

**RESEARCH ARTICLE** 



# Analysis of Seven Micronutrients in Breast Milk of Lactating Mothers from the Central Region of Ghana Using Epithermal Neutron Activation

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## Abstract

**Background:** Breast milk contains various micronutrients which nourishes a baby with nutrition, and therefore the endorsement for exclusive breastfeeding during the first six months after birth. Such micronutrients, if in excess, can have adverse effects on the baby.

**Objective:** The levels of seven micronutrients in breast milk obtained from 27 lactating mothers in the Cape Coast Metropolitan area have been determined using Epithermal Neutron Activation Analysis (ENAA). This technique was used because it is suitable for performing both qualitative and quantitative multi-nutritional analyses on samples, and offers accuracies and sensitivities superior to those attainable by other methods.

**Materials and Methods:** During the analysis, a- 3 mm thick flexible boron was used to cut-off thermal neutrons so as to assess epithermal neutrons, thereby creating an activation energy which measured the levels of micronutrients in the breast milk. The standard reference materials used were the International Atomic Energy Agency (IAEA)-336; IAEA-407, IAEA-350 and National Institute of Standard and Technology (NIST) USA SRM 1577b. The Relative standardization method was used in the quantification of the micronutrients, with an accuracy of about 94.7 %. The micronutrients are Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Manganese (Mn), Copper (Cu) and Iodine (I).

**Results:** Except for iodine which had levels below the recommended dietary allowance (RDA), the remaining micronutrients had levels above the upper limit of the RDA, with Manganese being the highest.

**Conclusion:** The levels recorded are directly linked to the food intake of the mothers, and therefore the need for pregnant and lactating mothers to be mindful about what they eat. Children could be exposed to metabolic disorders and diseases as a result of such high levels.

**Keywords:** Epithermal Neutron Activation Analysis (ENAA), Hazard Quotient (HQ), Lactating Mothers, Micronutrients, Recommended dietary allowable (RDA).

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# Introduction

Breast milk is the natural first food for babies. It provides their energy and nutrients, promotes sensory and cognitive development, and protects them against infectious and chronic diseases. Exclusive breastfeeding during the first 6 months of life promotes immunity and reduces infant mortality due to illnesses such as diarrhoea and pneumonia (Ochoa & Turin, 2014). Babies go through a rapid period of growth after birth and usually double in length and triple in weight within their first twelve months. Even when solid foods are introduced, breast milk remains an important source of nutrition for proper growth and development (World Health Organization, 2009). The

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Academy of Nutrition and Dietetics recommends that infants consume breast milk alone during the first six months of life, and breast milk with complementary foods from 6 to 12 months. Mothers who choose not to, or are unable to breastfeed can offer infant formula in place of breast milk. The decision to breastfeed or formula feed a baby could be personal or influenced by health issues such as HIV/AIDS infection. Weighing the pros and cons of breastfeeding or formula feeding can help make a decision for the baby (WHO, 1998). Infants from 0 to 6 months should take breast milk or infant formula on demand, to help meet their nutritional requirements (Felder et al., 2018). Carbohydrates are the main source of energy for the body while protein contributes to the energy used by the body, and is essential for the building of tissues of the growing body. Fat contributes to the energy requirements of the body, and helps in the absorption of fat-soluble vitamins such as Vitamin A, D, E and K. Vitamins and minerals assist in the general protection and functioning of the body (World Health Organization, 2009).

Micronutrients are vital for the wellbeing of the human body and derived from diet. Iodine (I) is important for the production of thyroid hormones mostly concentrated in the thyroid gland (Endocrineweb, 2017). Thyroid cells combine with iodine and the amino acid tyrosine to control metabolism. The leading preventable causes of brain damage, which is iodine deficiency, can significantly lower the intelligence Quotients (IQ) of a whole population. It has been estimated that countries could prevent the loss of intellectual capacity by 10 to 15 percentage points if young children, newly born babies and pregnant mothers receive enough iodine (Caulfield et al., 2006). There is the concern that the continuous occurrence of Iodine Deficiency Disorders (IDDs) among children in Ghana may hamper the objectives of the educational reform programmes and the nation's developmental efforts. Statistics indicates that about 81,200 babies are born annually with mental impairments as a result of iodine deficiency, resulting in stunted growth and low IQs, thereby impeding their learning abilities (Ghana News Agency, 2007). Breast milk is the best source of iodine for babies, and helps in development of their brain and nervous system. Infants up to 6 months need an iodine intake of 90 micrograms per day (Andersson & Braegger, 2021).

