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Quantification of Heavy Metals in Breast Milk Samples Sampled from Kilimani/Kidoti in Zanzibar

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Abstract

People of Zanzibar are perpetual users of tomatoes. Different varieties of tomatoes are regularly consumed in day-to-day meals in Zanzibar. These fruits are farmed under different settings varying from purely organic mode to extensive use of agrochemicals. It is well known that the use of agrochemicals may give rise to heavy metals contaminations with significant health impacts to consumers. The use of fertilizers and pesticides in some areas during cultivation may bring about individuals to eat tomatoes together with harmful heavy metals. However, no study has been done to find the quantity of heavy metals entered in consumers' bodies. The objective of this study was to quantify heavy metals present in breast milk of individual tomato consumers. The methodology used was sampling, processing, digestion and analysis. The instrument used for analysis was Atomic Absorption Spectrometer (AAS). Samples were collected, digested with HNO_3 and H_2O_2 in a semi–closed glass digestion apparatus. After cooling, volume was adjusted to 25 ml with distilled water and analyzed by AAS. Zinc detected by AA spectrometer ranged from 231 µg/l to 1466 µg/l of milk, with average of 900±457 µg/l. Cadmium ranged from 24.1 µg/l to 35.9 µg/l with average of 31.1±3.46 µg/l. Lead ranged from 32.4 to 1630 µg/l with average of 707±582 µg/l. The concentration of Al metal, which was detected in only one sample, was 0.91 µg/l. The results show that breast milk contains heavy metals contaminants necessary to be removed before infant feeding.

Keywords: fertilizers; pesticides; breast milk; zinc; cadmium; lead; aluminium; AA Spectrometer; quantify.

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1. Introduction

Inorganic pollutants, such as heavy metals, are potentially risky elements. A heavy metal is any metal or metalloid of environmental concern referred to the harmful effects of cadmium, mercury and lead, which are denser than iron. It applies to any other similarly toxic metal, or metalloid such as arsenic, regardless of density. Other similar commonly encountered heavy metals are selenium, silver, cadmium. The most toxic elements are lead, cadmium, mercury, arsenic, copper, cobalt, zinc, and some other metals [1].

One of the most important environmental problems currently is ground water contamination and between the wide diversity of contaminants affecting water resources, heavy metals receive particular concern considering their strong toxicity even at low concentrations [2]. This contamination in aquatic and soil environments threatens aquatic ecosystems, agriculture and human health. Also, these elements are accumulated in sediment and in the tissues of living organisms in the river. Aquatic plants absorb heavy metals from water column and pour water, incorporate into the food chain, and their levels increase through biological magnification [3]. Some kinds of toxic sediments kill benthic organisms, reducing the food available to larger animals such as fish. Some contaminants in the sediment are also taken up by benthic organisms in bioaccumulation. When larger animals feed on these contaminated organisms, the toxins are taken into their bodies, moving up the food chain in increasing concentrations in biological magnifications. As a result, fish and shellfish, waterfowl, and freshwater and marine mammals may accumulate hazardous concentrations of toxic chemicals [4].

Humans need nearly 72 trace elements for metabolic processes, including very low concentrations of heavy metals, such as Cu, Sn, V, Cr, Mo, Mn and Co. Most metals are toxics at high concentrations, while others provoke deleterious effects at low concentrations. Cd, Pd and V metals are pollutants of biological interest due to their toxicity [5].

