Micronutrients I_2, Fe^{2+}, Mg, K and Naconcentration in *Typhalatifolia*and *Cyperus papyrus* reedSaltsof Busia and Lugari regions of Western Kenya

Wangila Tsikhungu Phanice[1]; Tsimbiri Pamella[2]; Peter Nyongesa[3]; Mule Shadrack[4], Wati Nakhone Lenah[5], Kinyanjui Thomas[6]

[1]University of Kabianga, School of Science and Technology, P.O box 2030, Kericho, Kenya

[2]School of Health Sciences, Egerton University, P.O box 536, Njoro, Kenya

[3]Ministry of Labour, Occupational Health and Safety, P.O box 12242, Nakuru, Kenya

[4]University of Kabianga, School of Science and Technology, P.O box 2030, Kericho, Kenya

[5]Egerton University, Department of Soil Science, P.O box 536, Njoro, Kenya

[6]Egerton University, Chemistry Department, P.O box 536, Njoro, Kenya

Abstract: ***Background**: The concentrations of micronutrients namelyI_2, Fe^{2+}, Mg, K and Nawere determined in indigenous salt samples of Busia and Lugari regions of Western Kenya in 2013. **Methods**: pH measurement was done using a JENWAY pH meter model 3505 while Iodine determination was by use of sodium thiosulphate titrimetric method. Iron (II) was done using the Phenanthroline method. Sodium and Potassium Metals in Salt Samples was by a determined by flame photometer model 410 while magnesium was determined using an AAS machine. **Results**: The salt content of raw material do not exceed 1 and 10% in the ash obtained. T. latifoliacontains, respectively 4% of salt in the ash obtained and 0.003% of the raw material. The results indicate higher pH values, with an average of 10.53, for salts prepared from Busia, Cyperus papyrus (pH= 10.23-10.55)as compared to those from Lugari, Typha Latifolia (pH=9.56-9.76), with an average of 9.66. Salts from Cyperus papyrus species of Busia County had the highest moisture content, ranging from 4.88 % to 15.61% while those from Lugari County, Typha Latifolia salts had the lowest moisture content with a range of 0.53 % to 1.03 %. Lugari salt samples had high levels of iodine with a range of 0.5082 to 1.1435ppm while Busia salts had lower iodine levels in the range of 0.3049-0.4158 ppm. The average concentration of iodine in Typhalatifolia is higher than in Cyperus papyrus, with 0.937 ppm and 0.1802 ppm respectively. Sodium is higher than potassium in all the samples, and particularly for Busia samples with the highest Na concentrations. There is between 41.04 - 54.22 % more sodium in Lugari samples compared to the 70. 78 – 75.01 % in Busia samples. Iron concentrations are directly proportional with iodine, with higher levels of iron in C. papyrus (15.04-17.89 mg/g) than in T. latifolia(range of 4.61-11.25 mg/g).iodine is higher in Busia than in Lugari. Sodium, iron and iodine are apparently higher in Busia samples as compared to the salt samples from Lugari with 796.79, 1.789 and 3.173 ppm respectively as the highest concentrations for the elements. Sodium is the highest in each of the regions with 796.79 ppm in Busia while Lugari recorded up to 182.80 ppm as the highest. Iodine levels seemed to increase with increase in pH while there was no significant relationship with % moisture.***