Magnesium (Mg) promotes the health of children and adults, and is responsible for approximately 800 enzymatic functions in the human body. Babies up to the age of 6 months may require 30 mg per day. It is needed for DNA formation, helps children get a better sleep to conserve energy and aids in insulin and blood sugar regulation (Mayo Clinic, 2023).

An adequate amount of Copper (Cu) is important for the optimal functioning of the brain as its presence within certain enzymes in the brain helps form key neurotransmitters that allow brain cells communicate to one another (Svetlana et al, 2019). Babies up to the age of 6 months may require 0.20 mg per day (Food and Nutrition, 2001). The normal term infant is born with a generous store of copper in the liver making copper deficiency a rare event (Widdowson, Dauncey, & Shaw, 1974). Copper deficiency might be responsible for a resistant anemia in milk-fed infants because cow's milk is low in copper (Picciano, 1985).

Manganese (Mn) is involved in the formation of bones (Food and Nutrition, 2001) and aids in the action of some enzymes involved in carbohydrate metabolism (Lenntech, 2019). Babies up to the age of 6 months may require 0.003 mg of Mn per day (Food and Nutrition, 2001), with an unbalanced level (relatively high or low) resulting in poor brain development (Norton, 2023). Obesity, changes in hair colour, abnormal bone and cartilage function and growth retardation may be an adverse effect of Mn deficiency (Lenntech, 2019).

Sodium (Na) is an electrolyte/mineral that functions as a major ion of the extra cellular fluid and also aids nerve impulse transmission. Babies up to the age of 6 months may require 120.0 mg of Na per day (Health Supplements Nutritional Guide, 2017). A baby will have its entire sodium requirement from breast milk, and the need not to add additional salt, which is the main source of sodium, to the baby's food.

Potassium (K) works with sodium to control the body's water balance. This helps maintain a normal blood pressure, to the extent that a diet low in potassium and high in sodium will cause.

# Materials and Methods

Study Area: The study is quantitative research aimed at determining the level of seven micronutrients in the breast milk of volunteer mothers from 20 communities in the Central Region of Ghana. Two health facilities located in Cape Coast and Elmina were used due to their accessibility and high utilization by patients. These facilities provide both static and outreach child welfare services, and therefore attend to mothers from towns in and around them. Cape Coast and Elmina are metropolis of the Central Region of Ghana, along the Gulf of Guinea (Figure 1). The original inhabitants of the communities are mostly subsistence farmers and fishmongers (Hood, Vowotor, Nyarko, & Fletcher, 2011), with the volunteers being mainly petty traders, caterers, teachers, nurses, seamstresses, students, hairdressers, and some unemployed.

#### Sample Collection

*Inclusion Criteria*: The criteria for selection was that the mothers should have volunteered to be part of the study, should have had full term pregnancies, practicing exclusive breastfeeding and was within the first six months of the babies' lives.

The study utilised the services of trained midwifes and nurses who were service providers to these mothers, and were able to take the breast milk aseptically. Before the sample (breast milk) collection, the nipples and areolas of the breasts were cleaned with 70% methyl alcohol. The breast milk was collected by manual expression, was between 10-20 mL and delivered directly into sterile 60mL plastic vials labelled with codes and immediately stored on ice to prevent spoilage and contamination, as recommended by The Breastfeeding Network, 2014. The samples were transported to the storage point and stored in a freezer below -20oC. They were then transported in a vaccine carrier at the required temperature to the Ghana Research Reactor-1 (GHARR-1) facility at the Ghana Atomic Energy Commission, Kwabenya, Accra, for analysis. Multivariate Analysis was used to analyse the results. Data collection at the health facilities lasted for one month.