Heavy metals can cause serious health effects with varied symptoms depending on the nature and quantity of the metal ingested. They produce their toxicity by forming complexes with proteins, in which carboxylic (–COOH), amine ($–NH_2$), and thiol (–SH) groups are involved. These modified biological molecules lose their ability to function properly and result in the malfunction or death of the cells. When metals bind to these groups, they inactivate important enzyme systems or affect protein structure, which is linked to the catalytic properties of enzymes. This type of toxin may also cause the formation of radicals which are dangerous chemicals that cause the oxidation of biological molecules. The most common heavy metals that humans are exposed to are aluminium, arsenic, cadmium, lead and mercury. Aluminium has been associated with Alzheimer’s and Parkinson’s disease, senility and presenile dementia. Arsenic exposure can cause among other illness or symptoms cancer, abdominal pains and skin lesions. Cadmium exposure produces kidney damage and hypertension. Lead is a commutative poison and a possible human carcinogen while for mercury, toxicity results in mental disturbance and impairment of speech, hearing, vision and movement. In addition, lead and mercury may cause the development of autoimmunity in which a person’s immune system attacks its own cells. This can lead to joint diseases and ailment of the kidneys, circulatory system and neurons. At higher concentrations, lead and mercury can cause irreversible brain damage [2]. Fat solubility influences the storage of pesticides in the mother's tissues, their mobilization during lactation, the transfer of pesticides from the mother's blood plasma to the milk, and thus the total dose to the infant. The passage through several barriers is greatly accelerated if a substance is fat-soluble. The list of chemicals of concern includes metals such as lead, mercury and cadmium, and solvents and halogenated hydrocarbons that include many of the fat soluble pesticides, described as transferring to milk very efficiently due to the high proportion of fat in milk [6]. However, no study was done to establish the quantity of heavy metals in breast milk in Zanzibar. The objective of this study was to quantify heavy metals present in breast milk of tomato consumers from Zanzibar. The hypothesis of this research was that there is no significant publicized information on quantity of heavy metals present in breast milk of tomato consumers from Zanzibar. The present study has significantly addressed the information on quantity of heavy metals present in breast milk of tomato consumers from Zanzibar. Among the limitations of this research were the reagents used for digestion of samples should be analytical. The water used for dilution should be distilled and whenever possible should be double distilled. Milk samples should be produced by health women who lived in study area and ensured by farmer ate the tomato fruits. Milk samples should be processed as quickly as possible.

2. Materials and Methods

2.1 Study area

This study was conducted at Kilimani/Kidoti area in North A district of Zanzibar.

2.2 Apparatus

Beakers, conical flasks, measuring cylinders, centrifuge, Analytical balance, AA Spectrophotometer (iCE 300 Series), for analyzing breast milk samples.

2.3 Chemicals

Acetone, hexane, ethanol, ethyl acetate, nitric acid, hydrogen peroxide, distilled water and so on.

2.4 Sample collection

Breast milk samples were collected from human bodies of persons living the area who ate tomato fruits from the farm. For heavy metal determination, samples were collected from 10 healthy women who were living in the chosen area. The milk samples were taken using local naked and well washed personal hands into tubes at the Hygiene and Treatment Centre of Zanzibar. All the milk samples were stored at cold temperature in clean polyethylene containers [7].

2.5 Sample processing

About five grams of milk were placed into 50 ml centrifuge. About ten ml of 1:1 acetone:hexane were added and shaken for 1 min, then shaken for 3 min and then centrifuged for 5 min at 3500 rpm [8].

2.6 Sample extractions/digestion

Five ml of the milk samples was acid digested with 1.4 ml of HNO_3 and 1.4 ml of 30 % H_2O_2 in a semi–closed glass digestion apparatus. After cooling, volume was adjusted to 25 ml with dH_2O [7]. Simultaneously suspended particles were separated by filtration.

2.7 Heavy metals in samples

Thermo Scientific Atomic Absorption Spectroscopy with model of (iCE 300 Series) was used to determine the concentration of digested samples after treatment through different conditions of the parameters. The analysis was done at working conditions of the Atomic Absorption Spectroscopy for Lead, Cadmium and so on at wavelengths: Cd 228.8 nm, Pb 217 nm and other metals [9].

2.8 Sample introduction

The instrument was warmed up and then calibrated with standard solutions, a simple aliquot volume of 10 μL of samples, which obtained after digestion was injected in to graphite tube with the help of an auto-sampler, acetylene was used as a gas, deuterium background correction and a temperature program of the furnace was optimized to obtain the best signal during the atomization process, the instrumental parameters were adjusted according to the manufacturer's recommendation (Unicam-Atomic Absorption Methods Manual 1994). In order to monitor the contamination during the whole procedure of analysis, 5.00 g of water was treated the same as real samples for each batch of analysis as water samples procedural blank. Concentration of the metal ions present in the sample was determined by reading their absorbance using AAS and comparing it on the respective standard calibration curve. Then heavy metals were analyzed with TSAAS (Thermo scientific model iCE300Series)

2.9 Data analyses

The study generated a database of heavy metals analyses. In order to reveal a reliable outcome the resulting database needed to be analyzed properly. Data was analyzed using Microsoft Office Excel. The data were also expressed in term of descriptive statistics while the table was presented with Mean (average) values, variances, standard deviation and correlations. The graphs were drawn by using Microsoft Office Excel and correlations were found by using the same Office.

