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Metals profile of milled shrimps and the potential risk associated with their consumption

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Abstract
The presence of metals in milled shrimps sold on some major markets in Kumasi were investigated to ascertain their levels and the potential health risk they may pose to humans when ingested, due to the level of pollution in the marine environment where these shrimps are obtained from. The samples, which comprised of 30 composites, were analysed using x-ray florescence spectrometry and found to contain Co, Cr, Cu, Fe, K, Mo, Ca, Zn, As, Sr, and Zr with average concentrations of 4.09 mg kg^{-1}, 5.17 mg kg^{-1}, 25.14 mg kg^{-1}, 351.47 mg kg^{-1}, 9050.74 mg kg^{-1}, 4.08 mg kg^{-1}, 21984.48 mg kg^{-1}, 696.89 mg kg^{-1}, 8.99 mg kg^{-1}, 328.54 mg kg^{-1}, and 9.86 mg kg^{-1} respectively. Non-carcinogenic risk indicators analysed suggested a likelihood of health hazard when the milled shrimps are ingested, particularly concerning is the levels of arsenic determined. The arsenic may, however, be in organic form which will make it less of a concern. The levels of the metals could not be linked statistically to the milling process after comparing them to procured controls, which may suggest that these metals may have been picked up in the aquatic environment and/or prior to milling. There is a need, therefore, for action to reduce pollution and remediate the aquatic environment.

Keywords Toxic metals, Essential metals, Exposure, Health risk

Introduction

Seafood is enjoyed by many around the world because of its richness in essential nutrients for humans (Gharibzahedi and Jafari 2017). Among such foods are crustaceans such as shrimps from marine ecosystems which have been identified as a source of high-quality proteins, minerals, and vitamins (Kandathil et al. 2020). Food from the marine world has contributed to food security for millions around the world for millenniums, and the socio-economic importance of these foods cannot be overstated. The reliance on marine protein in Ghana was hedged at about 60% as of 2013 (Jones 2015; Kurekin et al. 2019). The increased dependence on shrimps was emphasized when a shrimp farm was established in Ghana at Ada Foah in 2013 (Dzidzornu 2018).

Shrimps sold on Ghanaian markets are mainly harvested from the Atlantic Ocean (Tuffour et al. 2020). Black tiger shrimps or giant tiger prawn, (*Penaeus monodon*), and pink shrimps (*Penaeus notialis*) are very popular on Ghanaian markets. Unfortunately, there are growing concerns of environmental pollution in and around oceans and seas where shrimps are fished (Van Dyck et al. 2016; Dodoo et al. 1998). The marine ecosystem is believed to be contaminated with varying levels of pollutants which include heavy metals due to natural

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and anthropogenic sources. (Appiah-Opong et al. 2021). Toxic metal contamination is prevalent in water, air and soil and are taken up by plants and animals (Pandey and Madhuri 2014; Kloke et al. 1984). Though some of these metals are essential nutrients for human metabolism (Tegegne 2015), their levels in the ocean have been significantly altered perhaps by human activities and some natural processes exposing living organisms to bioaccumulation, diseases, and/or even death from toxicity (Ali et al. 2019).

The contamination of the aquatic environments is a collective result of the natural make-up of land in and around the aquatic environments, runoffs, mining, the release of untreated or partially treated industrial waste among others. Garcia-Vazques et al. found in 2021 that Tuna caught from West African waters were more contaminated than those caught in South African and European waters (Garcia-Vazques et al. 2021).

Common heavy metals present in marine environments and associated with seafood are mercury, arsenic, cadmium, and lead (Aziz et al. 2023). These metals are either known to be toxic, carcinogens, and/or mutagens. Some heavy metals may cause serious problems to organ systems in humans and affect health even when ingested through food in trace quantities (Mahurpawar 2015).

There is therefore a global concern about marine pollution because it inadvertently affects the water, sediments and indigenous organisms living in them such as shrimps (Aziz et al. 2023). The bioaccumulation of toxic metals in organisms in these ecosystems could travel through food chains and be introduced to human populations who may depend on seafood to ensure their food security and balanced diet.

