Speciation of Lead, Iron and Cadmium in Selected Brands of Canned Sardine Fish Sold in Port Harcourt, Rivers State, Nigeria

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Abstract: Chemical Speciation is a significant factor that governs toxicity and mobility of heavy metals. Therefore knowledge of polar and non-polar forms of metals could be useful in predicting toxicity. In this research chemical species of lead, iron and cadmium in selected canned fish marketed in Nigeria were evaluated as polar form of nonpolar concentrations. Dry digestion was carried out on the sardine fish meal while wet digestion was applied to the fish oil. The metal concentration was determined using the Flame Absorption Spectrophotometer. The results show that the concentration of the trace metals in the fish meal and fish oil ranged from 13.12 ± 3.81 (Pb), 19.22 ± 4.83 (Fe), 2.50 ± 0.48 (Cd) mg/kg and 14.37 ± 4.34 (Pb), 15.54 ± 2.45 (Fe), 1.65 ± 0.4 (Cd) mg/kg respectively. Chemical speciation of fish meal and fish oil gave rise to varying amounts of polar and non-polar fractions. The polar fractions of fish meal and fish oil ranged between 0.82 ± 0.49 (Pb), 2.0 ± 0.48 (Fe), 0.27 ± 0.17 (Cd) mg/kg and 4.13 ± 1.47 (Pb), 5.93 ± 1.25 (Fe), 0.50 ± 0.17 (Cd) mg/kg respectively; non polar fractions of fish meal and oil ranged between 2.07 ± 0.73 (Pb), 2.23 ± 0.58 (Fe), 0.36 ± 0.08 (Cd) mg/kg and 10.13 ± 2.94 (Pb), 8.65 ± 1.63 (Fe), 1.45 ± 1.41 (Cd) mg/kg respectively. Results reveal that the non-polar species of metals were predominant in the canned fish analyzed.

Key words: Digestion, Extraction, Fish meal, Polar, Toxicity.

1. INTRODUCTION

Fish contains high protein content, low saturated fat, essential minerals and omega fatty acids that support good health. The total mineral content in the flesh of marine fish ranges from 0.6 to 1.5% wet weight [1]. In Nigeria, due to the insufficient domestic catches and aquaculture production, there is high dependence on large volumes of imports to satisfy local demand. These imported fishes are mostly frozen or canned fish. Canned fish are fish which have been processed, sealed in airtight container such as a sealed tin can, and subjected to heat. Boadi, et al., [2], stated that canned fish is subjected to lead contamination during canning process. Solder used in the manufacture of cans has been recognized as a source of lead contamination during canning [3]. The pH of the canned products, oxygen concentration in headspace, quality of coating and storage place may also influence metal levels in canned fish.

Metal toxicity, environmental mobility and tendency to be accumulated in living systems has been directly linked to the metals chemical forms. Knowledge of the total concentration gives only poor information about the potential risk. Thus, the need for speciation analysis arises from the necessity to determine the concentration of those species characterized by the highest toxicity, mobility etc. with respect to the other species of the same element. Metals may be present in an environmental compartment but yet, not bioavailable. Therefore, it is important that speciation of canned fish be periodically carried out [4].

Speciation can also be based on the polarity of the species. Polar species dissolve in polar solvents while non polar species dissolve in non-polar solvents. Generally, inorganic species are said to be polar while organic or organometallic species are said to be non-polar. Knowledge of the distribution of polar and non-polar species of metals in fish will help us to better understand the toxicoekinetics of these metals when it enters the human system. For instance, polar species will likely enter the blood plasma and brain fluid while non polar species will be found in the blood lipids [5, 6].
Levels of heavy metals concentration in canned fish has been widely reported and studied [7]. Work on the heavy metal contamination in canned fish marketed within Kumasi in the Ashanti region of Ghana for Pb, Zn, Fe, Cd, Mn and Hg, reported that the trace metals were within permissible levels in most of the canned fish analyzed [8]. Suhendran [9], stated that trace metals in canned fish must be monitored comprehensively and periodically with respect to the consumer’s health. Literatures abound on determination of total amount of heavy metals and not on the speciation of metals. However, Spayad et al., [10], reports that the different species of arsenic produce diverse toxicological effects in human, with inorganic forms being more toxic than organic ones. They stated that the species of arsenic found in fish are arsenobetaine (AsB) and arsenocholine (AsC), which are relatively less toxic to humans compared to the other species.