2.10 Data presentation

Data was presented by using table format data presentation. Rows and columns were used to show the statistical data. To simplify the results for easier visualization and understanding the data, graphic presentation was also used.

3. Results

Analysis of heavy metals in human breast milk detected presence of four metals including Zn, Al, Cd and Pb. The metal Al was only detected at detection frequency of 10% (in only one sample) of analyzed samples while

Zn, Cd and Pb were measured in all milk samples. The other metals were below the method detection limits. Table 1 Presents concentration of measured heavy metals. The concentrations ranges of, Zn, Cd and Pb were 231 - 1470 µg/l, 24.1 - 35.9 µg/l and 32.4 - 1630 µg/l respectively. Zn was measured at highest mean concentration of 900±457 µg/l followed by Pb (mean = 707±582 µg/l) and Cd (mean = 31.13±3.46 µg/l). The concentration of Al metal was 0.91 µg/l.

From literature the mean concentration of Pb and Cd in 100 samples of human breast milk from Iran was 0.0147 and 0.0121 ppm, respectively. The mean concentration of mercury (Hg) in 100 samples of human breast milk was 0.96 ppb. The maximum concentrations were found in breast milk of rural mothers [10].

From another research done in Ghana the mean level of Pb was 4.33 µg/l with a range up to 32.0 µg/l. The mean level of Cd was found to be 1.34 µg/l and range up to 12.30 µg/l. The mean arsenic concentration was 1.54 µg/l and ranging up to 6.22 µg/l [11]. In another study the mean ± standard deviation of Cd and Pb concentrations in human milk were 2.44 ± 1.47 µg/l (range 0.62 - 6.32 µg/l) and 10.39 ± 4.72 µg/l (range 3.18 - 24.67 µg/l), respectively [7]. The study was done in Iran and the objective was to analyze the concentrations of zinc and copper in infant formulas and human milk during prolonged lactation. Levels of these metals were examined in relation to selected parameters such as age, weight, height, education and occupation of mothers. The findings showed that the mean values of Zn and Cu in human milk were 2.95±0.77mg/l and 0.36±0.11 mg/l [12]. A study was done in Austria with the evaluation of the influence of mothers' lifestyles on Pb and Hg levels in breast milk. The results showed that breast milk had low Hg and Pb concentrations (Hg: 1.59 ±1.21 1µg/l; Pb: 1.63 ± 1.66 6µg/l) [13].

Comparing the results in literature and that obtained from this study the concentration of Cd and Pb were greater than the concentration of Cd and Pb quoted by above literatures.

