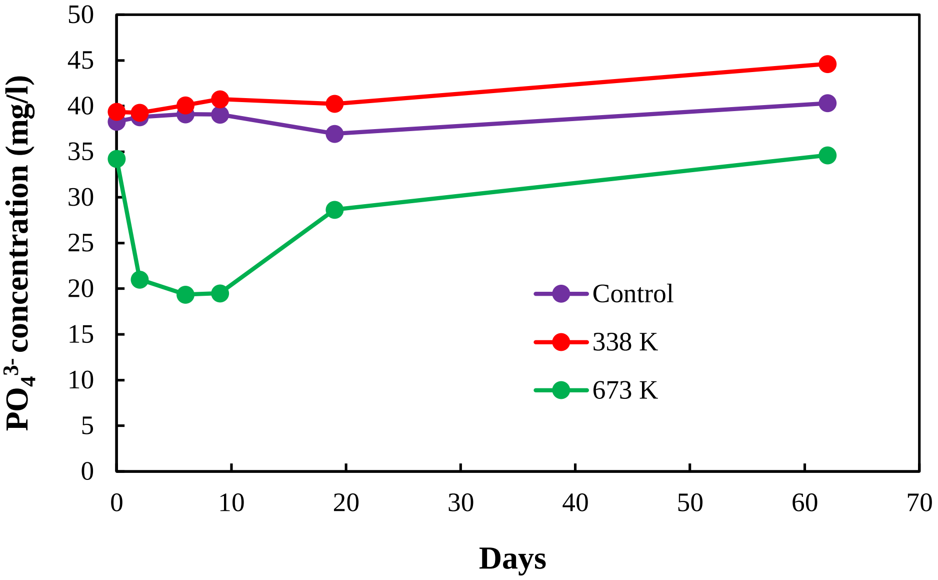

Fig. 4.30: Phosphate concentrations of the solutions after the regeneration of CBC (Experiment 2)

Table 4.15 shows the pH, alkalinity, and PO_4^{3-} concentrations of the solutions (Experiment 2). The pH and alkalinity of final solutions were increased as the same manner in Experiment 1.

Table 4.15: Solution pH, alkalinity, and PO_4^{3-} concentrations (Experiment 2)

		pH	Alkalinity (μeq/l)	PO_4^{3-} (mg/l)
	Initial Solution	7.30	409	0
Final solutions before regeneration	Control	8.17	904	38
	338 K regenerated	8.28	963	39
	673 K regenerated	8.23	1065	34
Final solutions after regeneration	Control	8.43	1296	40
	338 K regenerated	8.42	1360	45
	673 K regenerated	8.40	1549	35

The equation (2) took place during the adsorption, since phosphate was detected in the final solutions (Table 4.14).

SEM images of CBC used for the study were taken in two different stages to compare the surface, morphology, and size distribution. Figures 4.31, and 4.32 respectively show the SEM images of the control CBC, and 673 K regenerated CBC after the adsorption. SEM image of CBC before the adsorption was shown in Figure 4.14.