Figure 1: A map showing the towns of individual volunteer mothers used for the study

Epithermal Neutron Activation Analyses: During the analysis using ENAA (Figure 2), epithermal neutrons interact with the breast milk (target nucleus) via a non-elastic collision, resulting in the formation of a compound nucleus in an excited state. The excitation energy of the compound nucleus is due to the binding energy of the neutron with the nucleus. The compound nucleus will almost instantaneously de-excite into a more stable configuration through the emission of one or more characteristic prompt gamma rays. In many cases, this new configuration yields a radioactive nucleus which also decays by the emission of one or more characteristic delayed gamma rays, but at a much slower rate according to the unique half-life of the radioactive nucleus. Depending on the particular radioactive species, half-lives can range from a fraction of a second to several years (Ali, 2000).



Figure 2: A process of neutron capture followed by emission of gamma ray (Vowotor et al., 2020)

Most neutron energy distributions are quite broad and consist of three principal components: Thermal, Epithermal, and Fast. The thermal neutron component consists of low-energy neutrons (energies below 0.5 eV) in thermal equilibrium with atoms in the reactor's moderator, while the fast neutron component (energies above 0.5 MeV) consists of the primary fission, yielding neutrons having much of their original energy following fission (Ali, 2000).

ENAA technique uses only epithermal neutrons (energies between 0.5 eV to about 0.5 MeV) to induce (n, gamma) reactions. In a typical unshielded reactor irradiation position, the epithermal neutron flux represents about 2% the total neutron flux (Ehmann & Vance, 1993). In reactor activation, this ENAA technique is performed by enclosing samples in thermal neutron filters such as cadmium or boron, which removes thermal neutrons from the reactor neutron spectrum. This has been applied to a variety of sample matrices including geological and biological materials (Chisela, Gawlik, & Brätter, 1986). A typical reactor neutron energy spectrum showing the various components used to describe the neutron energy regions is presented in **Figure 3**.



Figure 3: A typical reactor neutron energy spectrum showing the various components used to describe the neutron energy regions (Vowotor et al., 2020).

Sample Preparation and Irradiation: 500 mg of each sample (breast milk) was measured into polyethylene vials using a micrometre pipette after shaking to ensure uniformity. The polyethylene capsule with diameter 1.2 cm and height 2.35 cm containing the liquid sample was then put into a bigger polyethylene capsule with diameter 1.6 cm and height 5.5 cm (Rabbit capsule) and smoothly heat-sealed with a soldering rod. This technique is known as Double encapsulation. The irradiation vials (capsules) used were pre-cleaned by first washing with distilled water, after which they were soaked in an acidic reagent for 24 hours and then rinsed in distilled deionised water. They were then further soaked in nitric acid for 24 hours, rinsed thoroughly with distilled deionised water and airdried in a clean fume hood.

To validate the procedure, various standard reference materials, namely IAEA-336 (trace and minor elements in Lichen), IAEA-407 (trace elements and methyl mercury in fish tissue), IAEA-350 (trace elements in tuna fish homogenate) and SRM 1577b (Bovine liver) were prepared and packed similarly as the samples (IAEA, 2003; IAEA, 2023; EVISA, 2010a; EVISA, 2010b). The samples were transferred to the irradiation sites through a pneumatic transfer system at a pressure of 60 psi. The irradiation was categorized according to the half-life of the element of interest and carried out in the Ghana Research Reactor-1 (GHARR-1) at the Ghana Atomic Energy Commission (GAEC). The reactor operates at 15 KW at a thermal flux of  $5 \times 1011$  ncm-2s-1, and uses a 3 mm thick flexible boron element to cut off thermal neutrons in order to assess only epithermal neutrons. 94.7% accuracy was achieved.

