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In-Vitro Analysis of Heavy Metals in Commercially Smoked Fish Sold in Kumasi, Ghana

Albert Kwamina Adjaye Aggrey[1], Emmanuel Agyapong Asare[1], Isaac Kwaku Attatsi[2], Emmanuel Dartey[1]

[1]Department of Chemistry Education, University of Skills Training and Entrepreneurial Development, Asante Mampong, Ghana
[2]Department of Environmental and Safety Engineering, University of Mines and Technology, Tarkwa, Ghana
Email: kwakuasareagyapong@yahoo.com

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Abstract

This study examined heavy-metal concentrations and gastric-phase solubility in three commonly sold smoked fish species: herring (*Sardinella aurita*), mudfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*): purchased from Atonsu, Kejetia, Asafo and Bantama markets in the Kumasi Metropolis, Ghana. A total of 120 fish from 20 vendors were dissected into heads, gills and fillets. Total concentrations of Cd, Pb, Cr, As and Hg were determined by Microwave Plasma-Atomic Emission Spectroscopy (MP-AES) after aqua-regia digestion. An artificial gastric fluid (pH 1.25, porcine pepsin, 37˚C, 24 h) was used to quantify the gastric-phase soluble fraction of each metal; the procedure did not include an intestinal phase and therefore does not measure intestinal absorption or whole-gastrointestinal bioaccessibility. Pb, Cr and As were detected in all three species; Cd was found only in mudfish; Hg was below the detection limit in every sample. In the raw fish, concentrations of Pb, Cr and As consistently exceeded WHO/FAO limits. After simulated gastric digestion, substantial percentages of these metals were released into the soluble fraction, and several gastric-phase concentrations (particularly Cr and As in herring and mudfish, and Pb in herring and tilapia) still exceeded the corresponding WHO/FAO limits. Cadmium and lead in the mudfish gastric filtrates remained below the limits. Dietary exposure was calculated solely from the gastric-phase soluble concentrations measured in the edible fillets. The resulting Hazard Indices were 1.274 for herring (above the threshold of 1.0), 0.570 for tilapia and 0.308 for mudfish (both below 1.0). Lead and chromium were the principal contributors to the elevated HI of herring. The findings indicate that regular consumers of smoked herring in Kumasi may be exposed to potentially unsafe levels of Pb and Cr via the gastric-soluble fraction of the edible muscle. Regular monitoring of smoked fish, improved control of possible contamination sources, and public education remain necessary to protect consumers.

Keywords

Heavy Metals, Smoked Fish, Bioaccessibility, *In-Vitro* Digestion, Hazard Index, Target Hazard Quotient, Kumasi Metropolis, Food Safety

1. Introduction

Fish provide a very important nutritional addition to the human diet, including high quality proteins, essential fatty acids, vitamins and minerals needed for normal physiological activities (Noreen et al., 2025). In addition to being a source of food, fish is necessary worldwide and is an inexpensive and widely available source of nutrition to consumers (Mishra, 2020; Selamoglu & Naeem, 2023). The combination of increasing global population and enhanced knowledge of the nutritional value of fish has led to a persistent growth in demand for fish and fish products (Noreen et al., 2025; Vilain & Baran, 2016). Currently, there are concerns about the safety of fish (Huntingford et al., 2006; Håstein et al., 2006; Samarajeewa, 2023). Aquatic ecosystems have been subjected to the anthropogenic inputs of heavy metals from industrial operations, mining activities, agriculture, and indiscriminate dumping (Ali et al., 2021; Sharma et al., 2024). Examples include the heavy metals Hg, Pb, As, and Cd detected in fish at levels above those established by the safety limits set internationally as defined by WHO and FAO (Dey et al., 2024; Kortei et al., 2020).

Extended intake of high doses of metals in food can disturb normal functions and play a role in negative health outcomes, such as neurodegenerative diseases, renal dysfunction, and carcinogenesis (Cheng et al., 2025). Fish caught in more highly industrialised and polluted waters have higher levels of these metals than those from less polluted areas (La Colla et al., 2018). In places where fish smoking is a common practice, contamination issues are further heightened, due to the fact that the choice of wood fuel used in smoking the fish further increases concentrations of heavy metals and other toxic compounds in the smoked fish (Sonone et al., 2021). Similar research conducted by Tümerkan (2022) confirmed that high heavy metals and other contaminants such as PCBs in smoked fish are generally due to exposure of fish to additional contaminants during the smoking process.

In Ghana, tilapia, herrings, and mudfish are consumed in large amounts, especially in urban areas, because of their availability in markets as reported by Overå et al. (2022). However, rapid industrial growth, mining activities, and increased application of agricultural chemicals in Ghana have raised concerns about the safety of fish as reported by Hasselberg et al. (2020) and Andoh-Odoom et al. (2024). A study done by Massresha et al. (2025) documented lethal concentrations of heavy metals in fish sourced from rivers and lakes, with most exceeding

WHO/FAO safety thresholds. For instance, a study conducted on fish by Tolkou et al. (2023) documented Hg and Pb concentrations far above WHO/FAO recommended concentrations, while studies conducted on fish from Lake Volta also documented dangerously high concentrations of Cd and As, attributed to improper industrial waste disposal and agricultural runoff (Magna et al., 2021). It is also reported that both large scale and illegal mechanised mining have contributed to the elevations of Hg and Pb in the area's water resources (Isah et al., 2025).

In recent years, the Ashanti Region, including the greater Kumasi metropolis, has seen great development and economic growth at an environmental cost. Mining activities, industrial effluents, and agrochemical runoff have burdened the surface and groundwater systems of the area with high concentrations of toxic metals such as Hg, Pb, Cd, and As (Padhiary & Kumar, 2024; Sharafi & Salehi, 2025). Fish, as aquatic biota, accumulate the dissolved metals through their gills and other body parts, thus, serving as an exposure conduit when consumed by humans (Rand et al., 2020). Not only that, the processing method-smoking-trusted by Ghanaians to preserve fish, has no mechanisms to rid the fish of the metals. Worse, according to Adeyeye and Oyewole (2016), the additives and fuels used in traditional smoking systems only introduce more chemical elements to the fish. As popular as smoked fish remains, their consumption, especially in the mining and agricultural hotspots, should be suspect (Sengar et al., 2025).