Keywords: micronutrients, Iron, sodium, iodine, potassium,indigenous reed salts

1. Introduction

Salt, chemically known as sodium chloride**,** sometimes called White Gold, is probably the oldest and the commonly used food additive. It improves the taste of foods and inhibits the growth of microorganisms. Salt was used in China, Ethiopia and New Guinea for religious rites. In Europe Salt was so important that it was used for rates and taxes and to pay salaries (Godelier, 1969; Khodakov*et al.*, 1989). Today most people become accustomed to the taste of salt so that its consumption increased despite of numerous diseases it contribute to raise like hypertension, cardiovascular and kidney diseases. Bibliographic sources indicated that in France for example it caused 25,000 deaths per year by hypertension (Alderman *et al.*, 1998). Because of the damage it does on the human health, many consumers and especially in Africa prefers vegetable salt from ash obtained by burning plants parts which is potassium rich. In Africa, vegetable or ash salts were used formerly in economic activities before colonialism came. In Central Africa, including Chad regions, vegetable salt is still traditionally produced and consumed either by alimentary habit or for therapeutic reasons. It is used as substitute of modern salt for atrophic gastritis, for icterus, to lower the blood pressure and as sedative against cough (Allaramadji, 2011). The traditional preparation of the vegetable salt is time consuming with low yielding quantity. Its chemical composition is also unknown. The salt production method proceeds largely by lixiviation but depends on the region. The plants used for preparation are mostly waste parts of cultivated plants like stems of maize, millet, sorghum and some widespread plants like *Hygrophila* species. Also false trunk of pawpaw or banana is used to produce vegetal salt (Allaramadji, 2011). The reports on chemical composition of vegetable salts are scarce. Zerries (1964) reported analyses of vegetable salts conducted by Martius in the Xingu river and by Sick in the British Guyana. Schmeda-Hirschmann (1994) analyzed four vegetable salts from the Papaguayan Chaco. Echeverri and Roman-Jitdutjaano (2011) have reported the chemical analyses of ash salts from 57 species

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used by the Witoto Indians of Amazon. Only little is known about ash salts from Africa.Since plants and animals are essential sources of micronutrients for man, it becomes necessary to monitor the levels in biological materials that are required by man for both dietary and medicinal purposes. This is because deficiency or excess of micronutrients can be factors of disease generation.

1.1 Study Aim

Even though a lot of phytochemical and bioactivity studies have been carried out on a number of medicinal plants in Ghana,[Bayor MT, Gbedema SY, Annan K,2009] not much has been reported on the heavy metal contents of these plants. This study therefore sought to establish the presence, quantity and prevalence of selected micronutrients (iodine, sodium, potassium and Fe II) and magnesium in salt samples from two species of reed plants, *T. latifolia* and *C. papyrus*., commonly used for preparation of salt and further finding use as a table salt, treatment of coughs among other uses.

2. Methods and Materials

2.1 Salt Sample Collection

Plant samples were collected at full growth stage from randomly selected sampling points in Ululo and Budimbidi in Busia region, and Matete and Lugari in Lugari region in Western Kenya. The samples were collected from January 2012 to June 2013 from Busia swamps and Nzoia river banks. Quadrats measuring 500 m x 500m were randomly mapped out from each sampling site, with five sampling spots at a distance of 50 m from each other within each quadrat. Reed Plant samples were randomly harvested using a stainless steel knife. Plants were collected from the five spots from each sampling sites of Ululo and Budimbidi soils in Busia region, and Matete and Lugari in Lugari regionof Western Kenya where *C. papyrus*and*T. Latifolia*reed plants, were collected from respectively.

2.2 Preparation and Preservation

All samples were collected and air dried in the sun for two weeks and burned to obtain ashes, filtered and evaporated to dryness to obtain salt crystals. About 2 kg salt samples were collected into polythene bags and transported to the laboratory for further preparation, treatment and analysis.The salt samples were homogenized by grinding using using a Retsch, Model PM400 agate ball miller. Finely ground samples were sieved with 2 mm mesh before being stored in labeled polythene bags at ambient temperature prior to analysis.

2.3 Sample Preparation and Analysis

Salt pH Measurement: To determine pH, an electronic analytical balance, Shimadzu type model AUY 120 was used to weigh 0.5000 g of each salt sample was dissolved in 20 ml of distilled water and made to 50 ml mark in a clean volumetric flask. The pH values of the sampled salts was determined using a JENWAY pH meter model 3505, and a combined glass electrode (Ag/AgCl; PHE 1004), calibrated with pH 7 and pH 4.01 buffers, allowing 5 minutes for the reading to stabilize at a temperature of 20.8^{o}C. Determination of pH for each sample was done in triplicate.

