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Assessment of Heavy Metals in the Atlantic Horse Mackerel (Trachurus Trachurus) from Cold Storage in Benin Metropolis, Nigeria

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Abstract
Heavy metals can contaminate fish and water which in turn present a risk to humans. The dearth of eco-toxicological data on the heavy metal content in Trachurus trachurus particularly from cold storage in Benin Metropolis, Nigeria, warranted this research, which was achieved via Atomic Absorption Spectrometric technique. The mean concentrations (mg/kg, wet weight) of heavy metals in T. trachurus by cold storage point ranged from 0.242 for Cd at Uselu to 52.98 for Zn also at Uselu with significant differences (p<0.05) in the mean concentrations of heavy metals in T. trachurus between cold storage points. The total toxicity of mixtures (TTM) value for heavy metals was 0.845 while the estimated annual intake (EAI) and estimated daily intake (EDI) of heavy metals peaked for Zn with values of 1.345 mg/person/year and 0.00368 mg/person/day respectively. The target hazard quotient (THQ) values ranged from 0.0000276 (Cd) to 0.0000135 (Zn) with a total target hazard quotient (TTHQ) value of 0.0000267 while the quota for heavy metals in T. trachurus ranged from 0.36% for Cd to 87.80% for Zn. The total heavy metal burden in T. trachurus by cold storage point ranged from 46.95 mg/kg at Oliha to 54.37 mg/kg at Uselu. T. trachurus from cold storage were safe for human consumption with regard to heavy metal levels as International thresholds for heavy metals in fish were not surpassed. It was proffered that continuous monitoring of the heavy metal status of this fish species is maintained in order to protect the health of potential consumers.

Keywords: Eco-toxicological, Heavy metals, Target Hazard Quotient, *Trachurus trachurus*

INTRODUCTION
Pollution of natural aquatic media with heavy metals which are released from geologic (natural) and anthropogenic (non-natural) sources, especially the latter, has remained a community health issue around the world (Roy *et al.,* 2022). Over the years there have been elevations in industrial, agronomical and agrarian practices including an upsurge in metropolitan sewage which continue to spew heavy metals into aquatic ecosystems (Noman *et al.,* 2022). The presence of heavy metals in the aquatic environment especially beyond natural levels is injurious to the health of natural aquatic resources, the most obvious of which are damages to the breathing apparatus of fish, lethality and eventual mortality. Heavy metals are known to accumulate in various environmental compartments and can devastatingly contaminate fish, water, soil and sediment which in turn present a risk to other bio-resources

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even at micro-levels (Kaviani, 2022). Furthermore, the unabated introduction of heavy metals into natural aquatic media disturbs developmental and reproductive processes of aquatic organisms including zooplankton, phytoplankton, shellfish and finfish especially against the background that these elements are non-biodegradable (Kumar *et al.,* 2022). The good health of populations around the world is very much dependent on the quality and wholesomeness of their diet hence the need to determine the levels of contaminants such as heavy metals in food (Yap *et al.,* 2021). In addition, hydrobionts such as fish have been used as indicators of environmental health while the determination of heavy metals in such species gives an inroad for predicting contamination in end-of-the-line consumers including man (Dione *et al.,* 2022).The Atlantic Horse Mackerel (*Trachurus trachurus*, Linnaeus 1758), is a marine, pelagic-neritic, oceanodromous and benthopelagic species that often shoals with juvenile Herrings and other species of *Trachurus* (e.g. *T. mediterraneus* and *T. picturatus*). This fish species feeds on crustaceans, small fishes and cephalopods and the countries with the largest catches are Netherlands and Ireland (Food and Agriculture Organization of the United Nations, 2011). The geographic distribution of *T. trachurus* includes the North-eastern Atlantic from Iceland to Senegal, Cape Verde Islands, Mediterranean and Marmara Seas. The fish species is utilized frozen, fresh, smoked, canned and dried salted. *T. trachurus* is an imported fish species in Nigeria that is commonly retailed in open markets with bulk purchases from cold storage facilities within Benin Metropolis, Edo state, Nigeria. There is paucity of eco-toxicological data with regard to the heavy metal content in this fish species specifically sourced from cold storage facilities in Benin Metropolis, Nigeria, which warranted this research. Wangboje *et al.,* (2017), however provided the heavy metal levels in *T. trachurus* sourced from open markets (Non-cold storage) in a rural community within Edo state, Nigeria. Cold storage facilities are essentially cold rooms where sub-zero temperatures are maintained for storing fish (Wangboje and Idemudia, 2022).The heavy metals of interest were Cadmium (Cd), Lead (Pb), Copper (Cu) and Zinc (Zn). According to Wang *et al.,* (2022), heavy metals such as Cd and Pb are non-essential elements that are extremely toxic with very long half-lives and both have deleterious effects on the health of humans and other mammals. On the flipside, Cu and Zn are essential elements needed for a myriad of chemical and metabolic processes in the human body. Copper and Zinc are often tested in the flesh of cultured fish while the pollution of water with heavy metals has been observed to be a threat to aquaculture (Al-Faiz *et al.,* 2022). Data generated from the study have been referenced to International thresholds for heavy metals in fish and is expected to serve as a guide for potential consumers.

