# COMPARATIVE DETERMINATION AND HEALTH RISK ASSESSMENT OF CADMIUM, CHROMIUM AND COPPER IN WILD AND AQUACULTURED CLARIAS GARIEPINUS, OREOCHROMIS NILOTICUS AND MICROPOGONIAS UNDULATUS SOLD IN GWAGWALADA AREA COUNCIL, FCT - NIGERIA

# DALLATU E. MUSA<sup>1\*</sup>, JUDE E. EMUROTU<sup>1</sup>, ATUMEYI A. UGBEDEOJO<sup>1</sup>, IBRAHIM ESTHER<sup>2</sup> AND ERNEST O. ONUGWU<sup>1</sup>

<sup>1</sup> Chemistry Department, Faculty of Science, Federal University, P.M.B. 1154, Lokoja, Kogi State, Nigeria. <sup>2</sup> Industrial Chemistry Department, Faculty of Science, Federal University, P.M.B. 1154, Lokoja, Kogi State, Nigeria.

# ABSTRACT

This study assesses the levels of copper (Cu), chromium (Cr), and cadmium (Cd) in wild and aquacultured Oreochromis niloticus (Tilapia), Micropogonias undulatus (Croaker), and Clarias gariepinus (Catfish) that are sold in Gwagwalada Area Council, Federal Capital Territory, Nigeria. A random sample of 500 g of each species of fish was taken from the markets in Dobi, Gwagwalada, and Zuba. The labels for the composite samples were: aquacultured catfish (Acf), aquacultured tilapia (Atf), aquacultured croaker (Acrf), wild catfish (Wcf), wild tilapia (Wtf), and wild croaker (Wcrf). Oven-dry weight and furnace procedures were used to determine the contents of moisture and ash. Wcf, Wtf, and Wcrf had moisture contents of  $4.25\pm0.354\%$ ,  $5.25\pm0.453\%$ , and  $4.50\pm0.000\%$ , respectively, according to the results, whereas Acf, Atf, and Acrf had moisture contents of  $7.01\pm0.230\%$ ,  $7.12\pm0.234\%$ , and  $6.70\pm0.210\%$ , respectively. For Wcf, Wtf, and Wcrf, the ash content was  $1.75\pm0.354\%$ ,  $1.64\pm0.231\%$ , and  $1.48\pm0.213\%$ , respectively. The possible health concerns linked with the consumption of these heavy metals were determined by measuring and comparing their concentrations. The results show that various fish species accumulate metals at different rates, which could have an impact on public health because extended exposure to these toxins can cause health problems.

Keywords: Cadmium (Cd), Chromium (Cr), Copper (Cu), Wild and Aquacultured Fish.

# 1. INTRODUCTION

<sup>1-7</sup> Define heavy metals as metallic elements whose atomic weights and densities are five times greater than that of water in other words, they are metals which possess a specific density of more than 5 g/cm<sup>3</sup> and adversely affect the environment and living organisms and according to <sup>7-9</sup> these metals are quintessential to maintain various biochemical and physiological functions in living organisms when in very low concentrations, however they become noxious when they exceed certain threshold concentrations. Heavy metals often contaminate bodies of water which are the habitats of fish and other aquatic lives through discharge/ disposal of industrial waste water (effluent) into ponds, streams, rivers, sea and oceans; run off of water / leachates from mining sites into these bodies of water; through feeds and washed off of heavy metal contained fertilizers/ pesticides by rains into bodies of water <sup>10-13</sup>. Bioaccumulation of metals in tissues of fish to toxic level could occur. These fish are subsequently harvested, processed and sold for nutritional, medicinal, industrial and many other uses.