Marine organisms are sensitive to toxic metals at significant levels in water and sediments. (Batvari et al. 2016). Shrimps and crabs are susceptible to low-level metal toxicity because they are invertebrates and tend to accumulate more toxic metals than fish and these metals become biomagnified as a result (Batvari et al. 2016). Heavy metal risk via consumption of fish has been widely studied but seafood species like shrimps have received much less heavy metal health risk research (Ortiz-Moriano et al. 2024).

Limited data exists as far as the metal content of shrimps from markets in Kumasi is concerned. There is therefore the need to ascertain the metals levels and potential risk associated with the consumption of milled shrimps marketed. The findings could offer insight into the extent of contamination of the aquatic ecosystem.

Materials and methods

Study area

The study was carried out in Kumasi (as seen in Fig. 1), the capital city of the Ashanti Region of Ghana. The city is in the middle belt of Ghana and elevated 250 to 300 m above sea level. Kumasi is situated between Latitude 6.35ºN and 6.40ºS and Longitude 1.30ºW and 1.35ºE. It is approximately 270 km north of the nearest coast in the national capital, Accra. It has a projected population of 1,989,062 accommodating about 36.2% of the region's population. Kumasi is a meeting point for snappy commercial activities as it provides the avenue for marketing products to consumers, not only from other parts of Ghana but also from the West African sub-region. The study area was chosen due limited literature on the metal content of milled shrimps in Kumasi. This information is important because milled shrimp is used in popular delicacies in the metropolis and is therefore important to determine the possible metal health risks which may result from its consumption.

Sample collection

Samples of machine-milled shrimps were randomly sampled from different vendors at ten markets in the Kumasi metropolis. In all, a total of thirty-three (33) samples were obtained, comprising of 30 milled dry shrimps and 3 un-milled dry shrimps to serve as control. Samples were collected into Ziploc bags and appropriately labelled with the location of sampling as indicated in Fig. 1.

Sample preparation

The samples were sundried for 3 days and further blended using a porcelain mortar and pestle to give finer particles to increase surface area. Ground samples were sieved through a 0.5 mm test sieve and independently stored in appropriately labelled transparent plastic Ziploc bags.

Laboratory analyses

Metals in the samples were screened using a Niton XL3t GOLD field portable X-ray fluorescence (FP-XRF) spectrometer based on the United States Environmental Protection Agency Method 6200 for metal analyses (US EPA, 2007). The FP-XRF spectrometer allows scanning for 25 elements, ranging from sulphur to uranium (ThermoFisher Scientific, 2023).). The metals screened include Molybdenum, Zirconium, Strontium, Uranium, Lead, Gold, Arsenic, Mercury, Zinc, Copper, Nickel, Iron, Manganese, Chromium, Vanadium, Titanium, Calcium, Potassium, Tin, Cadmium and Silver. The sample holder was half-filled (~3.0 g) with the pulverised sample, placed in the XRF and scanned for 180 s to obtain results. Triplicates of each sample were analysed, and the averages of the readings were recorded.

Quality control

The XRF analyser was calibrated with standard reference material (NIST SRM 2711). The porcelain pestle, mortar