MATERIALS AND METHODS

Description of study area

This research was carried out in Benin City (Latitude 6°20′ 00′′N and Longitude of 5 37′ 20′′ E) in Edo state, Nigeria (Fig. 1). The population of the City is estimated to be about 1, 782,000.The amount of rainfall in the City ranges between 1750 mm and 2000 mm annually with an average temperature of 34^{0}C. Further details of the study area have been published by Wangboje and Braimah (2022). The research focused on four purposely selected major districts of the City with cold storage facilities that sell the fish species of interest viz: Ekiosa, New Benin, Oliha and Uselu. The specific GPS information of the collection points are; Ekiosa (Latitude 6.1649° N and Longitude 5.6879° E), New Benin (Latitude 6.3448°N and Longitude 5.6340 °E), Oliha (Latitude 6.2298°N and Longitude 5.5409°E) and Uselu (Lattitude 6.2298°N and Longitude 5.6340°E).

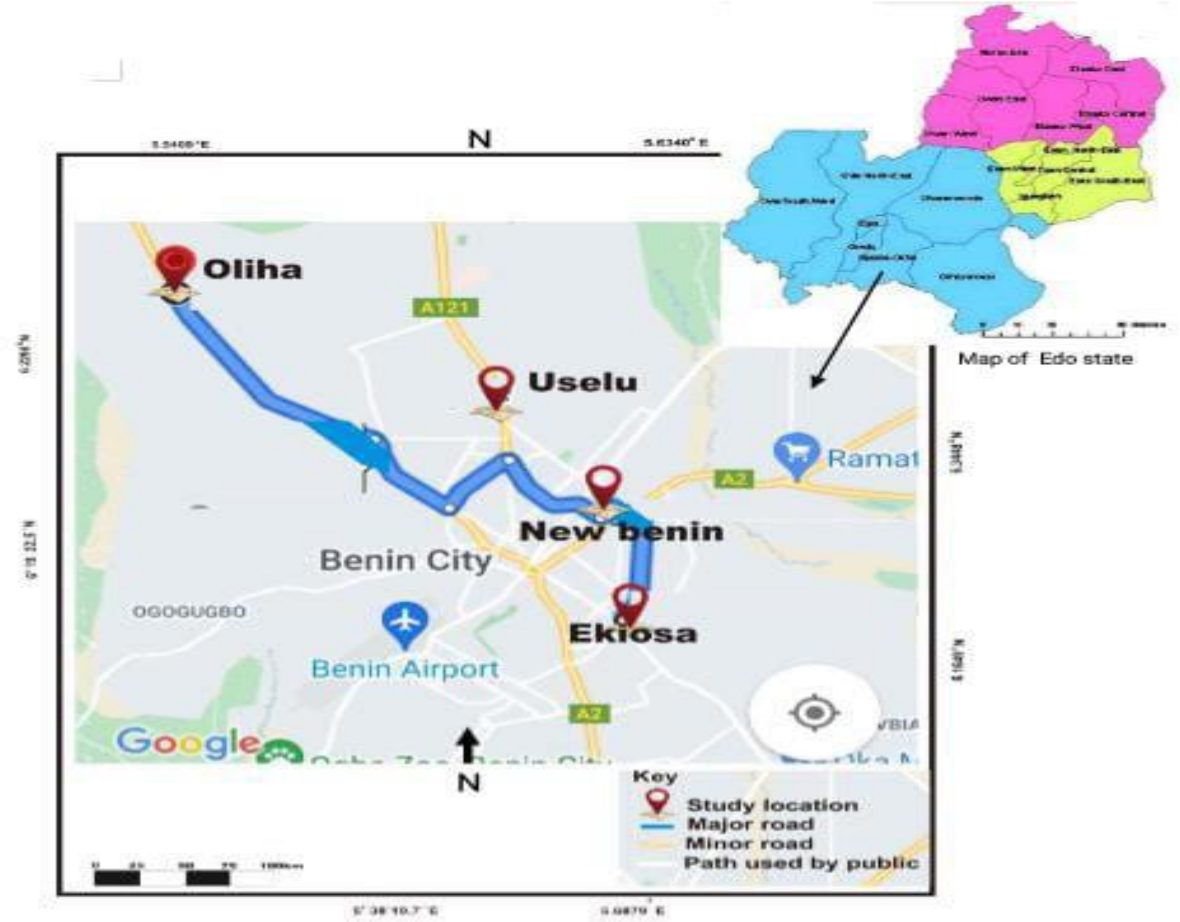

Fig. 1: Map of study area (Source: Google map, 2021)

Collection of fish samples

Fish samples of apparent similar size (n=64) were purchased from the aforesaid cold storage points between September and December 2021. September and October represented the wet months while November and December represented the dry months. Both wet and dry months were considered to determine the existence of temporal variations of heavy metal concentrations in samples of fish. Samples were placed in labeled zip-lock® bags and conveyed to the laboratory in a Thermocool® ice chest within 24 hours for further studies.