The situation is not unique to Ghana, as elevated heavy metals in smoked fish have been reported in studies from West and East Africa. Mohammed et al. (2024) found Pb and Cd in smoked fish samples at levels higher than those in the WHO/FAO guidelines. Al-Sulaiti et al. (2023) reported Hg content in fish from polluted water at equally high levels. Results from Nigeria and Kenya have shown greater metal burden in smoked fish than in fresh fish as reported by Robinson et al. (2025) and Onoja et al. (2025), indicating that elevated contamination can be traced to both the initial environment and the smoking process itself. Eboh et al. (2006) claimed that heavy metals levels in smoked fish are not properly monitored and regulated, and this could be a major factor contributing to the contamination reported. In the same vein, fish vendors have limited knowledge about this issue and are unaware that fish sold for daily consumption could be a source of toxic metals.

Despite the growing body of research on heavy metal contamination in fish, most studies have only reported total metal content in samples without considering the fraction of metals that would actually become available for absorption in the human body during digestion. This is important because total metal content does not indicate fraction of the metal the body absorbs as indicated by Milea et al. (2025) and Oroian et al. (2025). Consequently, the current study aimed to assess Hg, Pb, Cd, As, and Cr concentrations in selected smoked fish (tilapia, herrings, and mudfish) sold in the Ghanaian markets, determine percentage of metals that would be bioaccessible to consumers, to determine the potential health risk associated with consumption of smoked fish. In order to achieve the aims, the study sought to, determine Hg, Pb, As, Cd, and Cr contents in smoked fish sampled from Atonsu, Kejetia, Asafo, and Bantama markets in greater Kumasi, compare metal contents with permissible limits set by WHO/FAO, USEPA, and the China Food Safety Authority, apply an *in-vitro* gastrointestinal model to simulate human digestion and determine quantities of metals available for absorption after 24-hour digestion, compare quantities of metals in the filtrate with WHO/FAO acceptable safety thresholds, and use concentrations recorded in the *in-vitro* study to evaluate potential health risks consumers would likely endure.

By simulating digestion, this study bridges a critical gap in understanding the actual health risks associated with smoked fish consumption and provides evidence to compel authorities to tighten existing regulations or establish new ones to protect public health.

2. Methodology

2.1. Study Area

This study was conducted in selected markets located in the Greater Kumasi Metropolis (GKM) of the Ashanti Region, Ghana (**Figure 1**). Kumasi is located in the transitional forest region in south-central Ghana, approximately 270 km northwest of Accra, situated at latitude 6˚37'N to 6˚45'N and longitude 1˚35'W to 1˚41'W (Cobbinah & Amoako, 2012). The city is considered the commercial, industrial, and cultural centre of the Middle Region of Ghana. As of 2024, approximately 4 million people live in the Greater Kumasi Metropolitan area, making it one of the fastest-growing urban metropolis areas in West Africa (Enu et al., 2024). The study focused on four major markets: Kejetia (the largest market in West Africa), Asafo, Bantama, and Atonsu markets, which serve as major commercial hubs for smoked fish distribution in the region.

Figure 1. Map of the study area showing the selected market centres in the greater Kumasi metropolitan area (GKM).

2.2. Research Design and Sampling

This study employed a purposive sampling technique to select smoked-fish sellers who had been in the business for not less than five years, as they were considered experienced and knowledgeable enough to provide relevant information during sampling. A total of 120 smoked fish (40 herring *Sardinella aurita*, 40 mudfish *Clarias gariepinus*, and 40 tilapia *Oreochromis niloticus*) were purchased from 20 vendors across the four major markets (Atonsu, Kejetia, Asafo and Bantama) in the Greater Kumasi Metropolis. Sampling was conducted on a single occasion at each market. From each vendor, fish of the same species that had been exposed to the market environment for 1 - 3 days were selected in order to minimise additional contamination from handling and atmospheric deposition. After collection, fish of the same species from the same market were pooled by anatomical part (heads, gills and fillets) before homogenisation; same species from different markets were pooled as one, and different species were not mixed. Each homogenised composite was then analysed in analytical triplicate (i.e., three independent digestions and instrumental measurements of the same homogenate). The triplicate values reported throughout the Results section are therefore analytical replicates of the pooled market-level composites, not independent biological replicates of individual fish. Each fish type was put into separate locked-fresh bags to reduce cross-contamination, labelled and then placed in brown paper bags, and transported to the laboratory for preparation and analyses.

Catch location/production origin and detailed smoking history (wood type, kiln type, duration, and fuel source) were not available for the commercially obtained samples. All fish were purchased as already-smoked products from market vendors; therefore, it was not possible to determine whether the measured metals originated primarily from the aquatic environment in which the fish were raised or caught, from the smoking process itself, or from both. Consequently, any discussion of contamination sources in this study remains speculative and is limited to possible contributing factors reported in the literature.

2.3. Sample Preparation and Digestion

The different fish types were separated into heads, gills, and fillets. Each part was homogenized using Kenwood Blend-X Fresh Blender (Model: BLP41.AOCT) for 3 minutes at 10,000 rpm. Each homogenised sample was placed into a separate zip-lock plastic bags, labelled and stored in a dry place for analysis with MP-AES. For digestion, 1.0 gram of each homogenised sample was weighed into separate 250 mL Pyrex flat-bottom flasks, and 100 mL of freshly prepared aqua regia (3:1 HCl 37% w/w, HNO_3 70% w/w) was added. The flasks were heated on a programmable hot plate at approximately 108˚C to facilitate digestion. Digestion was completed when the reddish-brown colour of nitrogen dioxide (NO_2) fumes emission ceased. The digested samples were filtered through Whatman No. 42 filter paper into 50 mL volumetric flasks and then made to the volume with distilled water. All samples were analysed in triplicate using Microwave Plasma-Atomic Emission

Spectroscopy (MP-AES) in the Environmental Laboratory at the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.

