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Double Burden of Malnutrition: Toxic Metals in Breast Milk May Limit the Amounts of Micronutrients Available to Infants through Breast Milk

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Abstract

Background: The amounts of micronutrients in the diets of infants, and the factors that influence them needs to be monitored at the population level in order to avert detrimental developmental defects that impose lifetime-limitations on an infant's regulatory and defense systems. This study therefore, sought to evaluate if increasing levels of the toxic metals, Hg, Pb and Cd in breast milk will result in reducing amounts of the micronutrients Zn, Se and Cu in breast milk. **Methods:** Breast milk samples of 114 women living in two mining areas (57 women each) in Ghana, whose babies' amounts of breast milk intake at three months postpartum, and amounts of toxic metals had previously been determined in a prospective study, were analyzed for micronutrients by a combination of acid and microwave digestion, and quantifications were by two different modes (hydrogen and helium) of Octapole Reaction System Inductively Coupled Plasma Mass Spectrometer (7500 ce. Agilent), equipped with an ASX-510 Auto-sampler (Cetac). **Results:** All the breast milk specimen collected contained detectable amounts of Cu, Zn, both at levels less than have been previously reported, and Se. For specimen that did not contain Pb the amount of Se ranged from about 110 to 245 ng/g of milk, however, as the amount of Pb increased, the corresponding highest detected amount of Se reduced steeply, resulting in a right-angle triangle-shaped scatter plot. Similar relationships were observed between other toxic metals and micronutrients studied. A curve fitting regression analysis showed significant quadratic and cubic relationships between the amounts of Hg and Se, as well as between Pb, As and Cu. **Conclusion:** The results clearly suggest a double burden of malnutrition in these mining areas, where high loads of maternal toxic metals in breast milk, related significantly with a progressive reduction in the amounts of the micronutrients Cu and Se in breast milk, potentially reducing in infants' intake of these micronutrients.

Keywords

Toxic Metals, Micronutrients, Breastmilk, Mining, Infant Nutrition

1. Introduction

No other source compares to breast milk as a source of nutrients for the appropriate development of infants; for this reason and more, exclusive breast feeding is recommended for at least, the first six months of the life of a new born [1] [2]. After delivery, an infant has to develop and maintain his/her own regulatory, defense and other critical biological systems. Often, these developments involve the use of antioxidant systems, which are made up of a number of substances including micronutrients [3]. For example, selenium (Se), as part of the enzyme glutathione peroxidase is essential for proper functioning of a number of cellular defense mechanisms. Aside such direct benefits of micronutrients to infants, the biological interactions of some micronutrients with some toxic elements, result in protection against toxicity of these toxic elements [4] [5]. As indicated in a review by Zwolak and Zaporowska, [3], studies have suggested that selenium protects against the toxicity of arsenic and cadmium.

It is generally assumed that breast milk contains adequate amounts of micronutrients to satisfy the growing demands of healthy infants. However, maternal dietary habits, changes in dietary patterns and/or environmental exposures of mothers to elements, may have a significant impact on the concentrations of micronutrients in the breast milk they produce overtime [6] [7] [8] [9] [10]. Such impacts have been recorded for some micronutrients in human breast milk. These were mostly interpreted as either large inter-individual, inter-period [11], and inter-location differences in the concentrations of micronutrients in human breast milk. Yet still, whilst the concentration of some micronutrients vary with the stage of lactation, for others, their concentrations in human breast milk, to a large extent, are biologically regulated [11] [12]. Another factor that exerts a more influential impact on the amount and availability of micronutrients is the presence of toxic metals. For instance, environmental cadmium exposures have been indicated as resulting in Zn deficiency and disruption of the biological regulation of Zn, even though dietary supplies were not limited [13].

Therefore, following our earlier analysis of breast milk obtained from mothers living and working in mining communities in Ghana, for which As, Hg and Pb were above the WHO provisional tolerable daily intake (PTDI) values, for some of the breast milk samples [14], it was necessary for the further analysis of these breast milk samples to determine the amounts of micronutrients they contain. Therefore, this study sought to provide the first data on micronutrient concentration of breast milk and the corresponding quantified intake of micronutrient through breast milk of infants whose mothers were living in two mining areas in Ghana. Additionally, although the toxicity of some toxic elements have been reported to be reduced by micronutrients in some body fluids, not enough studies have been reported on the interaction of these when they co-exist in breast milk. Therefore, this study sought to evaluate, if the presence/co-existence of toxic metals in breast milk may potentially have a negative impact on the amounts of the micronutrients, Se (not biologically regulated), Zn and Cu which are biologically regulated.