a) **Moisture content:** About 0.5000 grams of each of the salt samples were weighed using an electronic analytical balance, Shimadzu type model AUY 120. The weighed salt samples were dried in a Memmert oven model UNB 500 at 105^{o}C for 8 hours and reweighed. The difference gave the moisture content of the weighed salt samples.
b) **Iodine determination:** An electronic analytical balance, Shimadzu type model AUY 120 was used to weigh 0.5000 grams of the reed plant salt samples into a 50 ml measuring cylinder. double-distilled water was added to dissolve the salt completely and made to 50 ml. the salt solution was transferred into a 50 ml conical flask with stopper; 1 ml of 2 N sulphuric acid was added to the salt solution, followed by 5 ml of 10% potassium iodide, giving the solution a yellow color. The flask was stoppered and put it in the dark for 10 minutes. The liberated iodine was titrated with sodium thiosulphate solution using 1ml of 1 % starch indicator near the end of the titration giving the solution deep purple color; the resulting solution was further titrated with sodium thiosulphate until the purple coloration disappeared and the solution becomes colorless; From the redox equations, based on the weight of the sample salt, 0.5 g, used to prepare the salt solution, the iodate content was calculated in mg of iodate per kg of the salt (ppm). This was repeated to obtain concordant results.
c) **Iron (II) content**: Phenanthroline method for total iron was used for determination of iron in the salt samples. The absorption spectrum for the 2.0 ppm standard solution was recorded against the reagent blank inthe range of 400 - 700 nm and used to obtain the spectrum: by plotting the absorbance as a function of the wavelength. The concentration of the given sample solution was determined with the help of the calibration curve. The ferrous ion present in the unknown sample solution was calculated by accounting for the dilution factor.
d) **Sodium and Potassium Metals in Salt Samples**:Solutions of 0.5 g of salts in 500 mL of solvent were used in analysis. Sodium and potassium were determined by flame photometer 410 using butane at 2.1 kg cm^{-2} pressure with 0.4 L min^{-1} rate. Analyses were conducted at Egerton University, Department of Chemistry.

2.4 Statistical Analysis

The data were based on three replicates and subjected to analysis of variance. Means were calculated and compared using t-test , standard errors of each individual nutrient of the samples were computed, and variations among the species were evaluated by least significance difference (LSD) at 5% level of probability ($p<0.05$). Data analysis was conducted using the SPSS Statistical Computer Package.

3. Results and Discussion

3.1 Salt content of Ash and Raw Material

Table 1 shows the percent of the salt content of ash and raw material.

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Table 1: Percent of salt obtained, calculated using ash and raw material

Plant species	*Region*	*Ratio of salt to ash, %*	*Ratio of salt to raw material, %*
T. latifolia	Lugari	4	0.003
C. papyrus	Busia	10	0.125

Table 1 shows that salt content of raw material do not exceed 1 and 10% in the ash obtained. The lowest salt in the raw material and in the ash is that of stems of *T. latifolia*followed by that of *C. papyrus*. The greatest salt contents of the two materials are that of *C. papyrus* (10% for ash and 0.125% for raw material). *T. latifolia*contains, respectively 4% of salt in the ash obtained and 0.003% of the raw material. The lowest ratio of salt in reed stems can be explained by the fact that they contain essentially carbohydrates like cellulose which burn in processing for ash production.The high percentage of salt from *C. papyrus* explains why it's still the most traditionally used plant in Busia region to produce indigenous salt. Echeverri and Roman-Jitdutjaano (2011) have mentioned another species of the genius, *Hygrophilaspinosa*, as one of the most plants used in Africa to produce ash salt. Bibliography reveals that salt contents of plant are generally low (http//www.alchimie-pratique.org/selplante.html). Most of the authors have found the ratio of 0.42% of raw fern, 0.55% of raw vine, 0.55% of raw willow and 0.15% of raw oak. All these data are higher than the results obtained in this study.

3.2 pH determination

The pH of samples from Busia and Lugari were determined and represented in the figure below 4 below.

