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Evaluation of Heavy Metal Contents and Potential Human Health Risk Assessment of Selected Canned Sardines Fish Sold in Yenagoa, Nigeria

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Abstract

Canned sardine fish is consumed regularly in all countries. The levels of heavy metals which are present in the environment could constitute a hazard to food security and public health. These can be accumulated in aquatic animals such as fish. In this study, selected heavy metals: Copper (Cu), Nickel (Ni), Chromium (Cr), Zinc (Zn), Cadmium (Cd), Manganese (Mn), Lead (Pb) and Iron (Fe) were evaluated in Sardines that are commonly consumed in Nigeria. Eight different brands of canned sardines were purchased in Yenagoa and were taken to the Laboratory for heavy metal analysis. Standard wet digestion procedure was adopted for sample preparation while Atomic Absorption Spectrophotometer (AAS) technique was adopted for metal analysis. The results show that heavy metal concentrations of Fe in the samples were above World Health Organization (WHO) and United States Environmental Protection Agency (USEPA) permissible limits, while Zn was below the permissible limits. However, Pb, Cu, Cd, Cr, Ni and Mn were below detectable limits in all the samples. Daily intake of metal (DIM) values in the sardine samples for Zn and Fe were significantly lower than the recommended daily intake and the upper tolerable daily intake levels. The Health risk index (HRI), Target hazard quotient (THQ) and Hazard Index (HI) for Zn and Fe in this study were less than 1 (<1) for both adult and children, therefore, the canned sardines does not pose a potential human health risk concern for the consumers. However, the high Fe concentrations in all the sardine samples may cause public health concern as they are above WHO and USEPA tolerable limits.

1. Introduction

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals are essential to maintain the metabolism of the human body. At higher concentrations these heavy metals can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination, high ambient air concentrations near emission sources, or intake via the food chain. A group of heavy metals are classified as non-essential, because they have no biological, chemical and physiological importance in man. After acute and chronic exposures, they causes a variety of adverse health effects to humans such as dermal changes, respiratory, pulmonary, cardiovascular, gastrointestinal, haematological, hepatic, renal, neurological, developmental, reproductive, immunologic, genotoxic, mutagenic, and carcinogenic effects (Mandal & Suzuki, 2002). Some heavy metals, such as cadmium and lead, injure the kidney and cause symptoms of chronic toxicity, including impaired organ function, poor reproductive capacity, hypertension, tumors, and hepatic dysfunction (Abou-Arab et al., 1996). Sub-lethal effects of heavy metals are of concern as they accumulate and are transferred through the food-chain to humans (Yilmaz & Yilmaz, 2007).

The human health risk assessment is the process requires identification, collection, and integration of information on the toxins and chemicals health hazards, exposure of human to the chemical and relationships between exposure, dose and adverse health effects in polluted environmental. On the other hand, a human potential health risk assessment includes hazard identification, dose-response assessment, exposure assessment and risk characterization steps (Sobhanardakani, 2017).

In recent years, concern about food quality has increased, particularly in those foods at risk of containing toxic elements and compounds that represent a risk to human health, such as persistent organic pollutants (POPs) and heavy metals. There is increasing concern about the quality of canned foods in several parts of the world. The determination of toxic elements in food has prompted studies on their toxicological effects. Heavy metals pose a common threat to all organisms in the ecosystem by forming an ever increasing accumulation through food chain. Humans may be exposed to harmful nonessential elements such as arsenic, silver, lead, mercury, cadmium, and nickel mainly through drinking water, consumption of fresh and processed foods and through occupational exposures (Ikem & Egiebor, 2005).

Fish consumption has increased simultaneously with the growing concern of their nutritional and therapeutic benefits. In addition to its important source of protein, fish typically have

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rich contents of essential minerals, vitamins and unsaturated fatty acids (Medeiros et al., 2012). The American Heart Association recommended eating fish at least twice per week in order to reach the daily intake of omega-3 fatty acids (Kris-Etherton et al., 2002). However, fish are relatively situated at the top of the aquatic food chain; therefore, they normally can accumulate heavy metals from food, water and sediments (Yilmaz et al., 2007; Zhao et al., 2012). The content of toxic heavy metals in fish can counteract their beneficial effects; several adverse effects of heavy metals to human health have been known for long time (Castro-Gonzalez & Mendez-Armenta, 2008). This may include serious threats like renal failure, liver damage, cardiovascular diseases and even death (Al-Busadi et al., 2011; Rahman et al., 2012). Therefore, many international monitoring programs have been established in order to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem (Meche et al., 2010).