Laboratory protocol and procedures

In the laboratory, fish samples were allowed to thaw out before being washed with distilled water to remove adhering debris. The identifies of fish samples were confirmed by applying key data sourced from the Food and Agricultural Organization of the United Nations (FAO) species identification sheets and the fish base website (www.fishbase.org). They were weighed whole (Mean weight: 50.14± 1.32 g) using a Scout Pro SPU402® electronic top loading scale while their total lengths (Mean length: 31.25±1.26 cm) were measured using a translucent ruler. Muscle tissues were excised with a stainless steel dissecting lancet from the flanks of fish specimens after descaling and oven dried at a temperature of 80^{o}C until constant weight was attained in a Surgifield-Uniscope® (SM 9023 model) laboratory oven. Each dried sample was milled separately using a porcelain mortar and pestle and kept in sealed vials prior to digestion. Four (4) g of the milled fish sample was homogenized with 50 mL of deionized water and placed into a 250 ml conical flask. Ten (10) mL of concentrated HCl and 1 mL of concentrated HNO_3 were added in succession (Chindah *et al.,* 2004). The mixture was heated in a steam bath to a thick yellow liquid before allowed to cool. The mixture was filtered into a 100 mL volumetric flask using No.42 Watman® filter paper and made up to mark with deionized water, which was prepared using a Labtech® ultra-pure water deionizer. The digest was stored in a 100 mL plastic reagent bottle ready for Atomic absorption spectrophotometer (AAS) analysis. Fish digests were analyzed for Cd, Cu, Zn and Pb by means of an Atomic Absorption Spectrophotometer (Unicam® 969 series) equipped with solar software using air acetylene flame. Concentrations of metals in fish were expressed in mg/kg (wet weight). Blanks, spiked samples and duplicate analyses were performed for all analytes as part of the quality assurance procedures. All reagents used were of analytical grade (SIGMA, U.S.A.). Ethically speaking, all fish samples used in this research were lifeless and frozen at the point of purchase. All adopted procedures conformed to standard scientific research guidelines including the ARRIVE® guidelines for experimental animals.

Total toxicity of mixtures (TTM) index for heavy metals

By applying the TTM index, one can determine if a mixture of heavy metals in a particular medium exceeds the quality guideline value for that medium (ANZECC/ARMCANZ, 2000).

TTM = $\Sigma\, {CI}/{GV1}$ ……………………………..Equation (1)

where**:** C1 = Concentration of the 'ith' component of mixture; GV1= Guideline value for the 'ith' component; TTM >1= The mixture has exceeded the Guideline value.

Estimated annual intake (EAI) and Estimated daily intake (EDI) of heavy metals

EAI (Mg/person/year) = $\text{Concentration of heavy metal in fish} * \text{Per capita figure} / \text{Adult body weight (Assumed to be 70kg)}$ ……………………………..Equation (2)

where: Per capita figure is 13.3 kg/person/year for Nigeria (Word Fish Center, 2021)

EDI (Mg/person/day) = ${EAI}/{365\ Days}$ ……………………………..Equation (3)

Target hazard quotient (THQ) for non-carcinogenic risk

The target harzard quotient (THQ) for non-carcinogenic risk was calculated using the following equation (USEPA, 2016).

THQ= ${Ed * Ef * EDI * Ct}/{At * Rfd}$ X 10^{-3} ……………………………..Equation (4)

where Ed is the exposure duration of 30 years, Ef is exposure frequency of 350 days/ year, Ct is a conversion factor of 0.208 to convert fresh wet weight to dry weight, "At" is the average time for the non-carcinogenic element i.e. 365 days/ year for 65 years or 23,725 days, Rfd is the reference dose in mg $kg^{-1}day^{-1}$ for heavy metals (Pb=0.01, Cd=0.005, Zn=0.25 and Cu=0.25) (USEPA 2011).

Total target hazard quotient (TTHQ) for non-carcinogenic risk

This is essentially the total of the individual heavy metal THQ values taking into cognizance that a synergistic effect of heavy metals may occur in nature (Paul *et al.,* 2021).

TTHQ = $THQ^1 + THQ^2 + THQ^3 + \ldots\ldots\ldots\ THQ^{ith}$ ………………………….Equation (5)

Statistical procedure

Statistical software (GENSTAT® version 13.3 for Windows) was used for analyzing generated data. One-way analysis of variance (ANOVA) was used to test for significant differences ($p < 0.05$) between mean values of heavy metals while the New Duncan Multiple Range Test was used to separate significant means. Microsoft Excel (for Windows 2010), was used for all graphical presentations.

RESULTS AND DISCUSSION

As presented in Table 1, the summary statistics for heavy metals in *T. trachurus* ranged from 0.0289 mg/kg for Cd to 7.077 mg/kg for Zn while the mean concentrations (mg/kg) of heavy metals in *T. trachurus* by cold storage point ranged from 0.242 for Cd at Uselu to 52.98 for Zn also at Uselu with observed significant differences ($p<0.05$) in the mean concentrations of heavy metals in *T. trachurus* between cold storage points (Table 2). The mean concentrations (mg/kg) of heavy metals in *T. trachurus* monthly-wise ranged from0.242 for Cd in May 2021 to 49.72 for Zn in November 2021 with no observed significant differences ($p>0.05$) in the mean concentrations of Zn and Pb in *T. trachurus* between months (Table 3).

Table 1: Summary statistics for heavy metals (mg/kg) in *Trachurus trachurus*

Heavy metal	Mean	Minimum	Maximum	Threshold
Cd	0.0289	0.01	0.06	0.05*
Cu	0.793	0.52	0.98	30*
Zn	7.077	5.23	10.22	30-75*
Pb	0.1604	0.06	0.24	0.30**

*FAO (1983) **CODEX Alimentarius (2015)

Table 2: Mean concentrations of heavy metal (mg/kg) in *Trachurus trachurus* by cold room point

Cold room point	Cd	Cu	Zn	Pb
Ekiosa	0.367 ± 0.0985^{b}	0.642 ± 0.138^{bc}	49.87 ± 2.358^{b}	0.258 ± 0.0996^{a}
New Benin	0.250 ± 0.0798^{a}	0.508 ±0.124^{a}	46.94 ±1.549^{a}	0.267 ± 0.0651^{a}
Oliha	0.342 ± 0.0900^{b}	0.592 ± 0.0996^{ab}	45.75 ± 1.511^{a}	0.267 ± 0.0651^{a}
Uselu	0.242 ±0.0515^{a}	0.725 ± 0.154^{c}	52.98 ± 1.226^{c}	0.425 ± 0.0622^{b}