Concentration of 900 µg/l Zn from breast milk of Kilimani, Zanzibar<<concentration of 2.95 mg/l Zn from human milk of Iran. Concentration of 31.1 µg/l Cd from breast milk of Kilimani, Zanzibar>>1.34 µg/l Cd from human breast milk of Ghana, 0.0121 ppm Cd from Iran, 2.44 µg/l Cd from Iran. Concentration of 707 µg/l Pb from breast milk of Kilimani, Zanzibar>>4.33 µg/l Pb from human breast milk of Ghana, 0.0147 ppm Pb from Iran, 10.4 µg/l Pb from Iran, 1.63 µg/l Pb from Austria. The reason behind for these differences probably may be the geographical and social nature of Zanzibar. People of Zanzibar Islands use fish and marine animals in their daily meals regardless whether they are safe or contaminated with heavy metals. The recommended dietary allowance (RDA) for zinc intake is recommended for infants (2–3 mg/day) and children (5–9 mg/day) [14]. Since the mean quantity of Zn in breast milk obtained in this research was 721 µg/l, the volume of breast milk in maternal uptake that an infant is supported to feed from mother breast so as to uptake this quantity is 2.77 – 4.16 litres/day and children is 6.93 – 12.5 litres/day. Since the children uptake these amount of breast milk from their mother's breasts they will also take Cd and Pb contaminants. Infant will uptake 80.2 – 121 µg of Cd and child will uptake 216 – 389 µg of Cd. Infant will also uptake 1960 – 2940 µg of Pb and child will uptake 5000 – 8840 µg of Pb. The biochemical function chosen was an increased excretion of coproporphyrin, and to keep Pb below 50 µg/l. It was suggested that the threshold limit value (T.L.V.) should be about 0.12 mg/m^3 or a little more in the case of a 40-hour week [15]. Since the mean concentration of Pb in breast milk is 707 µg/l and hence should be for a 566-hour week, which is impossible. By considering recommended tolerable levels of 3.57 mg /kg and 0.8-1.0 mg /kg for Pb and Cd respectively to infants through milk and the recommended desirable levels of 3-5 mg and 0.5-1.0 mg for Zn and Cu, respectively [16], then, reminding the infant is supported to feed from mother breast a volume of 2.77 – 4.16 litres/day and children is 6.93 – 12.5 litres/day, the level of recommended tolerable of Pb for infants will be 9.89 - 14.9 µg and the level of recommended tolerable of Cd for infants will be 2.22 – 3.33 µg. The Cd and Pb intake by any child at Kilimani will also very high to compare with the recommended Cd and Pb to be intake by infant and child and hence are above the health threshold values.

Table 1: Concentration of heavy metals in breast milk sampled from Kilimani/Kidoti

Sample ID/ Parameter	Zn	Al	Cd	Pb	Total Metal Load
	µg/l	µg/l	µg/l	µg/l	µg/l
MH-1	451	BDL	30.8	313	795
MH-2	1340	BDL	28.2	1040	2410
MH-3	1130	BDL	35.1	587	1750
MH-4	231	0.910	31.9	181	444
MH-5	690	BDL	29.2	945.0	1660
MH-6	1310	BDL	30.6	1630	2970
MH-7	479	BDL	33.6	54.7	567
MH-8	1320	BDL	24.1	1540	2880
MH-9	586	BDL	35.9	32.4	655
MH-10	1470	BDL	31.9	741	2240

The quantity of zinc is greater than ten times the quantity of cadmium as shown in the following Figure 1:

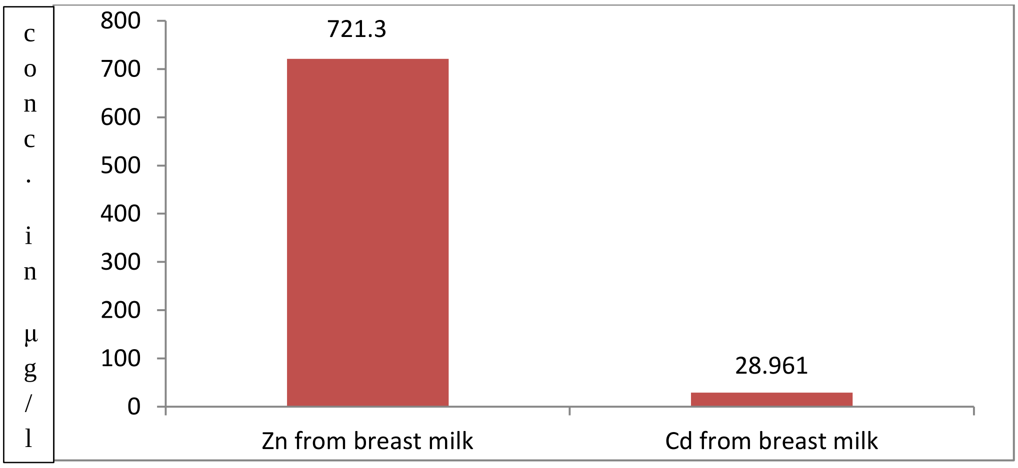

Figure 1: average concentration in µg/l of Zn and Cd in breast milk sampled at Kilimani

3.1 Correlation of heavy metals in breast milk with age of individuals

The following is ages of individuals who contributed in breast milk samples at Kilimani