2.4. *In-Vitro* Digestion Procedure

Artificial Gastric Fluid (AGF) was prepared to simulate human stomach conditions only. Sodium chloride was dissolved in 200 mL of distilled water; 7 mL of concentrated hydrochloric acid (37% w/w) was added slowly and the pH adjusted to 1.20. The pH was further adjusted to 1.25 by drop-wise addition of 1.0 M hydrochloric acid and the solution mixed thoroughly. Porcine pepsin (1.0 g, 2500 U mg^{-1}) was added and stirred until complete dissolution. The mixture was transferred to a 1 L volumetric flask and made to volume with distilled water. Conductivity of the prepared gastric fluid was determined with a two-point calibrated conductivity meter (Jenway 4510, Cole-Parmer Ltd.) and recorded as 13.50 ± 2.12 $mS{\cdot}cm^{-1}$. The gastric fluid was validated by confirming pH 1.25 at 37˚C and by testing pepsin activity (1 mL of fluid incubated with 2% haemoglobin at pH 2 and 37˚C for 10 min). The solution was stored in the dark and used within 24 h to prevent decomposition.

For the *in-vitro* assay, 100 mL of prepared gastric fluid was measured into separate 250 mL Pyrex flat-bottomed flasks. One gram of homogenised heads, gills or fillets (analytical triplicate) was added to each flask. The pH of the mixture was maintained at 1.25 throughout the incubation. Flasks were sealed with cotton wool and incubated at 37˚C for 24 h on a temperature-programmable hot plate with intermittent gentle shaking to simulate gastric motility. After incubation the contents were filtered through Whatman No. 1 filter paper. The filtrates, which represent the gastric-phase soluble fraction of the metals, were collected into labelled Teflon containers. The filtrates were then digested with 100 mL of freshly prepared aqua regia at approximately 108˚C until clear solutions were obtained, cooled, filtered through Whatman No. 42 filter paper, transferred into 50 mL volumetric flasks, made to volume with distilled water and analysed by MP-AES. The identical procedure was applied to herring, mudfish and tilapia samples.

The procedure simulated only the gastric phase of digestion. No intestinal phase (bile salts, pancreatin or neutral-to-alkaline pH) was included. Consequently, the concentrations reported for the filtrates are the gastric-phase soluble fractions of Cd, Pb, Cr, As and Hg; they do not represent intestinal absorption or whole-gastrointestinal bioaccessibility. Results are therefore interpreted strictly as the quantity of each metal solubilised under simulated stomach conditions.

2.5. Calculation of Gastric-Phase Soluble Concentrations and Percentage Released

All filtrate concentrations were expressed on a fish-tissue basis ($mg{\cdot}kg^{-1}$) using the following conversion:

$$C_{\text{gastric}} = C_{\text{measured}} \times \frac{V_{\text{final}}}{m_{\text{sample}}} \quad (1)$$

where:

C_{measured} = metal concentration (mg·L^{-1}) determined by MP-AES in the final 50 mL volumetric flask.

V_{final} = 0.050 L (final volume after aqua-regia digestion and make-up).

m_{sample} = 0.001 kg (1.0 g of homogenised fish tissue originally added to the gastric fluid).

Thus, a measured value of x mg·L^{-1} in the 50 mL flask corresponds to 50x mg·kg^{-1} in the original fish tissue.

The percentage of each metal released into the gastric-phase soluble fraction relative to the matching total-metal concentration was calculated as:

$$\%\ \text{released} = \frac{C_{\text{gastric}}}{C_{\text{total}}} \times 100 \tag{2}$$

where C_{total} is the total metal concentration previously determined in the corresponding raw (undigested) sample of the same anatomical part and species. These percentages are reported alongside the absolute concentrations in the Results section to substantiate statements concerning the extent of metal solubilisation under simulated gastric conditions.

2.6. Health Risk Assessment Models and Equations

Potential health risks associated with heavy metal exposure through smoked fish consumption were estimated using established dietary exposure assessment models. The Estimated Daily Intake (EDI) of each metallic contaminant was calculated using the formula:

$$\text{EDI} = (C \times \text{IR})/\text{BW} \tag{3}$$

where C is the metal concentration in smoked fish (mg/kg), IR is the daily ingestion rate of smoked fish (0.021 kg/day), and BW is the average body weight of the exposed population (60 kg).

The Target Hazard Quotient (THQ) for each metal was calculated using the formula:

$$\text{THQ} = \text{EDI}/\text{RfD} \tag{4}$$

where RfD refers to the oral reference dose (mg/kg body weight/day) as determined by WHO. The following RfDs were used: Pb (0.0004 mg/kg/day), Cr (0.001 mg/kg/day), As (0.0003 mg/kg/day), and Cd (0.001 mg/kg/day). THQ less than 1 indicates no likely risk to those eating the fish species for that metal, whiles THQ above 1, indicates the possibility of adverse effects. Hazard Indices (HIs) were calculated by summing all THQs for the metals.

2.7. Quality Assurance

To ensure the accuracy and precision of the analytical method, certified reference materials (e.g., ERM-BB422 Fish Muscle and DORM-5 Fish Protein) were co-analysed with the respective samples. All samples were prepared and analysed in triplicate using the same procedures and under the same conditions to ensure consistency and comparability of results. The artificial gastric juice was validated by checking its pH at 37˚C and testing pepsin activity prior to use.

3. Results and Discussion

3.1. Quality Control

To ensure the accuracy and precision of the analytical method, certified reference materials (ERM-BB422 Fish Muscle and DORM-5 Fish Proteina) were co-analysed with the respective samples. The recovery efficiencies obtained for the analysed metals were within the acceptable range of 85% - 110%, confirming the reliability of the digestion and analytical procedures. All samples were prepared and analysed in triplicate using the same procedures under the same conditions to ensure consistency and comparability of results. The artificial gastric juice was validated by checking its pH at 37˚C and testing pepsin activity prior to use.

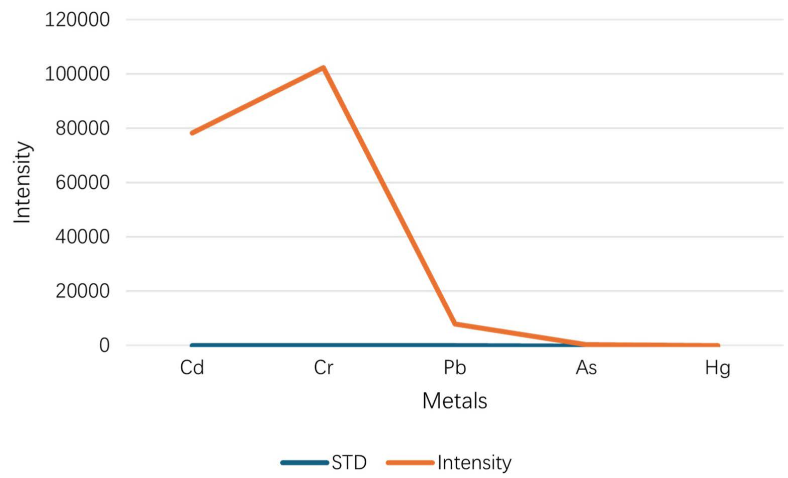

Figure 2. The calibration curves for the metals.

