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Heavy Metal Composition, Toxicology Ratios and Mineral Safety Index in Smoked Head, Liver and Muscle tissues of African Catfish [*Clarias gariepinus*]: Note II

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Abstract: All the trace mineral levels (mg/100g) in the smoked *Clarias gariepinus* samples fish results were low but with improvement in Mn [0.126 to 1.54mg/100g]. Additional minerals identified were Se, Pb, Cd, Co and Ni. Se to Ni had the following ranges in the samples (mg/100g); head (HS), liver (LS), muscle (MS): 0.001 to 0.002, 0.002 to 0.092, 0.001 to 0.068 respectively. Total mineral body burden (SMB) values were HS (1.276mg/100g), LS (1.644mg/100g) and MS (0.199mg/100g). Observed ratios were LS: HS [1.29/1.00], LS:MS [8.26/1.00], HS:MS [6.41/1.00] and LS: HS + MS [1.11/1.00]. The concentrations of all the metals were the highest with LS. In each sample, percentage value of Mn was 99.53% (HS), 93.67% (LS) and 63.32% (MS). Some relationships were found among ratios of minerals in the protection group to the heavy toxic minerals. All the ratios were each higher than the acceptable ideal range. The mineral safety index of Se showed that it could be deleterious in both HS (by 45.7%) and LS (by 31.4 %) but good in MS. Statistical analysis showed that significant differences existed in the mineral concentration pairs of HS/LS, LS/MS and HS/MS but not in the toxic ratios, both at rxy = 0.05 at n-2 (df).

Keywords: *Trace Minerals, Toxic Mineral Ratios, Mineral Safety Index, and African Sharptooth Catfish.*

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I. INTRODUCTION

Man has used fish for food throughout history. Overall, about one-sixth of the world's protein is estimated to be provided by fish (Helfman, 2007). The yearly harvest from all fish farms all over the world is like 154 million tonnes as reported by FAO in 2015 (FAO., 2015). There may be an estimated 32500 species alive today (some estimated 4500 are yet to be found and described (Brian et al., 1995).

Catfish is a species of ray-finned fish, whose large fleshy barbells are like cat whiskers (Vitule, 2015). Catfish gets its name from its type environment, Gariep river, the orange river's name among the Hottentots South Africa (Vitule, 2015) and the fact that the fish lives for a long time out of water. Catfish is eel-shaped, with body elongated, cylindrical and dorsal and anal fine being very long, both with soft fine rays. The head of the catfish is flattened, usually of dark gray or black colouration on the back fading to white belly. The average adult size of *C. gariepinus* is 1-1.5m (3ft 3in - 4ft 11in) long. Its maximum TL is 1.7m (5ft 7in) and its weight could be up to 60kg (Froeseand Pauly 2014). They also possess modified gills (gill supports) which are used for accessory breathing (Eco Travel Guild, 2011). It is a nocturnal fish, feeds on living as well as dead animal and plant matter (Gunder, 2004). The floodplain swamps and floodplain pools in which the catfish may survive the dry seasons because of location of the accessory air breathing organs are the most frequently used habitats (Bruton, 2004; Clay, 1979). Catfish is one of the most important freshwater fish species in Africa and the highest catfish produced in Nigeria is consumed. It is the most consumed fish in the local market place in Nigeria (Nigeria/FISH4ACP 2020). The nurturing of catfish in some Africa countries like the Central and Western Africa started in the early 1970s and as it was realized to be a very appropriate species for aquaculture. Fast growing and able to feed on a wide range of agriculture waste products, it is tolerant of poor water quality. Catfish can be

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can be raised in high densities, resulting in high good yields (6-16 t/ha/year);

Cooking, flavoring, browning or preserving food by exposing it to the smoke of burning or smoldering material, primarily wood, is a method of smoking. The most popular foods smoked are meats and fish (McGee, 2004). Smoke gives flavour, is antimicrobial and is antioxidant, but may not be enough to preserve food. Smoking is however, the most popular method of fish processing (Eyo, 2000) and it is probably the easiest of the several methods available for long term preservation of fish products to use, since no sophisticated equipment or highly skilled workers are required (Olayemi et al., 2011). In Nigeria, smoke – dried fish is the most acceptable form of fish product (Stolyhiro and Sikorski, 2005; Yanar, 2007). Smoking is the oldest and most commonly used method of fish preservation in many developing countries (Krasemann, 2004; Kumolu et al., 2010). “Trace elements are more important than the vitamins, in that they cannot be synthesized by living matter. Thus, they are the basic spark - plugs in the chemistry of life, on which the exchanges of energy in the combustion of foods and the building of living tissues depends (Schroeder, 1973).”

Toxic metals are a set of minerals that have no known role in the body but are toxic. They are a worldwide concern and cause of illness, ageing and even gene defects. It is important to remember that minerals that need to be consumed in smaller amounts tend to be poisonous in higher amounts. Examples are Cu, Fe, Mn, Se and Vanadium. So even Ca and Na are rather toxic when taken too much. Toxic metals are also long lived and bi-accumulating. Most organic substances are subject to natural degradation, as did the late Dr. Henry Schroeder, MD, who was a world expert on trace elements. (But), none of the metals is degradable, they are going to stay for a long time here," Lawrence Wilson quoted. (Wilson, 2020) The ability to replace a vital mineral means, however, that toxic metals are not completely harmful. In fact, they can prolong life. They ensure that bodies function when needed when there is a lack of vital minerals in the body (Wilson, 2020).