*Relative Standardization:* In the relative standardization method, a standard chemical (index std) of known mass, Wstd, of the element is co-irradiated with the sample of unknown mass Wsam. When the sample to be irradiated is a short-lived radionuclide, both the standard and sample are irradiated separately under the same conditions, usually with a monitor of the same neutron fluence rate, and both counted in the same geometrical arrangements with respect to the gamma-ray energy. It is then assumed that the neutron flux, cross section, irradiation times and all other variables associated with the counting are constant for the standard and the sample at a particular sample-to-detector geometry. With this assumption, the neutron activation equation reduces to equation 1 as:

$$\rho_{sam} = \frac{\left| \left( \frac{P_A}{t_c} \right) \right|_{sam} \left[ \rho CDW \right]_{std}}{\left[ \frac{P_A}{t_c} \right]_{std} \left[ CDW \right]_{sam}}$$

where (PA/tc)std and (PA/tc)sam are the counting rates for the standard and sample respectively, std and sam are the counting concentrations of the standard and element of interest respectively, Cstd and Csam are the counting factors for standard and sample, Dstd and Dsam are the decay factors for the standard and sample respectively.

*Qualitative and Quantitative Analysis:* The qualitative analysis determined the levels of the seven micronutrients in the breast milk samples by isolating the spectra peaks, assigning corresponding radionuclides and identifying the micronutrients present. This was done using an ORTEC EMCAPLUS Multichannel Analyzer (MCA) Emulation software. A Microsoft Window-based software, MAESTRO, was used for spectral analysis (Serfor-Armah et al, 2018). This software identifies the various photo peaks, estimates and works out the areas under them.

The quantitative analysis involves the calculation of the areas under the peaks of identified micronutrients and converting them into concentrations using an appropriate software or equation(s) (Jean & Alfassi, 1994). The counting of the induced radioactivity was done using a PC-based y-ray spectrometer which consists of an n-type high purity Germanium (HPGe) detector (model GR2518), coupled to a computer-based Multichannel Analyzer via electronic modules, and an amplifier (model 2020, Canberra Industries Incorporated). The relative efficiency of the detector is 25%, with an energy resolution of 1.8 KeV at y-ray energy of 1332 KeV of 60Co. The other quantitative measurements were carried out using Equation 1. The detection limits (DL) of the micronutrients and the nuclear data used to undertake the ENAA have been summarized in Table 1.

## Results

Measured levels of the micronutrients were directly linked to the types of foods taken in by the volunteer mothers. **Table 2** shows some of the foods consumed by the mothers and the individual micronutrients they contain.

Table 2: Micronutrients contained in some foods consumed by mothers involved in the study

Micronutrient	Foods
Na	Shrimp, Soup, Ham, Vegetable Juice, Salad
	Dressing, Sandwiches, Dried Meats, Sauces,
	Breads, Canned Meats, Poultry and Seafood,
	Biscuits, Baked Beans, Sausage, Bacon
	(Healthline, 2023: a)
Mg	Wheat, Spinach, Almonds, Cashews, Peanuts,
	Dark Chocolate, Legumes, Vegetables (Peas,
	Cabbage, Green Beans, Avocado, Banana, salm-
	on, mackerel, tuna (Steen, 2020)
K	White Beans, Potatoes and Sweet Potatoes,
	Beets, Spinach, Tomato Sauce, Oranges and
	Orange Juice. (Healthline Media, 2023)
Ca	Yogurt, Sardines, Salmon, Beans, Almonds,
	Some Leafy Greens like Broccoli, Parsley, Spin-
	ach, Lettuce, Cabbage, "Kontomire", etc, Milk,
	Lemon, and Orange. (Healthline, 2023: b)
Mn	Pineapple, Spinach, Lima Beans, Sweet Pota-
	toes, Brown Rice, Soybeans, Corn, Turmeric
	(Food and Nutrition, 2021)
Cu	Organ meats (liver), Oysters, Lobster, Squid,
	Mushrooms, Nuts and Sesame Seeds, Cashew
	Nuts, Almonds, Sunflower Seeds, Leafy Greens,
	Dark Chocolate. (Healthline, 2023)
I	Yogurt, Iodized Salt, Shrimp, Tuna, Eggs
	(Healthline, 2019)
1	