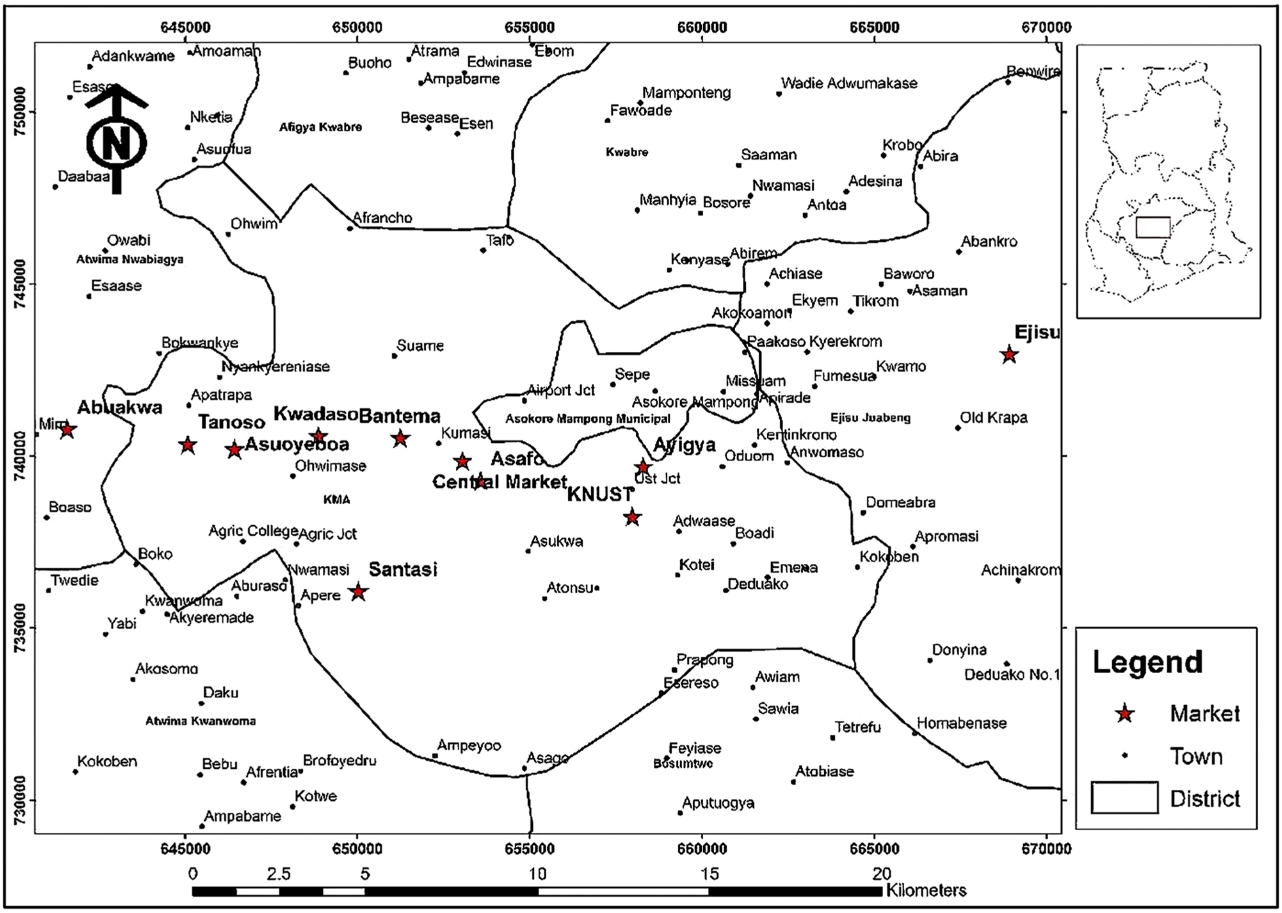

Fig. 1 A map of the study area showing sampling locations

Table 1 Sample Location and Coding

SAMPLE LOCATION	MARKET CODE
Ayigya	AYIG
Kwadaso	KWAD
Santasi	SANT
Tanoso	TANO
Central Market	CENT
Abuakwa	ABUA
KNUST Campus	KNUST
Bantama	BANT
Asafo	ASFO
Asuoyeboa	ASYE

and all other glassware and tools were soaked in HNO_3 and rinsed with $KMnO_4$ and water before and after use.

Statistical analysis

Pearson Correlation analysis was used to determine the correlations between the various metals detected and t-test was used to determine any significant difference in the heavy metals found in milled samples and those in the control samples. These analyses were done with the help of GraphPad Prism 8 and Microsoft office excel.

Health risk assessment

The Hazard Index (HI) and Hazard Quotient (HQ) have been suggested by various researchers to calculate the potential health risk brought on by the consumption of heavy metals by humans when using the standard reference dose (RfD) (Chary et al. 2008; Chien et al. 2002). The health risks were thus determined.

Hazard quotient (HQ) was calculated using:

$$\text{Estimated daily intake } (EDI) = \frac{\text{C} \times \text{EF} \times \text{ED} \times \text{FIR}}{\text{WAB} \times \text{TA} \times 1000} \quad (1)$$

Where:

C is the metal concentration (mg/kg).

E_D is the exposure duration (70 years).

E_F is the exposure frequency (365 days/year).

F_{IR} is flour ingestion rate (100 g/person/day) (Dayal, 2013).