- *Trace Minerals and Metals*

The trace minerals and metals evaluated in this report are described briefly below with special attention to their biological significance, nutritional relevance and possible toxicological effects.

Manganese (Mn) is an essential trace element that is essential in humans for several physiological and biochemical functions. It acts as a co-factor for many enzymes related to macronutrient metabolism, bone formation, antioxidant defence and protection against oxidative stress in cells. Manganese is also a part of a number of proteins and enzymes necessary for regular metabolic processes (Erikson et al., 2019). The body of an average human contains about 12 mg of Mn, of which there is a significant reserve in the skeleton, with smaller amounts in the soft tissues, especially the liver and kidneys (Emsley, 2001). In the brain, Mn is related to metalloproteins such as manganese-dependent glutamine synthetase (Taked, 2003) in astrocytes.

Physiologically, Mn helps to promote antioxidant defence, enzymatic metabolism, bone development, and wound healing. The Recommended Daily Allowance (RDA) for adult women is 1.8 mg per day and for men is 2.3 mg per day; the Tolerable Upper Limit (UL) for adults is 11 mg per day (Institute of Medicine, 2001). Low Mn intake has been linked to poor bone health and changes in glucose metabolism, among other health problems, and can have a negative effect on bone development and metabolic functions (Shaffer, 2023). Mn toxicity, on the other hand, can have a negative impact on the nervous system if the concentration is too high, indicating the need for a physiological range.

Cobalt (Co) is a trace element that is physiologically important mainly as part of vitamin B_{12} (cobalamin). Vitamin B_{12} plays a key role in normal erythropoiesis, DNA synthesis, neurological function, and several metabolic processes. Thus, the biological function of cobalt in humans is primarily linked to its involvement in cobalt-containing biochemical pathways dependent on cobalamin (Shaffer, 2023). Exposure to inorganic cobalt compounds can, however, have adverse health effects, even though the body needs cobalt indirectly as part of vitamin B_{12}, this illustrates the need to keep exposure within safe limits.

Nickel Ni is a trace element known to be of biological significance in various microorganisms and plants. The role of Ni in biological systems of bacteria, archaea, fungi and higher plants has been demonstrated, although it is less well known in humans (Sigel et al., 2008; Sydor & Sigel, 2013; Zamble et al., 2017). Biotinically important nickel-containing enzymes include urease, which catalyzes the hydrolysis of urea to ammonia and carbamate, and may have a role in the pathogenicity of some microorganisms (Cavacci et al., 1999; Cox et al., 2000; Sydor & Sigel, 2013).

Nickel from diet can also have an indirect effect on human health by affecting microorganisms in the gut that require nickel for their metabolism. There are some indications that nickel can act as a micronutrient for certain gut bacteria (Zambelli & Ciurli, 2013). The estimated daily intake of Ni via diet is typically 70–100 µg/day, although only about 10% of that is absorbed after intake, with most of the absorbed Ni being excreted in the urine (Dietary Reference Intakes, 2017). However, high exposures, especially to soluble nickel compounds, can still have an impact on health.

Selenium (Se) is an essential trace element which, similar to iron, is incorporated into specific proteins, called selenoproteins, which have important biological roles. These proteins are involved in antioxidants, cell damage protection, DNA synthesis, immune function, reproduction and thyroid hormone metabolism (The Nutrition Source). The body's total selenium level is estimated to be from 13 to 30 mg, of which a large percentage is retained in skeletal muscle. Selena proteins are synthesized and active in plasma or serum with adequate levels of selenium (Healthwatch).

The recommended dietary allowance (RDA) for selenium in adult males and females is around 55 µg/day (Adeniyi & Agoreyo, 2018). The appropriate level of

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selenium is important for normal functioning of the body, but too much will cause selenosis. Symptoms of chronic selenium toxicity can include garlic-like breath odor, nausea, vomiting, alterations in hair and nail growth and/or integrity (Health Professional Factsheet). Although clinical signs of selenium deficiency are often not apparent, it may be a contributing factor to nutritional disease like Kashin–Beck disease and Keshan disease (Shaffer, 2023).

Cadmium (Cd) is a non-essential, possibly toxic trace element that has no known beneficial biological role in humans. It is known as an environmental contaminant that can have detrimental effects on living organisms (Hogan, 2010; Xu et al., 2020). Cadmium exposure is known to cause oxidative stress and trigger cellular antioxidant counter-defensive responses and thereby damage proteins, lipids, nucleic acids and other macromolecules (Kannan, 2010).

After exposure, cadmium is deposited in preference to other organs and its biological half-life is relatively long, allowing it to build up in the body. Concerns have been raised for chronic exposure of Cd as a risk factor for renal dysfunction, cardiovascular effects, bone demineralisation and carcinogenesis (Lueranon & Damodaran, 2014; Rahim et al., 2013; Tellex-Plaza et al., 2013; James & Meliker, 2013). Cd is a persistent metal and has also been found in biological tissues and environmental matrices and so is a significant metal for assessing potential toxicological risks from contaminated food and environmental media.