The aim of this research is therefore to investigate selected trace metals, Pb, Cd and Fe levels in canned fish marketed in Port Harcourt and their speciation as polar and non-polar fractions. The specific objectives is twofold namely; determination of total trace metals in canned fish meal, and fish oil and then determination of the polar and non-polar fractions of the trace metals found in them. The importance of such a study lies in the fact that there are no regulations on the amount of heavy metals in canned fish here in Nigeria.

2. MATERIALS AND METHODS

2.1. Sampling

The canned fish used in this work were obtained from supermarkets in Port Harcourt, Rivers State, Nigeria. The brands used were Geisha Sardine (G), Titus Sardine (T) and Queens Sardine (Q). Twenty-seven canned sardines were grouped into three brands to give nine samples per group, labeled G1 (n=3), G2 (n=3), G3 (n=3), T1 (n=3), T2 (n=3), T3 (n=3), Q1 (n=3), Q2 (n=3), Q3 (n=3). After opening the canned sardines, oil was drained out as a separate set of sample; thus, there were nine samples of sardine fish and nine samples of sardine oil.

2.2. Sample Storage/Treatment

The oil sample was stored in a freezer at below 4°C while the fish meal sample was weighed and immediately transferred to an oven. It was dried in the oven at 50°C until a constant weight was recorded. The dried fish was brought out and homogenized with a mortar and pestle. The dried fish was then stored in an air tight container until extraction and/or digestion was carried out.

2.3. Determination of Physicochemical Characteristic of the Sardine Sample

The pH and conductivity were determined according to the method of ASTM D3838-80 with slight modification according to method of [11]. One (1.0) g of each sample was weighed and transferred into a 250 ml beaker and 100 ml of distilled water was added and stirred for 1 hour. Samples were allowed to stabilize and then pH measured using an electronic pH/Conductivity meter, Jenway 430 Model. The same samples were further used for electrical conductivity (EC) of the fish and results read off in μS. The constant weight of a beaker was obtained and thereafter the whole wet sardine fish sample was weighed into the beaker and transferred to the oven for 4 hrs at 70°C. The sample was cooled and weighed. This was continued until a constant weight was obtained. Moisture content was estimated thus,

\[
\% \text{ Moisture content} = \left( \frac{W_1 - (W_3 - W_2)}{W_1} \right) \times 100
\]

Where \( W_1 \) = weight of wet sardine sample, \( W_2 \) = constant weight of crucible

\( W_3 \) = weight of sample + beaker after drying and cooling [12].

2.4. Determination of the Total Metal Content in Sardine Fish Meal

2 g of the dried fish sample was weighed into a 250 mL conical flask and 15 mL of concentrated nitric acid was added. The conical flask was covered with a watch glass until most of the sample had dissolved by standing overnight. The solution was heated on a hot plate at about 70°C until all the yellowish brown fumes are ended. After cooling, small portions of deionized hot water was added to dissolve the precipitate left. The sample was filtered with No. 541 Whatman filter paper into a 100 mL beaker. The filtrate was made up to the 100 MI mark with deionized water and then taken to the AAS for analysis.
2.5. Extraction Procedure for Fish Meal

2.5.1. Methanol Extraction

5 g of the dried and grounded fish meal was collected in a 100 mL beaker and 25 mL of methanol was added and left to stand for 24 hrs. After 24 hrs, the sample was filtered with Whatman filter No. 541, and the filtrate (methanol extract) was left in the open until digestion was carried out. The methanol extract is the polar fraction of the sample.