In the last few decades, the concentrations of heavy metals in fish have been extensively studied in different parts of the world (Elnabris et al., 2013). Most of these studies concentrated mainly on the heavy metals in the edible part (fish muscles). However, other studies reported the distribution of metals in different organs like the liver, kidneys, heart, gonads, bone, digestive tract and brain. This study was aimed at evaluating heavy metal contents and potential human health risk assessments of selected canned sardines fish sold in Yenagoa, Nigeria.

2. Material and methods

2.1 Sample Collection

Eight (8) samples of canned sardines' fish were purchased at Yenagoa, Bayelsa State, Nigeria and were taken to the laboratory for toxic metal analysis. The samples were designated as; SR, TS, OA, DR, LJ, NA, CA and VA.

2.2 Samples Preparation and Procedure

Wet digestion method was used in the preparation of the Sardine samples for heavy metal analysis. 5 ml of analytical unit (sample) was weighed into digestive tube and 20 ml of digestion acid at ratio 1: 3: 1: 1: (HNO₃ + H₂SO₄ + HCl + HClO₄) was added. This was latter digested using FOSS TECATOR Digester Model 210 at 250°C for 1 hour at the first instance and continued until a clear solution was obtained in a fume cupboard. The clear solution was filtered into a 100 ml volumetric flask and completed to the mark with de-ionised water.

2.3 Determination of toxic metals

All digested samples were analyzed in triplicate using Atomic Absorption Spectrophotometer (Buck 210). Standard for each element under investigation was prepared in mg/l/100g and the limit standard concentration for each element was adhered to according to the BUCK Scientific instruction and the results obtained were compared with World Health Organization standards for the metal limits for human consumption.

2.4 Quality assurance protocol and statistical analysis

Appropriate quality assurance procedure and precautions were carried out to ensure reliability of the results. Samples were carefully handled to avoid contamination. Glassware and sample containers were soaked in 1 mol/L HNO₃ for 48 h and rinsed with ultrapure water and the reagents were of analytical grade. Precision and accuracy of the analytical procedure was also

investigated by carrying out recovery experiments. Accuracy of the digestion procedures was verified by examination of the recovery data, spiking analyzed samples with aliquots of metal standards and then reanalyzing the samples. The percentage recovery was greater than 95% with the percent relative standard deviations less than eleven, indicating good accuracy and precision. The results were expressed as mean ± standard (SD) using SPSS Statistic 17.0.

2.5 Health risk assessment

The potential health risks of heavy metal consumption through sardines were assessed based on the daily intake of metal (DIM) (Chary et al., 2008), health risk index (HRI) (Jan et al., 2010), and the target hazard quotient (THQ) (Wang et al., 2005; Storelli, 2008). The daily intake of metals (DIM) was calculated to averagely estimate the daily metal loading into the body system of a specified body weight of a consumer. This will inform the relative availability of metal. This does not take into cognizance the possible metabolic ejection of the metals but can easily tell the possible ingestion rate of a particular metal. The estimated daily intake of metal in this study was calculated based on the formula below:

$$DIM = \frac{C_{metal} \times C_{factor} \times C_{food\ intake}}{Body\ average\ weight} \quad (1)$$

Where,

C metal is the heavy metal conc. in sardine (mg/kg),

C factor is the conversion factor,

C food intake is the daily intake of sardine.

The conversion factor of 0.085 was used in this study, daily sardine intake of 65 g/day for adult and 28.35 g/day for children, while the average body weight used was 65 kg for adult and 24 kg for children in this study (Oguntona, 1998).