According to **Figure 2**, the calibration curves for the determination of Cd, Cr, Pb, As, and Hg were prepared using standard solutions at concentrations ranging from 0.01 to 2.0 mg/L. The calibration standards for each metal produced the following intensity responses: Cd (1000 μg/L, 78,000 intensity), Cr (1000 μg/L, 102,000 intensity), Pb (1000 μg/L, 8000 intensity), As (1000 μg/L, 2000 intensity), and Hg (1000 μg/L, 2000 intensity). The correlation coefficients (R^2) for each metal exceeded 0.995, indicating good linearity across the concentration range tested.

3.2. Heavy Metal Concentrations in Raw Smoked Fish (*Sardinella aurita*)

Concentrations of Cd, Pb, Cr, As, and Hg were determined in herring samples in triplicate and reported as mean ± SD. The results are presented in **Table 1**.

Cd and Hg were not detected in all parts of the raw smoked herring as presented (**Table 1**). Pb, Cr, and As concentrations ranged from 1.126 ± 0.37 mg/kg in gills to 2.475 ± 0.41 mg/kg in heads (for Cr). All Cr concentrations exceeded the WHO/FAO acceptable level of 0.05 mg/kg. Pb concentrations ranged from 1.439 ± 0.14 mg/kg in heads to 2.247 ± 0.16 mg/kg in fillets, while As ranged from 1.769 ± 0.04 mg/kg in fillets to 2.114 ± 0.06 mg/kg in gills. All concentrations of Pb, Cr, and As exceeded the permissible limits set by WHO/FAO for fish and fish products.

Table 1. Mean concentration of metals in raw smoked herring (*Sardinella aurita*).

Sample	Cd	Pb	Cr	As	Hg
Heads	BDL	1.439 ± 0.14	2.475 ± 0.41	1.793 ± 0.01	BDL
Gills	BDL	1.879 ± 0.2	1.126 ± 0.37	2.114 ± 0.06	BDL
Fillets	BDL	2.247 ± 0.16	1.475 ± 0.32	1.769 ± 0.04	BDL
WHO/FAO	0.10	0.2	0.05	0.05	0.50
USEPA	0.10	0.30	NS	NS	1.00
China	0.10	0.50	NS	0.10	0.50

BDL = Below Detection Limit, NS = Not Specified.

Metal concentrations above WHO/FAO recommendations indicate that the fish are not wholesome for human consumption and pose potential health risks to consumers. Elevated Pb levels are particularly concerning as Pb is a neurotoxin that causes damage to soft tissue and bones and can result in permanent brain damage, especially in young children and pregnant women. Excessive Cr and As levels are also associated with serious health effects including respiratory problems, skin disorders, and various cancers.

The possible sources of this contamination include industrial discharge of untreated or poorly treated wastes into water bodies, mining effluents entering rivers and streams, agricultural runoff from farmlands, and coastal pollution through urban storm water runoff and solid waste disposal along the coast of Ghana. The smoking process itself may also contribute to the elevated metal concentrations through the use of contaminated wood fuel during the smoking process.

3.3. Mean Concentration of Metals in Raw Smoked Mudfish (*Clarias gariepinus*)

Concentration of the metals of interest in each part of the raw smoked mudfish was recorded in triplicate and the data reported in the form mean ± std deviation and results are presented (Table 2).

As indicated in Table 2, Hg was not detected in any parts of the raw smoked mudfish. Cd levels in all parts were below the 0.10 mg/kg recommended limit, ranging from 0.037 ± 0.01 mg/kg in fillets to 0.089 ± 0.01 mg/kg in gills. Pb concentrations exceeded WHO/FAO limits (0.20 mg/kg) in all parts, ranging from 0.819 ± 0.21 mg/kg in heads to 1.639 ± 0.13 mg/kg in fillets. Cr concentrations ranged from 1.010 ± 0.13 mg/kg in gills to 1.545 ± 0.13 mg/kg in fillets, all exceeding the 0.05 mg/kg WHO/FAO limit. As concentrations ranged from 1.600 ± 0.14 mg/kg in heads to 2.212 ± 0.12 mg/kg in gills, all exceeding the 0.05 mg/kg WHO/FAO limit.

Table 2. Mean concentration of metals in raw smoked mudfish (*Clarias gariepinus*).

Sample	Cd	Pb	Cr	As	Hg
Heads	0.065 ± 0.01	0.819 ± 0.21	1.156 ± 0.11	1.600 ± 0.14	BDL
Gills	0.089 ± 0.01	1.539 ± 0.13	1.010 ± 0.13	2.212 ± 0.12	BDL
Fillets	0.037 ± 0.01	1.639 ± 0.13	1.545 ± 0.13	1.888 ± 0.19	BDL
WHO/FAO	0.10	0.20	0.05	0.05	0.05
USEPA	0.10	0.30	NS	NS	1.00
China	0.10	0.50	NS	0.10	0.50

BDL = Below Detection Limit, NS = Not Specified.

Metal concentrations exceeding WHO/FAO recommendations indicate that the mudfish are highly contaminated and therefore unfit for human consumption. Consumers are at increased risk of harmful health effects due to exposure to excessive Pb, Cr, and As levels. The high levels of these metals in mudfish indicate severe contamination of aquatic ecosystems, likely from mining activities, industrial effluents, and agricultural runoff entering the water bodies where the fish are caught. Additionally, fish smokers may have contributed to these high concentrations through the use of wood fuel containing significant levels of these metals during the smoking process.

3.4. Mean Concentration of Metals in Raw Smoked Tilapia (*Oreochromis niloticus*)

The raw smoked tilapia was separated into head, gills and the fillets. The parts were digested, analyzed in triplicate and the mean concentration are presented (**Table 3**).