T_A is the average exposure time ($E_F \times E_D$).

W_{AB} is the average body weight (70 kg for adults).

If $T_A = (E_F \times E_D)$, then Eq. 1 reduces to.

$$EDI = \frac{\mathrm{C} \times \mathrm{FIR}}{\mathrm{WAB} \times 1000} \quad (2)$$

Risk of each metal

The potential non-carcinogenic effects of each metal were evaluated by the Hazard Quotient (HQ) using Eq. (2). An HQ value < 1 indicates no significant risk of non-carcinogenic effects for the exposed consumers. The probability of non-carcinogenic effects increases with increasing HQ value (i.e., HQ > 1).

$$\mathrm{HQ} = \frac{EDI}{RfD} \quad (3)$$

Where RfD is the oral reference dose.

The RfD value for arsenic is 3×10^{-3} mg kg^{-1}.

Results and discussion

Concentration of metals

The levels of essential metals and toxic metals identified and quantified in milled shrimp sold on markets as food ingredient are presented in Tables 1 and 2.

The XRF analyser detected on average the presence of essential metals in the order, Ca > K > Fe > Zn > Cu > Cr > Co > Mo (Table 2). Calcium was found to have the highest levels, and this may be attributed to the exoskeleton of shrimps that is predominantly calcium as dissolved calcium is absorbed by shrimps in the aquatic environment for exoskeleton development, growth, and mobility (Xu et al. 2020). Calcium is an essential metal due to its role in bone and teeth formation, regulation of nerve and muscle function, and various enzyme activation (Burton and Foster 1988; Soetan et al. 2010).

The tissues of invertebrates have a high tendency to accumulate higher levels of zinc due to the presence of sulphide-transporting protein with zinc at its active sites. Also, zinc acts as a precursor in most enzymatic activities in most invertebrates. All but samples from Santasi had zinc concentrations that were within the allowed limit of zinc in seafood set by the United States department of health (2000) as 200 mg/kg.

The concentration of Cu in the milled shrimps were also within permissible limits of 80 mg/kg of copper set by the United States department of health ,2000. Chromium was not detected in most samples as it was below the detection limit of the instrument used except for milled shrimp samples from the Ayigya, Santasi, Central Market and the KNUST markets. Chromium, one of the most prevalent elements in the earth's crust and saltwater, can be found in a variety of oxidation states in the environment, mainly CrO, trivalent (+3) and hexavalent (+6) chromium. Trivalent chromium found in food and supplements is essential and of low toxicity. Chromium levels detected were above the International Atomic Energy Agency (IAEA) limits for Cr in shrimps (0.7 mg kg^{-1}). This finding was contrary to the work done by Fatema et al., 2015, which detected Cr levels below IAEA permissible limits.

Some of the copper, chromium and molybdenum detected may be attributed to the wearing from the body of the mills used for milling the shrimps which are predominantly made from stainless steel. Cobalt was recorded in all samples. These results agree with the findings of Mitra et al. 2010, who reported that the crustaceans concentrate cobalt from the surrounding medium, but this varies among species. Bantama had the highest cumulative concentration of essential metals followed by Santasi and Kwadaso with KNUST having the least cumulative load as shown in Fig. 2.

The concentrations of toxic metals in samples analysed were found to be in the order, Sr > Zr > As (Fig. 3). The range of toxic metals as noted in the shrimps obtained from the ten markets in Kumasi were BDL to 15.87 mg kg^{-1} for As, 255.71 to 437.63 mg kg^{-1} for Sr, and 2.83 to 25.34±6.82 mg kg^{-1} for Zr. The least levels of the toxic metals were found in the samples obtained from the Asafo, Kwame Nkrumah University of Science and Technology, and Bantama markets, while the highest levels of the toxic metals were found in the milled shrimps obtained from the Bantama, and

Table 2 Essential metal load in milled shrimp sold on the various markets in Kumasi