The health risk index (HRI) was calculated using the formula below:

$$HRI = \frac{DIM}{RFD} \quad (2)$$

The THQ was calculated using the formula below:

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times WAB \times TA} \times 10^{-3} \quad (3)$$

Where:

EF is the exposure frequency (350 days/year)

ED is the exposure duration (60 years, equivalent to the average lifetime of the Nigerian population for adult and 20 years for children were adopted)

FIR is the food ingestion rate (sardine consumption values is 65 g/person/day) (Oguntona, 1998);

C is the metal concentration in the sardine (mg/kg);

RFD is the oral reference dose (Zn and Fe values were 0.300 and 0.700 mg/kg/day, respectively) (USEPA IRIS, 2006).

WAB is the average body weight (65 kg for adults and 24 kg for children was adopted in this study) (Oguntona, 1998) and

TA is the average exposure time for non-carcinogens (ED × 365 days/year). If the THQ value is greater than 1, the exposure is likely to cause obvious adverse effects.

Calculation of hazard index: Hazard index is used to evaluate the potential risk to human health when more than one toxic metal is involved. Hazard index was calculated as the sum of target hazard quotients (THQs) (Abou-Arab, 2001). Since different

pollutants can cause similar adverse health effects, it is often appropriate to combine THQs associated with different substances (Al-Jassir et al., 2005) as seen in equation 4.

$$HI = \Sigma THQ (THQ1 + THQ2 + THQ3 \dots THQn) \quad (4)$$

3. Results

3.1 Heavy Metal contents

Heavy metal contents in the sardine samples are shown in Table 1. The highest mean value of Zn was TS (0.14±0.001 mg/kg) and

lowest was VA (0.03±0.001 mg/kg). Highest Fe mean value was 2.67±0.001 mg/kg (TS) and lowest was NA (1.34 ±0.001 mg/kg). Meanwhile, Cu, Ni, Pb, Cd, Mn and Cr were below detectable limits.

3.2 Potential health risk assessment

The DIM values of Zn and Fe from the sardine samples were below the permissible tolerable daily intake limits as shown Tables 2 and 3 for both adult and children. Health risk index, Target hazard quotient (THQ) and hazard index (HI) values were less than 1 (Tables 2 and 3).

Table 1 Heavy Metal Contents of Various Sardines Sold in Yenagoa

Sample Code	Heavy metals (mg/kg)							
	Cu	Zn	Ni	Pb	Cd	Fe	Mn	Cr
SR	BDL	0.11±0.001	BDL	BDL	BDL	1.45±0.001	BDL	BDL
TS	BDL	0.14±0.001	BDL	BDL	BDL	2.67±0.001	BDL	BDL
OA	BDL	0.05±0.001	BDL	BDL	BDL	1.93±0.001	BDL	BDL
DR	BDL	0.09±0.0009	BDL	BDL	BDL	2.30±0.001	BDL	BDL
LJ	BDL	0.06±0.001	BDL	BDL	BDL	1.90±0.001	BDL	BDL
NA	BDL	0.05±0.001	BDL	BDL	BDL	1.34±0.001	BDL	BDL
CA	BDL	0.07±0.001	BDL	BDL	BDL	1.70±0.001	BDL	BDL
VA	BDL	0.03±0.001	BDL	BDL	BDL	1.62±0.001	BDL	BDL
USEPA	1.30	5.00	0.02	0.015	0.005	0.3	0.05	0.05
WHO	2.0	5.00	0.02	0.01	0.003	0.3	0.10	0.05

BDL<0.001; BDL: Below Detectable Limit, WHO: World Health Organization, USEPA: United States Environmental Protection Agency, Values are Mean±SD triplicate determination