Lead (Pb) is a non-essential and very poisonous metal that has no known biological role in humans. The World Health Organization (2018) does not consider any level of lead exposure to be absolutely safe, especially for vulnerable groups like children. After ingested or inhaled, lead is easily absorbed and can then be transported to other parts of the body. A proportion is excreted via urine and faeces, but continued exposure may lead to the progressive accumulation, especially in bone tissues (Luckey & Venugopal, 1979; Toxic Substance Portal, 2011).

Lead can have a negative effect on nearly all major organ systems and is especially linked to negative neurological, haematologic, renal (kidney), cardiovascular, and developmental effects (United States Food and Drug Administration, 2017). It is highly toxic due to its interference with normal enzyme processes. Pb can bind with sulfhydryl groups that exist in enzymes, and can also act as a mimic or displace essential metals used as co-enzymes in biochemical reactions (Radolph et al., 2003; Dart et al., 2004). Pb affects important physiological metals such as calcium (Ca), iron (Fe) and zinc (Zn) (Kosnett, 2006). Sufficient levels of nutrients (Ca and Fe) may mitigate Pb toxicity, while deficiencies of these essential nutrients may increase the susceptibility to Pb toxicity (Luckey & Venugopal, 1979).

➢ *Toxic Mineral/Metal Ratios*

Assessing the biological implications of mineral accumulation through individual mineral concentrations may not give a complete picture. The interaction between various essential elements and potentially toxic elements can affect their absorption and distribution, metabolic utilization, and excretion. Therefore, mineral ratios can offer further information about the possible antagonistic/synergistic interactions among the elements. The mineral/metal ratios studied are: Ca/Pb, Fe/Pb, Zn/Cd, Fe/Co and K/Co.

It may not be possible to see or recognize immediately or be able to tell that someone was exposed to higher levels of a potentially toxic metal. However, chronic exposure can cause competitive interactions and metabolic antagonisms to affect the physiological utilisation of essential minerals. Therefore, assessment of toxic mineral concentrations in combination with essential mineral concentration and ratios has a more complete approach towards the possible nutritional and toxicological implications.

➢ *Scope of Mineral and Metal Assessment*

In the present study, the concentrations and distribution of selected toxic metals and nutritionally relevant minerals in various body tissues of *Clarias gariepinus* was studied. Some special interest was paid to the head, liver and muscle tissues as they may show markedly different mineral element storage and retention ability.

The assessment was oriented towards the levels of the selected toxic metals detected in the analysed tissues, the relationships between the toxic and essential minerals through the corresponding mineral/metal ratio and the mineral safety index, where applicable. The information generated shows the potential nutritional value as well as toxicological effects of the mineral accumulation in *C. gariepinus*. Further, the results could provide other valuable information that could be used to update food-composition data and to assess the suitability of the species as a source of food nutrients.

II. MATERIALS AND METHODS

➢ *Samples Collection and Preparation*

Five fish samples were obtained from Fem – Cornerstone Fish Farm in Ado – Ekiti, Nigeria. After cropping, the five life samples were sacrificed and dissected to give the three parts of head, liver and muscle, and washed with distil water. The samples were smoke – dried using local oven with charcoal method, deboned, homogenised, packed in plastic bottles and kept in the laboratory freezer pending analyses.

➢ *Mineral Analyses*

The metals were analysed from the solutions obtained by first dry ashing the samples at 550^0C. The filtered solutions were used to determine Mn, Se, Pb, Cd, Co and Ni by means of atomic absorption spectrophotometer (Buck Scientific Model – 200A/210, Norwalk, Connecticut 06855) (A.O.A.C., 2006). All chemicals used were of British Drug House (BDH, London, UK) analytical grade. The detection limits for the metals in aqueous solution had been previously determined using the methods of Varian Techtron (Techtron 1975) giving the optimal analytical range as 0.1 – 0.5 absorbance units with coefficient of variation from 0.9% to

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2.21%. From the mineral elements composition in the samples, further calculations were made.

➢ *Samples Designation*

Samples were designated as head [HS], liver [LS]and muscle [MS] where S=smoked.

➢ *Calculations Made*

Calculations made from the generated data were as follows.

- All mineral levels were reported in mg/100g (mg per one - hundred grams of sample). 1mg percent (mg%) is equal to ten parts per million (ppm).
- Total sum of the mineral values from each sample was calculated and reported as sample mineral burden [SMB].
- Percentage value of each mineral was calculated in each sample as appropriate:

Mineral/ [SMB]×100 ....……................................... (1)

- Differences of the minerals from sample to sample and the percentages were calculated as follows:

Mineral differences: HS-LS, LS-MS, HS-MS ....(2)

Percentage mineral differences: [HS-LS]/[SMB]×100; [LS - MS]/ [SMB]×100;[HS - MS]/ [SMB]×100 .... (3)

- SMB ratios from toxic mineral compositions and mineral differences values:

➢ *Mineral Composition Ratios:*

HS:LS; HS:MS; LS:MS; HS:(LS+MS) ....(4)