Table 2

MH	1	2	3	4	5	6	7	8	9	10
Age in years	24	27	40	30	22	27	44	40	39	23

The trend of levels of Zn from breast milk with respect of the age of tomato consumers is shown in the following Figure 2:

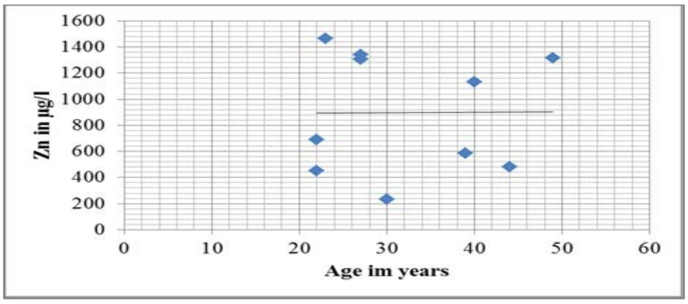

Figure 2: a graph of concentration of Zn in Breast milk Samples Sampled from Kilimani against ages of the individual consumers

Correlation = - 0.197

The concentration of Zn in breast milk of consumers increased slightly with the increase of the age of consumers. Since the feeding of tomatoes is dynamic and human body undergoes bioaccumulation, the quantity of Zn probably is used in some quantity for metabolic process in human bodies. Bioaccumulation will cause the increase of quantity of Zn and may become harmful or toxic if maximum level of allowed quantity in human body is exceeded. Below is Figure 3 indicating the relation between Cd concentrations from milk and ages of milk producers:

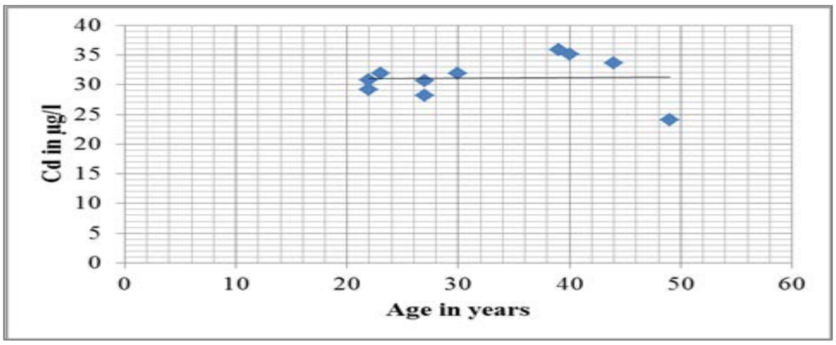

Figure 3: a graph of concentration of Cd in Breast milk Samples Sampled from Kilimani against ages of the individual consumers

Correlation = 0.0218

The graph above shows that concentration of Cd in breast milk increased slightly with the increase of the age of consumer. Since the feeding of tomatoes is dynamic and human body undergoes bioaccumulation the quantity of Cd will increase and may become harmful or toxic if maximum level of allowed quantity in human body is surpassed.

Figure 4 shows a correlation of concentrations of Pb with ages of individual tomato consumers as follows:

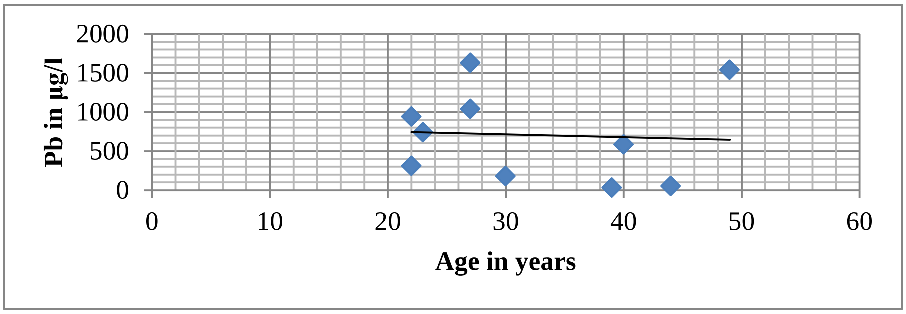

Figure 4: a graph of concentration of Pb in Breast milk Samples Sampled from Kilimani against ages of the individual consumers

Correlation = - 0.064

The graph above shows that concentration of Pb in breast milk of consumers decreased slightly with the increase of the age of consumer. Since the feeding of tomatoes is dynamic and human body undergoes bioaccumulation the quantity of Pb will increase and may become harmful or toxic if will pass the maximum level of allowed quantity in human body.