MARKET	[Zn] mg/kg	[Ca] mg/kg	[Mo] mg/kg	[K] mg/kg	[Fe] mg/kg	[Cu] mg/kg	[Cr] mg/kg	[Co] mg/kg
AYIG	48.12	11831.03	6.05	4692.64	246.83	26.61	5.69	6.06
KWAD	68.84	32734.87	5.69	10572.03	348.91	26.73	BDL	5.69
SANT	6461.04	27044.31	BDL	13019.21	178.07	35.61	8.65	BDL
TANO	61.57	21317.85	5.39	8295.21	271.07	34.29	BDL	5.39
CENT	56.49	17170.42	5.03	5659.78	1457.74	41.85	28.47	5.03
ABUA	71.47	24217.73	4.06	8377.87	182.74	BDL	BDL	4.07
KNUST	39.80	5266.64	5.21	5266.64	208.44	22.23	8.88	5.27
BANT	63.51	47009.89	3.35	20278.19	155.07	23.96	BDL	3.37
ASFO	49.92	18409.28	2.77	8277.59	142.53	19.41	BDL	2.77
ASYE	48.17	14842.81	3.22	668.21	323.34	20.68	BDL	3.25

Where BDL means below detection limit

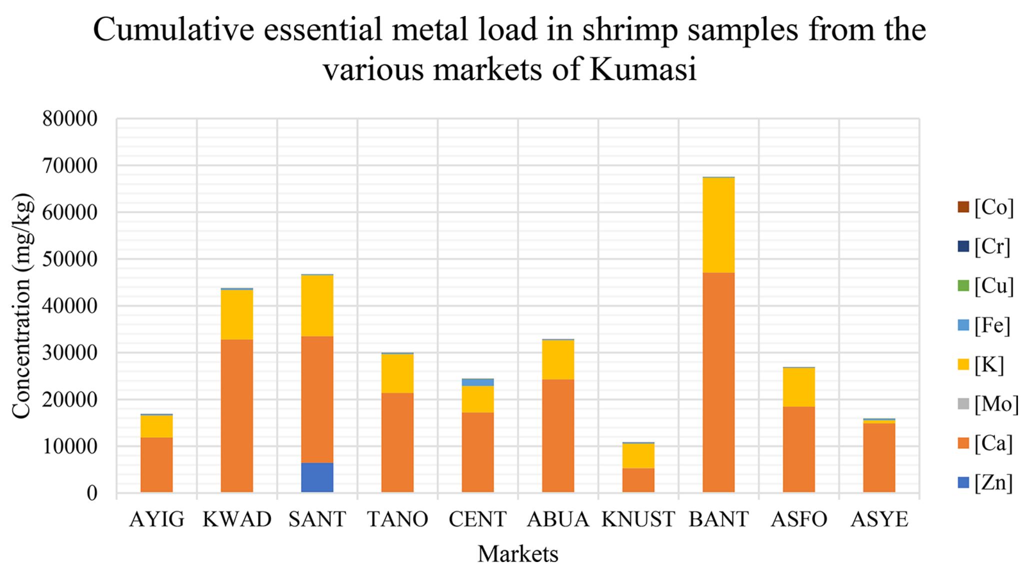

Fig. 2 Cumulative essential metal load in Shrimp samples

Table 3 Toxic metal load in milled shrimps sold in Kumasi

MARKET	[Zr] mg/kg	[Sr] mg/kg	[As] mg/kg
AYIG	8.85	292.52	8.48
KWAD	13.57	319.81	15.07
SANT	6.86	324.33	5.61
TANO	4.45	330.32	9.54
CENT	8.45	300.65	12.35
ABUA	16.43	287.33	7.65
KNUST	6.02	255.71	9.84
BANT	2.83	371.35	15.87
ASFO	5.84	365.72	BDL
ASYE	25.34	437.63	5.53

Where BDL means below detection limit

Asuoyeboah markets, with the Asuoyeboah recording the highest for both Sr and Zr (Table 3). The wide variation in quantities of toxic metals found in the milled shrimps may be a confirmation of bioaccumulation of these metals in the tissues of the shrimps, which depends on their exposure to these metals in their habitat. This influence is more than other factors of exposure, such as mode of fishing, storage, processing, and distribution. Several studies have confirmed the bioaccumulation of toxic metals in seafoods. Seafoods appears to be good sources of strontium (Lill et al. 2014). High strontium levels bioaccumulate in the tissues of shrimps through intake by feeding. Filter feeding shellfish which shrimp is an example of, preferentially concentrate strontium above their natural abundance. Brannon and Rao in 1979 concluded that concentrations of strontium are