2. Methods

2.1. Study Design

2.1.1. Study Location

Bases on three indicators as at the time of the study (January 2012 to November 2014; 1) a high occurrence of mostly unregulated small scale mining, 2) frequent reports of high levels of toxic metal pollution in the environment of municipalities and 3) observance of a high number of pregnant women and nursing mothers participating in small scale mining activities in two municipalities, Tarkwa and Obuasi, were purposively selected for this study. For each municipality, a hospital was randomly selected from among 3 hospitals for the study, these were, 1) the Dompime Health Centre, which served Dompime and three other surrounding gold mining communities in the Tarkwa Municipality of the Western Region and 2) the Mangoasi Community Hospital in the Obuasi Municipality of the Ashanti Region, which served Mangoasi and seven other surrounding gold mining communities.

2.1.2. Study Population and Sampling Procedure

At each of the two health facilities, breast-feeding mothers whose babies were between 2 and 3 months old and were resident within the study communities were recruited from among mothers attending the Maternal Health Care Clinics: they were identified using the Standard Case Record Forms. Among these, those who met the following inclusion criteria were included in the study; breast-feeding mothers-baby pairs who had been attending anti-natal clinic at the two selected hospitals, had indicated they were going to attend post-natal clinics at the same hospitals or could be reached by the research team within the study communities, who had intended to continue breastfeeding for 12 months and had intended to continue living in the communities for a year from the time of recruitment (January 2012). However, following were excluded from the study; mothers of twins, mothers who had complications or needed further medical attention after delivery, babies who had medical issues post-partum, mothers and infants who were HIV positive and mothers who stopped breast feeding. It should be noted that, although a large proportion of the records were reviewed, the exact total number of Standard Case Record Forms reviewed and the total number of women invited to participate were not recorded. Specifically, 73 and 64 mothers at Dompime Health Centre and Mangoasi Community Hospital respectively were recruited (137 mother-baby pairs in all); however, for each of the selected hospitals, 57 mother-baby pairs were reached at 3 months follow-up time, making a total of 114 mother-baby pairs.

2.2. Data and Specimen Collection

At enrolment, data on mother and infant demographic characteristics and locator information were collected with a structured questionnaire, by a one-on-one interview-based questionnaire administration (detail reported by Bansa *et al.*, [14]). Participating mother-baby pairs were subsequently followed-up when the babies attained the ages of 3, 6, 9 and 12 months for specimen collection. Samples of the following biological specimen were collected, breast milk, hair and urine.

2.3. Research Ethics

In line with the principles of research ethics, the prospective study sought for and obtained ethical clearance from the Ethics Review Committee of the Ghana Health Service as well as institutional approval for the conduct of the study in a Ghana Health Service health facility. The submitted protocol included the performance of the assessment of micronutrients in breast milk samples. During the prospective study, the mothers were provided with all the information they required to take an informed decision on their participation. Informed consent for the mothers, and parental consent for their babies were provided by enrolled participants. Privacy was ensured during the completion of the questionnaire and the confidentiality of the data collected was ensured by using codes to identify participants' completed questionnaires and specimens.

2.4. Determination of Se, Cu and Zn in Breast Milk

Of the breast milk sample collected from each mother, 0.15 g was weighed into quartz tubes followed by the addition of 1 mL of 65% HNO_3 (suprapur) and 1 mL of 30% H_2O_2 (suprapur). The resultant solution was subjected to closed vessel microwave digestion at maximum power (1500 W): ramp to 130˚C for 10 min., ramp to 200˚C for 10 min., held for 20 min. and then cooled for 20 min. Following the subsequent equilibration of the solution to room temperature, it was quantitatively transferred into polyethylene graduated tubes and made-up to 20 mL with Mili-Q water. This whole procedure was repeated with Mili-Q water in place of breast milk sample, and the resultant solution used as blank. Where necessary, primarily based on appearance, digested solutions were diluted before measurements of concentrations of micronutrients were taken with two different modes (hydrogen and helium) of Octapole Reaction System (ORS) Inductively Coupled Plasma Mass Spectrometer (7500 ce. Agilent) equipped with an ASX-510

Auto-sampler (Cetac). Three central points of the spectral peaks were used for the quantification of all isotopes of each element. The instrumental parameters and conditions were: nebulizer Micro Mist, spray chamber Scott-type, spray chamber temperature of 5˚C, plasma gas flow rate of 15 L/min, carrier gas flow rate of 0.8 L/min, make-up gas flow rate of 0.1 L/min, nebulizer pump at 0.1 rps. RF power of 1500 W, reaction cell gases: H2 4 mL/min and He 4 mL/min, isotopes monitored 75 As, 111 Cd, 206 Pb, 207 Pb, and 208 Pb. In respect of quality control, tuning of the instrument was done daily, using a solution containing Li, Mg, Y, Ca, Ti and Co and external calibration was used for quantification.