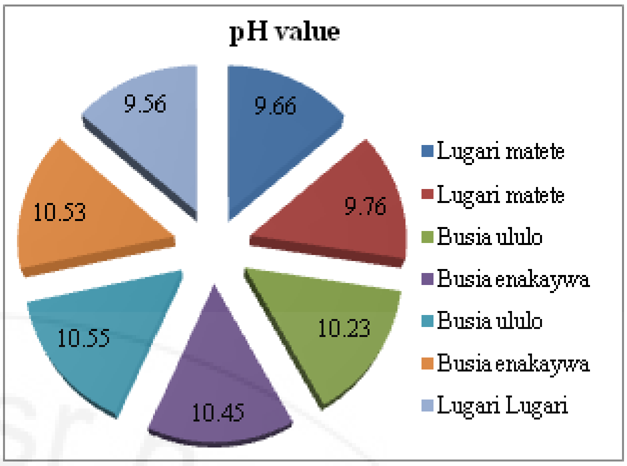

Figure 1: pH values of *Cyperus papyrus* and *Typha Latifolia*salts

The results indicate higher pH values, with an average of 10.53, for salts prepared from *Busia*, *Cyperus papyrus* (pH= 10.23-10.55)as compared to those from Lugari, *Typha Latifolia* (pH=9.56-9.76), with an average of 9.66. The above results were tabulated as in figure 4, and Busia salts had higher pH values above 10, an average of 10.44**.** The pH of the salt is very much influenced by the type and quantity of impurities present, and this may in turn affect the stability of the iodine compounds (Biber *et al*, 2002). From the study it is clear that salt samples collected from Busia County had higher pH values, more alkaline, as compared with Lugari salt samples. Busia samples had pH ranging from 10.23 to 10.55, an average of 10.44, while samples from Lugari County had a range of 9.56 to 9.76, an average of 9.66.

3.3 Moisture Contents

The salt samples processed from two reed species, *Typha Latifolia*, of Lugari and the Busia *Cyperus papyrus* species were compared for their moisture contents and the results are represented in table 5 below.

Table 1: Moisture Content

County source	*Plant species*	*Sampling site*	*No. of samples*	*Mean moisture content in grams*	*Range*	*% moisture content*
Lugari	*Typha Latifolia*	matete	2	0.0053	0.0053-0.0053	0.53
Lugari	*Typha Latifolia*	Lugari	2	0.0104	0.0100-0.0108	1.04
Busia	*Cyperus papyrus*	enakaywa	2	0.0867	0.0569-0.1156	8.67
Busia	*Cyperus papyrus*	ululo	2	0.1561	0.1559-0.1564	15.61
Busia	*Cyperus papyrus*	enakaywa	2	0.1115	0.1067-0.1163	11.15
Busia	*Typha Latifolia*	Enakaywa	2	0.0488	0.4410-0.5350	4.88
Lugari	*Typha Latifolia*	matete	2	0.0061	0.0055-0.0068	0.61

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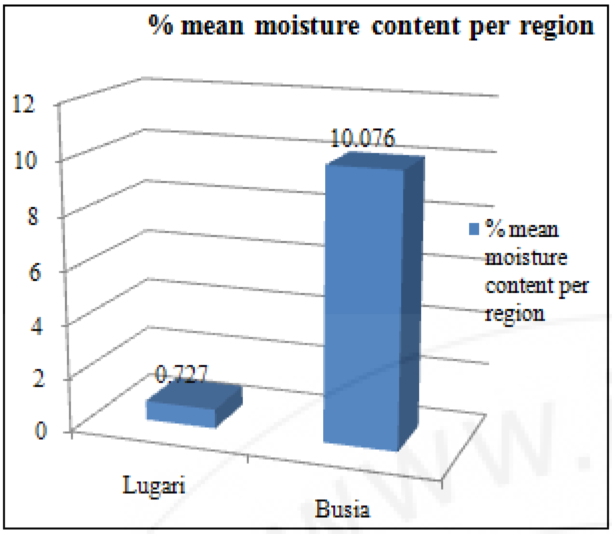

Figure 2: % mean moisture content per region

Salts from *Cyperus papyrus* species of Busia County had the highest moisture content, ranging from 4.88 % to 15.61% with the highest 15.61 % having been obtained from Enakaywa village, Butula division while those from Lugari County, *Typha Latifolia* salts had the lowest moisture content with a range of 0.53 % to 1.03 %, the highest 1.03 % coming from Matete division. *Cyperus papyrus* salts reported a mean % moisture content of 10.076% compared to *Typha Latifolia* salts with 0.727. Salts obtained from *Cyperus papyrus* reed plants that thrive in swampy areas have higher moisture contents than those from *Typha Latifolia* obtained from rivers mainly, as is the case with Busia and Lugari salt samples respectively.