Mean values with similar letters on the same column are not significantly different ($p>0.05$)

Table 3: Mean concentrations of heavy metal (mg/kg) in *Trachurus trachurus* by months

Month	Cd	Cu	Zn	Pb
September 2021	0.308 ±0.0669^{ab}	0.625 ± 0.1220^{a}	48.94 ± 3.475^{a}	0.317 ± 0.0937^{a}
October, 2021	0.308 ±0.0900^{ab}	0.742 ± 0.1620^{b}	48.15 ± 2.951^{a}	0.342 ± 0.0996^{a}
November 2021	0.242 ± 0.0793^{a}	0.567 ± 0.1440^{a}	49.72 ± 3.768^{a}	0.258 ± 0.0900^{a}
December 2021	0.342 ± 0.1240^{b}	0.533 ± 0.0778^{a}	48.72 ± 3.088^{a}	0.300 ± 0.1130^{a}

Mean values with similar letters on the same column are not significantly different ($p>0.05$)

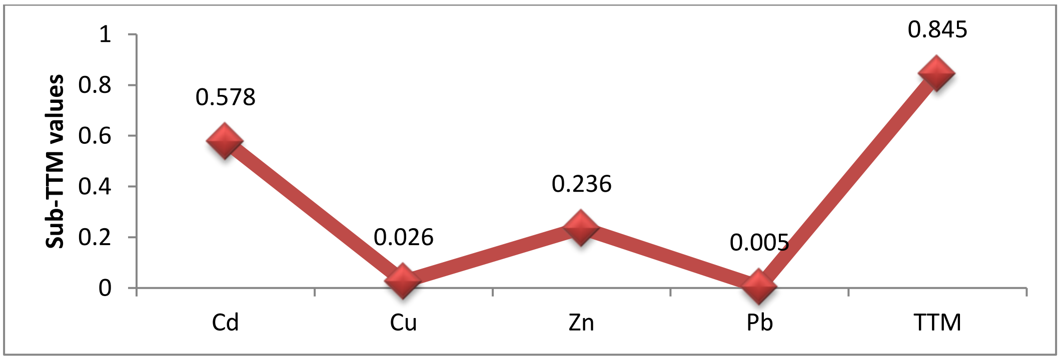

Fig. 2: Total toxicity of mixtures (TTM) value for heavy metals in *Trachurus trachurus*

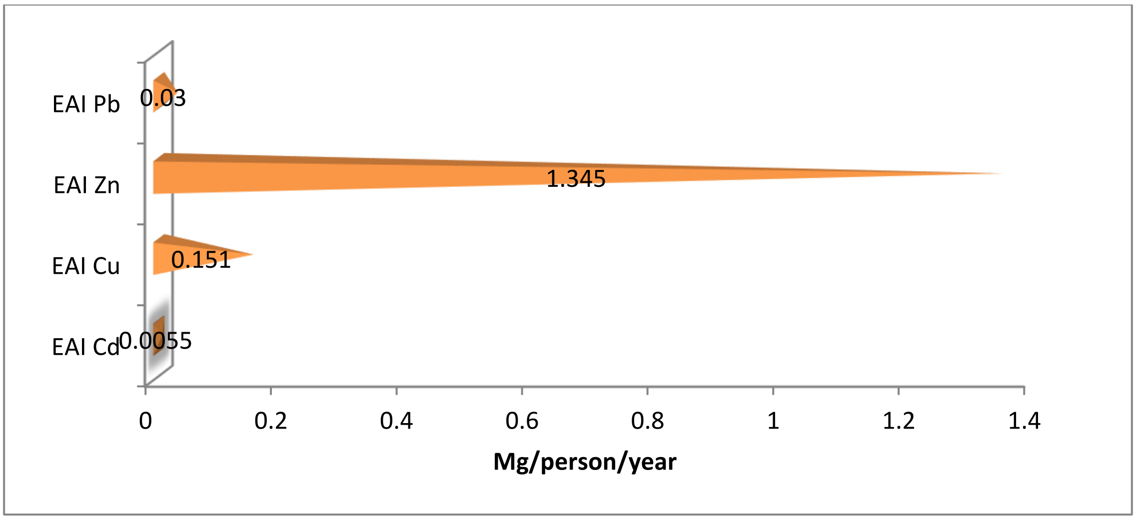

Fig.3: Estimated annual intake (EAI) values for heavy metals in *Trachurus trachurus*

The observed TTM value for heavy metals in *T. trachurus* was 0.845 as shown in Fig.2 while the EAI and EDI values were at a peak for Zn with values of 1.345 mg/person/year (Fig. 3) and 0.00368 mg/person/day (Fig. 4) respectively. The THQ values ranged from 0.0000276 (Cd) to 0.0000135 (Zn) with a TTHQ value of 0.0000267 (Fig.5) while the quota for heavy metals in *T. trachurus* ranged from 0.36% for Cd to 87.80% for Zn (Fig. 6). As presented in Fig. 7, the total heavy metal burden in *T. trachurus* by cold storage point ranged from 46.95 mg/kg at Oliha to 54.37 mg/kg at Uselu while the temporal total (mg/kg) for heavy metals in *T. trachurus* ranged from 49.54 in the month of October to 50.78 in the month of November as shown in Fig. 8. The quota of heavy metals in *T. trachurus* by specific cold storage point ranged from 0.44% (Cd) at Uselu to 97.85 % (Zn) at New Benin (Fig.9).