2.5. Determination of Se, Cu, and Zn in Urine

Into a previously acid-washed and appropriately labelled 100 ml polytetraflouroethylene (PTFE) Teflon bombs, 5 µL of urine sample from a participant (one each for mothers and infants) was added. To this, 6 mL of concentrated nitric acid (HNO_3, 65%), 3 mL of concentrated hydrochloric acid (HCl, 35%) and 0.25 mL of hydrogen peroxide (H_2O_2, 30%) were added working in a fume chamber. The solution was loaded on a microwave carousel and the vessel caps secured tightly using a wrench. The complete assembly was microwave irradiated for 26 minutes using milestone microwave lab station ETHOS 900, INSTR: MLS-1200 MEGA. After digestion, the Teflon bombs were cooled in a water bath to reduce internal pressure and allow volatilized material to re-stabilize. The volume of the digest was made-up to 20 mL with double distilled water and assayed for the presence of the micronutrients using VARIAN AA 240FS-Atomic Absorption Spectrometer in an acetylene-air flame. Duplicates of samples, blanks (deionized water) and reference standards for the elements of interest (internal positive controls), were digested in the same manner as described for the urine samples. Reference standards used were from Fluka Analytical, Sigma-Aldrich Chemie GmbH, Switzerland.

2.6. Data Management and Analysis

As part of a prospective study, deuterium enrichment data were entered into a pre-made Microsoft Excel data sheet and deuterium excretion curve was plotted for mother and baby according to the model provided by the Nutritional and Health-Related Environmental Studies Section of the International Atomic Energy Agency (IAEA). Curves fitting and calculation of endpoints (human milk intake, the baby intake of fluids other than human milk intake and mother's and baby's body composition) were performed using pre-made Microsoft Excel data sheet (Human milk intake template FTIR calcs.xls). These calculated endpoints and other collected data (demographic, anthropometric, dietary intake) were transferred to a SPSS 20.0 database and analyzed. The data on micronutrients for the 3 months follow-up are presented in the paper. Geometric mean of the micronutrients (in breast milk, and urine) were compared. Categorical data were described as distributions of subgroups represented as percentages of total number of participants at each follow-up time. A curve fitting analysis to determine the relationship between the amounts of toxic metals and micronutrients detected in breast milk collected from participating mothers was performed.

3. Results

3.1. Demographic Information

With a mean age of 27.5 (95% CI of 26.4 - 28.6) years, 83.5% of the mothers were married. Furthermore, as at the time of this study, most of them (79.8%) were having secondary education, 59.5% were self-employed and 12.3% wage-employed, (Table 1). Irrespective of the nature of their houses, 41.1% of the mothers owned the house they lived in.

3.2. Amounts of Micronutrients

As depicted in Table 2, the amount of copper (Cu) detected in the breast milk of lactating mothers, 3 months postpartum, ranged between 0.41 and 131.96 μg/L of breast milk, with a median of 2.12 μg/L of breast milk and a geometric mean of 2.05 μg/L of breast milk. For the micronutrient selenium (Se), its amounts varied between 109.28 and 295.88 μg/L of breast milk, with a median of 171.65 μg/L of breast milk and a geometric mean of 173.52 μg/L of breast milk. For Zinc (Zn), the variation in its amounts range between 3.20 and 26.08 μg/L of breast milk, with a median of 11.91 μg/L of breast milk and a geometric mean of 11.51 μg/L of breast milk.

Using the amount of breast milk consumed per day by each infant, which ranged between 0.33 Kg/day and 2.16 Kg/day (detail reported by Bansa *et al.*, [14]), an infant's intake of each micronutrient was calculated relative to its body weight; the summary of these is reported as Table 3. The intake of Cu by the participating infants ranged between 0.05 and 0.63 μg/Kg body weight/day, with a median of 0.23 μg/Kg body weight/day and a geometric mean of 0.200 μg/Kg body weight/day. The amount of Se intake were much higher, ranging between

Table 1. Distribution of the socio-demographic characteristics of the mothers.

Demographic information	Categories	Number, n (%); (N = 114)
Marital status	Married	95 (83.5)
	Living without a partner	19 (16.5)
Educational Status*	No formal education	9 (7.5)
	Alternative education	4 (3.9)
	Secondary	91 (79.8)
	Post-secondary	10 (8.8)
Employment status*	Unemployed	32 (28.2)
	Wage employed	14 (12.3)
	Self employed	68 (59.5)
Home ownership	Rented	67 (58.9)
	Own property	47 (41.1)

None of the women had stopped school at the Primary level nor were formally employed.

Table 2. Amounts of micronutrients detected in mothers' breast milk.

Descriptive parameters	Amount of micronutrients in breast milk at 3 month postpartum, (μg/L of breast milk#)		
	Se	Cu	Zn
Geometric mean	173.52	2.05	11.51
SD	87.56	15.46	7.27
95% CL (low)	153.29	0.00	9.83
95% CL (high)	193.74	5.62	13.19
Median	171.65	2.12	11.91
Minimum	109.28	0.41	3.20
Maximum	295.88	131.96	26.08
WHO study of micronutrients in human breast milk; median (min – max) (μg/L)	21.7 (16.4 - 38.2)^	258 (208 - 381)^	1.23 (0.81 - 2.77) mg/L^
	20.5 (10.4 - 37.1)*	202 (57 - 638)*	2.16 (0.88 - 3.87) mg/L*

#1 g of human breast milk is equivalent to 0.970 mL. ^Nigeria. *DR Congo (Zaire).