Table 3. Mean concentration of metals in raw smoked tilapia (*Oreochromis niloticus*).

Sample	Cd	Pb	Cr	As	Hg
Heads	BDL	1.249 ± 0.22	2.275 ± 0.18	1.525 ± 0.14	BDL
Gills	BDL	1.690 ± 0.23	1.925 ± 0.20	1.846 ± 0.17	BDL
Fillets	BDL	2.057 ± 0.19	1.609 ± 0.19	1.820 ± 0.13	BDL
WHO/FAO	0.10	0.20	0.05	0.05	0.50
USEPA	0.10	0.30	NS	NS	1.00
China	0.10	0.50	NS	0.10	0.50

BDL = Below Detection Limit, NS = Not Specified.

Cd and Hg were below detection limit in all parts of the raw smoked tilapia (**Table 3**). Pb concentrations ranged from 1.249 ± 0.22 mg/kg in heads to 2.057 ±

0.19 mg/kg in the fillets, all exceeding WHO/FAO, USEPA, and China Food Safety limits. Cr concentrations ranged from 1.609 ± 0.19 mg/kg in the fillets to 2.275 ± 0.18 mg/kg in the heads, all exceeding the 0.05 mg/kg WHO/FAO limit. As concentrations ranged from 1.525 ± 0.14 mg/kg in the heads to 1.846 ± 0.17 mg/kg in the gills, all exceeding WHO/FAO limits.

Metal concentrations exceeding WHO/FAO recommendations indicate that tilapia sold in the Ghanaian market contain substantial levels of contamination and are not safe for human consumption. The elevated Pb levels are particularly concerning as Pb exceeded the USEPA and China Food Safety Authority limits in all parts by substantial margins. This level of contamination may have occurred due to poor management of environmental monitoring and a lack of knowledge on the part of smoked fish outlets about the sources of metal contamination. Possible sources include discharge of untreated industrial and mining effluents into water bodies, agricultural runoff, and the use of contaminated wood fuels during the smoking process.

3.5. Discussion of Findings for Raw Smoked Fish Analysis

The data from the raw smoked fish analysis reveal significant contamination of all three species with Pb, Cr, and As, with concentrations far exceeding WHO/FAO permissible limits. The detection of Pb, Cr, and As in herring, mudfish, and tilapia, coupled with the absence of Hg in all samples and Cd only in mudfish, indicates a pattern of contamination that is consistent with studies from other West African countries (Kwaansa-Ansah et al., 2019; Nyantakyi et al., 2021). The presence of Cd only in mudfish may be attributed to the benthic feeding habits of mudfish, which spend more time in contact with contaminated sediments where Cd tends to accumulate (Hama et al., 2025; Nyantakyi et al., 2021).

The Pb in the herring (1.439 - 2.247 mg/kg), mudfish (0.819 - 1.639 mg/kg), and tilapia (1.249 - 2.057 mg/kg) exceeded the WHO/FAO permissible limit of 0.20 mg/kg by substantial margins. These findings are particularly concerning given that Pb is a well-established neurotoxin that causes damage to soft tissue and bones and can result in permanent brain damage, especially in young children (Kwaansa-Ansah et al., 2019; Nyantakyi et al., 2021). The elevated Pb levels in all fish parts, particularly in fillets, suggest that consumers are directly exposed to unsafe levels of Pb. The source of this contamination is likely attributable to industrial discharge, mining effluents, and agricultural runoff entering water bodies, as documented by Sankhla et al. (2016) in the Ghanaian context. The smoking process may further exacerbate contamination through the use of contaminated wood fuel, as reported by Adherr et al. (2022) and Hasselberg et al. (2020).

Cr in the raw fish samples were alarmingly high, with herring heads recording the highest at 2.475 ± 0.41 mg/kg, followed by tilapia heads at 2.275 ± 0.18 mg/kg. All samples exceeded the WHO/FAO limit of 0.05 mg/kg. While Cr(III) is considered an essential trace mineral for metabolism, the Cr(VI) oxidation state poses the greatest toxicity as it can easily cross cell membranes and causes harm through conversion to lower valence states (Wise Jr. et al., 2022). The presence of Cr at these elevated levels indicates severe contamination of aquatic ecosystems, likely from industrial activities such as electroplating, pigment production, and wood preservatives (Zhigalenok et al., 2025). The high Cr observed across all fish parts, particularly in the heads, suggest accumulation through gill uptake from contaminated water.

As concentrations in the raw fish samples ranged from 1.525 ± 0.14 mg/kg in the heads of tilapia to 2.212 ± 0.12 mg/kg in the gills of mudfish, all exceeding the WHO/FAO limit of 0.05 mg/kg. As is a known carcinogen associated with skin lesions, cardiovascular disease, nerve damage, and various cancers (Martinez et al., 2011; Speer et al., 2023). The elevated As levels found in this study are consistent with findings from other studies in Ghana, particularly from Lake Volta, where dangerously high concentrations of As were attributed to improper industrial waste disposal and agricultural runoff (Gbogbo et al., 2017; Mawuko, 2025). The presence of As in all fish parts suggests widespread contamination of water bodies from natural sources (volcanic eruptions, erosion) and anthropogenic activities such as mining, smelting, and pesticide use as indicated by Malik et al. (2023) and Raut et al. (2025).

The absence of Hg in all samples is a notable finding, contrasting with studies that have reported Hg contamination in fish from polluted water bodies (Sackey et al., 2024). This may indicate that the water bodies where these fish were sourced have not been significantly impacted by Hg contamination, or that Hg levels were below the detection limit of the analytical method used (BDL < 0.01 mg/kg). However, this does not diminish the seriousness of Pb, Cr, and As contamination observed.

3.6. Percentage Contribution of Metals to Total Metal Burden

For herring, Cd and Hg were not detected. Pb contributed 25.21% in the heads, 36.70% in the gills, and 40.92% in the fillets to the metal burden. Cr contributed 43.37% in the heads, 21.99% in the gills, and 26.86% in the fillets. As contributed 31.43% in the heads, 41.30% in the gills, and 32.21% in the fillets. The total metal burden was 16.317 mg/kg, with As having the highest overall contribution.