Table 2 Results of Daily intake of metal, Health risk index, Target hazard quotient and Hazard index of heavy metals in sardine for adults (65kg)

adults (65kg)							
Sample code	DIM		HRI		THQ		HI
	Heavy Metal		Heavy metal		Heavy metal		
	Zn	Fe	Zn	Fe	Zn	Fe	
SR	0.0041	0.054	0.014	0.08	1.53E-4	8.66E-4	1.02E-3
TS	0.0052	0.099	0.017	0.14	1.95E-4	1.59E-3	1.79E-3
OA	0.0019	0.072	0.006	0.10	6.97E-5	1.15E-3	1.22E-3
DR	0.0033	0.085	0.011	0.12	1.25E-4	1.37E-3	1.49E-3
LJ	0.0022	0.070	0.007	0.10	8.36E-5	1.14E-3	1.22E-3
NA	0.0019	0.049	0.006	0.07	6.97E-5	8.01E-4	8.71E-4
CA	0.0026	0.063	0.009	0.09	9.75E-5	1.02E-3	1.11E-3
VA	0.0011	0.060	0.004	0.09	4.18E-5	9.68E-4	1.01E-3

DIM; Daily intake of metal; HRI; Health risk index; THQ; Target hazard quotient; HI; Hazard Index

Table 3. Results of Daily intake of metal, Health risk index, Target hazard quotient and Hazard index of heavy metals in sardine for Children (24kg)

Sample code	DIM		HRI		THQ		HI
	Heavy Metal		Heavy metal		Heavy metal		
	Zn	Fe	Zn	Fe	Zn	Fe	
SR	0.011	0.145	0.037	0.21	4.15E-4	2.34E-3	2.75E-3
TS	0.0141	0.268	0.047	0.38	5.28E-4	4.32E-3	4.85E-3
OA	0.0050	0.194	0.016	0.28	1.88E-4	3.12E-3	3.31E-3
DR	0.0090	0.231	0.030	0.33	3.39E-4	3.72E-3	4.06E-3
LJ	0.0060	0.191	0.020	0.27	2.26E-4	3.07E-3	3.29E-3
NA	0.0050	0.135	0.016	0.19	1.88E-4	2.16E-3	2.35E-3
CA	0.0070	0.170	0.023	0.24	2.64E-4	2.75E-3	3.01E-3
VA	0.0030	0.163	0.010	0.23	1.13E-4	2.62E-3	2.73E-3

DIM; Daily intake of metal; HRI; Health risk index; THQ; Target hazard quotient; HI; Hazard Index

4. Discussion

4.1 Heavy metal contents

Some factors, such as water chemistry, duration of exposure of fish to contaminants in water, concentrations of contaminants in water column, feeding habit of fish, contamination of fish during handling and processing, quality of canned fish and shelf life of canned fish can affect in the level of contaminants in fish.

However, the metal levels in canned fishes is influenced by the pH of the canned product, oxygen concentration in the headspace, the quality of the lacquer coatings of canned products, quality of coating and also storage place (Tahán et al., 1995; Hosseini et al., 2013). The metals accumulation varies greatly between both fish species and/or fish tissues. Generally, fish could translocate the large quantities of toxic heavy metals in the liver, gill, and also muscle tissues (Sobhanardakani et al., 2012). Contaminants in fish can pose a health risk to the fish

themselves, to their predators, and to humans who consume them (**Burger & Gochfeld, 2005**).

Iron is an essential mineral and is the most abundant transition element, and probably the most well-known metal in biologic systems especially plays an important role in the human physiology. Iron deficiency causes anemia, reducing cognitive function and also physical work capacity. Whereas, high intake of this element may be the cause of organ failure (**Mol, 2011; Hussein & Khaled, 2014; Stancheva et al., 2014; Wheal et al., 2016**). All the Fe concentrations in the samples analyzed were above USEPA and WHO recommended permissible limits of 0.3 mg/kg. These high Fe concentrations might be as a result of the cans used for packaging the fish. These findings are in agreement with the reports of **Khalid & Samir, (2016)**.

Zinc is known to be involved in most metabolic pathways in humans and zinc deficiency can lead to loss of appetite, and other health problems. Zinc is widespread among living organisms, due to its biological significance. The maximum zinc level permitted for fish is 50mg/kg according to Food Codex. The UK required nutritional intake (RNI) ranges set by COMA for zinc are 5.5-9.5 mg/kg/day for adult males and 4.0- 7.0 mg/day for adult females. Zinc is an essential element with a recommended daily allowances ranging from 5 mg for infants to 15 mg for adults. Too little zinc can cause health problems, but too much zinc is also harmful. Harmful health effects generally begin at levels in the 100 to 250 mg/day range (**RAIS, 2007**). The present results showed that all the Zn concentrations in the samples analyzed were below USEPA and WHO recommended permissible limits. This result is in accordance with the findings of **Rustu, (2016)**, while Cu, Ni, Pb, Cd, Mn and Cr were below detectable limits.