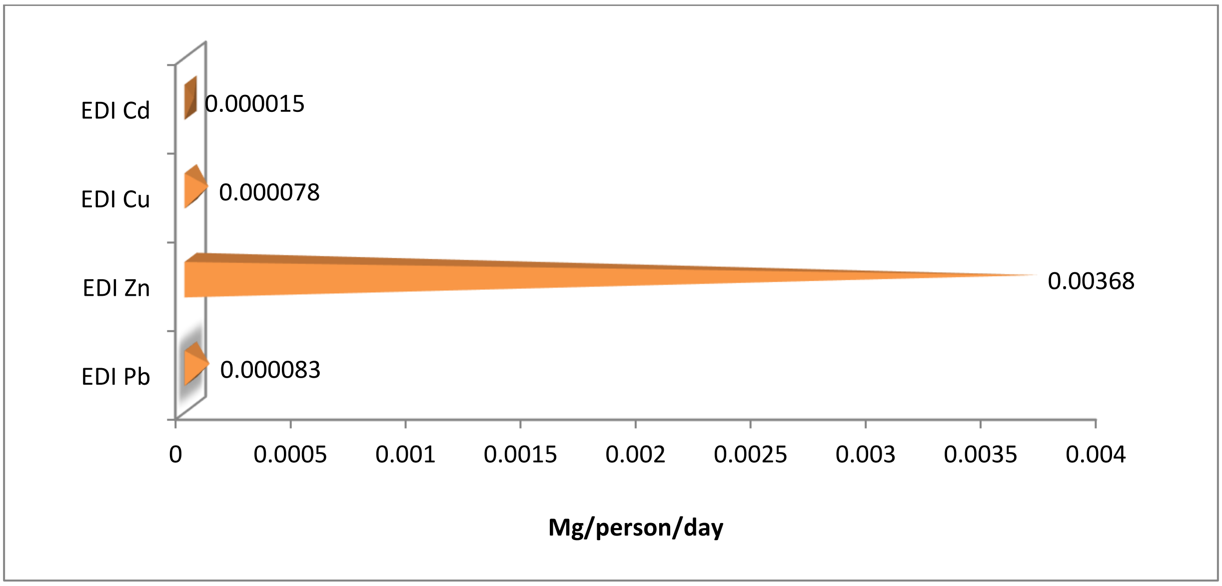

Fig.4: Estimated daily intake (EDI) values for heavy metals in *Trachurus trachurus*

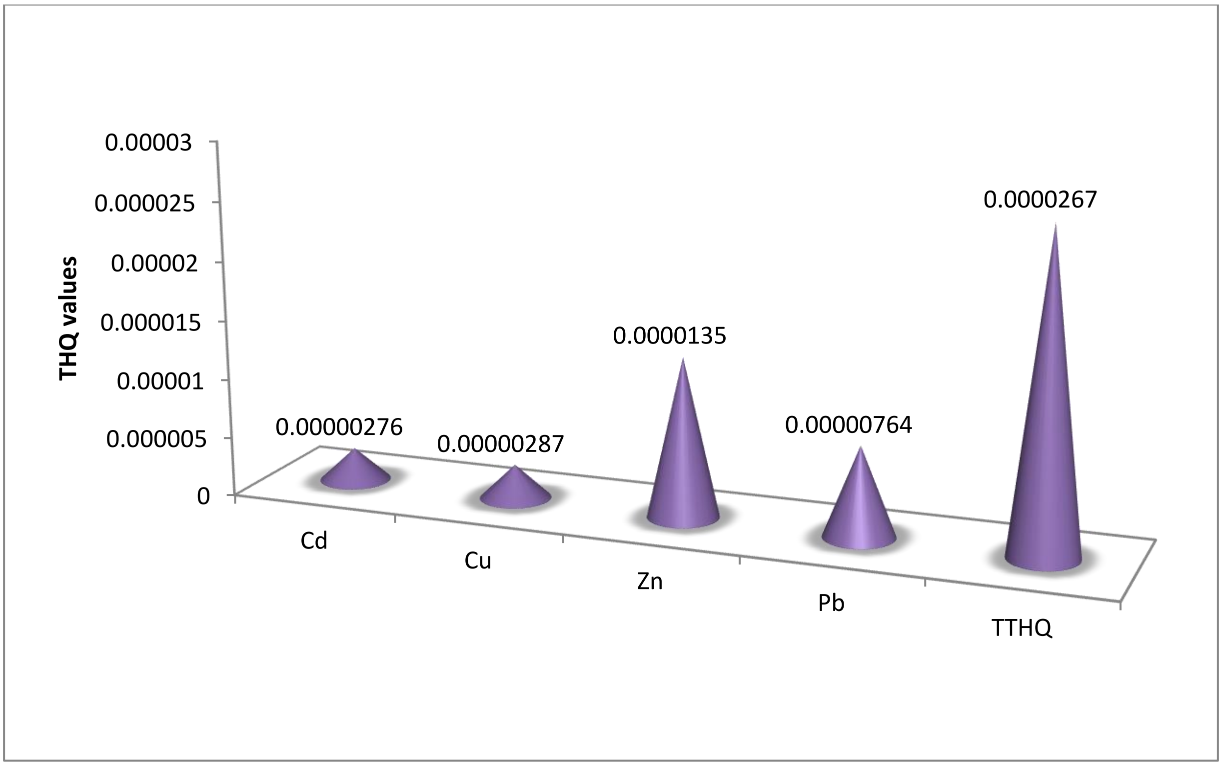

Fig.5: Target hazard quotient (THQ) and Total target hazard quotient (TTHQ) values for heavy metals in *Trachurus trachurus*