Table 3. Estimated amounts of micronutrients intake by infants through human breast milk.

Descriptive parameters	Amount of micronutrients consumed by infants, (μg/Kg body weight/day)		
	Se	Cu	Zn
Geometric mean	18.52	0.200	1.155
SD	13.702	0.167	1.002
95% CL (low)	15.356	0.161	0.923
95% CL (high)	21.686	0.239	1.386
Median	19.32	0.23	1.16
Minimum	9.23	0.05	0.17
Maximum	41.90	0.63	4.01
WHO PTDI*	-	500	300 - 1000

Note that the average weight of the 3 months old infants who participated in this study was 6.37 (95% CI of 6.17 - 6.57) Kg. *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2011. http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=4197.

9.23 and 41.90 μg/Kg body weight/day. The median and geometric mean intakes of Se were 19.32 μg/Kg body weight/day and 18.52 μg/Kg body weight/day respectively. The amounts of Zn intake through breast milk was estimated to have varied between 0.17 and 4.01 μg/Kg body weight/day, with a median and geometric mean of 1.16 μg/Kg body weight/day and 1.155 μg/Kg body weight/day respectively.

The amounts of these micronutrients excreted through urine by both the mothers and infants were very similar (**Table 4**). While the amount of Cu excreted

Table 4. Amounts of micronutrients excreted through babies' urine.

	Amounts of micronutrients in the urine, (mg/L)					
	Se		Cu		Zn	
Descriptive parameters	Mothers	Infants	Mothers	Infants	Mothers	Infants
Geometric mean	0.004	0.004	0.029	0.027	0.004	0.004
SD	0.003	0.003	0.028	0.029	0.003	0.003
95% CL (low)	0.003	0.003	0.023	0.021	0.003	0.003
95% CL (high)	0.005	0.004	0.036	0.034	0.005	0.005
Median	BDL	BDL	0.04	0.03	BDL	BDL
Minimum	BDL	BDL	0.01	0.01	BDL	BDL
Maximum	0.008	0.007	0.10	0.11	0.07	0.008

ranged between 0.01 and 0.1 mg/L of urine, the amounts of Se and Zn excreted ranged between, below detection limits (BDL) and 0.007, BDL and 0.008 mg/L of urine respectively. Table 4 presents the descriptive statistics of the amounts.

3.3. Toxic Elements and Micronutrients

Overall, it was noticed that all the breast milk samples collected contained detectable amounts of all the micronutrients studied (Figures 1-3), and the toxic metals As and Hg. However, Pb and Cd were not detected in some samples.

As depicted in Figure 1(a), the amounts of Se detected in breast milk that had no detectable amount of Pb varied widely, ranging from about 110 ng/g of breast milk to 245 ng/g of breast milk. However, for breast milk samples with amount of Pb less than 10 ng/g of breast milk, the range of the amount of Se was from 150 to about 270 ng/g of breast milk. As the amount of Pb increased, the corresponding highest detected amount of Se was reducing steeply, resulting in a right-angle triangle-shaped scatter plot. A similar shape was observed for the scatter plot for the amounts of Se and Cd (Figure 1(b)). Specifically, the amount of Se in breast milk without Cd varied within a narrow range; between about 110 and 250 ng/g of breast milk. However, as the amounts of Cd increased, the maximum amount of Se reduced steeply, forming a right-angle triangle-shaped scatter plot. Conversely, the relationship between the amounts of Se and As, and Se and Hg, as shown by the scatter plots (Figure 1(c) and Figure 1(d)), both seem to show that as the amount of toxic metals increased, the amount of Se also increased to a highest and then reduced subsequently.

The output for a curve fitting analysis, as shown in Table 5, indicates that there were no significant linear, quadratic, cubic and exponential (and other fitting curve tested in SPSS, data not shown) relationship between Se and Pb, as well as with Cd, and As.