Despite the fact that heavy metals have some useful applications like the use of arsenic compounds as rat poisons and insecticides under strict control 14, organoarsenic compounds are added to poultry feed to prevent disease and improve weight gain 15. Mercury is extensively used in thermometers, barometers, pyrometers, hydrometers, mercury arc lamps, fluorescent lamps and as a catalyst <sup>9</sup>, it is also being used in pulp and paper industries, as a component of batteries and in dental preparations such as amalgams<sup>16</sup>. According to<sup>17</sup>, chromium finds its applications in industries such as metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production and pulp and paper production. About three-fourths of cadmium is used in alkaline batteries as an electrode component, the remaining part is used in coatings, pigments and platings and as a plastic stabilizer <sup>18</sup>. These metals have a history of being harmful to human health, even at low quantities and they can build up in the body over time, creating serious health issues for instance, lead exposure has been reported by 9, 19 to harm the brain system, kidneys and reproductive system, 20-22 report that cadmium exposure can result in lung and prostate cancer, kidney damage and brittle bones 23. Nausea, mouth ulcers, skin ulcers, skin rashes, vomiting, diarrhea and arthritic pain have been reported by 9, <sup>24</sup> as symptoms for higher amounts of aluminium in human body, its exposure is probably a risk factor for the onset of Alzheimer disease (AD) in humans, as hypothesized by 25 and other complications associated with aluminium toxicity are lung problems, anemia, impaired iron absorption, nervous system problems <sup>24</sup>. The inorganic forms of arsenic such as arsenite and arsenate are reportedly said to be highly carcinogenic causing cancer of lungs, liver, bladder and skin 26-<sup>28</sup>. Cadmium and its compounds are being classified as group 1 carcinogens for humans by the International Agency for Research on Cancer 29 and can cause both acute and chronic intoxications <sup>30</sup>. Chromium(VI) compounds, such as

calcium chromate, zinc chromates, strontium chromate and lead chromates, are highly toxic and carcinogenic in nature <sup>31</sup>. <sup>32</sup>reported that iron can catalyze the reactions involving the formation of radicals which can damage biomolecules, cells, tissues and the whole organism. Iron poisoning has always been a topic of interest mainly to pediatricians <sup>32</sup>. Children are highly susceptible to iron toxicity as they are exposed to a maximum of iron-containing products <sup>33</sup>. Lead poisoning is a classic disease and the signs that are observed in children and adults are mainly pertaining to the central nervous system and the gastrointestinal tract <sup>34</sup>. <sup>35</sup>.

Heavy metals bio- accumulate in living organisms over time causing various damages, diseases and disorder such as gastrointestinal and kidney dysfunction, nervous disorders, skin lesions, vascular damage, birth defects thus, heavy metals contamination in fishes can pose serious risks to consumers. Analysis of wild *Clarias gariepinus* (catfish), *Oreochromis niloticus* (tilapia fish) and *micropogonias undulatus* (croaker fish) sold in these three markets of Gwagwalade Area Council for cadmium (Cd), chromium (Cr) and copper (Cu) by <sup>7</sup> showed some levels of heavy metals contamination hence the need to check levels of these metals in both wild and aquacultured *Clarias gariepinus* (catfish), *Oreochromis niloticus* (tilapia fish) and *Micropogonias undulatus* (croaker fish) sold in the three major markets of Gwagwalada Area Council, FCT – Abuja and compare their levels of contamination.

Analyzing levels of heavy metals in fish according to <sup>7</sup>, allows for an assessment of potential dietary exposure to these metals. By understanding the concentration of these metals in fish, researchers and regulatory bodies can estimate the extent of heavy metal intake by individuals, helping in risk assessment and mitigation strategies <sup>7</sup>. Regulating the amounts of heavy metals in food and water sources has been attempted. For instance, the World Health Organization (WHO) has established restrictions on the allowable levels of heavy metals in food, supplements and drinking water <sup>36</sup>. The European Union has also created guidelines for the highest allowed levels of heavy metals in food <sup>37</sup>.

This study assesses levels of cadmium (Cd), chromium (Cr) and copper (Cu) in some wild and aquacultured fish sold in three major markets of Gwagwalada Area Council, FCT – Abuja. This was achieved through interview of fish vendors in the markets as to whether the fish are wild fish or not; wild fish samples collection from the markets; aquacultured fish samples collection from fisheries within the study areas; sample preparation; heavy metal analysis using atomic absorption spectrophotometer (ASS) and comparison of results obtained from samples analysis, and with safety standard specifications. Findings of this study are useful to the society, humanity, researchers since its contribution lies in enhancing food safety, protecting human health, advancing scientific knowledge and promoting responsible agricultural practices.