Table 1: Detection limits of the micronutrients and the nuclear data used to undertake the ENAA

Element	Radioisotope	Gamma Ray	Half-life	Irradiation	Counting Time	DL (μg/g)
		Energy (keV)		Time (min)	(min)	
Na	<sup>24</sup> Na	2754	15 hr	10	10	0.001
Mg	<sup>27</sup> Mg	1014.4	9.46 min	10	10	0.1
K	<sup>42</sup> K	1524.6	12.4 hr	10	10	0.01
Ca	<sup>49</sup> Ca	3084.5	8.72 min	10	10	1.0
Mn	<sup>56</sup> Mn	1810.7	2.58 hr	10	10	0.0001
Cu	66Cu	1039.2	5.1 min	10	10	0.01
Ι	<sup>128</sup> I	440.9	25 min	10	10	0.0001

Table 3: Summary of the levels of the micronutrients measured in the breast milk

	Sodium (Na)	Magnesium (Mg)	Potassium (K)	Calcium (Ca)	Manganese (Mn)	Copper(Cu)	Iodine(I)
Min	294.4000	132.4000	1359.0000	408.1000	0.0018	0.2235	0.0290
Max	2797.0000	426.7000	5225.0000	1497.0000	1.1150	1.0199	0.1445
Mean	1098.6259	309.2481	2438.0741	697.4037	0.4028	0.6944	0.0613
SD	36.4403	23.7186	176.349	35.2538	0.0384	0.0079	0.0102

Assessment According to Levels: Measured levels of the micronutrients in the breast milk are shown in Figure 4, and a summary of the statistics presented in Table 3.



Figure 4: Measured levels of the seven micronutrients in the breast milk

The measured levels were within the following intervals: Na: 294.4-2797.0 mg/kg; Mg: 132.4-426.7 mg/kg; K: 1359.0-5225.0 mg/kg; Ca: 408.1-1497.0 mg/kg; Mn: 0.0018-1.115 mg/kg; Cu: 0.2235-1.0199 mg/kg; and I: 0.029-0.1445 mg/kg. In terms of abundance, they can be arranged in the order: K > Na > Ca > Mg > Cu > Mn> I. The low standard deviation (SD) values obtained indicate that the spatial distribution of the individual micronutrients in their breast milk was uniform.

Assessment According to Recommended Dietary Allowable: Recommended dietary allowable (RDA) is the dietary requirement for a micronutrient. It is an intake level which meets a specific criterion for adequacy, thereby minimizing risk of nutrient deficit or excess (Health Canada, 2022). Exclusively breastfed babies take in an average of 750 ml or 708.738 g per day between the ages of 1 month and 6 months (Bonyata, 2019). If the level of a micronutrient in milk per day (RDA) is expressed in mg/d, and the average intake of milk per day represented by M g/d, the level of micronutrients in the breast milk represented by N mg/g can be expressed in equation 2 as:

(Vowotor et al, 2011);	
N = RDA / M	(Equation 2)

The RDA levels and their upper limits, and those measured using data from the breast milk for the various micronutrients are shown in Table 4. Also in Table 4 are the Allowable daily intake values, ADI, calculated from the Mean Measured levels, MMC using equation 3.

$$ADI = MMC \times RDA / RDA level$$
 (Equation 3)

Assessment According to Health Risk Estimation: To estimate the health effects, hazard quotient (HQ), the estimated lifetime average daily dose of each micronutrient is compared to its Reference Dose (RfD). The reference dose represents an estimated daily consumption level that is likely to be without deleterious effects in a lifetime, based on the equation (4) detailed in the US. EPA handbook (Chao-yang et al., 2018):

The hazard quotient (HQ) = ED/RfD(Equation 4)

where, ED = Estimated Dose and RfD = Reference Dose.

HQ obtained for the various micronutrients are also presented in Table 4.