However, there were significant quadratic ($R^2 = 0.144$; $p = 0.019$) and cubic

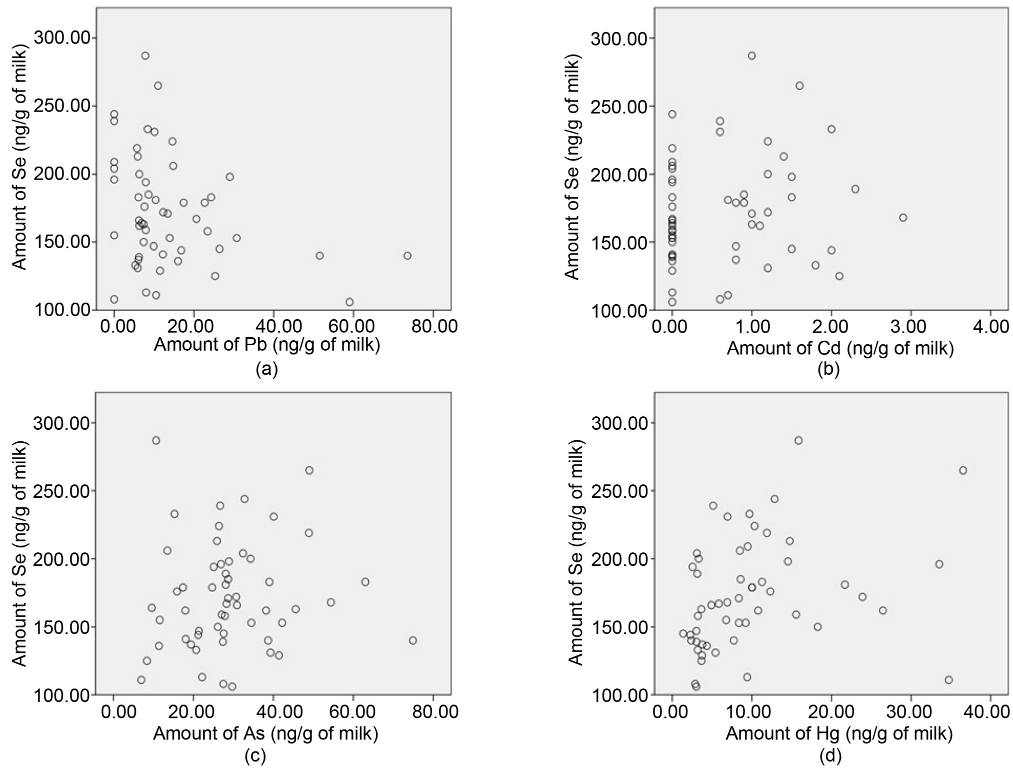

Figure 1. The amounts of Se ingested by infants through breast milk in relation to the amounts of (a) Pb, (b) Cd, (c) As and (d) Hg in the breast milk.

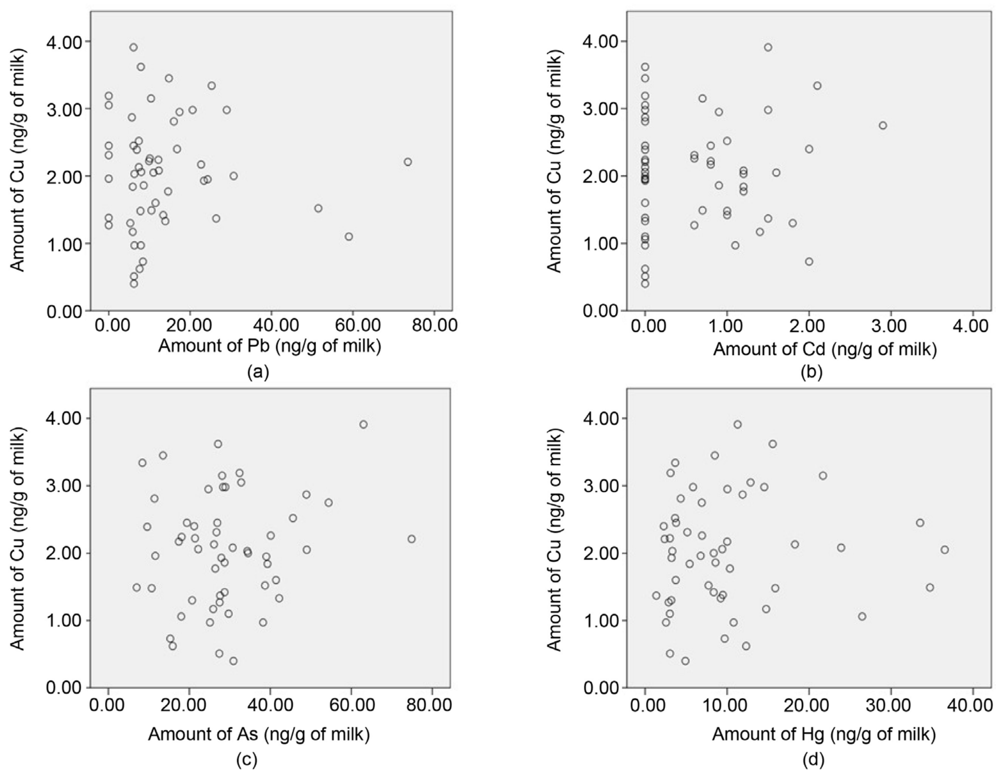

Figure 2. The amounts of Cu ingested by infants through breast milk in relation to the amounts of (a) Pb, (b) Cd, (c) As and (d) Hg in the breast milk.