The study involved the collection of representative wild and aquacultured cat, tilapia and kroaker fish samples randomly from three major markets and fisheries in Gwagwalada Area Council, preparation of samples and laboratory analysis of the samples using Atomic Absorption Spectrophotometer (AAS).

# 2. MATERIALS AND METHOD

# 2.1 Apparatus/ materials and instruments

Assorted glass wares, crucibles with lids, crucible tongs, laboratory knife, spatula, pestle and mortal and weighing balance; oven (AS ONE 1-5197-01 FC-1000), pH meter (MSP43), Atomic Absorption Spectrophotometer (AAS) (JFS/99/23/0004hine ED-XRF Analyzer) and Muffle Furnace (GPC AMS2750E) were used.

## 2.2 Reagents

Analytical grade nitric acid (HNO<sub>3</sub>), hydrochloric acid (HCl), hydrogen peroxide ( $H_2O_2$ ), buffer tablets of pH 4.7 and 9, potassium hydroxide (KOH), and sodium carbonate ( $Na_2CO_3$ ) were used. Standards of cadmium (Cd), chromium (Cr) and copper (Cu) were also used. These reagents were purchased from BDH.

### 2.3 Description of study area

Gwagwalada has an area of 1,043 km<sup>2</sup> and a population of 157,770 at the 2006 census. It is projected to have a 6.26% growth between 2020 and 2025, the largest increase on the <u>African</u> continent. The latitude of Gwagwalada, Nigeria is 8.950833, and the longitude is 7.076737. Gwagwalada, Nigeria is located at *Nigeria* country in the *Towns* place category with the gps coordinates of 8° 57' 2.9988" N and 7° 4' 36.2532" E. Figures 1a and b are maps of Federal Capital Territory, Abuja showing Gwagwalada Area Council and map of Gwagwalada Area Council showing the sampling areas: Dobi, Gwagwalada and Zuba <sup>38</sup>.



**Figures 1a and b:** Maps of Federal Capital Territory Abuja showing Gwagwalada Area Council and map of Gwagwalada Area Council showing the sampling areas: Dobi, Gwagwalada and Zuba. **Source**<sup>39</sup>.

### 2.4 Samples and sampling

The samples were cat, tilapia and croaker fish. About 500 g each of the wild fish were randomly bought on 15th November, 2023 from fish vendors in Dobi, Zuba and Gwagwalada markets into washed and rinsed – labeled (Wcf, Wtf and Wcf for wild cat fish, wild tilapia fish and wild croaker fish respectively) plastic sampling containers, covered and transported to laboratory for analysis. Similarly, 500 g each of the aquacultured fish were randomly bought on 15th December, 2023 from fisheries in Dobi, Zuba and Gwagwalada towns into washed and rinsed - labeled (Acf, Atf and Acf for aquacultured cat fish, aquacultured tilapia fish and croaker fish respectively) plastic sampling containers, covered and transported to laboratory for analysis.

# 2.5 Sample preparation

This was done in line with the method reported by  $^{40-42}$ . Substances other than fish were identified and removed from the samples and the samples were washed, rinsed with distilled – deionized water in order to remove dust and any other particles adhered to the samples which might contain heavy metals.

### 2.6 Determination of moisture content

This was done according to the oven dry basis method procedure reported by  $^{43.50}$ . About 3.0 g of pulverized dried samples was weighed into a dried and weighed crucible as W<sub>1</sub>. This was placed in an oven and heated at 105 °C for 30 minutes. It was removed, allowed to cool in desiccators and weighed as W<sub>2</sub>. The crucible with its content was again transferred back to the oven, heated, removed after 30 minutes, allowed to cool and weighed – this was repetitively done until constant or stable values for mass of crucible with content after cooling was obtained as W<sub>3</sub>. The percentage moisture content was calculated using the formula

% Moisture content = 
$$\frac{Mass of Moisture}{mass of sample} \times 100$$

This was done in triplicates.