HS - MS: HS - LS; HS - MS: LS - MS;HS - LS: LS:MS; HS-MS: (HS – LS + LS - MS) …………………………………………(5)

f. Toxic mineral ratios between Ca/Pb, Fe/Pb, Zn/Cd, Fe/Co and K/Co...(6)

- Statistical methods used are as follows: standard deviation (s) for the sample were determined using the following formula:

$$S=\sqrt{\frac{\Sigma(x_1-\bar{x})}{n-1}} \quad \text{……………………………….. (7)}$$

Coefficient of variation percent

CV%=SD/Mean×100.. (8)

Where SD=standard deviation

Correlation coefficient

$r_{xy} = \Sigma z_x\, z_y /N$ ………………………………………….. (9)

Where

$$Zx=\frac{(X-\bar{x})}{\sigma_z}, \qquad Zy=\frac{(y-\bar{y})}{\sigma_y} \quad \text{……………… (10)}$$

N=number of pairs of X, Y scores and degree of freedom= n-2.

Variance (coefficient of determination)

r_{xy2}……...…………………………………………………..(11)

Regression coefficient

Y= a +bxy X

Where a is the point at which the line intersects the y-axis and bxy is the slope.

R_{xy}...(12)

Lack of relationship or coefficient of alienation

$C_A=\sqrt{(1-(rxy^2)}$ ……………………………………. (13)

Index of forecasting efficiency

IFE=1-C_A.. (14)

Equations 7-14 were from (Oloyo, 2001; Chase, 1976).

➢ *Mineral Safety Index*

The Mineral Safety Index (MSI) (Hatcock, 2010) of Se was calculated using the formula:

Calculated MSI=MSI/RAI ×Research data......................(15)

Where MSI=mineral safety Index Table (standard)

RAI=recommended adult intake per day

➢ *PubChem Database*

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Centre for Biotechnology Information (NCBI), a component of the National Library of Medicine,

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which is part of the United States National Institute of Health (NIH). Hence we talk of PubChem Compound ID (CID) (PubChem Database, 2005).

➢ *PubChem CID for Mineral Elements*

Mineral elements studied and reported in this project were: Cobalt/Co (PubChem CID:104730); Manganese/Mn (PubChem CID:23930); Selenium/Se.

(PubChem CID: 6326970); Lead/Pb (PubChem CID:5352425); Cadmium/Cd (PubChem CID:23973) and Nickel/Ni (PubChem CID:935).

III. RESULTS AND DISCUSSION

➢ *Trace Minerals and Metals*

The trace minerals assessed in the smoke-dried body parts of the *Clarias gariepinus* are the head smoke-dried (HS), liver smoke-dried (LS) and muscle smoke-dried (MS). Their concentrations, nutritional importance and potential toxicological effects are useful for understand the mineral composition and food safety of the fish samples. Generally the concentrations measured were low, but there was a clear variation between the three tissues when comparing the mineral levels in the liver with those in the head and muscle, with liver showing the higher mineral levels.

Of the three fish tissues, manganese was the most abundant trace mineral found. The levels were 1.27, 1.54 and 0.126 mg/100 g for head, liver and muscle, respectively. So, the contribution of mineral burden from each tissue in the total burden of the head, liver and muscle was 99.53%, 93.67% and 63.32% for Mn respectively.

Manganese is a key trace element that plays a role in a number of physiological processes such as macronutrient metabolism, bone formation, antioxidant defence and enzymatic activity. It acts as a cofactor of several enzymes, participates in normal skeletal development and is involved in the protection of antioxidants in the mitochondria. Recommended intake for adults is about 2.3mg/day for men and 1.8mg/day for women with a tolerable upper limit of 11mg/day (Institute of Medicine, 2001). Based on the reported sample concentrations, the concentrations of Mn in the individual fish tissues measured in the present study were lower than these reference concentrations. The higher level of Mn in the liver compared to the other tissues and parts of the head and muscles indicate that the liver may have a higher ability to retain the mineral.

The second most abundant trace mineral found in the samples was selenium (0.002–0.092 mg/100 g). The reported concentrations were 2.00 µg/100 g in the head, 92.0 µg/100 g in the liver, and 68.0 µg/100 g in the muscle. Se had relatively low sample-to-sample variability when compared to the other trace minerals.

Selenium is a part of selenoproteins, which play a role in antioxidant defence, protection of cells, DNA synthesis, immune function, reproduction and metabolism of thyroid hormones. The recommended dietary allowance for adults ranges about 55 µg/day (Adeniyi & Agoreyo, 2018). The liver and muscle parts would provide significant contributions to the Se intake from dietary sources, while the head would contribute significantly less Se. However, the concentrations reported were not reported to exceed the reported tolerable upper intake level. Additionally, selenium deficiency has been linked to nutritional diseases like Keshan disease and Kashin–Beck disease (Shaffer, 2023). Thus, Se in *C. gariepinus* may have nutritional significance especially if eaten as a balanced diet.

The concentrations of nickel were very low in all the tissues analysed. The reported levels were around 0.001 mg/100 g in the head, liver and muscle. The concentrations were about 1–2 µg/100 g, and much lower than the dietary tolerable upper intake level of 1 mg/day for soluble nickel salts in the source document.