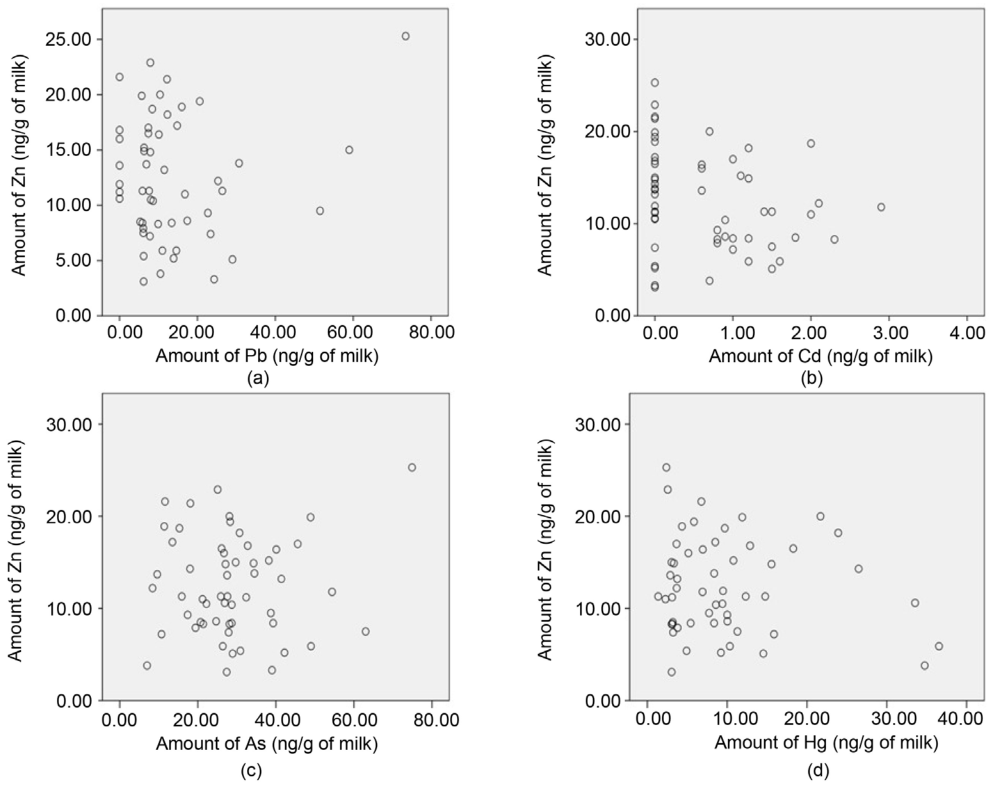

Figure 3. The amounts of Zn ingested by infants through breast milk in relation to the amounts of (a) Pb, (b) Cd, (c) As and (d) Hg in the breast milk.

(R^2 = 0.0.178; p = 0.020) relationships between the amounts Se and Hg. The amount Cu ranged from 1.30 to 3.30 ng/g of breast milk for breast milk samples that had no detectable amount of Pb. For the amount of Pb between 1.00 and 5.00 ng/g of breast milk, the corresponding amount of Cu varied between 0.40 and 4.00 ng/g of breast milk.

The indicating scatter plot (Figure 2(a)) shows that as the amount of Pb increased above 5.00 ng/g of milk, the highest amount of Cu detected reduced steeply, forming a right angle triangle shaped scatter plot. However, as shown in Figure 2(b), the amount of Cu, in breast milk that had no detectable amount of Cd, ranged between 0.40 and 3.60 ng/g of breast milk. As the amount of Cd increased, the change in the amount of Cu did not follow any noticeable pattern. Similarly, with an increase in the amount of As (Figure 2(c)), and Hg (Figure 2(d)) respectively, the changes in the amount of Cu did not follow any noticeable pattern.

Consequently, the curve fitting analysis revealed that the amount of Cu in breast milk samples had a significant linear (R^2 = 0.275; p < 0.0001), quadratic (R^2 = 0.453; p < 0.0001), Cubic (R^2 = 0.545; p < 0.0001) and exponential (R^2 = 0.170; p = 0.002) relation with the amount of Pb. Additionally, the amounts of Cu and Cd showed a significant cubic relationship (R^2 = 0.155; p = 0.032). However, the amounts of Cu and As, as well as Cu and Hg did not show any significant relationship (Table 5).

Table 5. Model characteristics for the curve fitting analyses for the relationship between toxic metals and micronutrients in breast milk at 3 months post-partum.