### 2.7 Preparation of standard and working standards

This was done in line with the procedures reported by <sup>51, 52</sup>. 100 ppm solution (stock) of each heavy metal was prepared in 100 cm<sup>3</sup> using distilled – deionized water, made up to mark and labeled as such. Thereafter, dilution principle equation,  $C_1V_1 = C_2V_2$  was used to prepare working standards of 2.0 ppm, 4.0 ppm, 6.0 ppm, 8.0 ppm and 10.0 ppm for each metal in 100 cm<sup>3</sup> and labeled respectively.

# 2.8 Thermal digestion (ashing) of samples

The ash content was determined using the ignition method in line with the procedure reported by <sup>43,49</sup>. A crucible was thoroughly washed and pre-heated in a muffle furnace at 450 °C. About 10.0 g of pulverized sample was weighed and placed in the pre-heated cooled and weighed crucible and then reweighed. The crucible was covered with its lid and then placed in the muffle furnace. Its temperature was allowed to rise to 550 °C and the ashing was carried out for three hours after which crucible was removed from the furnace, allowed to cool in a desiccator and reweighed. The percentage ash content was calculated using the formula:

% Ash content = 
$$\frac{\text{Mass of ash}}{\text{mass of dried sample}} \times 100$$

This was done in triplicates.

### 2.9 Preparation of sample solutions

Ash of each sample was dissolved and filtered into 100 cm<sup>3</sup> volumetric flask with distilled – deionized water, made up to mark and labeled according to the procedure reported by  $^{50, 52}$ .

# 2.10 Atomic absorption spectrophotometric determination of heavy metals

This was done in line with the procedures reported by <sup>50, 52</sup>. Hollow cathode lamp of each heavy metal was fixed and its wavelength was set. The instrument

was switched on for fifteen minutes in order to stabilize it followed by the compressor to supply air at a regulated pressure. The fuel, acetylene was then on and regulated. The ignition control knob was pressed for flame to alight.

A blank, 0.0 mg/ L or ppm was introduced and aspirated into the flame. The blank control was adjusted to set zero absorbance or 100 % transmittance and working standards of the heavy metal in question were introduced and aspirated, agreeable absorbance readings were recorded against their concentrations (ppm). Similarly, sample solutions were respectively introduced, aspirated and their absorbance were recorded. Absorbance of samples and concentration of Cd, Cr and Cu in the samples were read up from the atomic absorption spectrophotometer's monitor.

### 2.11 Health risk assessment

This was done by adopting the procedure reported by <sup>53</sup>. Potential health risks associated with long term consumption of fish contaminated with heavy metals were assessed by calculating the average daily dose (ADD) of heavy metals, hazard index (HI), target hazard quotient (THQ) and non-carcinogenic risk (NCR). Table 4 shows the parameters that characterized the ADD.

$$ADD = \frac{Ci \times IR \times EF \times ED}{BW \times AT}$$
(1)

Where Ci is metal concentration in fish, IR is ingestion rate, EF is exposure frequency, ED is exposure duration, BW is body weight of consumer and AT is average time. The health risk was assessed in relation to its non-carcinogenic as well as carcinogenic effects based on the calculation of ADD estimates and defined toxicity according to the following relationships <sup>54-55</sup>.

Table 4. Input parameters to characterize ADD value

| Exposure parameters | Symbols | Units     | Value       |
|---------------------|---------|-----------|-------------|
| Concentration       | С       | mg / kg   | Table 3     |
| Ingestion rate      | IR      | g/day     | 2.2         |
| Exposure frequency  | EF      | days/year | 365         |
| Exposure duration   | ED      | Years     | 70          |
| Adult BW            | BW      | Kg        | 70          |
| Child BW            | BW      | Kg        | 16          |
| Average time        | AT      | Years     | 25,550 days |
| Source 54, 55       |         |           |             |

Source 54, 5

Estimation of non-carcinogenic risk of heavy metals consumption was determined using target hazard quotient values. Target hazard quotient is a ratio of the determined dose of a contaminant to oral reference dose considered detrimental. If the ratio is greater than or equal to 1, an exposed population is at risk. The non-carcinogenic risk of heavy metals was calculate using Eq. 2:

$$Hazard Quotients(HQ) = \frac{ADD}{RfD}$$
(2)

Where ADD is average daily dose and RfD is reference dose.