2.5.2. Hexane Extraction

The residue from (a) above was dried to constant weight (to completely remove methanol) and then collected in a fresh 100 mL beaker. 25 mL of hexane was added to the residue and left to stand for 24 hrs. Then the sample was filtered and the filtrate (hexane extract) was left in the open until digestion. The hexane extract is the non polar fraction of the sample. The solvent in extract (a) and (b) was not allowed to evaporate to dryness before digestion was carried out [13].

2.5.3. Digestion of the Fish Meal Extract

1 mL of concentrated nitric acid was added to the extract and heated to a low volume in a beaker, making certain that the sample does not boil and that no portion of the beaker is allowed to go to dryness. The heating continues with small addition of the nitric acid until the digest is light in colour or does not change appearance with continuous heating. Any precipitate formed was dissolved with small quantity of hydrochloric acid. On completion of digestion, small amount of deionized water was added and stirred. The solution was filtered and the final volume of the filtrate was adjusted and stirred. The solution was filtered and the final volume of the filtrate was adjusted to 50 ml with deionized water. The sample was then taken to the AAS for analysis.

2.6. Determination of the Total Metal Content in the Fish Oil

The oil sample was removed from the freezer, melted in a water bath at 30°C and mixed thoroughly before sampling. Samples were prepared for analysis by wet digestion (acid digestion) as outlined by Anwar et al., [14]. Duplicate samples 1.0 g was weighed into separate conical flasks. 5 mL of concentrated nitric acid was then added and the contents heated at 70 - 80°C for 2 – 3 hours, on a hot plate. Heating was continued at about 150°C overnight, 3-5 mL of concentrated sulphuric acid and 30% hydrogen peroxide (each) was added occasionally and continuous heating further allowed to completely the organic matter. All contents of the flask were evaporated and the semi-dried mass was dissolved in a small amount (5 mL) of deionized water, filtered through Whatmann no. 42 filter paper, and made up to a final volume of 25 mL in volumetric flask with 2N nitric acid.

2.7. Extraction of Fish Oil

1 g of the sardine fish oil was weighed into a conical flask, then 15 ml each of methanol and hexane was weighed into it. This mixture was shaken together and allowed to stand for 24 hrs. it was then transferred into a separating funnel and two layers were formed. The lower layer, which represented the hexane fraction, was separated from the upper layer (methanol fraction). The extracts were kept in an open for 24 hrs to allow some of it to evaporate and to achieve reduction in volume. Then, digestion was carried out and the digestate was made up to 25 ml with distilled water.

2.8. Instrumentation

The flame atomic absorption spectrophotometer (FAAS) Buck Scientific Model 205 with acetylene gas was used in the determination of the heavy metals. Concentration of metals was calculated with reference to a standard curve. Calibration of the instrument was done by preparing standard solutions of the metal in concern by diluting their Certified Reference Material (CRM). The standard concentration prepared were 0.1 mg/l and 0.01 mg/l for the points to draw the calibration curve. Table 1 shows the instrumental conditions used for the analysis of the sample and standards.
Table 1. Instrumental Conditions used for the Analysis of Samples and Standards

| Metal | Wavelength (nm) | Slit width (nm) | Lamp Current (mA) | Fuel/Oxidant | Flame colour | Detection limit | Signal Output |
|-------|-----------------|-----------------|-------------------|--------------|--------------|----------------|---------------|
| Pb    | 283.3           | 0.7             | 1.5               | Acetylene/Air| Lean blue    | 0.08           | 100%          |
| Fe    | 248.3           | 0.2             | 1.5               | Acetylene/Air| Lean blue    | 0.05           | 100%          |
| Cd    | 228.9           | 0.7             | 1.5               |              |              | 0.01           | 100%          |

2.9. Analytical Quality Assurance (AQA)

All glasswares were soaked overnight in 10% (v/v) nitric acid, followed by washing with 10% (v/v) hydrochloric acid and rinsed with double deionized water and dried before use. All other quality control measures were carried out according to [15].