Micronutrient	Toxic metal	Model Characteristics, R^2 (p value)			
		Linear	Quadratic	Cubic	Exponential
Se	Pb	0.026 (0.235)	0.095 (0.071)	0.097 (0.149)	0.021 (0.290)
	Cd	0.000 (0.910)	0.040 (0.339)	0.048 (0.465)	0.000 (0.932)
	As	0.021 (0.296)	0.021 (0.565)	0.027 (0.691)	0.027 (0.224)
	Hg	0.021 (0.296)	**0.144 (0.019)**	**0.178 (0.020)**	0.017 (0.348)
Cu	Pb	**0.275 (0.000)**	**0.453 (0.000)**	**0.545 (0.000)**	**0.170 (0.002)**
	Cd	0.038 (0.151)	0.065 (0.168)	**0.155 (0.032)**	0.027 (0.225)
	As	0.000 (0.900)	0.002 (0.948)	0.008 (0.941)	0.000 (0.894)
	Hg	0.009 (0.502)	0.018 (0.633)	0.025 (0.733)	0.000 (0.929)
Zn	Pb	0.00 (0.163)	0.000 (0.995)	0.094 (0.160)	0.000 (0.911)
	Cd	0.055 (0.081)	0.081 (0.107)	0.098 (0.161)	0.024 (0.256)
	As	0.001 (0.787)	0.003 (0.828)	0.029 (0.671)	0.001 (0.827)
	Hg	0.003 (0.693)	0.037 (0.378)	0.067 (0.321)	0.000 (0.984)

Figure 3(a) shows a narrow range in the amount of Zn (about 11 to 22.0 ng/g of breast milk) in breast milk with no detectable amounts of Pb. Although there was a reduction in the amount of Zn, with an increase in the amount of Pb, beyond 60.0 ng of Pb/g of breast milk, the amount of Zn increased. Conversely, the amount of Zn showed little variation over a wide range for milk samples for which Cd was not detected (Figure 3(b)). However, as the amount of Cd increased, the highest detected amount of Zn generally reduced. On the other hand, the distribution of the amount of Zn in breast milk that had As did not show any noticeable pattern (Figure 3(c)), just as that for the amount of Zn in breast milk that had Hg (Figure 3(d)). The curve fitting analysis also did not show any significant relationship between Zn and each of these toxic metals.

4. Discussion

Evidence, which suggests the maternal load of toxic metals, detectable in breast milk, may negatively affects the amounts of micronutrients that the breast milk contains, and therefore the amounts of same, which infants may obtain through breast milk [13], was the bases for this study. Although genetic, other biological and socioeconomic factors influence the amounts of micronutrients in breast milk, the environmental influences are often more important [9] [15]. Additionally, although the interaction of these elements (toxic and micronutrient) have been studied and reported for water, different body organs and body fluids, particularly blood, the interaction between these elements may defer depending on the body target, because different proteins, which are the carriers of these elements, are present in these different targets. This study wherefore, analyzed breast milk specimens collected from lactating mothers living and working in two mining areas in Ghana; these specimens have been previously shown to contain As, Hg, Pb at levels above the WHO provisional tolerable daily intake (PTDI) values, and Cd at levels below the PTDI [14]. In respect of the current micronutrient study, the amounts of Cu and Zn were used because these are thought to be biologically regulated [11], and also that their levels in breast milk may be affected by the presence of these toxic metals. On the other hand, Se was studied for the following reasons; 1) its amount in breast milk is not biologically regulated [11], 2) it is detectable in breast milk with good reliability [9], and 3) its amount may also be influenced differently by the presence of toxic metals in breast milk. Therefore, these served as an indicators for the potential effect of toxic metals on micronutrient concentration in breast milk. Considering the amounts of these micronutrients, in the breast milk sample collected at 3 months postpartum, it is clear that the median amount of Cu detected in this study, 2.12 (range 0.41 - 131.96) µg/L of milk, was over 100 fold lower than those reported by a WHO study of breast milk collected at, three months postpartum which reported a median of 258 (range of 208 - 381) µg/L and 202 (range of 57 - 638) µg/L for lactating mothers in Nigeria and the Democratic Republic of Congo respectively [9]. Furthermore, human breast milk is said to usually contain 200 - 400 µg of Cu/L [16] [17]. In respect of the median amounts of Se detected in this study, which was 171.65 (range 109 - 295) µg/L of breast milk, it was observed to be about 10 fold more and 10 fold less than the median amounts reported for Nigeria 21.7 (range 16.4 - 38.2) µg/L and the Democratic Republic of Congo 1.23 (range 0.81 - 2.77) mg/L respectively by the same WHO study. However, it remains within the expected range for mature human milk, which has been stated to contain selenium in the range 2.6 - 200 µg/L [8]. Similarly, the median amounts of Zn detected in this study, which was 11.91 (range of 3.20 - 26.08) µg/L of milk, were about 10 folds more than the median amount of 1.23 (range 0.81 - 2.77) mg/L reported for Nigeria and the 2.16 (range 0.88 - 3.87) mg/L reported for the Democratic Republic of Congo by the WHO study [9].

Other studies have indicated that concentration of zinc in human milk falls during lactation, from about 2.5 µg/mL in the first month, to about 0.9 µg/mL at 3 months after birth [10] [16] [17].