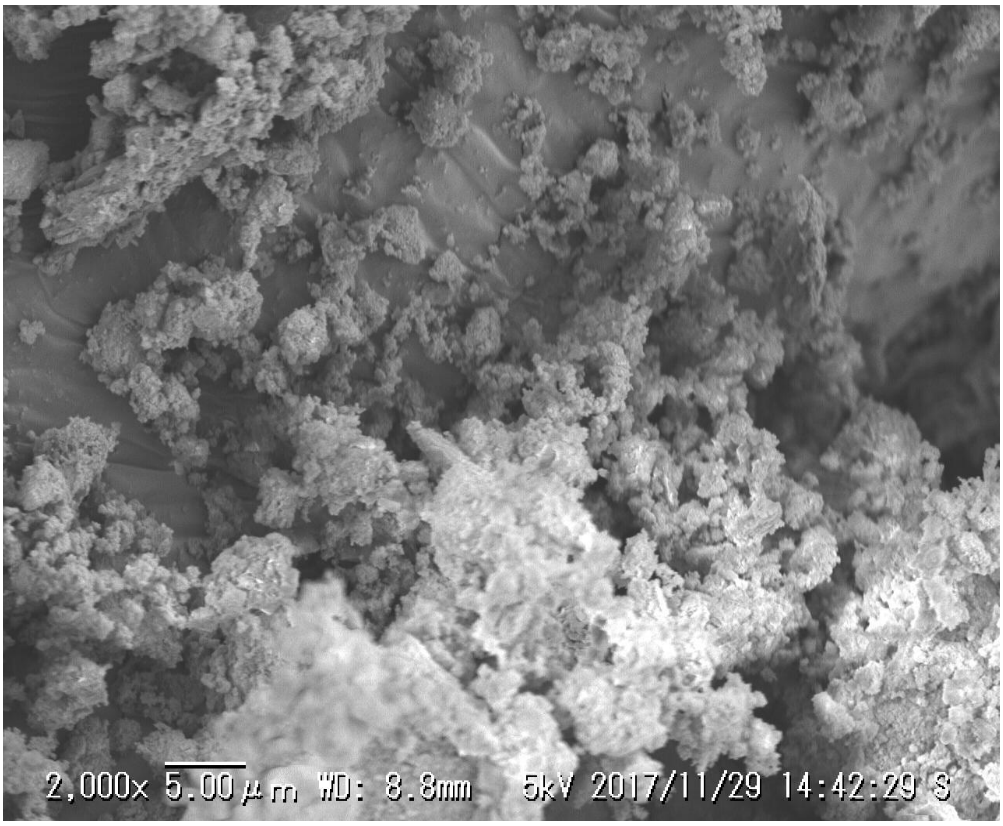

Fig. 4.31: SEM image of the control CBC (Experiment 2)

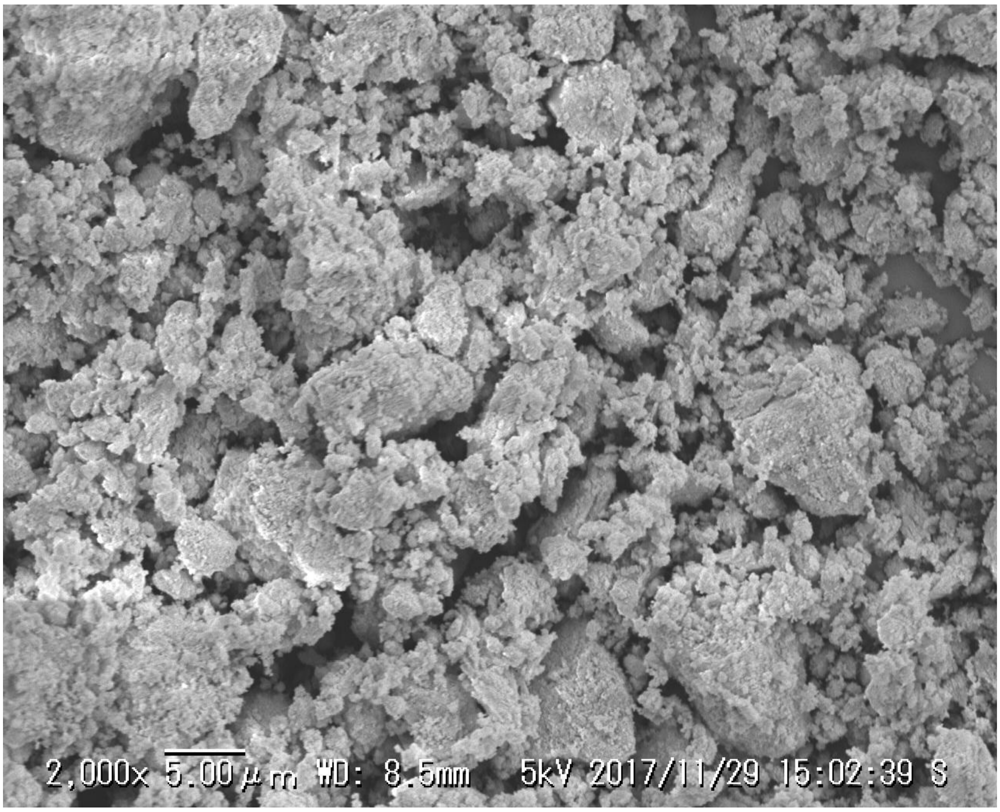

Fig. 4.32: SEM image of the 673 K regenerated CBC (Experiment 2)

According to the Figures, it was clear that the CBC regenerated to 673 K (Figure 4.32) shows denser arears than control CBC (Figure 4.31), and CBC before the adsorption (Figure 4.14). It was concluded that the unopen pores in CBC may activated during the heat regeneration of CBC, and F^- may adsorb to those pores during the adsorption after regeneration.