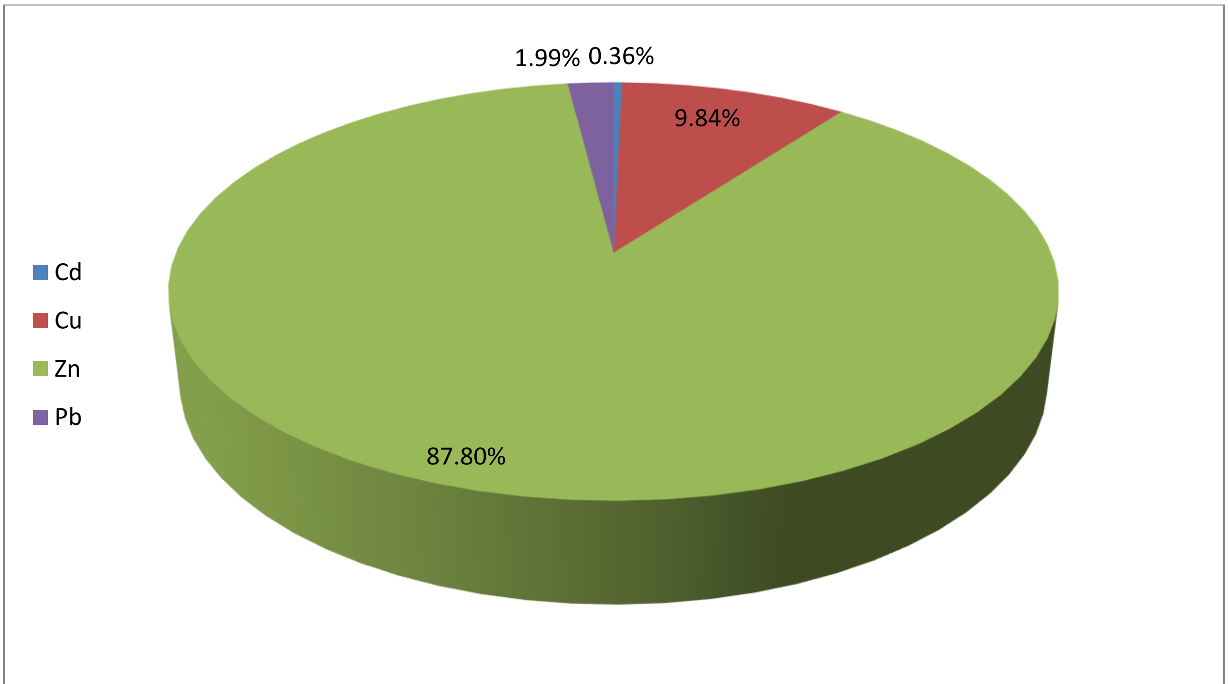

Fig.6: Quota of heavy metals in *Trachurus trachurus*

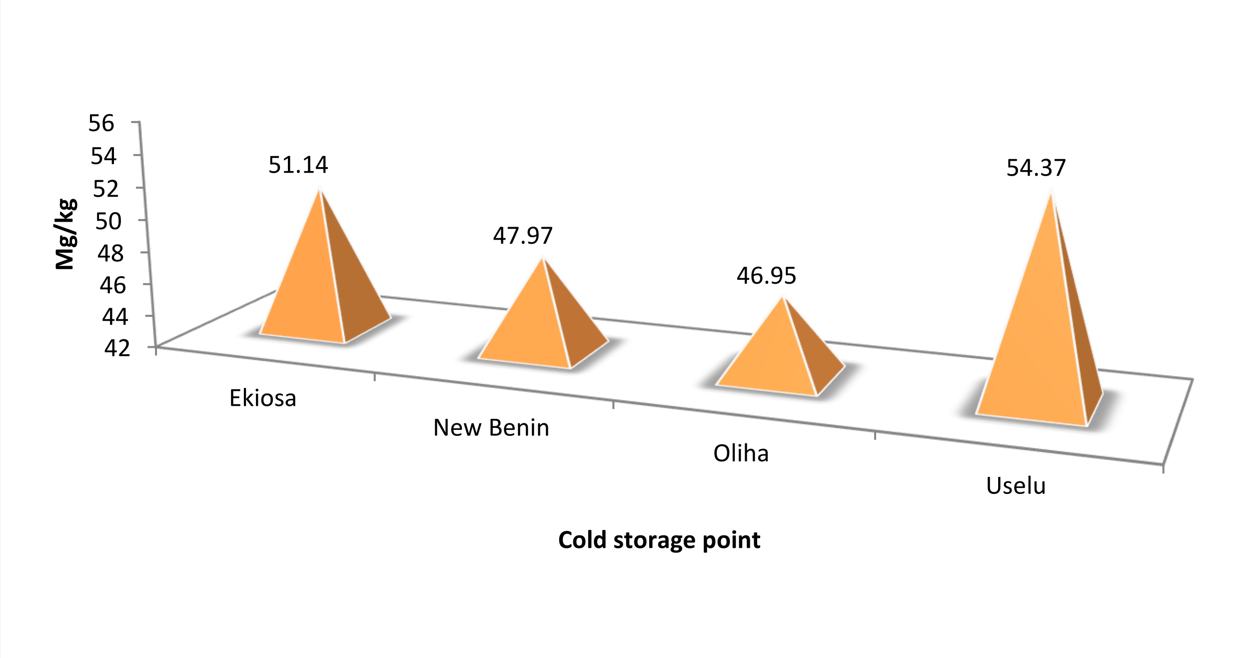

Fig. 7: Total heavy metal burden in *Trachurus trachurus* by cold storage point

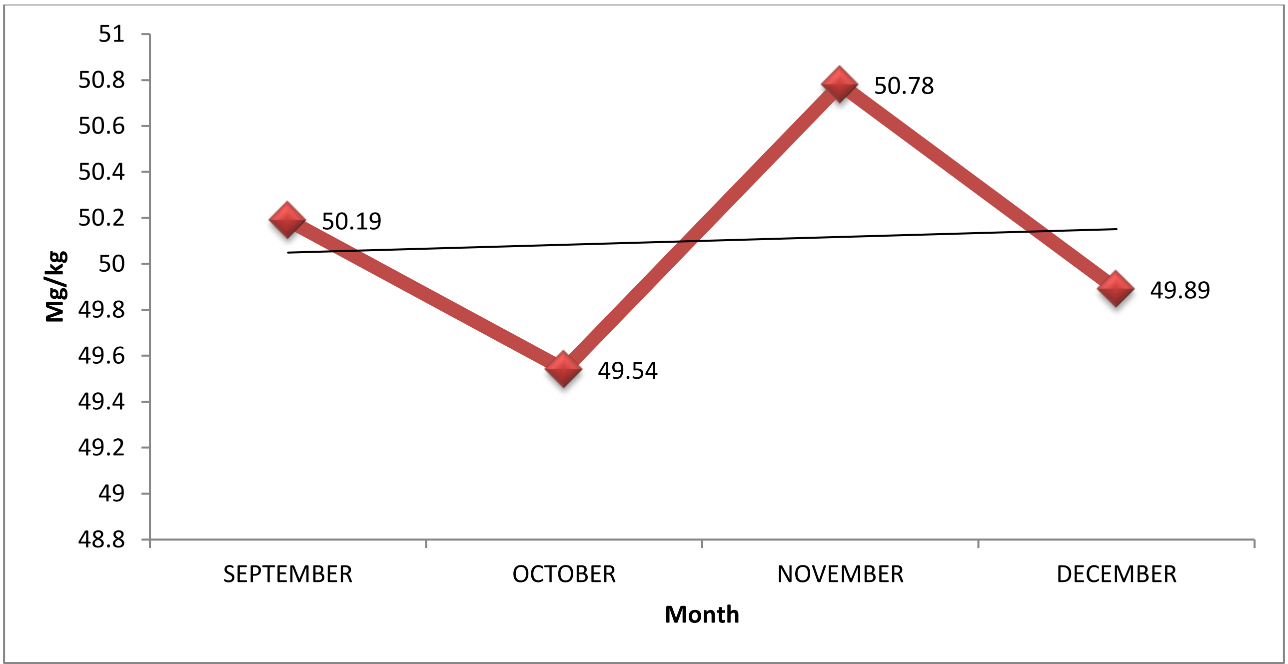