3.2 Correlation of heavy metals in breast milk

Figure 5 shows a trend relationship of Cd and Zn concentrations from milk as follows:

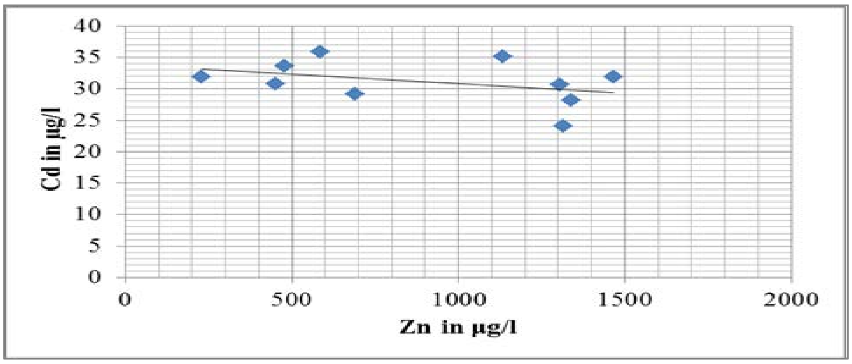

Figure 5: a graph of concentration of Cd in Breast milk Samples Sampled from Kilimani against concentration of Zn of the individual consumers

Correlation = - 0.392

The quantity of Cd decreased with the increase in concentration of Zn in breast milk of consumers. Cd is useless, harmful and toxic in human body. Both Cd and Zn undergo bioaccumulation in human body hence the trend shows that there could be extra sources of Zn for which consumers take in. Also uptake rate of Zn may be different from that of Cd.

In Figure 6 correlation of Pb concentration and Zn concentration from breast milk is indicated as follows:

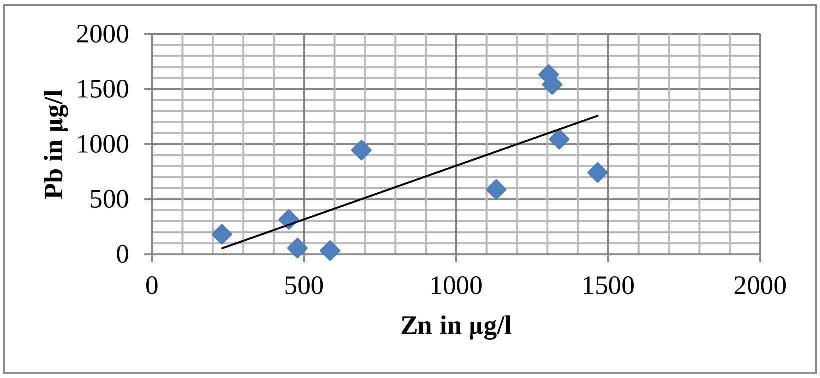

Figure 6: concentration of Pb in breast milk samples sampled from Kilimani against concentration of Zn of the individual consumers

Correlation = 0.764

This graph also shows a good trend of increasing concentration of Pb in breast milk of tomato consumers with the increasing of concentration of Zn in breast milk of tomato consumers. It seems that both Pb and Zn are produced by the same sources and generally the uptake of these heavy metal elements by breast milk is similar in rate.

Correlation of of Pb and Cd concentrations is well shown in Figure 7 as follows below:

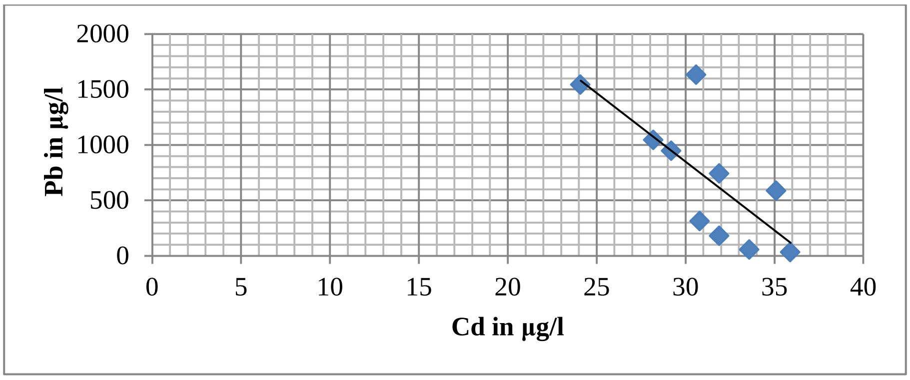

Figure 7: concentration of Pb in breast milk samples sampled from Kilimani against concentration of Cd of the individual consumers