Nigri et al. was also studied regeneration of cow bone char and investigated that the best regenerating temperature was 673 K as we found in our study. They reported that diffusion of fluoride ions inside to the bone char pores was observed during the heat regeneration. This phenomenon may occur during our study also since adsorbed fluoride in exhausted CBC was not evaporated as HF as mentioned by Kaseva. Nigri et al. reported that the fluoride adsorption by regenerated bone char was fitted to pseudo second order kinetic model. The adsorption capacity was represented by Freundlich, Redlich-Peterson, and Sips isotherms indicating the formation of heterogeneous layers on the surface of bone char. The formation of fluoridated hydroxyapatite was also observed in their study (Nigri et al. 2017). It was concluded that the unopen pores in CBC may activated during the heat regeneration of CBC in our study instead of the above mentioned phenomenon. Further, release of SO_4^{2-} from CBC may result void spaces in CBC and F^- may adsorb to those void spaces also during the adsorption.

4. Conclusions

Defluoridation of drinking water is essential in order to avoid potential human health risks from fluoride contaminated water. CBC was selected as a low cost, and efficiency raw material in removing fluoride from drinking water. Eight particle sizes of CBC were investigated to find out the best particle size of CBC, and it was found to be that the particles with the diameter of 10 µm was the best particle size of CBC. Finer sized CBC was investigated for fluoride removal from drinking water in relation to its adsorption capacity using a laboratory scale CBC column filter. The particles with a diameter of 106-212 µm was selected as the finer particle size. Finer sized CBC showed an unusual adsorption capacity of 11.2 mg F^-/g CBC, higher than that previously reported. The XRD patterns, SEM images, and BET surface area of CBC were evaluated to investigate the high performance of finer sized CBC. The XRD patterns, SEM images, and BET surface area of CBC showed that the structure of CBC before and after the fluoride adsorption was similar. The smaller radius of finer sized CBC enhanced the mechanism of fluoride adsorption by ion exchange.

The CBC regenerated to 673 K yielded the highest fluoride adsorption capacity confirming that the 673 K is the best regenerating temperature. The structure of CBC was not changed during the regeneration. Further the regeneration of CBC is possible, and it could be also used as a defluoridation technique.

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GENERAL CONCLUSIONS

Sri Lankan water quality was analyzed in relation to the public health, and chronic kidney disease of unknown etiology (CKDu) in Sri Lanka. 1304 well water samples were analyzed for pH, fluoride, nitrate, hardness, aluminum, and manganese concentrations, and 1435 well water samples were analyzed for arsenic, cadmium, lead, and chromium concentrations. Spatial distribution maps were created for the pH, fluoride, nitrate, hardness, aluminum, and manganese concentrations to interpret the analyzed results. Most of the well water was contaminated with fluoride, and hardness. 20% of the wells were recorded to have fluoride concentrations of more than the Sri Lankan standard of 1.0 mg/l. 42.2% of well water was very hard which showed the hardness concentration more than 180 $CaCO_3$ mg/l. Fluoride causes dental fluorosis, and skeleton fluorosis. Further, fluoride, and hardness supposed to be causes for CKDu which was highly prevalent in Sri Lanka. Therefore, profound attention should be given to minimize the fluoride, and hardness contamination in Sri Lankan drinking water. There is a potential to risk human health from nitrate, aluminum, manganese, and arsenic since some of the well water exceeded the WHO guidelines although prominent health hazardous were not reported so far in Sri Lanka.

Rice samples, and human urine samples collected from CKDu-endemic and non-endemic areas were analyzed for arsenic, cadmium, lead, and chromium contents. There was no significant difference in the arsenic, cadmium, lead, and chromium contents in rice, and human urine between CKDu-endemic, and non-endemic areas indicating that arsenic, cadmium, lead, and chromium in rice, and human urine are not possible causes of CKDu. Furthermore, the relationship between L-FABP concentration, and arsenic, cadmium, lead, and chromium in human urine was analyzed. No relationships were found between them. Groundwater samples collected from CKDu-endemic areas were analyzed for pesticides. Pesticides were not detected in groundwater, hence; pesticides in groundwater is not a possible cause for CKDu. Rice samples collected from both CKDu-endemic, and non-endemic areas were analyzed for pesticides. Pesticides were not detected in rice, hence; pesticides in rice is not a possible cause for CKDu.

Chicken bone char (CBC) was investigated as a low cost, and efficiency raw material in removing fluoride from drinking water. The best carbonizing temperature for the preparation of CBC was investigated and it was found to be 873 K. Performance of larger sized CBC was investigated using a community based CBC filter using particles with a diameter of 5-10 mm established at Wilgamuwa, Sri Lanka. It showed an adsorption capacity of 2.45 mg F^-/g CBC. Performance of finer sized CBC was investigated using a laboratory scale CBC column filter using particles with a diameter of 106-212 µm. It showed an unusual adsorption capacity of 11.2 mg F^-/g CBC, higher than that previously reported. The smaller radius of finer sized CBC enhanced the mechanism of fluoride adsorption by ion exchange. CBC was found to be an efficiency, and effective media in the defluoridation of drinking water. The regeneration of CBC is possible and it could be also used as a defluoridation technique. The CBC regenerated at 673 K yielded the highest fluoride adsorption capacity confirming that 673 K is the best regenerating temperature among 673 K, 773 K, and 873 K.