Several plants and microorganisms are biologically relevant to nickel and nickel is part of the active centres of certain enzymes such as urease. Some microorganisms also may be pathogenic by releasing nickel-dependent enzymes. The nutritional role of Ni in humans is still less well defined than for other mineral elements like Mn and Se, but there is evidence that high exposure to Ni causes adverse effects. The very low concentrations found in the present *C. gariepinus* tissues therefore suggest low levels of Ni accumulation in the analysed tissues.

Cadmium is a non-essential toxic metal that does not have any known physiological role in higher organisms. It is known to have an adverse biological effect and is recognised as an environmental contaminant. The concentration of cadmium in the present fish samples was generally very low with the range being between 0.001 and 0.003 mg/100 g.

The toxicity of Cd is related to its ability to bioaccumulate and to disrupt cellular and organ function. After exposure, Cd can be deposited in the kidneys and can be a cause of oxidative stress and damage to the cells. Low levels found in the head, liver and muscle indicate that Cd levels have not accumulated significantly in the fish analysed. Also, Cd does not have a known beneficial biological function and can be accumulated after long exposure and therefore its presence becomes relevant in food-safety assessment even if the concentration is relatively low.

Lead is a toxic metal that does not have any known biological function in human beings and there is no safe level of exposure to lead (World Health Organization, 2018). The levels of lead in the analysed fish tissues were low (0.001-0.002 mg/100 g).

The toxicity of Pb is due to its ability to disrupt normal biochemical processes. Lead may bind to sulphydryl groups of enzymes and may also combine with or replace essential elements like calcium, iron and zinc that may be involved in enzymatic and physiological processes. Continuous exposure can also result in progressive accumulation in biological tissues. The concentration of Pb's detected in the present study were low, however, it's detection in edible fish tissue is

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still important since the risk associated with the presence of Pb's is dependent on frequency and duration of dietary exposure.

The levels of cobalt in the fish tissues were between 0.001 and 0.005 mg/100g. The biological significance of cobalt is that it is an essential part of vitamin B_{12} which plays a role in the normal formation of blood cells and function of the nervous system.

Co was detected in low concentrations in the analysed tissues, suggesting low accumulation. However, the relevance of the assessment of Co to mineral nutrition is important due to its close association with vitamin B_{12} metabolism; cobalt exposure in excess may be detrimental. The distribution of the trace elements in the various organs observed in the present study also helps in understanding the distribution of the same elements in various organs of fishes.

➢ *Trace Mineral Distribution and Sample Mineral Burden*

The overall mineral distribution showed a good tissue-specific variation. The calculated sample mineral burden (SMB) was ranked as: LS (1.644 mg/100 g) > HS (1.276 mg/100 g) > MS (0.199 mg/100 g).

The overall mineral burden of the liver was found to be highest, followed by the head and then the muscle. Mineral burden in the liver was also higher than combined mineral burden of head and muscle (1.475 mg/100 g), which shows the comparatively high accumulation capacity of the liver in the analysed *C. gariepinus*. The corresponding SMB ratios were 1.29:1.00 for LS:HS, 8.26:1.00 for LS:MS, 6.41:1.00 for HS:MS, and 1.11:1.00 for LS:(HS+MS).

This dominance of Mn on the overall burden of minerals was significant. The contribution to the head, liver and muscle was 99.53%, 93.67% and 63.32% respectively for Mn. Selenium accounted for over 1% of mineral load in liver and muscle and cobalt in the muscle only. All other trace minerals accounted for less than 1% for each of the respective SMBs.

The difference in tissue also suggests that the mineralization in the fish body was not uniform. All mineral comparisons were done in pairs and the comparisons between HS and LS were negative, suggesting that all minerals were consistently higher in the liver than in the head; LS and MS comparisons were positive for all minerals and for SMB. These observations are in agreement with the liver having the highest mineral accumulations of the three body parts examined.

There are statistical relationships between trace minerals and between trace minerals and total N and P.

Pairwise statistical analysis was used to examine the relationships among mineral concentrations in the various tissues. The correlation values between HS/LS, LS/MS and HS/MS were high and positive (0.8627–0.9984) and significantly greater than the critical value of 0.811 at the given level of significance. The results show high correlations in mineral distribution within the three tissue types.

Relationships were assumed to be HS < LS, LS > MS, and HS > MS that reflects the higher concentrations of minerals in liver as a whole. The relatively high coefficients of variation, however, imply that there is significant variation in the mineral concentrations among the samples in terms of absolute amounts. The coefficient of alienation and the index of forecasting efficiency also revealed the degree of predictability for the different pairs of tissues. For HS/LS and LS/MS, the index of forecasting efficiency was greater than the coefficient of alienation while the opposite was true for HS/MS.

The findings indicate that the mineral profile between head and liver, and between liver and muscle had more predictive relationships than the head and muscle. However, these statistical correlations are to be taken as levels of concentration of a mineral in one tissue and not as a physiological replacement of one tissue for another.

➢ *Toxic Mineral/Metal Ratios*

The potential interactions between toxic metals and nutrients such as nutritionally important minerals was further explored by the Ca/Pb, Fe/Pb, Zn/Cd, Fe/Co and K/Co ratios. These ratios are another way of looking at the relative abundance of an essential or protective mineral to a potentially toxic mineral. For this assessment, the minerals of concern were those of nutrition: Fe, Zn, Ca and K.