4.2 Health risk assessment

To assess the health risk of the consumers of both adult and children due to heavy metal intake from sardines consumption, the daily intake of metals (DIM), health risk index (HRI), target hazard quotient (THQ) and hazard index (HI) were calculated from equations 1, 2, 3 and 4 respectively and the results are presented in Tables 2 and 3. The DIM results both for adult and children were compared with the recommended daily intake of metals and the upper tolerable daily intake level (UL) established by the Institute of Medicine for people between the ages of 19 to 70 years (**FDA, 2001; Garcia-Rico, 2007**). It is very clear that daily intake of metals in sardine samples for Zn and Fe are significantly lower than the recommended daily intake of Zn and Fe (8 mg day⁻¹ person⁻¹) and the upper tolerable daily intake level (UL) of Zn (40 mg day⁻¹ person⁻¹) and Fe (18 mg day⁻¹ person⁻¹) (**USEPA, 2010**). The HRI for Zn and Fe from this study were less than 1 (HRI < 1) for both adult and children. Generally, HRI < 1 means that the exposed population is safe of metals health risk while HRI > 1 means the reverse (**Khan et al., 2008**). The consumers are therefore at no risk of Zn and Fe was also reported by **Tsafe et al. (2012)**.

The THQ is a ratio between the measured concentrations and the oral reference dose, weighted by the length and frequency of exposure, amount ingested and body weight (**Tsafe et al., 2012**). The parameter defines the exposure duration and the risk with that period. The THQ values of Zn and Fe due to sardine consumption for the populace (adults and children) of the study are shown in Tables 6 and 7. In this study, the THQ in Zn and Fe is far less than 1 in the entire sardine samples for both adult and children, therefore, it does not pose health risk concern. Meanwhile, Hazard Index (HI) is the calculation which shows when a population is at risk. From the results in the present study, it was observed that the combined HI values for all the samples under study were less than (<) 1 which indicates that

there are no potential health risk to those consuming these sardines.

5. Conclusion

Heavy metal contamination in canned foods has been an important topic. Facility modernization and quality manufacturing are required to prevent heavy metal contamination in sardines and other canned products and thus the possible health hazards to the consumer. A long-term and/or excessive consumption of foods containing heavy metals above the tolerance levels has a hazardous impact on human health. Since canned foods are widely consumed, they contribute a large fraction to the heavy metals intake and, therefore, strict control of these elements is advisable. For this reason, the steps in all processes must be monitored for preventing the contamination by heavy metals. Also, this present study confirms the fact that all the brands of sardines fish analyzed were safe for human consumption as there was no potential health risk to consumers. However, the shelf life of these brands needs to be reduced to avoid oxygen intake by rusted cans and result to leaching of alloyed materials into the food.

Declaration of interest

The authors report no conflicts of interest.