Fig. 8: Temporal total for heavy metals in *Trachurus trachurus* from cold storage with trendline

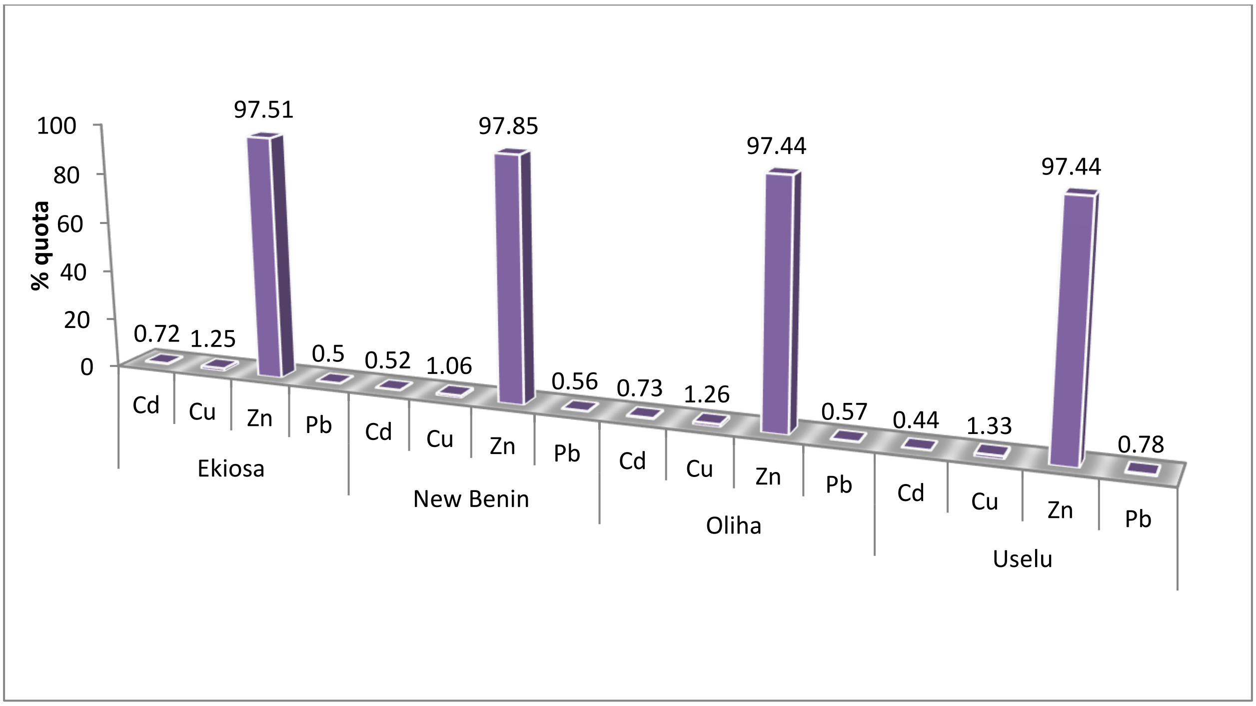

Fig. 9: Quota of heavy metals in *Trachurus trachurus* by specific cold storage point