3.4 Iodine content in salts samples

Results from figure 2 below indicate the high levels of iodine in Lugari county samples as seen, with a range of 0.5082 to 1.1435ppm. Busia salts had lower iodine levels in the range of 0.3049-0.4158 ppm. *Typha Latifolia* is a bioaccumulator of iodine as compared to*Cyperus papyrus.* Equally from Busia region, Enakaywa salts from*Cyperus papyrus* reeds species did not show the presence of iodine. For iodine consumption therefore *Typha Latifolia* salts are more recommended.

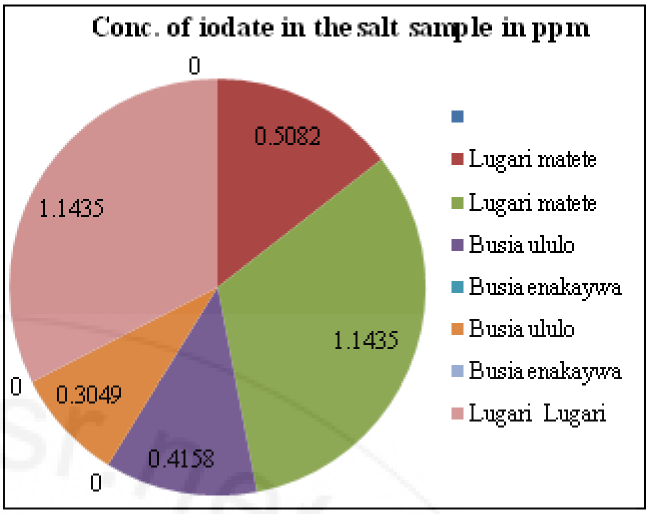

Figure 3: Average concentration of iodate in the salt sample

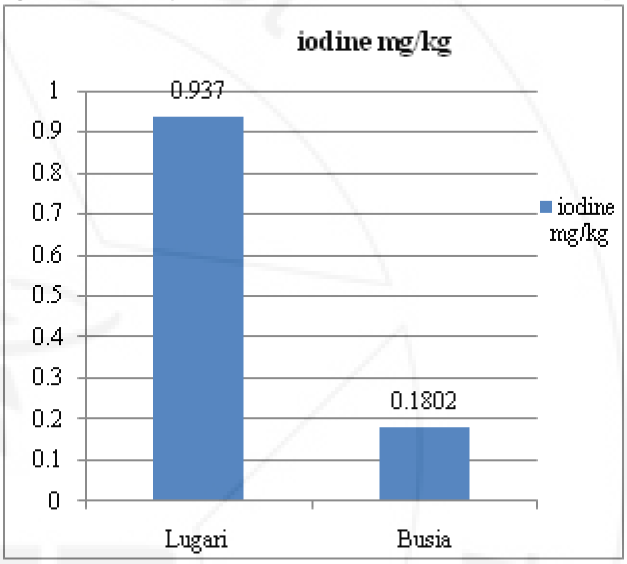

Figure 3: Average iodine concentration per region in mg/kg

The average concentration of iodine in *Typhalatifolia* is higher than in *Cyperus papyrus*, with 0.937 ppm and 0.1802 ppm respectively.Total salt iodine concentrations were mainly in the range 0.36 – 1.14 mg kg^{-1}, lower than the worldwide average of 5 mg kg^{-1} (Fleming, 1980; Ure and Berrow, 1982) and the UK average of 9.2 mg kg^{-1} (Whitehead, 1979). There is need to increase the iodine levels in the salts from the two regions of western Kenya.