2.10. Statistical Analysis

Data obtained from this study were analyzed using the statistical package for social sciences (SPSS) version 18.0 for windows. Independent sample t-test was used to compare means, and values were considered significant at \( p < 0.05 \). Results were reported as mean ± n SDV for triplicate analysis where appropriate.

3. RESULTS AND DISCUSSION

Table 2. Physicochemical characteristic of the sardine sample

| Sample brand | pH  | % Moisture | Colour   |
|--------------|-----|------------|----------|
| TITUS        | 5.85| 63.25      | Brownish |
| GEISHA       | 6.2 | 59.69      | Light grey|
| QUEEN        | 6.3 | 60.20      | Brownish |

The pH value of a canned fish is a direct function of the free hydrogen ions present in it. The pH recorded for the various type canned fish ranged from 5.85 to 6.60 (Table 2) and are classified as low-acid foods with a pH value greater than 4.6.

The moisture content of the fish samples which, in some samples, was as high as 60%. The moisture loss, recorded after drying the sample, will therefore not guarantee an efficient toxicological analysis of the sample when the standard limit used is on a wet weight basis.

Table 3. Percentage extraction of the metals in the fish meal (%)

| Sample ID | Pb  | Fe   | Cd   |
|-----------|-----|------|------|
| T 1       | 20  | 23.2 | 30   |
| T 2       | 15  | 18.4 | 35   |
| T 3       | 32  | 24.7 | 26.7 |
| G 1       | 20  | 22.6 | 20   |
| G 2       | 25  | 21.7 | 26   |
| G 3       | 28.2| 34   | 24   |
| Q 1       | 14.7| 18   | 24   |
| Q 2       | 14.8| 7.8  | 20   |
| Q 3       | 14.1| 23.6 | 22.8 |

Key:

\( T1,T2,T3: \#1,\#2,\#3 \) Titus sardine fish meal samples respectively

\( G1,G2,G3: \#1,\#2,\#3 \) Geisha sardine fish meal samples respectively

\( Q1,Q2,Q3: \#1,\#2,\#3 \) Queen sardine fish meal samples respectively

The extraction efficiency of sardine fish range of 7.8% to 36% recorded here are low and it implies that most of the metals in the fish are not leachable, thus, are locked in the fish matrix and are found in the residue after extraction. However, the extraction of the fish oil is more efficient with the solvent used; recording almost 100% extraction in most cases since no residue was left over.
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Table 4. Mean trace metal concentration (mg/kg) in polar (PF) and non polar (NPF) fractions