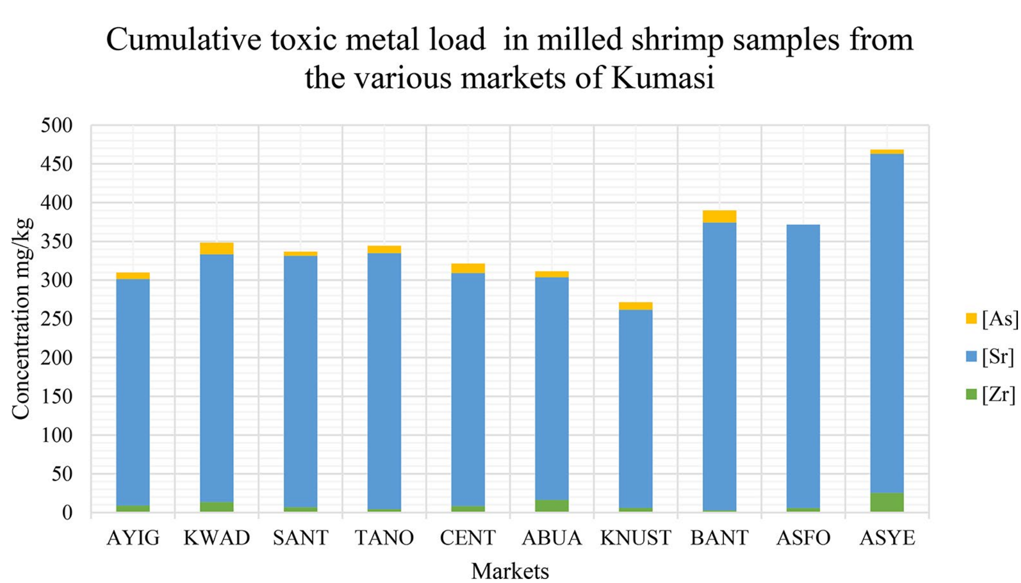

Fig. 3 Cumulative toxic metal load in Shrimp samples

Table 4 Correlation coefficient matrix of metals in milled shrimp samples ($r = 95\%$)

	Co	Cr	Cu	Fe	K	Mo	Ca	Zn	As	Sr	Zr
Co	1.00										
Cr	-0.01	1.00									
Cu	0.00	0.56	1.00								
Fe	0.26	**0.89**[a]	0.54[b]	1.00							
K	-0.46[b]	-0.41[b]	0.01	-0.31	1.00						
Mo	**1.00**[a]	-0.01	0.00	0.27	-0.46[b]	1.00					
Ca	-0.29	-0.42[b]	-0.01	-0.17	**0.93**[a]	-0.29	1.00				
Zn	**-0.79**[a]	0.09	0.32	-0.15	0.30	**-0.78**[a]	0.15	1.00			
As	0.43[b]	-0.04	0.19	0.24	0.47[b]	0.43[b]	0.57[b]	-0.44[b]	1.00		
Sr	-0.41[b]	-0.31	-0.01	-0.15	0.30	-0.41[b]	0.30	-0.03	-0.10	1.00	
Zr	0.00	-0.10	-0.42[b]	0.03	-0.36	0.00	-0.21	-0.15	-0.44[b]	0.41[b]	1.00

[a]*Correlation is significant at the 0.05 level (two-tailed),* $P < 0.05$.

[b]*Correlation is not significant at the 0.05 level (two-tailed),* $P > 0.05$.

Table 5 Metal levels in control samples in mg kg^{-1}

control	Co	Cr	Cu	Fe	K	Mo	Ca	Zn	As	Sr	Zr
1	2.69	BDL	16.99	213.88	9763.24	3.29	29368.89	48.01	BDL	402.08	BDL
2	4.21	BDL	9.42	BDL	10591.88	4.21	16987.14	40.97	BDL	222.18	7.12
3	3.29	BDL	51.81	355.89	6105.27	2.69	67378.79	58.53	8.91	1035.26	BDL
Average	3.40	BDL	26.07	189.92	8820.13	3.40	37911.61	49.17	2.97	553.17	2.37
SD	0.63	0.00	18.46	146.28	1949.28	0.63	21440.82	7.22	4.20	348.71	3.36