For mudfish, the cumulative heavy metal burden was 13.599 mg/kg. As had the highest contribution (41.92%), followed by Pb (29.39%), Cr (27.28%), and Cd (1.404%). Hg was not detected. The fillets had the highest Pb burden (41.01%), while As was fairly evenly distributed across all parts.

For tilapia, the total metal burden was 15.996 mg/kg. Cr contributed 36.32%, As 32.45%, and Pb 31.23%. Pb was highest in fillets (41.17%), Cr was highest in heads (39.16%), and As was evenly distributed across all parts.

3.7. *In-Vitro* Assessment of Filtrates Obtained from Smoked Fish Samples

Smoked herring sample testing was completed via the *in-vitro* analysis to determine the amount of Cd, Pb, Cr, As, and Hg that would eventually enter the bodies of people who consumed the smoked herring over a 24-hour period. The mean metal concentrations reported as mean ± standard deviation (**Table 4**) were obtained from the analysis of the filtered sample of the smoked herring after being completed as a part of this analysis for all the *in-vitro* analyses.

Table 4. Mean concentration of metals in smoked herring filtrate (*Sardinella aurita*).

Sample	Cd	Pb (mg/kg)	% released	Cr (mg/kg)	% released	As (mg/kg)	% released	Hg
Head	BDL	0.082 ± 0.02	5.7%	1.062 ± 0.011	42.9%	0.108 ± 0.030	6.0%	BDL
Gill	BDL	0.087 ± 0.016	4.6%	1.240 ± 0.016	110.1%*	0.213 ± 0.019	10.1%	BDL
Fillet	BDL	0.888 ± 0.022	39.5%	0.979 ± 0.021	66.4%	0.132 ± 0.017	7.5%	BDL
WHO/FAO	0.10	0.20	–	0.05	–	0.05	–	0.50
USEPA	0.10	0.30	–	NS	–	NS	–	1.00
China	0.10	0.50	–	NS	–	0.10	–	0.50

BDL = Below Detection Limit.

According to **Table 4**, after 24-hour simulated gastric digestion, Pb, Cr and As remained in the gastric-phase soluble fraction of all herring parts, while Cd and Hg were below detection. Pb concentrations in the filtrates ranged from 0.082 ± 0.02 mg·kg^{-1} in the heads (5.7% of total Pb) to 0.888 ± 0.022 mg·kg^{-1} in the fillets (39.5% of total Pb). The fillet value exceeded the WHO/FAO limit of 0.20 mg·kg^{-1}. Cr was extensively solubilised: 42.9% of total Cr was released from the heads (1.062 ± 0.011 mg·kg^{-1}), essentially all of the Cr from the gills (1.240 ± 0.016 mg·kg^{-1}; 110.1%), and 66.4% from the fillets (0.979 ± 0.021 mg·kg^{-1}). All three gastric-phase concentrations far exceeded the WHO/FAO limit of 0.05 mg·kg^{-1}. As was less readily released: 6.0% from heads (0.108 ± 0.030 mg·kg^{-1}), 10.1% from gills (0.213 ± 0.019 mg·kg^{-1}) and 7.5% from fillets (0.132 ± 0.017 mg·kg^{-1}). Nevertheless, every gastric-phase As concentration still exceeded the WHO/FAO limit of 0.05 mg·kg^{-1}. These results show that a substantial gastric-phase soluble fraction of Pb, Cr and As remains after simulated stomach digestion. Because the assay did not include an intestinal phase, the values indicate the quantity of metal that becomes available for potential subsequent absorption rather than the amount actually absorbed into the bloodstream.

3.8. Mean Concentrations of Metals in Smoked Mudfish Filtrates

The mean metal levels obtained in the filtrate of the smoked mudfish samples in the *in-vitro* studies were also calculated and presented in the form mean ± standard deviation (**Table 5**).

In the gastric-phase soluble fraction of mudfish, Cd and Pb remained below WHO/FAO limits in all parts, whereas Cr and As exceeded the 0.05 mg·kg^{-1} limit. Cd release was high in heads (98.5%; 0.064 ± 0.013 mg·kg^{-1}) and fillets (91.9%; 0.034 ± 0.016 mg·kg^{-1}) but lower in gills (40.4%; 0.036 ± 0.013 mg·kg^{-1}). Pb release was modest (9.2% - 18.2%), giving gastric-phase concentrations of 0.149 - 0.150 mg·kg^{-1}. Cr release was low (4.6% - 6.9%), yet the absolute gastric-phase concentrations (0.070 - 0.071 mg·kg^{-1}) still exceeded the WHO/FAO limit. As release was also limited (5.4% - 7.4%), but the resulting concentrations (0.119 - 0.120 mg·kg^{-1}) exceeded both the WHO/FAO limit and the China Food Safety limit of 0.10 mg·kg^{-1}. Thus, although only a small percentage of the total Cr and As present in the raw mudfish were solubilised under gastric conditions, the absolute amounts released were still high enough to exceed international safety thresholds. These figures represent the gastric-phase soluble fraction only and do not equate to intestinal absorption or systemic uptake.

Table 5. Mean concentration of metals in smoked mudfish filtrate (*Clarias gariepinus*).

Sample	Cd (mg/kg)	% released	Pb (mg/kg)	% released	Cr (mg/kg)	% released	As (mg/kg)	% released	Hg
Head	0.064 ± 0.013	98.5%	0.149 ± 0.012	18.2%	0.071 ± 0.011	6.1%	0.119 ± 0.017	7.4%	BDL
Gills	0.036 ± 0.013	40.4%	0.150 ± 0.017	9.7%	0.070 ± 0.011	6.9%	0.119 ± 0.014	5.4%	BDL
Fillets	0.034 ± 0.016	91.9%	0.150 ± 0.012	9.2%	0.071 ± 0.012	4.6%	0.120 ± 0.017	6.4%	BDL
WHO/FAO	0.10	–	0.20	–	0.05	–	0.05	–	0.50
USEPA	0.10	–	0.30	–	NS	–	NS	–	1.00
China	0.10	–	0.50	–	NS	–	0.10	–	0.50

BDL = Below Detection Limit.

3.9. Mean Concentrations of Metals in Smoked Tilapia Filtrates

The mean metal levels obtained in the filtrate of smoked tilapia samples in the *in-vitro* studies were also calculated and presented in the form mean ± standard deviation (**Table 6**).