| Sample       | Pb (Range (mean ± S.E)) | Fe (Range (mean ± S.E)) | Cd (Range (mean ± S.E)) |
|--------------|-------------------------|-------------------------|-------------------------|
| FISH MEAL    |                         |                         |                         |
| TITUS (PF)   | 0.4 – 2.3 (1.03 ± 0.63) | 1.2 – 3.4 (2.1 ± 0.66)  | 0.2 – 0.3 (0.23 ± 0.34) |
| TITUS (NPF)  | 1.7 – 7.6 (3.8 ± 1.91)  | 0.4 – 3.4 (2.0 ± 0.87)  | 0.1 – 0.5 (0.37 ± 0.13) |
| GEISHA (PF)  | 0.5 – 1.3 (0.8 ± 0.24)  | 1.7 – 3.6 (2.5 ± 0.56)  | 0.2 – 0.4 (0.3 ± 0.06)  |
| GEISHA (NPF) | 1.0 – 1.8 (1.33 ± 0.24) | 2.8 – 4.1 (3.43 ± 0.38) | 0.3 – 0.5 (0.4 ± 0.06)  |
| QUEENS (PF)  | 0.03 – 1.8 (0.62 ± 0.59)| 0.6 – 1.9 (1.4 ± 0.41)  | 0.1 – 0.5 (0.27 ± 0.12) |
| QUEENS (NPF) | 1.03 – 1.17 (1.09 ± 0.04)| 0.1 – 2.3 (1.27 ± 0.56) | 0.2 – 0.4 (0.3 ± 0.06)  |
| FISH OIL     |                         |                         |                         |
| TITUS (PF)   | 1.2 – 2.07 (1.58 ± 0.26) | 1.4 – 3.10 (2.44 ± 0.52) | 0.13 – 0.61 (0.38 ± 0.14) |
| TITUS (NPF)  | 8.41 – 21.7 (15.16 ± 3.86) | 4.30 – 9.52 (6.39 ± 1.60) | 0.2 – 1.30 (6.39 ± 3.97) |
| GEISHA (PF)  | 1.98 – 4.41 (3.45 ± 0.75) | 8.05 – 15.53 (10.56 ± 2.49) | 0.5 – 0.63 (0.54 ± 0.04) |
| GEISHA (NPF) | 4.02 – 9.65 (6.62 ± 1.63) | 8.38 – 17.30 (11.96 ± 2.73) | 1.0 – 1.3 (1.13 ± 0.09) |
| QUEENS (PF)  | 1.20 – 10.69 (7.37 ± 3.41) | 3.71 – 6.20 (4.82 ± 0.73) | 0.2 – 1.25 (0.58 ± 0.33) |
| QUEENS (NPF) | 2.10 – 13.06 (8.60 ± 3.33) | 7.08 – 8.42 (7.61 ± 0.55) | 0.5 – 1.0 (0.83 ± 0.17) |

PF – polar fraction, NPF – non polar fraction

Table 5. Mean trace metal concentration (mg/kg) in the fish meal and oil

| Sample       | Pb (mean ± S.E) | Fe (mean ± S.E) | Cd (mean ± S.E) |
|--------------|-----------------|-----------------|-----------------|
| TITUS Fish meal | 19.0 ± 5.77    | 18.50 ± 5.20    | 2.0 ± 0.58      |
| Fish Oil     | 17.68 ± 3.99   | 9.38 ± 1.54     | 1.35 ± 0.52     |
| GEISHA Fish meal | 8.60 ± 1.94   | 24.17 ± 5.50    | 3.0 ± 0.29      |
| Fish Oil     | 10.85 ± 2.68   | 23.55 ± 4.88    | 1.92 ± 0.25     |
| QUEEN Fish meal | 11.72 ± 3.73  | 15.0 ± 3.79     | 2.5 ± 0.58      |
| Fish Oil     | 14.57 ± 6.36   | 13.68 ± 0.94    | 1.67 ± 0.44     |

3.1. Metals in Fish Meal and Fish Oil

Figure 1, shows that the total lead concentration in the fish meal ranges from the lowest value of 7.5 mg/kg in sample G1 and G2 to the highest value of 30.5 mg/kg in sample T3. Fe concentration ranged from 9 mg/kg in Q2 to 34 mg/kg in G1, while the Cd concentration ranges from 1 mg/kg in T1 to 3.5 mg/kg in G1 and Q3. Generally, the total concentration of Pb and Cd reported in this work is found to be higher than the levels reported by Suhendran [15,16].

Figure 1 also shows the trace heavy metal concentration in the fish oil of the canned sardine. The concentration range of Pb was found to be from 3.75 mg/kg to 25.75 mg/kg; Fe: 7.0 mg/kg to 33 mg/kg and Cd: 0.5 mg/kg to 2.5 mg/kg. When compared with each other the metal concentration in fish meals were generally higher than in the oils except for fish oil of GEISHA and QUEEN sardine in which Pb was significantly higher.