SD = standard deviation

Table 6 EDI of metals in milled shrimp via consumption in mg kg^{-1} day $^{-1}$

Market	Co	Cr	Cu	Fe	K	Mo	Ca	Zn	As	Sr	Zr
AYID	0.009	0.008	0.038	0.353	6.704	0.009	16.901	0.069	0.012	0.418	0.013
KWAD	0.008	BDL	0.038	0.498	15.103	0.008	46.764	0.098	0.022	0.457	0.019
SANT	BDL	0.012	0.051	0.254	18.599	BDL	38.635	9.230	0.008	0.463	0.010
TANO	0.008	BDL	0.049	0.387	11.850	0.008	30.454	0.088	0.014	0.472	0.006
CENT	0.007	0.041	0.060	2.082	8.085	0.007	24.529	0.081	0.018	0.430	0.012
ABUA	0.006	BDL	BDL	0.261	11.968	0.006	34.597	0.102	0.011	0.410	0.023
KNUST	0.008	0.013	0.032	0.298	7.524	0.007	7.524	0.057	0.014	0.365	0.009
BANT	0.005	BDL	0.034	0.222	28.969	0.005	67.157	0.091	0.023	0.531	0.004
ASFO	0.004	BDL	0.028	0.204	11.825	0.004	26.299	0.071	BDL	0.522	0.008
ASYE	0.005	BDL	0.030	0.462	8.669	0.005	21.204	0.069	0.008	0.625	0.036
AVERAGE	0.006	0.007	0.036	0.502	12.930	0.006	31.406	0.996	0.013	0.469	0.014
PMTDI				0.800		0.200		0.300	0.003		

PMTDI = Permitted Maximum Tolerable Daily Intake (World Health Organization 1982; World Health Organization 1983)

Table 7 Hazard quotient of metals in milled shrimp samples

Market	Fe	Mo	Zn	As	HI
AYID	0.441	0.045	0.230	4.000	4.716
KWAD	0.623	0.040	0.327	7.333	8.323
SANT	0.318	0.000	30.767	2.667	33.751
TANO	0.484	0.040	0.293	4.667	5.484
CENT	2.603	0.035	0.270	6.000	8.908
ABUA	0.326	0.030	0.340	3.667	4.363
KNUST	0.373	0.035	0.190	4.667	5.264
BANT	0.278	0.025	0.303	7.667	8.273
ASFO	0.255	0.020	0.237	0.000	0.512
ASYE	0.578	0.025	0.230	2.667	3.499
MEAN	0.628	0.030	3.320	4.333	8.311

highly significant, bearing to the fact that shrimps absorb strontium in their habitat, especially during their moulting stages.

The level of arsenic in the milled samples was found to be higher than those found in crabs and shrimp samples from the Mediterranean Coast at Damietta region studied by Abd-Elghany et al. (Abd-Elghany, 2020).

The high levels of As are not alarming as the arsenic content in fish and shellfish are usually organic compounds that have low toxicity (World Health Organization 2019). Contrary to this, Tsuji et al. (2019) states that As is a non-threshold element (even small doses may provide cancer risk). Currently, the risk associated with arsenic from seafood can only be assessed based on the inorganic components as there is insufficient data on organic forms of arsenic (Taylor et al. 2017).

In comparing the accumulative capacity of essential and toxic metals, it was observed that the levels of Ca, K and Fe were higher than strontium while Zn and Cu were higher than Zr and As. Festa and Thiele (2011) explains this as being the result of enzymatic and respiratory processes and relatively high levels of these metals needed to carry out these biological functions. Additionally, these necessary metals seem to diffuse passively, most likely as a soluble complex due to gradients created by membrane surface adsorption. (Mitra et al. 2010). Enough work has not been done on Strontium and Zirconium permissible levels.

Statistical analysis

The findings of the Pearson's correlation analysis, which was used to identify the pairwise correlations within the data, are displayed in Table 4. Strong positive correlations (r > 0.7) between Fe/Cr (0.89), Mo/Co (1.00), and Ca/K values were discovered (0.93). Strong negative correlations (r > 0.7) between the Zn/Co (-0.79) and Zn/Mo levels were discovered (-0.78).