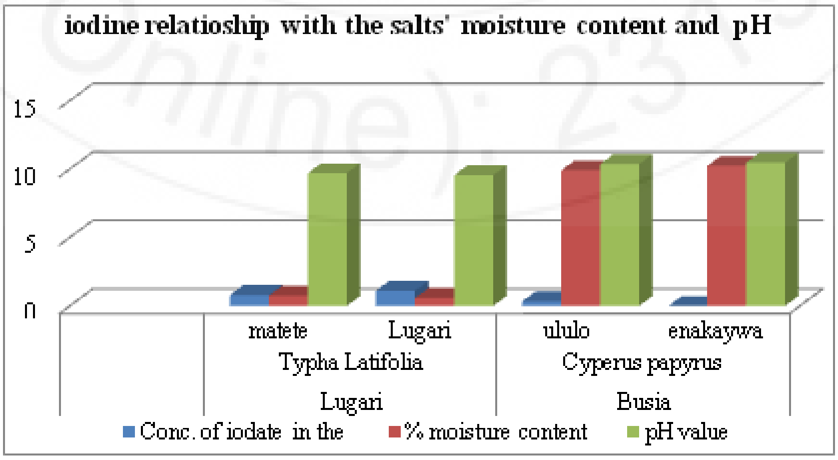

Figure 4: iodine relatioship with the salts' moisture content and pH

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The pH values are inversely proportional to the iodate content in the salt samples, meaning that the alkaline condition contributes to the loss of iodine from the salts, as in the figure below. Equally, where pH values are high and the % moisture high, there is no iodate present in the salt. The situation is such that the iodine content of the salt samples is inversely proportional to the pH as well as the moisture content.Salt samples from Lugari County had the highest iodate concentration ranging from 0.5082 to 1.1435 ppm, with the highest from Lugari division and the lowest from Matete division while Busia had a range of 0.3049 to 0.4158 ppm.*Cyperus papyrus* salts from Busia County had lower iodate content as compared to *Typhalatifolia* salts in Lugari County while other samples had no iodate (NP). Of the two villages sampled, samples from Enakaywa village did not contain any amount of iodate. Elemental iodine readily sublimes and is then rapidly lost to the atmosphere through diffusion. The presence of moisture and some hygroscopic impurities and metal ions impurities such as iron accelerates the loss of iodine (Diosady*et al,* 1998). As is the case for moisture content, it is clear that the salts from Busia recorded the lowest iodate contents, which is true with the moisture contents that were high. It is therefore important to prepare this salt to ensure lower moisture levels so as to have higher iodate contents. Moisture is naturally present in the salt, or is abstracted from the air by hygroscopic impurities such as magnesium chloride. Other evidence of the magnitude of iodine losses from iodized salt come from studies assessing the efficacy of stabilizing compounds in local salt. Findings from control samples without stabilizers suggest considerable variation, in iodine stability in spite of differences in experimental design. Diosady *et al,* (1998) found that coarse salt iodized with iodate at "normal" room temperature and humidity showed iodine losses of 15 % at 3 months, increasing to 20 % at 12 months. Samples containing a stabilizer calcium carbonate did not appear to have lost any iodine over an 18 month period under these conditions (Diosady *et al,* 1998). Noting that indigenous salts do not contain such stabilizers may possibly result in greater.Plant salts from Lugari County contain higher levels of iodate, on average 2.03 mg/kg, as compared to plant salts from Busia, with an average concentration of o.4920mg/kg (ppm). While plant salts from UluloVillage of Busia County contain some iodate, plant salts from Enakaywa village in Busia had no iodate. Also noted was the high iodine content in Lugari than in Busia salt samples. There is an inverse proportionality between iodine and % moisture contents in both the two regions.Although some of the plant salts contain iodate the amount is very low compared to the recommended levels and therefore this salt requires iodisation.

3.5 Sodium, Potassium and Magnesium Concentration in Salt Samples

Salt samples were analyzed for the presence of sodium, potassium and magnesium and data represented in table 6 below. The results were further compared with the pH and moisture contents for respective salt samples.