References

1. Abou-Arab, A.A.K., Ayeshe, A.M., Amra, H.A. & Naguib, K. (1996). Characteristic levels of some pesticides and heavy metals in imported fish. *Food Chem* 57(4), 487-492.
2. Abou-Arab, A.A.K. (2001). Heavy metal contents in Egyptian meat and the role of detergent washing on their levels. *Food Chem Toxicol* 39, 593-599.
3. Al-Busaidi, M., Yesudhasan, P., Al-Mughairi, S., Al-Rahbi, W.A.K., Al-Harthy, K.S., Al-Mazrooei, N.A. & Al-Habsi, S.H. (2011). Toxic metals in commercial marine fish in Oman with reference to national and international standards. *Chemosphere* 85(1), 67e73.
4. Al-Jassir, M.S., Shaker, A. & Khaliq, M.A. (2005). Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh City, Saudi Arabia. *Bull Environ Contam Toxicol*. 75, 1020-1027.
5. Burger, J. & Gochfeld, M. (2005). Heavy metals in commercial fish in New Jersey. *Environmental Research* 99(3), 403-412. <https://dx.doi.org/10.1016/j.envres.2005.02.001>
6. Castro-Gonzalez, M.I. & Mendez-Armenta, M. (2008). Heavy metals: implications associated to fish consumption. *Environ Toxicol Pharmacol* 26, 263e71.
7. Chary, N.S., Kamala, C.T. & Raj, D.S.S. (2008). Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicology and Environmental Safety* 69, 513-524.
8. Elnabris, K.J., Muzyed, S.K. & El-Ashgar, N.M. (2013). Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza Strip (Palestine). *J Assoc Arab Univ Basic Appl Sci* 13, 44e51. <http://www.sciencedirect.com/science/article/pii/S1815385212000302-item1#item1>.
9. FDA (Food and Drug Administration) (2001). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Report of the Panel on Micronutrients. National Academy Press, Washington, DC Food and Drug Administration, Dietary supplements, Center for Food Safety and Applied Nutrition.
10. Garcia-Rico, L., Leyva-Perez, J. & Jara-Marini, M.E. (2007). Content and daily intake of copper, zinc, lead, cadmium, and mercury from dietary supplements in Mexico. *Food Chemistry and Toxicology* 45, 1599-1605.
11. Hosseini, S.V., Aflaki, F., Sobhanardakani, S., Tayebi, L., Babakhani-Lashkan, A. & Regenstien, J.M. (2013). Analysis of mercury, selenium and tin concentrations in canned fish marketed in Iran,