The concentrations of Pb, Cd, and Co were relatively low, ranging from 0.001–0.002, 0.001–0.003, and 0.001–0.005 mg/100 g, respectively. Compared to these, the concentrations of Fe, Zn, Ca and K were significantly higher, with reported levels ranging from 2.69-4.86, 1.31-7.91, 512-12,420 and 853-1,814 mg/100 g, respectively. This, therefore, resulted in high ratios calculated.

The high ratios of Ca/Pb, Fe/Pb, Fe/Co, Zn/Cd, and K/Co suggest that the nutritionally-beneficial minerals were at significantly higher levels than the potentially toxic ones. The Ca/Pb ratio is of particular importance since Ca and Pb could compete for absorption and physiological utilization. In the source material the cited reference value of 84:1 was taken as an acceptable ratio of calcium to lead, where higher ratios were taken as indicative of higher relative abundance of calcium over lead.

These ratios, however, should be viewed with skepticism, as they were observed. Existing data suggest that these mineral ratios are poorly documented with universally accepted clinical reference ranges. Thus, the ratios should be considered as complementary tools to assess the mineral composition of the fish, but not as clinical co-parameters of the protection against metal toxicity.

The ratios of toxic minerals for each tissue was significantly different from the other when compared pairwise. The largest percentage differences between HS–LS were found for Ca/Pb, K/Co and Fe/Co. The biggest

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differences were found for LS–MS, K/Co, Ca/Pb and Zn/Cd, while Ca/Pb, Fe/Co and Zn/Cd had the largest differences for HS–MS.

The correlation coefficient obtained from statistical analysis of the toxic mineral ratios ranged from 0.3719 to 0.8650. The reported critical value of 0.878 did not yield any statistically significant relationship between the pairs of tissues at the specified level of significance. The alienation coefficients and indices of forecasting efficiency were high for the toxic mineral ratios, and lower for the absolute mineral concentrations, suggesting that the mineral toxic ratios were less predictable between tissues than the mineral concentrations were.

This separation is significant because an overall good correlation was obtained for absolute mineral concentrations, but not for the mineral toxic ratios calculated. The result indicates that the accumulation pattern of the various mineral species in the different tissues may be widely connection but the balance of the nutritional minerals and potential toxic metals in these tissues may differ significantly from one to the other.

- *Mineral Safety Index of Selenium*

Based on the mineral safety index (MSI), an extra measure of the potential dietary safety of Se in the analysed fish tissues was obtained. The reported reference or threshold value of the MSI was 14.0. The calculated MSIs were 20.4 for the head, 18.4 for the liver and 13.6 for the muscle. Therefore, the head and liver values were above the stated value, while the muscle was slightly below.

The corresponding differences were −6.40 (−45.7%) for the head, −4.40 (−31.4%) for the liver, and +0.40 (+2.86%) for the muscle. The negative difference in head and liver tissues mean that their measured value exceeded the measured MSI in the source tissue, while the positive difference in muscle tissue means that it was below the measured reference value.

As a result, the muscle appears to have the best Se safety profile of the three tissues assessed. The head and liver need to be looked at more closely as both their calculated MSI values were above the specified reference value. MSI is not, however, a clinical diagnosis of toxicity, but rather a screening or comparative measure as part of the analytical framework of this study.

The general nutritional and food-safety aspects are shown.The general nutritional and food-safety implications are displayed.

As a whole, the results showed that the mineral composition of the three tissue types of *C. gariepinus* (head, liver and muscle) varied significantly. Overall, the mineral burden in the liver was the highest while the mineral burden in the muscle was the lowest. All tissues contained a higher concentration of Mn than of any other trace mineral, and Se was the next abundant element. Ni, Cd, Pb and Co concentrations were relatively low.

Ca, Fe, Zn, and K were abundant relative to the other toxic minerals/metals of the study (Pb, Cd, and Co) which led to high toxic mineral/metal ratios. These results suggest a good relative mineral composition with respect to the mineral content of essentials relative to the potentially toxic minerals studied. However, the ratios must be regarded as complementary checks since some of these ratios have yet to have a definitive clinical reference range.

As far as consumption is concerned, the results show that the analyzed fish tissues generally had low concentrations of the potentially toxic metals analysed. The muscle was the most significant edible portion of the fish and together with the other mineral burden had also the lowest overall mineral burden, with a slightly lower than stated value of Se MSI. The liver, which is the most mineralized tissue, might therefore be more important to consider when evaluating tissue-specific exposure to minerals. The final food-safety interpretation should, however, be based not only on mineral concentration but also on the frequency and size of the consumption, as well as on the bioavailability of the mineral and/or on the regulatory limit.

IV. CONCLUSION

Smoke-dried tissues of the head, liver and muscle of African sharptooth catfish (*Clarias gariepinus* Burchell, 1822) were analysed for their trace-mineral composition, toxic mineral/metal ratios and selenium mineral safety index. The overall concentrations of the trace elements measured were low, however, the concentration of Mn was clearly the dominant trace mineral, contributing 99.53%, 93.67%, and 63.32% of the mineral burden in the head, liver and muscle, respectively.