Correlation = - 0.736

This graph shows a trend of increasing concentration of Pb in breast milk of tomato consumers with the decreasing concentration of Cd in the breast milk of tomato consumers. It seems that both Pb and Cd are produced by the same sources and generally the uptake of these elements in breast milk is different in rate. The uptake rate of Pb is higher than uptake rate of Cd. Both Cd and Pb are harmful to human body.

3.3 Trend of heavy metals in breast milk samples with times (trips) of feeding

The following is the number of times (trips) of breast feedings

Table 3

MH	1	2	3	4	5	6	7	8	9	10
No of trips	1	4	12	5	2	2	11	8	11	2

Figure 8 indicates the correlation coefficient of concentration of Zn in milk and number of times of breast feeding of mothers as follows:

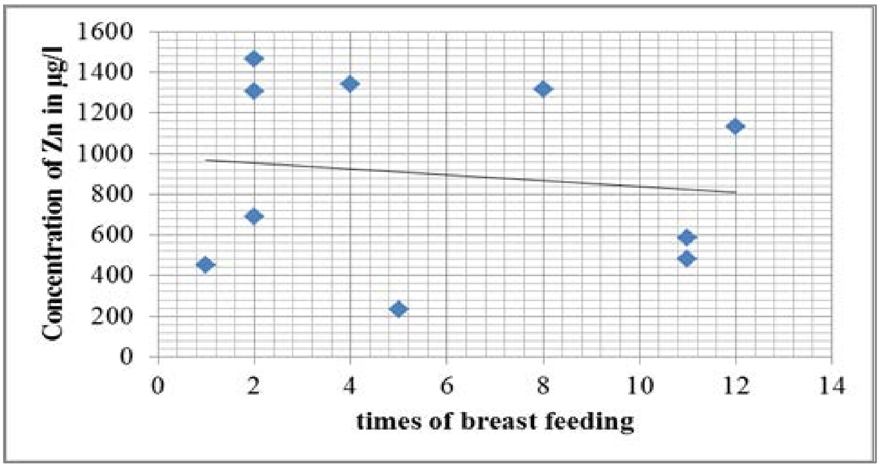

Figure 8: a graph of concentration of Zn in Breast milk Samples Sampled from Kilimani against times of breast feeding

Correlation = - 0.138

The concentration of Zn in breast milk of tomato consumers decreased with the increase the times (trips) of breast feeding.

Figure 9 experiences the occurrence of trend of Cd concentration in milk with respect to the number of times of breast feeding of mother as follows.

Concentration of Cd in breast milk increased with the increase times (trips) of breast feeding

Relation of quantity of Pb in breast milk with number of times of breast feeding is shown in the following Figure 10.

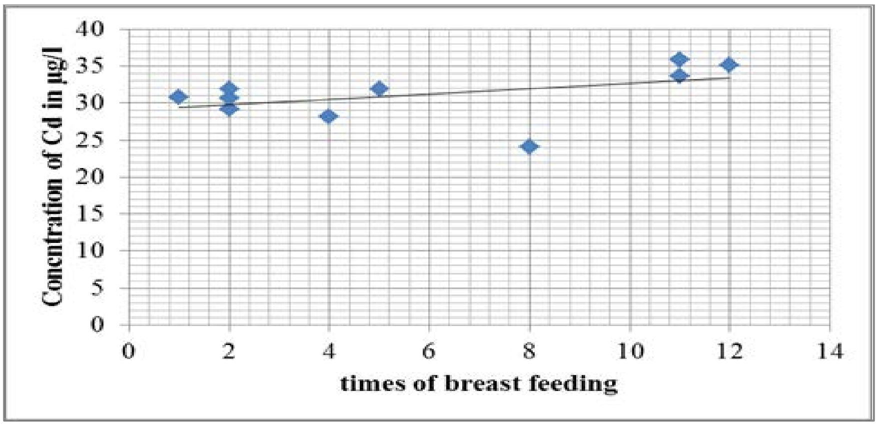

Figure 9: a graph of concentration of Cd in Breast milk Samples Sampled from Kilimani against times of breast feeding