These variations, particularly in the amount of Se, may be explainable, as has been indicated by other studies, as a result of the difference in maternal intake of

Se through food, [18]. This influence through food intake may further be enhanced by the fact that, the micronutrient content of food stuffs varies due to variations in the geochemical environment where the food stuffs are grown [18] [19] [20]. Furthermore, the extent of the consumption of certain foods, within a locality, particularly those that are noted as rich in the said micronutrient, undoubtedly could explain the variations in the amounts of the Se in different areas. These facts have been stated as the reasons for the different recommended national dietary allowable intake of Se in different countries [18]. Although the following was a limitation of this study, which is, food consumption data were not collected and evaluated as part of this study, due to difficulties in obtaining them, it is not uncommon for such data not to be available as part of such a study, as was for the cited WHO study [9].

This study however, evaluated another important, but often not studied factor, which is the interaction between these elements; that may contribute to the amounts of micronutrients in breast milk. The findings presented in this study, **Figure 1(a)** and **Figure 1(b)** and the curve fitting relationships represented by the R^2 and p values in **Table 5**, suggest that the amounts of Se in these breast milk specimens, if at all, were weakly/poorly and non-significantly related to the increasing amounts of Pb, As and Cd.

However, the amount of Hg was weakly related to, and significantly influenced the amount of Se in breast milk, showing a significant quadratic (R^2 = 0.144; p = 0.019) and cubic (R^2 = 0.178; p = 0.020) relationship. A study, has shown a significant negative exponential relationship between Se and Hg in blood samples of persons living in a mining area in China [21]. Due to the biological interactions between Se and Hg, which indicate that Hg binds to biomolecules/proteins bond to Se (Selenoproteins) to form a coordinated complexes [21] [22], and that Hg sequesters Se [23], it is understandable that co-presence of Se and Hg in breast milk will have a reducing effect on the amount of Se and also its availability to infants who ingest such breast milk. Additionally, since the amount of Se is not biologically regulated, and the influence of the presence of a toxic element is by competitive binding, the extent of the influence will depend on the relative amounts of Se and the toxic metal [23]. Therefore, the influence, even if it is possible, may not be evident once Se is present in relatively higher amounts [15] [22]. Based on the understanding of the biological interactions between Se and Hg, Se has been used in the reduction of the amount and toxicity of Hg by giving Se supplementation to Hg exposed persons/organisms [21] [22] [24] [25] [26]. However, in this case, maternal Se that should have been present in breast milk and transferred to the breastfeeding infant (particularly at 3 months postpartum, where exclusive breast feeding is recommended and Se is important for mental development) was lost by the increasing amounts of Hg in breast milk.

However, in respect of the amount of Cu, which is biologically regulated [27], the findings presented as **Figure 2(a)**, **Figure 2(b)** and in **Table 5**, show that its amount was influenced by the amounts of Pb. This is evident by the significant linear, quadratic, cubic and exponential regression relationship (**Table 5**). More importantly, the cubic relationship indicates that about 54.5% of the change in the amount of Cu were associated with changes in the amount of Pb ($R^2 = 0.545$; $p < 0.0001$). Furthermore, the cubic relationship between the amounts of Cu and Cd, which also was significant ($p = 0.032$) indicated that 15.5% of the changes in Cu was associated with changes in the amount of Cd. In respect of the other biologically regulated micronutrient, Zn, influence on its amount was not suggested by the findings on each of the toxic elements; the scatter plots and regression relationship did not suggest any meaningful association. Other studies have indicated that Zn alone did not influence the accumulation of Cd in liver, but rather may have enhanced the influence of Fe on the accumulation of Cd [28]. In all, these assessments, may be limited, in that they present a simpler form of the interactions, which may be more complex than presented, since the effect of the toxic metals on each other, as they co-occur in the breastmilk (multiple regression relationship), as well as the effect of the co-presence of the micronutrients on each other in the breastmilk, were not considered. Furthermore, this study was not specifically powered to determine these relationships and a more complex form thereof, which is a limitation that is likely to make some of the relationships not obvious or clear.

5. Conclusion

This study has provided the first quantitative data on the intake of Se, Cu and Zn through breast milk, by infants whose mothers were living in mining areas in Ghana, and these do not indicate a status of micronutrient-malnutrition. Additionally, the findings suggest that a mother's load of Hg, on one hand, and that of Pb or Cd on the other, when any of these co-exist beyond certain amounts with Se and Cu respectively in breast milk, may negatively influence the amounts of Se and Cu the in breast milk. This will thereby potentially limit an infant's maximum amounts of these micronutrients ingestible through breast milk. We therefore recommend further studies that will assess these relations at different time-points post-partum and from different geographical areas. Additionally, protein binding study will add more insight to the interactions between these elements and the proteins in breast milk.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Authors' Contributions

DKB, RB, and TA contributed equally to the conception of study and proposal development. DKB, ECB-A, DD, AMD, RB, and TA participated in the conduct of the study. AKA, DB, ECB-A, DD, and AMD participated in data management. AKA conceived the manuscript, conducted the statistical analyses and drafted of the manuscript. All the authors contributed to the review and approval of the final manuscript for submission.

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List of Abbreviations

FTIR, Fourier Transform Spectroscopy.

PTDI, Provisional Tolerable Daily Intake.