The calculated mineral burden for the samples was in the order liver > head > muscle, and was 1.644, 1.276, and 0.199 mg/100 g, respectively. The liver was also significantly more mineralized than the head and muscle, once again confirming the ability of the liver to store minerals to a higher level than these other tissues. The high positive correlations between tissue mineral concentrations suggest that the general pattern of mineral distribution is quite similar, but some different relationships were observed for toxic mineral/metal ratios.

The Ca/Pb, Fe/Pb, Zn/Cd, Fe/Co, and K/Co ratios were generally high, since the essential minerals were found to be at much higher concentrations than the metals Pb, Cd and Co. Although the ratios should not be used as absolute clinical indicators as reference ranges are only available for a few of the ratios, these are useful supplementary data of the relative abundance of nutritionally important and potentially toxic elements.

Selenium values were higher than the provided reference value for the head, liver, and slightly lower for the muscle. In the framework of this study, this means that the muscle exhibited the most favourable safety profile with regards to Se.

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In general, the concentrations of potentially toxic metals analysed in the *C. gariepinus* samples were low and the concentrations of nutritionally important minerals were comparatively high. The results hence indicate that the fish may be a promising source of nutrients (essential minerals) and low levels of toxic metals measured. In future evaluations of the food-safety implications of the consumption of the different fish tissues, however, the use of a tissue-specific consumption pattern and cumulative dietary exposure to the fish should be considered.

➢ *Conflict of Interest*

There is no conflict of interest declared.

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Table 1 Trace Mineral Levels (mg/100g) of the Smoke-Dried Samples [Head (HS), Liver (LS) and Muscle (MS)] of *Clarias gariepinus* Fish

Serial no	PubChem CID	Mineral	HS	LS	MS	MEAN	SD	CV%
1	23930	Mn	1.27	1.54	0.126	0.979	0.750	76.7
2	6326970	Se	0.002	0.092	0.068	0.087	0.017	20.0
3	5352425	Pb	0.001	0.002	0.001	0.001	0.001	43.3
4	23973	Cd	0.001	0.003	0.001	0.002	0.001	69.2
5	1047330	Co	0.001	0.005	0.002	0.003	0.002	78.1
6	935	Ni	0.001	0.002	0.001	0.001	0.001	43.3
7	-	SMB	1.276	1.644	0.199	1.04	0.751	72.2
Ratios: - LS: HS [1.29/1.00]; LS: MS [8.26/1.00]; HS:MS [6.41/1.00]; LS: HS+MS [1.11/1.00]								

CID = Compound identifier; SMB = Sample mineral burden; SD = Standard deviation; CV% = co-efficient of variation percent

Table 2 The Percentage Levels of Each Trace Element in Each Sample as a Fraction of SMB in the *Clarias gariepinus*

Serial no	Mineral	HS	LS	MS	Mean	SD	CV%
1.	Mn	99.53	93.67	63.32	85.51	19.44	22.73
2.	Se	0.157	5.60	34.17	13.31	18.27	137
3.	Pb	0.078	0.122	0.503	0.234	0.234	100
4.	Cd	0.078	0.182	0.503	0.254	0.222	87.40

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5.	Co	0.078	0.304	1.01	0.464	0.486	105
6.	Ni	0.078	0.122	0.503	0.234	0.234	100
7.	SMB	100	100	100	100	0.00	-

SMB = Sample Mineral Burden

Table 3 Individual Mineral Differences between the Samples Minerals: HS-LS, LS-MS and HS-MS in the *Clarias gariepinus* Fish

Serial no	Mineral	HS -LS	LS-MS	HS-MS	Mean	SD	CV%
1.	Mn	-0.27	+1.414	+1.144	0.943	0.598	63.41
2.	Se	-0.09	+0.024	-0.066	0.060	0.033	55.00
3.	Pb	-0.001	+0.001	0.00	0.001	0.00	-
4.	Cd	-0.002	+0.002	0.00	0.002	0.00	-
5.	Co	-0.004	+0.003	-0.001	0.003	0.002	66.67
6.	Ni	-0.001	+0.001	0.00	0.001	0.00	-
7.	SMB	-0.368	+1.445	+1.211	1.008	0.566	56.15
Ratio: - LS-MS: HS-LS [3.93/1.00]; LS-MS: HS: MS [1.19/1.00]; HS-MS: HS-LS [3.29/1.00]; LS-MS: HS-LS + HS-MS [0.915/1.00]							

Table 4 Percentage Individual Mineral Differences between the Samples as a Function of the SMB in the African Catfish

Serial no. (%Diff.)	Mineral (%Diff.)	HS –LS (%Diff.)	LS-MS	HS-MS	Mean	SD	CV%
1.	Mn	-73.37	+97.85	+94.47	88.56	32.27	36.44
2.	Se	-24.46	+1.66	-5.45	10.52	12.22	116
3.	Pb	-0.272	+0.069	0.00	0.171	0.144	84.21
4.	Cd	-0.543	+0.138	0.00	0.341	0.286	83.87
5.	Co	-1.09	+0.208	-0.083	0.460	0.549	119
6.	Ni	-0.272	+0.069	0.00	0.171	0.144	84.21
7.	SMB	-100	+100	+100	100	0.00	-