Except for Iodine, levels of the other micronutrients were found to be high compared to the calculated levels of the RDA (Table 4). Looking at the difference between the RDA values and the measured/calculated RDA values, a negative sign (-), as in the case for iodine, denotes a value below the recommended RDA, while a positive sign (+), as obtained for the rest, denotes levels above the recommended RDA. HQ < 1 suggests unlikely adverse health effects, whereas HQ > 1 suggests the probability of adverse health effects (Liu et al., 2018). HQ values > 1 have been highlighted. Table 5 lists the side effects in consuming these micronutrients in excess quantities.

Pearson correlation was calculated and presented on Table 6. It showed a normal distribution in the levels of micronutrients consumed. The variables are continuous and exhibited a linear relationship. Though 95%

Table 4: RDA, their Upper limits and levels calculated (mg/kg), as well as HI calculated for the micronutrients for the breast milk

Micronutrient	Na	Mg	K	Ca	Mn	Cu	I
RDA	120.0	30.0	400.0	200.0	0.003	0.20	0.09
RDA Upper Level	169.3	42.3	563.4	281.7	0.0042	0.28	0.13
Mean Measured Level	1098.6259	309.2481	2438.0741	697.4037	0.4028	0.6944	0.0613
Average Daily Intake from Level	778.71	219.32	1730.97	495.14	0.29	0.5	0.04
Difference Between the RDA &	+ 658.71	+ 189.32	+ 1330.97	+ 295.14	+ 0.287	+ 0.3	- 0.05
Measured RDA							
HQ	6.49	7.31	4.33	2.48	95.9	2.48	0.47
RDA Values – (Andersson & Braegger, 2021).							

Table 5: The Seven micronutrients and the side effect when consumed in excess quantities

Element	Adverse Effect of Excessive Consumption
Na	Dark, yellow urine with a strong smell is a very common sign in babies with high sodium intake, weight gain, child is
	unusually thirsty for water, blood pressure increases, arterial blood vessels increase in thickness, predisposing the child to
	cardiovascular disease (Sinrich, 2019).
Mg	Large doses might cause too much magnesium to build up in the body, causing serious side effects including an irregular
	heartbeat, low blood pressure, confusion, slowed breathing, coma, and death (WebMD, 2023a).
K	Diarrhoea, arrhythmias, lethargy and abdominal distention are the most common manifestations of high potassium levels
	in babies (NHS, 2022).
Ca	Symptoms and signs of neonatal hypercalcemia include anorexia, reflux, gastroesophageal nausea, vomiting, lethargy or
	seizures or generalized irritability, and hypertension (Dysart, 2019).
Mn	High levels can wreak havoc on your baby's immature metabolic systems (WebMD, 2023b)
Cu	Vomiting, abdominal pain, sleep disorder, weakness, and damage to the liver and kidney. Dr. Paul Eck found that elevated
	tissue Cu is associated with homosexual desire (Nolan, 1983)
Ι	Low or High iodine causes thyroid gland inflammation, including goiter (an enlarged thyroid gland) and thyroid cancer
	((Alexander, et al., 1961);(Delange & Bürgi, 1989); NIH, 2022).

Table 6: Correlation coefficients of the levels of the seven micronutrients in breast milk

	Na	Mg	K	Ca	Mn	Cu	I	
Na	1							
Mgr <sup>2</sup>	0.465*	1						
-	0.01							
Kr <sup>2</sup>	0.035	-0.284	1					
	0.861	0.152						
Car <sup>2</sup>	0.459*	0.905**	-0.304	1				
	0.016	0.000	0.124					
Mnr <sup>2</sup>	0.250	0.836**	-0.214	0.733**	1			
	0.208	0.000	0.285	0.000				
Cur <sup>2</sup>	0.832**	0.545**	0.192	0.531**	0.389*	1		
	0.000	0.003	0.337	0.004	0.045			
$Ir^2$	-0.171	0.002	-0.175	0.020	-0.371	-0.269	1	
	0.393	0.993	0.384	0.920	0.057	0.174		
*Correlation is significant at the 0.05 level (2-tailed)								

confidence level was used to ascertain the strength of their relationship, there are other strongly correlated micronutrients with high coefficients of determination, hence cannot be ruled out (Pallant, 2020).