Table 6. Mean concentration of metals in smoked tilapia filtrate (*Oreochromis niloticus*).

Sample	Cd	Pb (mg/kg)	% released	Cr (mg/kg)	% released	As (mg/kg)	% released	Hg
Head	BDL	0.518 ± 0.019	41.5%	0.040 ± 0.011	1.8%	0.040 ± 0.013	2.6%	BDL
Gills	BDL	0.801 ± 0.017	47.4%	0.024 ± 0.013	1.2%	0.055 ± 0.012	3.0%	BDL
Fillets	BDL	0.568 ± 0.014	27.6%	0.034 ± 0.011	2.1%	0.052 ± 0.012	2.9%	BDL
WHO/FAO	0.10	0.20	–	0.05	–	0.05	–	0.50
USEPA	0.10	0.30	–	NS	–	NS	–	1.00
China	0.10	0.50	–	NS	–	0.10	–	0.50

BDL = Below Detection Limit.

In tilapia, Cd and Hg were not detected in any gastric filtrate. Pb was the dominant metal in the soluble fraction: 41.5% of total Pb was released from heads (0.518 ± 0.019 mg·kg^{-1}), 47.4% from gills (0.801 ± 0.017 mg·kg^{-1}) and 27.6% from fillets (0.568 ± 0.014 mg·kg^{-1}). All three values exceeded the WHO/FAO limit of 0.20 mg·kg^{-1}. Cr release was very low (1.2% - 2.1%), producing gastric-phase concentrations (0.024 - 0.040 mg·kg^{-1}) that remained below the WHO/FAO limit. As release was similarly limited (2.6% - 3.0%); concentrations were slightly above the 0.05 mg·kg^{-1} limit in gills and fillets but below it in heads. The data indicate that Pb is far more readily solubilised under simulated gastric conditions than Cr or As in tilapia. The reported concentrations are gastric-phase soluble fractions only; they do not represent the fraction that would be absorbed across the intestinal epithelium or the full gastrointestinal bioaccessibility.

3.10. Discussion of Findings for *In-Vitro* Bioaccessibility Study

The *in-vitro* digestion study provides critical insights into the actual health risks consumers face, as it simulates the amount of metals that would be released from the fish matrix and become available for absorption in the human body. The findings reveal that while simulated digestion reduced metal concentrations compared to raw samples, substantial quantities of Pb, Cr, and As remained bioaccessible.

For herring, the bioaccessible Pb ranged from 0.082 ± 0.02 mg/kg in the heads to 0.888 ± 0.022 mg/kg in the fillets. The fillet Pb concentration exceeded 0.20 mg/kg WHO/FAO limit, indicating that consumers who eat herring fillets are exposed to unsafe levels of Pb even after digestion. Cr remained highly bioaccessible, with all filtrate concentrations exceeding the WHO/FAO limit by 94% - 96%. This means that Cr is not bound tightly to the fish tissue and is readily released during digestion, posing a significant health risk. As also remained bioaccessible at levels exceeding WHO/FAO limits, with 0.108 - 0.213 mg/kg detected in the filtrates.

The high bioaccessibility of Cr and As in herring is particularly concerning because these metals are known to cause serious health effects, including respiratory problems, skin disorders, and cancers (Balali-Mood et al., 2021). The fact that these metals are not removed or bound during digestion suggests that consumers are directly exposed to these contaminants.

For mudfish, the *in-vitro* results showed that while Cd and Pb remained below safety limits, Cr (0.070 - 0.071 mg/kg) and As (0.119 - 0.120 mg/kg) exceeded WHO/FAO limits. The bioaccessible As levels also exceeded the China Food Safety limit of 0.10 mg/kg, indicating that even with more lenient standards, the contamination poses a risk. The chronic As exposure documented in this study could increase the risk of cardiovascular disease, cognitive developmental issues in children, and skin, bladder, and lung cancers (Martinez et al., 2011; Speer et al., 2023).

For tilapia, the *in-vitro* findings were mixed. Pb remained the major concern, with all filtrates exceeding WHO/FAO limits (0.518 - 0.801 mg/kg). This indicates that Pb is highly bioaccessible in tilapia, and regular consumption would expose consumers to unsafe Pb levels. However, Cr levels in all tilapia filtrates were below WHO/FAO limits, suggesting that Cr in tilapia may be less bioaccessible compared to herring and mudfish. As levels were slightly above the WHO/FAO limit in the gills and fillets but below in the heads, indicating relatively lower risk compared to herring and mudfish.

3.11. Health Risk Estimation and Computation

Target Hazard Quotient (THQ) and Hazard Index (HI) for Smoked Fish

Dietary exposure was calculated exclusively from the gastric-phase soluble concentrations measured in the fillets, the portion of the fish that is actually consumed. Concentrations from heads and gills were excluded because no mass-weighted edible-fish concentration or consumption pattern justifying their inclusion was available.

The Estimated Daily Intake (EDI) of each metal was calculated as:

$$EDI = (C \times IR)/BW$$

where C is the gastric-phase soluble concentration in the fillet ($mg \cdot kg^{-1}$), IR is the daily ingestion rate of smoked fish ($0.021\ kg \cdot day^{-1}$), and BW is the average body weight (60 kg).

The Target Hazard Quotient (THQ) for each metal was calculated as:

$$THQ = EDI/RfD$$

where RfD is the oral reference dose (Pb 0.0004, Cr 0.001, As 0.0003, Cd 0.001 $mg \cdot kg^{-1} \cdot day^{-1}$). The Hazard Index (HI) is the sum of the individual THQs. The results are presented in Table 7.

Table 7. Target hazard quotients and hazard indices for smoked fish species.

Fish species	Metal	Gastric-phase concentration ($mg \cdot kg^{-1}$)	EDI ($mg \cdot kg^{-1} \cdot day^{-1}$)	THQ	HI	Risk level
Herring	Pb	0.888	0.000311	0.777	1.274	Potential risk (HI > 1)
	Cr	0.979	0.000343	0.343		
	As	0.132	0.000046	0.154		
Tilapia	Pb	0.568	0.000199	0.497	0.570	No immediate risk (HI < 1)
	Cr	0.034	0.000012	0.012		
	As	0.052	0.000018	0.061		
Mudfish	Cd	0.034	0.000012	0.012	0.308	No immediate risk (HI < 1)
	Pb	0.150	0.000053	0.131		
	Cr	0.071	0.000025	0.025		
	As	0.120	0.000042	0.140		

Note: EDI = ($C \times 0.021\ kg\ day^{-1}$)/60 kg, RfDs used: Pb = 0.0004, Cr = 0.001, As = 0.0003, Cd = 0.001 $mg \cdot kg^{-1} \cdot day^{-1}$, Only fillet (edible muscle) gastric-phase soluble concentrations were used. Heads and gills were excluded and HI > 1 indicates potential non-carcinogenic risk under the assumptions of the model.