![Figure 1. Bar plot of Mean trace metal concentration (mg/kg) in the fish meal and oil](image-url)
3.2. Extraction Efficiency

The extraction efficiency of the solvents used in this work is represented by the percentage extraction as shown in Table 3, which gives percentage extraction range of 7.8% of Fe in Q2 to 35% of Cd in T2 of the dried fish sample. This low extraction efficiency, is likely due to the inability of the solvent to extract most of the metals from the fish tissue. These metals are bound with some proteins in the fish tissue by forming complexes with them; an example is metallothioneins in animal tissue. Metallothioneins are cystein-rich metal binding proteins involved in the detoxification of metals and metabolism of essential metals, and are found in various organs of animals. However, these bound metals can be effectively released by digesting the fish tissue. On the other hand, the solvent extraction of the canned fish oil was found to be more efficient than the fish meal [17].

The extraction efficiency may also have been influenced by the choice of solvent used. However, careful considerations were given before the choice of hexane and methanol as the solvents used. Hexane is a non-polar solvent with a boiling point of 69°C; dielectric constant of 1.88; density, 0.655 g/ml and dipole moment, 0.00 D. Methanol, on the other hand, is a polar protic solvent with a boiling point of 65°C; dielectric constant, 33; density, 0.791 g/ml and dipole moment of 1.70 D. Other considerations bordered on safety issues which include toxicity, flammability, radioactivity.

3.3. Lead

The mean concentration of lead in the polar and non-polar fraction of the canned fish samples are presented in Table 4; lead concentration in the polar fraction ranged from 0.62 mg/kg in QUEEN fish meal to 7.37 in QUEEN fish oil. Meanwhile, lead concentration in the non-polar fraction ranged from 1.09 mg/kg in QUEEN fish meal to 15.10 mg/kg in TITUS fish oil. From table 4, the total mean concentration of lead in the fish meal of the various brands, TITUS, GEISHA and QUEEN is given as 19 mg/kg, 8.6 mg/kg and 11.72 mg/kg respectively; this represents an order of TITUS > QUEEN > GEISHA. Extraction efficiency of lead ranges from 14.1% (Q1) – 32% (T3). Also, from table 4, the mean concentration of lead in the sardine fish oil of the various brands, TITUS, GEISHA and QUEEN is given as 17.68 mg/kg, 10.85 mg/kg and 14.57 mg/kg respectively; representing a concentration order of TITUS > QUEEN > GEISHA.

Generally, it was observed that the lead concentration in the non-polar fractions were higher than the polar fractions. This implies that most of the species of lead found in the sardine fish meal sample were organic or non-polar in nature, while the inorganic or polar lead species were limited. Also, QUEEN sardine fish meal extract had the lowest lead concentration, although it recorded higher concentration in its fish oil. The total metal concentration of lead in canned fish and oil reported in this work is found to be higher than the levels reported by may researcher [18]. Also, the lead concentration in this work is higher than the [19] standard of 0.3 mg/kg and [20] standard of 2.0 mg/kg in fish. However, these standard values are based on the wet weight of fish but this work was based on the dry weight of fish, due to the extraction necessity. According to the [20] the legal limit of lead in dried fish in the United Kingdom is 5.0 ppm. The lead concentration of sardine fish and oil obtained in this work still falls short of this value, thus there seem to be a small degree of contamination, though verification with a dry weight based standard is necessary.

FAO/ WHO, [22] stated that, inorganic lead compounds are classified by the IARC as probably carcinogenic to humans. Thus consumption of contaminated fish poses a risk to the consumer. Also, organic lead species like tetraethyl lead, which is toxic to humans, is likely to be found in the fish sample analyzed.