Table 2: Sodium, Potassium and Magnesium Concentration in Salt Samples

Reed species	*Sampling area*	*sample no.*	*pH*	*% Moisture content*	*conc. in mg/g*			
					Na	*K*	*Mg*	*Na/K ratio*
T. latifolia	Lugari	1	9.66	0.53	3658.8	2157.3	3.023	1.6960
	Lugari	2	9.76	1.03	4349.4	2489.6	3.472	1.7470
	Lugari	7	9.56	0.61	4349.0	1991.1	3.190	2.1842
	Busia	3	10.23	8.67	5336.5	1559.1	2.810	3.4228
C. papyrus	Busia	4	10.45	15.61	7285.7	1908	3.153	3.8185
	Busia	5	10.55	11.15	7967.9	1991.1	2.470	4.0018
	Busia	6	10.53	4.88	7227.2	1908	3.170	3.7878

n = 12; Weight of salt sample used= 0.5 g

From the results in table 6 above, the level of sodium is higher than potassium in all the samples, and particularly for Busia samples with the highest Na concentrations. There is between 41.04 - 54.22 % more sodium in Lugari samples compared to the 70. 78 – 75.01 % in Busia samples. Currently dietary guidelinesin the US recommended limiting salt intake to 1.5-2.4 grams of sodium per day while the American Heart Association suggests 1.5 gram limit. For a frame reference, one tea spoon of regular table salt contains about 2.3 grams of sodium. The balance between sodium and potassium may decide whether the salt consumption will ultimately be harmful or helpful. A normal Na/K ratio is between 2.5:1 and 4:1. A high ratio is associated with Zn and/or Mg deficiency, or due to the presence of toxic metals. Cadmium, Mercury, Nickel, Fe toxicity can elevate sodium levels and cause a high Na/K ratio (Guyton, 1981). comparing the salt samples' Na/K ratio with the recommended ratio of 2.5:1 and 4:1, it can be observed that Lugari samples are slightly below the 2.5:1 ratio lower limit while for Busia salts processed from *Cyperus p.* they meet the recommended limit of 2.5-4 for Na: 1 for K. there seems to be more toxicity in Lugari samples or that Zn/Mg levels are to be thoroughly investigated since these have an effect on the Na/K ratio in salts.

It is apparent from the results that the sodium levels increased with increase in pH and % moisture of the salt. For instance, Lugari had pH range of 9.56 to 9.76 and its sodium content was 124.32 to 182.80 ppm respectively, while Busia's pH range was 10.23 to10.55 for sodium content which was 533.65 and 798.79 ppm respectively. The moisture content also varied directly with amount of sodium, with Lugari having the lowest moisiture content and recording lower sodium content, where a percent of 0.53 moisture sample had 241.32ppm of sodium while a % moisture of 1.03 sample had sodium level of 182.80 ppm. This trend was also observed with the salt samples from Busia.

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3.6 Iron and Iodine Concentrations in the Salt

Samples were analysed for the presence of iron and iodine and results presented in table 7 below and further compared with the moisture levels.

Table 3: Iron and Iodine Concentrations in the Salt

Sampling area	sample no.	% Moisture content	Concentration in mg/g	
			Fe	I
Lugari	1	0.53	11.25	1.903
Lugari	2	1.03	6.503	0.846
Lugari	7	0.61	4.61	0.846
Busia	3	8.67	17.89	2.33
Busia	4	15.61	15.04	ND
Busia	5	11.15	16.3	3.173
Busia	6	4.88	15.04	ND

n = 12; Weight of salt sample used= 0.5 g

4. Conclusion

The chemical analysis shows that all the four salts are potassium rich and sodium poor. They also contain minor elements which play important roles in the metabolism of human organism. These salts can be used as dietetic salts as substitute of the industrial salt which contains mostly sodium chloride especially for persons who have health problems like hypertension, cardiovascular and kidney diseases.Plant salts from Lugari County contain higher levels of iodate, on average 2.03 mg/kg, as compared to plant salts from Busia, with an average concentration of o.4920mg/kg (ppm). While plant salts from Ululovillage of Busia County contain some iodate, plant salts from enakaywa village in Busia had no iodate. Also noted was the high iodine content in lugari than in Busia salt samples.there is an inverse proportionality between iodine and % moisture contents in both the two regions.By comparing the salt samples' Na/K ratio with the recommended ratio of 2.5:1 and 4:1, it can be observed that Lugari samples are slightly below the 2.5:1 ratio lower limit while for Busia salts processed from *Cyperus p.* they meet the recommended limit of 2.5-4 for Na: 1 for K, hence ideal for use.

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