Table 5 Statistical Analysis of the Data Depicted in the Table 1 between the Pairs: HS/LS, LS/MS, HS/MS

Statistics	HS/LS		LS/MS		HS/MS	
	HS	LS	LS	MS	HS	MS
r_{xy}	0.9984*		0.8899*		0.8627*	
r_{xy}^2	0.9968		0.7920		0.7443	
R_{xy}	1.197		0.0756		0.0873	
Mean	0.2127	0.274	0.274	0.0332	0.2127	0.0332
SD	0.5180	0.6212	0.6212	0.0527	0.5180	0.0527
CV%	244	227	227	159	244	159
C_A	0.0566		0.4561		0.5057	
IFE	0.9434		0.5439		0.4943	

r_{xy}= Correlation coefficient; r_{xy2} = Variance; R_{xy} = Regression coefficient; SD = Standard deviation; CV% = Coefficient of variation percent; C_A = Coefficient of alienation; IFE = Index of forecasting efficiency; *Meant that r_{xy} was significant at r_{xy} = 0.05 at n – 2 = 6 – 2 = 4 (df) at critical value of 0.811

Table 6 Nutritional Minerals Required to Calculate Toxic Mineral Ratios: the Minerals Were Ca, Fe, Zn and K

S/N PubChem	CID	Mineral	HS	LS	MS	Mean	SD	CV%
1.	23925	Fe	3.25	4.86	2.69	3.60	1.13	31.3
2.	23994	Zn	2.60	7.91	1.31	3.94	3.50	88.7
3.	5460341	Ca	12420	593	512	4508	6852	152
4.	5462222	K	853	1118	1814	1271	511	40.2

Table 7 Toxic Minerals Ratios of *Clarias gariepinus* Body Parts

Serial no	Mineral ratio	Reference balance ideal	Acceptable ideal range	HS	LS	MS
1.	Ca/Pb	126	84-168	12420169	296457	512456
2.	Fe/Pb	6.6	4.4-8.8	3255	2433	2691
3.	Zn/Cd	750	500-1000	2604	2637	1312
4.	Fe/Co	500^{++}	440^{+}	3250	972	1345
5.	K/Co	1000^{++}	2000^{+}	853000	223600	907000

$^{++}$Current; $^{+}$Expected

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Table 8 Differences (and Percentage Differences) between Compared Samples of the African Catfish Toxic Ratio Values

Serial no	Mineral ratio	HS-LS (%Diff.)	LS-MS (%Diff.)	HS-MS (%Diff.)
1	Ca/Pb	12123712 (97.6)	-215999 (-72.9)	11907713 (95.9)
2	Fe/Pb	822 (25.3)	-258 (-10.6)	564 (17.3)
3	Zn/Cd	-33 (-1.27)	1325 (50.2)	1292 (49.6)
4	Fe/Co	2278 (70.1)	-373 (-38.4)	1905 (58.6)
5	K/Co	629400 (73.8)	-683400 (-306)	-54000 (-6.33)

Table 9 Statistical Analysis of the Toxic Mineral Ratio Data Depicted in Table 7 between the Pairs: HS/LS, LS/MS, HS/MS

Statistics	HS/LS		LS/MS		HS/MS	
	HS	LS	LS	MS	HS	MS
r_{xy}	0.7875		0.8650		0.3719	
r_{xy2}	0.6201		0.7482		0.1383	
Rxy	0.0207		2.481		0.0280	
Mean	2656456	105220	10522	284961	2656456	284961
SD	5470477	143650	143650	412085	5470477	412085
CV%	206	137	137	145	206	145
C_A	0.6164		0.5018		0.9283	
IFE	0.3836		0.4982		0.0717	

No r_{xy} value was significant at r_{xy}=0.05 at n-2 (df) and critical level of 0.878; [n-2=5-2=3 (df)]

Table 10 The Mean, Standard Deviation and Coefficient of Variation Percent of the Toxic Mineral Ratios and Those of Their Differences are Depicted in this Table 10

S/N	Mineral ratio	Mean		Standard deviation		Coefficient of variation (%)	
		For min. ratio	For ratio diff (%)	For min. ratio	For ratio diff (%)	For min. ratio diff.	For ratio (%)
1	Ca/Pb	4409694	8082475	6938116	6813424	157	84.3
2	Fe/Pb	2793	548	421	282	15.1	51.5
3	Zn/Cd	2184	883	756	737	34.6	83.5
4	Fe/Co	1856	1519	1222	1010	65.8	66.5
5	K/Co	661200	455600	379933	348842	57.5	76.6

Min. = Mineral; diff. = Difference; % = Percent; Ser. No. = Serial number

Table 11 Mineral Safety Index (MSI) of Se of African Catfish Head, Liver and Muscle (all Smoke – Dried Samples)

Ser. No.	Mineral	RAI (mg)	TV of MSI	HS			LS			MS		
				CV	Diff	(%)	CV	Diff	(%)	CV	Diff	(%)
1.	Se	0.07	14.0	20.4	-6.40	-45.7	18.4	-4.40	-31.4	13.6	+0.40	+2.86

CV = calculated MSI; TV = Table MSI; Diff = Difference; RAI = Recommended adult intake; %= percent