- Environmental Monitoring and Assessment* 185(8), 6407-6412. <https://dx.doi.org/10.1007/s10661-012-3033-y>
12. Hussein, A. & Khaled, A. (2014). Determination of metals in tuna species and bivalves from Alexandria, Egypt. *Egyptian Journal of Aquatic Research* 40, 9-17. <https://dx.doi.org/10.1007/s10661-012-3033-y>
 13. Ikem, A. & Egiebor, N.O. (2005). Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama. *Journal of Food Composition and Analysis* 18, 771-787. <https://dx.doi.org/10.1016/j.jfca.2004.11.002>
 14. Jan, F.A., Ishaq, M., Khan, S., Ihsanullah, I., Ahmad, I. & Shakirullah, M. (2010). A comparative study of human health risks via consumption of food crops grown on wastewater irrigated soil (Peshawar) and relatively clean water irrigated soil (lower Dir). *Journal of Hazard Materials* 179, 612-621.
 15. Khalid, N.J. & Samir, L.S. (2016). Determination of some heavy metals in canned Sardines fish from Iraqi markets. *J. Fac. Med. Baghdad* 58(4), 387-391.
 16. Khan, S., Lin, A., Zhang, S., Huc, Q. & Zhu, Y. (2008). Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term waste water irrigation. *Journal of Hazardous Material* 152, 506-515.
 17. Kris-Etherton, P., Harris, W. & Appel, L. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106, 2747e57.
 18. Mandal, B.K. & Suzuki, K.T. (2002). Arsenic round the world: A review. *Talanta* 58, 20 1-235.
 19. Meche, A., Martins, M.C., Lofrano, B.E.S.N., Hardaway, C.J., Merchant, M. & Verdade, L. (2010). Determination of heavy metals by inductively coupled plasma-optical emission spectrometry in fish from the Piracicaba River in Southern Brazil. *Microchem J* 94, 171e4.
 20. Medeiros, R.J., dos-Santos, L.M., Freire, A.S., Santelli, R.E., Braga, A.M.C.B., Krauss, T.M. & Jacob, S.C. (2012). Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. *Food Control* 23, 535e41. <https://dx.doi.org/10.1016/j.foodcont.2011.08.027>
 21. Mol, S. (2011). Levels of Heavy Metals in Canned Bonito, Sardines, and Mackerel Produced in Turkey. *Biological Trace Element Research* 143, 974-982. <https://dx.doi.org/10.1007/s12011-010-8909-5>
 22. Oguntona, T. (1998). Green Leafy Vegetables. In: Osagie, A.U. and O.U. Eka (Eds.). *Nutritional Quality of Plant Foods*. Post Harvest Research Unit, Department of Biochemistry, University of Benin, Benin City, Nigeria, pp: 120-133. ISBN: 978-2120-02-2.
 23. Rahman, M.S., Molla, A.H., Saha, N. & Rahman, A. (2012). Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. *Food Chem* 134(4), 1847e54.
 24. RAIS, The Risk Assessment Information System (2007). http://rais.ornl.gov/tox/rap_toxp.shtml (accessed 15 January 2008).
 25. Rustu, I. (2016). A study on heavy metal content of Sardine (sardine Pilchardus) caught in the Dardanelles. *Journal of Geography and Geology*, 8(3), 35-41.
 26. Sobhanardakani, S. (2017). Tuna fish and common kilka: Health risk assessment of metal pollution through consumption of canned fish in Iran. *Journal of Consumer Protection and Food Safety* 12(2), 157- 163. <https://dx.doi.org/10.1007/s00003-017-1107-z>
 27. Sobhanardakani, S., Tayebi, L., Farmany, A. & Cheraghi, M. (2012). Analysis of trace elements (Cu, Cd and Zn) in muscle, gill and liver tissues of some fish species using anodic stripping voltammetry. *Environmental Monitoring and Assessment* 184(11), 6607-6611. <https://dx.doi.org/10.1007/s10661-011-2445-4>
 28. Stancheva, M., Makedonski, L. & Peycheva, K. (2014). Determination of heavy metal concentrations of most consumed fish species from Bulgarian Black Sea coast. *Bulgarian Chemical Communications* 46(1), 195-203.
 29. Storelli, M.M. (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food Chemistry and Toxicology* 46, 2782-2788.
 30. Tahán, J.E., Sanchez, J.M., Granadillo, V.A., Cubillan, H.S. & Romero, R.A. (1995). Concentration of total Al, Cr, Cu, Fe, Hg, Na, Pb, and Zn in commercial canned seafood determined by atomic spectrometric means after mineralization by microwave heating. *Journal of Agricultural and Food Chemistry* 43, 910- 915. <https://dx.doi.org/10.1021/jf00052a012>
 31. Tsafe, A.I., Hassan, L.G., Sahabi, D.M., Alhassan, Y. & Bala, B.M. 2012. Evaluation of Heavy Metals Uptake and Risk Assessment of Vegetables Grown in Yargalma of Northern Nigeria. *Journal of Basic and Applied Science Research* 2(7), 6708-6714.
 32. USEPA, (2010). Integrated Risk Information System (IRIS), United States Environmental Protection. Available online: <http://www.epa.gov/iris/index.html> (accessed on 4 May 2016).
 33. US-EPA IRIS, (2006). United States, Environmental Protection Agency, *Integrated Risk Information System*. <<http://www.epa.gov/iris/subst>>.
 34. Wang, X., Sato, T., Xing, B. & Tao, S. (2005). Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Science and Total Environment* 350, 28-37.
 35. Wheal, M.S., DeCourcy-Ireland, E., Bogard, J.R., Thilsted, S.H. & Stangoulis, J.C.R. (2016). Measurement of haem and total iron in fish, shrimp and prawn using ICP-MS: Implications for dietary iron intake calculations. *Food Chem* 201, 222-229. <https://dx.doi.org/10.1016/j.foodchem.2016.01.080>
 36. Yilmaz, A.B. & Yilmaz, L. (2007). Influences of sex and seasons on levels heavy metals in tissues of green tiger shrimp (Penaeus semisulcatus de Hann, 1844). *Food Chem* 101, 1664-1669.
 37. Yilmaz, F., Ozdemir, N., Demirak, A. & Tuna, A.L. (2007). Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. *Food Chem* 100, 830e5.
 38. Zhao, S., Feng, C., Quan, W., Chen, X., Niu, J. & Shen, Z. (2012). Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. *Mar Pollut Bull* 64, 1163e71.