Currelation = 0.442

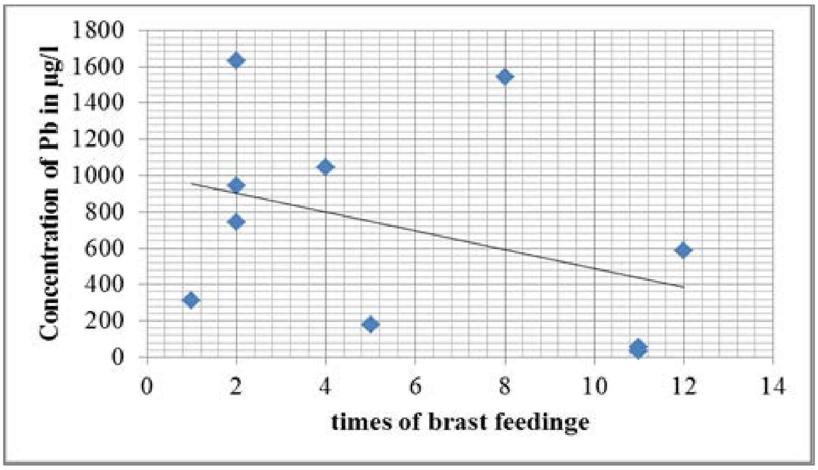

Figure 10: a graph of concentration of Pb in Breast milk Samples Sampled from Kilimani against times of breast feeding

Correlation = - 0.383

Concentration of Pb in breast milk decreased with the increase times (trips) of breast feeding.

Breast feeding is very important for offspring since the breast milk contains sufficient nutrients for children.

Also it is a way of transfer of contaminants including heavy metals which are dangerous for child health. The four detected heavy metals in breast milk samples indicate to what extent the contaminants can be transferred from mother's body to the child's body and cause healthy defect of child simply by sucking mother's breast. The total metal loads show the total quantity of heavy metals that can be accepted by child or infant by sucking 1 litre of breast milk from the mother's breast.

4. Conclusion

Breast milk samples from fruit consumers show positive detection of some heavy metals. Zn, Al, Cd and Pb were detected. The quantity of Zn, Cd and Pb intake by any child at Kilimani will be very high to compare with the recommended Zn, Cd and Pb to be taken by infant and child and hence will be above the health threshold values.

5. Recommendation

Further studies, as a quick and more accurate method for qualitative and quantitative determination of heavy metals in breast milk of fruit consumers should be done especially in Electro analytical chemistry using computers to read and analyze the results. Analysis of nutrients and contaminants in breast milk should be done as quickly as possible once after reproducing offspring and before breast feedings of infants.

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6. Appendix

6.1 Definitions

6.1.1 Recommended tolerable levels

Is the maximum level of continuing daily nutrient intake that is likely to pose no risk to the health of most of those in the age group for which it has been established.

6.1.2 Threshold limit value (T.L.V.)

Is a time-weighted average of the concentration of the hazardous agent in the atmosphere.

6.1.3 The maximum level of allowed quantity

Is the highest limit of quantity of substance allowed so as being less harm to the human body.

This is the greatest *quantity* or *amount* possible, assignable, allowable and so on. It is an upper limit *allowed* or allowable by law or regulation**.**

It is also the greatest quantity or degree reached or recorded; the upper limit of variation.

The Codex maximum level (ML) for a contaminant in a food or feed commodity is the maximum concentration of that substance recommended by the Codex Alimentarius Commission to be legally permitted in that commodity.

6.1.4 Meaning of the terms (units)

μg/l = microgram per litre = one millionth gram per litre.

ppm = part per million, for example μg/g = microgram per gram.

ppb = part per billion, for example ng/g = Nona gram per gram.