The heavy metal rank profile in *T. trachurus* from cold storage took the order Zn>Cu>Pb>Cd. This observation gives an indication that Zn was perhaps bioaccumulated the most by this fish species from its parent water body while the opposite could be justified for Cd. It is pertinent to note that metals exist in different forms or species and that each form exhibits a different bioaccumulation potential and indeed bioavailability, all of which could influence the eventual heavy metal load in fish. Fish species are known to readily accumulate heavy metals from their ambient aquatic media and the severity of such accumulation increases with the influx of industrial and municipal effluents (Banerjee *et al.,* 2022). The accumulation of heavy metals in fish has also been tied to the water layer they live with dermersal fish exhibiting a higher accumulation profile compared to those who thrive in the middle and upper layers of water (Huang, *et al.,* 2022). Significant differences ($p<0.05$) were observed in the mean concentrations of all the heavy metals in *T. trachurus* between the cold storage points indicating that the fish species may have been sourced from different suppliers or importers. No significant differences ($p>0.05$) were observed in the mean concentrations of Zn and Pb in *T. trachurus* between months suggesting a negligible variation in the concentrations of these metals in fish between months for the study period. The trendline within the temporal total (TT) for heavy metals further corroborated this observation, although the highest TT was observed to be in the month of November. The observed TTM value of 0.845 was clearly below unity suggesting that the guideline values for heavy metals in fish were not exceeded. The guideline values adopted in this research were sourced from FAO and CODEX Alimentarius. The latter contains threshold information on heavy metals jointly produced by the World Health Organization (WHO) and FAO. The EAI and EDI values according to Wangboje and Idemudia (2022), always follow the order of the rank profile of heavy metals in fish and would mean in this case that potential consumers would be receiving a higher dose of Zn and a lower dose of Cd in their fish diet. The observed THQ values in this study were all below unity including the TTHQ indicating that the fish species is safe for human consumption and does not present an immediate risk to potential consumers especially in terms of oncological risk. The suitability of *T. trachurus* for human consumption was further validated by the observation that the individual thresholds for heavy metals in fish were not exceeded especially for Cu and Zn which were undoubtedly below their respective limits. The mean concentrations of Cd and Pb were close to their respective limits but still attained a safe profile. The dominant presence of Zn in *T. trachurus* was highlighted by the quota value of well over 85% on a general note while a quota value of well over 95% was observed for the same metal in *T. trachurus* according to specific cold room points. Zinc is an essential element needed for a host of chemical reactions in the body and is a known immune system booster but could lead to elemental toxicity when consumed in excess. For example, Zn levels much above the recommended daily allowance (RDA) of 15 mg/Zn/day causes nausea, epigastric pain, lethargy, fatigue, anemia, neutropenia, impaired immune function and adverse effects on the ratio of low-density-lipoprotein to high-density-lipoprotein (LDL/HDL) cholesterol (Fosmire, 1990). Observations surrounding the heavy metal burden in *T. trachurus* according to cold storage points revealed that overall, Uselu point and Oliha point had the highest and lowest heavy metal burden respectively. This observation could serve as an Advisory to potential consumers in the sense that it would be better to buy *T. trachurus* from the Oliha point as a result of the obvious lower heavy metal burden compared to the other cold storage points. In another research carried out by Wangboje *et al.,* (2017) on the heavy metal content in *T. trachurus* from open markets in a rural community (Okada), in Edo state, Nigeria, far higher mean concentrations of Zn (41.65 mg/kg) and Cu (15.82 mg/kg) were observed while Cd was below detection limit. However, the mean concentration of Pb (0.01 mg/kg) in their research was lower than what was obtainable in this study. It is pertinent to note that the above scenario is a direct comparison between fish obtained from the open market and fish sourced from cold storage. Yap *et al.,* (2021) determined the levels of heavy metals in frozen fish imported by fishery companies in Republic of Benin, West Africa and observed much lower mean concentrations of Zn (0.25 mg/kg), Pb (0.01 mg/kg), Cu (0.03 mg/kg) and Cd (0.006 mg/kg) in *T. trachurus* when compared to this study. Ozuni *et al.,* (2012), investigated the heavy metal (Hg, Cd, Cr and Pb) content in *T. trachurus* from Tirana local markets in Albania and observed that Pb and Cr were below detection limits in samples of fish while the mean concentrations of Mercury (Hg) and Cd were 0.07 mg/kg and 0.05 mg/kg respectively. Since the aforesaid mean concentrations fell below acceptable limits, the workers concluded that *T. trachurus* was safe for human consumption within the Tirana community.

CONCLUSION

It has been established that *T. trachurus* particularly sourced from cold storage facilities in Benin metropolis, Nigeria, are safe for human consumption with regard to heavy metal levels. However in order to ensure that heavy metals do not at any time exceed thresholds and safety levels which could be detrimental to consumers over time, continuous monitoring of the heavy metal content in *T. trachurus* is advised. This research is considered to be one of such monitoring efforts, although future monitoring could entail a longer period of sampling in order to establish particular patterns and trends as they may arise.

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