PUBLICATIONS

1. **H. M. Ayala S**. **Herath**, Kazusa Kubota, Tomonori Kawakami, Shiori Nagasawa, Ayuri Motoyama, S. K. Weragoda, G. G. Tushara Chaminda & S. K. Yatigammana. (2017) Potential Risk of Drinking Water to Human Health in Sri Lanka. *Environmental Forensics*, 18 (3), 241-250.
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2. **H. M. Ayala S**. **Herath**, Tomonori Kawakami, Shiori Nagasawa, Yuka Serikawa, Ayuri Motoyama, G. G. Tushara Chaminda, S. K. Weragoda, S. K. Yatigammana & A. A. G. D. Amarasooriya. (2018) Arsenic, Cadmium, Lead, and Chromium in Well Water, Rice, and Human Urine in Sri Lanka relation to Chronic Kidney Disease of unknown etiology. *Journal of Water and Health*, 16 (2).
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3. **H. M. Ayala S**. **Herath**, Tomonori Kawakami & Masamoto Tafu. (2018) Regeneration of Exhausted Chicken Bone Char (CBC) to Optimize its usage in the Defluoridation of Drinking Water. *Journal of Ecotechnology Research,* 18 (3), 39-46.
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4. **H. M. Ayala S**. **Herath**, Tomonori Kawakami & Masamoto Tafu, The Extremely High Adsorption Capacity of Fluoride by Chicken Bone Char (CBC) in Relation to its Finer Particle Size.

5. **H. M. Ayala S**. **Herath**, Tomonori Kawakami & Masamoto Tafu, Heat Regeneration of Bone Char for a Sustainable Use in Fluoride Removal from Drinking Water.

PRESENTATIONS IN ACADEMIC SYMPOSIUMS

1. Pilot Scale Experiment of Fluoride Removal from Well Water in Sri Lanka by Chicken Bone Char
H.M. Ayala S. Herath, A.A.G.D. Amarasooriya, S.K. Weragoda, Kawakami Tomonori
23rd Symposium on Apatite, Toyama, Japan, 11th December 2014

2. Fluoride Removal from Drinking Water in Sri Lanka by Chicken Bone Char
H.M. Ayala S. Herath, A.A.G.D. Amarasooriya, S.K. Weragoda, Kawakami Tomonori
9th International Forum on Ecotechnology, Hotel OACity Kyowa, Miyako Island, Okinawa, Japan, 20th - 23rd December 2014

3. A Community Scale Filter of Chicken Bone Char for Fluoride Removal from Drinking Water in Sri Lanka
H.M. Ayala S. Herath, A.A.G.D. Amarasooriya, S.K. Weragoda, Kawakami Tomonori
The 49th Annual Conference of Japan Society on Water Environment, Ishikawa, Japan, 16th March 2015

4. Community Based Fluoride Removal Filter with Chicken Bone Char in Sri Lanka
H.M. Ayala S. Herath, A.A.G.D. Amarasooriya, S.K. Weragoda, K. Tomonori
6th international conference on Structural Engineering and Construction Management 2015, Earl's Regency Hotel, Kandy, Sri Lanka, 11th -13th December 2015

5. The Extremely High Adsorption Capacity of Fluoride by Chicken Bone Char (CBC) in Relation to Its Finer Particle Size
H. M. Ayala S. Herath, Tomonori Kawakami, Masamoto Tafu
12th International Forum on Ecotechnology, Toyama International University, Toyama, Japan, 2nd – 3rd December 2017

6. Fluoride removal from drinking water by contact precipitation by using bone char
Takahashi Haruna, Yamauchi Homare, Kawakami Tomonori, **H.M. Ayala S. Herath**
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7. Fluoride removal from drinking water by contact precipitation by using bone char

Takahashi Haruna, Yamauchi Homare, Kawakami Tomonori, **H.M. Ayala S. Herath**

The 49th Annual Conference of Japan Society on Water Environment, Ishikawa, Japan, 16th March 2015

8. Fluoride removal from drinking water by contact precipitation technique using chicken bone char

Takahashi Haruna, Yamauchi Homare, Kawakami Tomonori, **H.M. Ayala S. Herath**

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