When t-tests were run, it was discovered that the values of the control samples shown in Table 5 were not substantially different from the milled samples obtained from the markets. T-tests with a 95% confidence interval produced P values for cobalt, chromium, copper, iron, potassium, molybdenum, calcium, zinc, arsenic, strontium, and zirconium of 0.5220, 0.3313, 0.9119, 0.4911, 0.9338, 0.5106, 0.1031, 0.5732, 0.0643, 0.0502, and 0.0856, respectively. This is an indication that the various metals were picked up from the aquatic environment because it has been widely published that the aquatic environments are polluted by anthropological activities such as industrial waste, sewages, agricultural practices, mining, and dredging. (Rai 2008; Naser 2013; Dixit et al. 2015; Kumar et al. 2024).

The average EDI trend for the metals was Ca > K > Zn > Fe > Sr > Cu > Zr > As > Cr > Co = Mo (Table 6). Except for zinc and arsenic, the concentrations of the elements and the total amount consumed each day were generally lower than the recommended daily intake. The estimated daily intake of Chromium, iron, zinc, and arsenic reported in this work is higher than that in shrimps in Saint Martin Island, Bangladesh as reported by Baki et al. in 2018 (Baki et al. 2018). Baki reported the EDIs of 0.0037 and 0.00236 for iron and zinc, with chromium and arsenic being below detection limit. Liu et al. (2019) also reported lower EDIs for shrimps obtained from Laizhou Bay, China (Liu et al. 2019).

Risk indices

Risk indicators were obtained for Fe, Mo, Zn and As (Table 7). Hazard Quotient for all samples that were detected were far greater than one (HQ >> 1), except for samples obtained from Asafo market. This is an indication of a significant non-carcinogenic health risk to consumers. The Hazard Quotient, however, does not translate to the probability of adverse health effect. The HI values for the metals in milled shrimp samples obtained from the markets all show a likely health risk to consumers, with Asafo market being the exception. Zinc assessment in samples from Santasi market with HQ value of 30.767 indicated a very significant health risk to consumers. It is exclusively important to note that an HQ > 1 does not necessarily mean that adverse effects will occur but just a form of awareness that the cumulative effect of, and/or continuous exposure to these metals may be harmful (Niki et al. 2015). For to the lack of oral reference doses for the other metals, their HQ values were not computed.

Conclusions

The study evaluated the concentrations of metals in shrimps and the risk associated with the consumption of the toxic metals from ten markets in the Kumasi Metropolis, Ghana. The essential metals identified were Ca, K, Zn, Fe, Cu, Cr, Co, and Mo. Non-essential and toxic metals were As, Sr and Zr respectively. Iron, molybdenum, and zinc were generally within the allowable limits of intake of 0.8 mg kg^{-1} day^{-1}, 0.2 mg kg^{-1} day^{-1} and 0.3 mg kg^{-1} day^{-1} respectively while Arsenic was above the permissible level of 0.003 mg kg^{-1} day^{-1}. However, Arsenic in shrimp is likely organic in nature, which is known to be less toxic. Hazard Quotient values calculated for As were all greater than one indicating the risk of possible health effects upon accumulation. Public health education by respective agencies is therefore recommended to protect the consumers from the potential health hazard and more work needs to be done to reduce the pollution of aquatic environments.

Abbreviations

BDL	Below detection limit
C	Concentration
CENT	Central
E_D	Exposure duration
EDI	Estimated daily intake
E_F	Exposure frequency
F_{IR}	Flour ingestion rate
HI	Hazard index
HQ	Hazard quotient
IAEA	International Atomic Energy Agency
KNUST	Kwame Nkrumah University of Science and Technology
RfD	Standard reference dose
T_A	Average exposure time
W_{AB}	Average body weight
XRF	X-ray fluorescence

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Author contributions
MAN: Originator of the research idea, experimental design, supervision of research and draft of the manuscript. DA: Data Acquisition, data interpretation and draft of manuscript. ISQ: Data Acquisition, data interpretation and draft of manuscript. SOY: Data interpretation and draft of manuscript. MD: Experimental design, supervision of research and review of manuscript.

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Data availability
All data generated or analysed during this study are included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
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