For smoked herring the Hazard Index was 1.274, exceeding the threshold value of 1.0. Lead (THQ = 0.777) and chromium (THQ = 0.343) were the main contributors, while arsenic contributed a smaller share (THQ = 0.154). An HI value greater than 1.0 indicates a potential non-carcinogenic risk under the assumptions of the model. The elevated contribution of lead and chromium is consistent with earlier reports of high non-carcinogenic risk associated with these metals in smoked fish from West African markets (Bello et al., 2026).

For smoked tilapia the Hazard Index was 0.570, below the threshold of 1.0. Lead accounted for the largest portion of the index (THQ = 0.497), with chromium and arsenic contributing only minor amounts. Regular consumption of smoked tilapia fillets is therefore not expected to pose an immediate non-carcinogenic risk on the basis of the calculated HI, although the predominance of lead remains a point of concern given its well-documented toxicity even at low exposure levels (Yaradua et al., 2022).

For smoked mudfish the Hazard Index was 0.308, well below the threshold of 1.0. Arsenic and lead were the principal contributors, yet the overall index remains comfortably under the level of concern. Similar low HI values for certain smoked freshwater species have been reported in other African studies when only the edible muscle was considered (Ogundiran et al., 2024).

It should be emphasised that these HI values are based solely on the gastric-phase soluble fraction of each metal. They represent a conservative estimate of potential exposure rather than a direct measure of the amount absorbed into the bloodstream, because the *in-vitro* procedure did not include an intestinal phase. Nevertheless, the elevated HI for herring demonstrates that a meaningful quantity of lead and chromium remains soluble under simulated stomach conditions and is therefore available for possible subsequent absorption. Continued monitoring of smoked fish sold in Kumasi markets, together with measures to reduce contamination along the production chain, remains advisable (Yar et al., 2025).

3.12. Overall Interpretation and Public Health Implications

The overall findings of this study paint a disturbing picture of heavy metal contamination in smoked fish sold in Kumasi markets. The detection of Pb, Cr, and As at concentrations far exceeding WHO/FAO limits in all three fish species indicates that the aquatic ecosystems from which these fish are sourced are heavily contaminated. The persistence of these metals in bioaccessible forms after simulated digestion confirms that consumers are not only exposed to these metals but are likely to absorb them into their bloodstream. The fact that Cr and As remained highly bioaccessible in herring and mudfish, even after digestion, suggests that these metals are not tightly bound to fish tissue and are readily released during digestion.

The health risk assessment further confirms the severity of the situation, with herring and tilapia both having HI values above 1.0, indicating significant non-carcinogenic health risks. The high THQ values for Cr in herring and Pb in tilapia are particularly concerning, as these metals are known to cause serious health effects, including cancer, neurological damage, and developmental problems. The findings of this study are consistent with previous research in West Africa that has reported elevated heavy metals in smoked fish and associated health risks (Bello et al., 2026).

The implications for public health are substantial. The three fish species studied are among the most commonly consumed in Ghana, particularly by low-income populations who rely on smoked fish as an affordable source of protein. The high levels of contamination and associated health risks suggest that a significant portion of the Ghanaian population may be exposed to unsafe levels of Pb, Cr, and As through their diet. The lack of regular monitoring and enforcement of existing regulations has allowed this situation to persist, as noted by Kwaansa-Ansah et al. (2019) and Hasselberg et al. (2020).

4. Conclusion and Recommendations

4.1. Conclusion

This study determined the total and gastric-phase soluble concentrations of Cd, Pb, Cr, As and Hg in 120 smoked herring (Sardinella aurita), mudfish (Clarias gariepinus) and tilapia (Oreochromis niloticus) obtained from 20 vendors in four Kumasi markets. Lead, chromium and arsenic were detected in all three species, cadmium only in mudfish, and mercury was below detection in every sample; total concentrations of Pb, Cr and As consistently exceeded WHO/FAO limits. Simulated gastric digestion released substantial percentages of these metals into the soluble fraction, and several gastric-phase concentrations (particularly Cr and As in herring and mudfish, and Pb in herring and tilapia) still exceeded the corresponding limits, while cadmium and lead in mudfish filtrates remained below them. Because only the gastric phase was simulated, the reported values represent the fraction available for potential subsequent absorption rather than measured intestinal uptake. Dietary-exposure assessment based solely on the edible fillets yielded Hazard Indices of 1.274 for herring (above the threshold of 1.0), 0.570 for tilapia and 0.308 for mudfish; lead and chromium were the main contributors to the elevated herring HI. The market-based design cannot distinguish environmental from smoking-related contamination sources. Regular monitoring, enforcement of food-safety standards, and education of processors and consumers remain necessary to reduce potential long-term health risks.

4.2. Recommendations

Based on the findings of this study, it is prudent that Regulatory Authorities such as Food and Drugs Authority, Environmental Protection Agency, establish and enforce maximum permissible limits for Pb, Cr, and As in smoked fish. Conduct regular and random sampling of smoked fish from major markets and publish the results for consumers to make informed decision. It is also prudent to provide education and training on the health hazards of heavy metal contamination, the best types of wood to use as fuel during smoking process, adopt improved kilns and depuration techniques to reduce metal loads among others.

Author Contributions

Albert Kwamina Adjaye Aggrey conceptualised and designed the study, undertook the investigation, performed the data analysis, interpreted the findings, and drafted the manuscript. Emmanuel Agyapong Asare contributed to the refinement of the conceptualized framework and made substantial contributions to the experimental study design, data analysis and manuscript preparation. Isaac Kwaku Attatsi and Emmanuel Dartey contributed to the critical review and revision of the manuscript. All authors critically reviewed and approved the final version of the manuscript for publication.

Conflicts of Interest

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

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