3.4. Iron

The range of total iron concentration in the different brands of sardine fish meal was 9 mg/kg – 34 mg/kg, the lowest concentration found in Q2 whereas the highest concentration was found in G1. The total mean concentration of iron in the sardine fish meal of the various brands, TITUS, GEISHA and QUEEN was 18.50 mg/kg, 19.65 mg/kg and 15.0 mg/kg respectively; thus an iron concentration order of GEISHA > TITUS > QUEEN could be observed. The mean concentration of Fe in the polar fraction of fish meal ranged from 1.4 mg/kg in (QUEEN) to 2.5 mg/kg (GEISHA) while the iron concentration in the non-polar fraction ranged from 1.27 mg/kg (QUEEN) to 3.43 mg/kg (GEISHA). The total mean concentration of Fe in the sardine fish oil of the various brands, TITUS, GEISHA and QUEEN was...
3.5. Cadmium

The concentration of cadmium was generally lower in all the analyzed samples compared to the concentrations of lead and iron. The range of total cadmium concentration in the different brands of sardine fish meal was 1.0 mg/kg – 3.5 mg/kg, the lowest concentration found in Q2 while the highest concentration was found in G3 and T1. The total mean concentration of cadmium in the sardine fish meal of the various brands, TITUS, GEISHA and QUEEN is given as 2.0 mg/kg, 3.0 mg/kg and 2.5 mg/kg respectively; thus, a cadmium concentration order of GEISHA > QUEEN > TITUS. The cadmium concentration in the polar fraction of fish meal ranged from 0.23 mg/kg (TITUS) to 0.30 mg/kg (GEISHA) whereas the cadmium concentration in the non-polar fraction of fish meal ranged from 0.3 mg/kg (QUEEN) to 0.4 mg/kg (GEISHA). From Table 5, the extraction efficiency of cadmium in the fish sample ranged from 20% (G1 & Q2) to 35% (T2). The total mean concentration of cadmium in the sardine fish oil of the various brands, TITUS, GEISHA and QUEEN is given a 1.35 mg/kg, 1.92 mg/kg and 1.67 mg/kg respectively; an order of GEISHA > QUEEN > TITUS. The polar fraction of the fish oil recorded a cadmium concentration range of 0.38 mg/kg (TITUS) – 0.58 mg/kg (QUEEN), while non-polar fraction range of 0.83 mg/kg (QUEEN) – 6.39 mg/kg (TITUS). The concentration of cadmium was higher in the non-polar fraction than polar fraction, implying that there are more organic compounds of cadmium than inorganic compounds in the samples analyzed. Some of the cadmium concentrations were found to exceed the recommended EU limit of 0.1 mg/kg and 0.5 mg/kg. The high values of total trace metal concentration in this study can be attributed to two factors. First, a relatively higher level of contamination of the sardine fishes may have been used in this study. The source of contamination could be from the fish source water or from the canning process and storage [25]. The pH of the canned products, oxygen concentration in the head space, quality of coating and storage place may also control metal levels in canned fishes. Generally a lower pH leads to more availability of metals through leaching from the metal can material or from other sources. Also, has reported that solder used in the manufacture of cans has been recognized as a source of lead contamination during canning [26].

Secondly, the nature of the sample used in the metal analysis could account for the differences in metal concentration. The reference journals/ work listed above used wet fish sample, thus, the metal concentration calculations were on a wet weight basis. Also, most of the standard limits quoted here are based on wet weight. In contrast, the fish samples in this work were dried before digestion and analysis. The drying was necessary to concentrate the metals in the fish and also aid in the efficient extraction of the fish samples with the polar and non-polar solvents.

4. CONCLUSION

Some of the metal concentration observed in this work exceeded the recommended limits, but an effective toxicological analysis cannot be concluded due to the absence of standard limit values based on dry weight for correlation. However, there is an obvious contamination of the sardine samples with heavy metals, especially lead. Metal concentration in the non-polar fraction was generally higher than in the polar fraction, although, iron is slightly evenly distributed. This implies that the commonly found metal species in sardine fish and oil is organic in nature. This knowledge is significant because the distribution and absorption of metals in the human body is somewhat dependent on their polarity. It is
therefore recommended that in depth work be carried to compile data for metal contents of canned fish to be used in setting up regulatory standards here in Nigeria.

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