# Discussion

An important source of micronutrients is our foods, processed water and groundwater discharges (Kelly & Moran, 2002). Accordingly, it is important to characterize each distinct source and determine its contribution to mother and baby. Correlation coefficients between the seven micronutrients and their respective significance at 95% significance level in the breast milk are in Table 6. The interpretation of the strength of the correlation coefficients usually depends on the researcher as suggested by the guidelines from Rumsey 2010. In this study, values between are considered strong correlations. As the significance of rho is strongly influenced by the sample size, smaller sample sizes do not reach statistical significance as compared to larger sample sizes which may even be statistically significant at small (weak) correlations (Pallant, 2020). Even though 4 correlations were strong, 3 were statistically significant. This means that we can exude 95% confidence that the correlation between Ca and Mg is strongly correlated with a coefficient of 0.905.

As expected, there was a moderately strong correlation

between Na and Mg, as the main source of Na is salt. Salt is one of essential commodities mined and exported from the study area and used for seasoning in most of the foods consumed by participants in this study. Some participants said they normally eat bread made from wheat (rich in Mg and salt as in Table 2) for breakfast. Other salted foods rich in Mg and consumed by participants are soups made from vegetables, seafood (salmon, mackerel, tuna), beans and salted roasted peanuts with banana.

There was a strong correlation of 0.905 between Na and Ca. This can be attributed to the intake of salted foods rich in Ca such as sardine, salmon, beans and leafy greens, present in most mothers' daily diets like yam and palava sauce. Chocolate which is rich in Cu is made from Cocoa. Ghanaians generally consume a lot of cocoa products which are typically spiced with salt. Other foods rich in Cu like lobsters, squid, oysters and organ meats such as liver and kidneys are also seasoned with salt before used for meals. This can explain the correlation between Na and Cu.

Mg showed a strong correlation of 0.905, 0.836 and 0.545 with Ca, Mn and Cu respectively. Mg and Ca-rich foods like the legumes (gari and beans) and soups made from seafoods are heavily consumed by the mothers.

Manganese is an essential micronutrient which participates in the action of many enzymes, lack of which causes testicular atrophy, and excess is toxic (Encyclopaedia Britannica. 2023). It exhibited a strong correlation of 0.733 and 0.389 with Ca and Cu respectively. Foods like beans and spinach are a good source of Mn and Ca. Seafoods such as oysters, lobster and squid are rich in Cu and usually used in preparing stews which are taken with brown rice, rich in Mn. The correlation of 0.531 between Ca and Cu may come from the consumption of green leafy vegetables such as broccoli, parsley, spinach, lettuce, cabbage, Kontonmire and the likes. Mothers usually use these vegetables in preparing nutritious sauces.

## Conclusion

Diseases affecting people in their later years of lives may be as a result of foods served them due to lack of education on nutrition by their mothers in their childhood. From Table 4, only iodine had levels in the normal RDA range, with the remaining micronutrients recording high levels. With such high levels, mothers will be exposing their children to metabolic disorders and unexplained diseases in their future lives without knowing the cause. Na is found in salt, which is used virtually in all foods. Its accumulation in the body therefore starts right from childhood, and may be the cause of the unexplained hypertension in early adulthood.

#### Declarations

Acknowledgment: The authors wish to express their sincere appreciation to the Ghana Health Service (GHS) and the directors of the two health facilities for allowing the study to be carried out there. Appreciation also goes to the volunteer mothers, nurses and technicians who collected data, and the staff of Ghana Atomic Energy Commission at Kwabenya-Accra, for their assistance in running the tests and analysing of the samples.

*Ethical Approval and Consent to Participate:* The Ghana Health Service Ethical Review Committee gave us the approval to carry out this study, and issued us a certificate with number GHS-ERC-02/05/15 as a document for their approval. Breastfeeding mothers participated voluntarily, and were accorded the needed respect, dignity and confidentiality as samples taken were a part of their body issue.

*Conflict of interest:* The authors declare that they have no competing interests.

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