Hazard index is used to estimate the potential human health risk when more than one heavy metal is consumed. HI was calculated as the sum of HQs.

$$HI = (THQi + THQii + THQiii + THQn) \sum THQ$$
(3)

The individual metal toxicity responses (dose response) are  $5.0 \times 10^{-4}$  for Cd,  $3.0 \times 10^{-3}$  for Cr and  $4.2 \times 10^{-2}$  for Cu all in mg/kg/day as the Oral Reference Dose (RfD) <sup>54, 55</sup>. The risk assessments of a mixture of chemicals, the individual HQs are summed to form hazard index (HI): According to <sup>56</sup> an HI / HQ > 1 means an unacceptable risk of non-carcinogenic effects on health, whilst HI / HQ < 1 means an acceptable level of risk.

The ILCR for exposure to carcinogenic heavy metals was evaluated using the following equation:

### $ILCRm = EDIm \times CSFm$ (4)

Where  $CSF_m = Cancer Slope Factor (<math>\mu g/kg_{bw}/day$ )<sup>-1</sup> for each carcinogenic heavy metal according to <sup>57, 58</sup>.

The estimation of the risk due to exposure to multiple carcinogenic elements was determined by summing the individual ILCRs (Eq. 5)

$$ILCRtot = \sum m = 1nLTCRm$$
(5)

According to the <sup>59</sup>, an ILCR value greater than  $1 \times 10^{-4}$  indicates a high risk of developing cancer over a human lifetime, whereas values between  $1 \times 10^{-6}$  and  $1 \times 10^{-4}$  are considered an acceptable range for cancer risk. Health Canada instead suggests a lower value ( $1 \times 10^{-5}$ ) as the maximum safety threshold for cancer risk <sup>60</sup>.

# 2.12 Quality assurance

All apparatus/ glass wares were washed and rinsed with distilled – deionized water, all instruments were calibrated before use, analysis and measurements were done in replicates and, analytical weighing balance was used. Adequate precautions were also taken when handling samples to prevent cross-contamination.

### 3. RESULT AND DISCUSSION

#### 3.1 Moisture content

Tables 1 shows moisture content of wild and aquacultured cat, tilapia and croaker fish respectively.

Table 1. Moisture content of dried sampled fishes

| Samples | Moisture content (%) |  |
|---------|----------------------|--|
| Wcf     | 4.250±0.354          |  |
| Acf     | 7.010±0.230          |  |
| Wtf     | 5.250±0.453          |  |
| Atf     | 7.120±0.234          |  |
| Wcrf    | 4.500±0.000          |  |
| Acrf    | 6.700±0.210          |  |

Table notes:

Wcf = Wild cat fish Acf = Aquacultured cat fishs Wtf = Wild tilapia fish Atf = Aquacultured tilapia fish Wcrf = Wild croaker fish

Acrf = Aquacultured croaker fish

The data in Table 1 are graphically presented in Fig. 1.



Figure 1. Bar chart for % moisture content of the sampled fish

Result for moisture content presented in table 1 shows lower % moisture content in the wild cafish Wcf  $(4.250\pm0.354 \%)$  followed by wild croaker fish, Wcf  $(4.500\pm0.000 \%)$  and wild tilapia fish (Wtf) has the higher moisture content  $(5.250\pm0.453 \%)$  among the wild species of fish however, % moisture content in the sampled aquacultured fish species is high above those of the wild fish as the aquacultured catfish (Acf) contained  $7.010\pm0.230 \%$  moisture; aquacultured tilapia fish (Atf) containe  $7.120\pm0.234 \%$  and aquacultured croaker fish (Acrf)

contained 6.700±0.210 % moisture. Drying is removal of water from fish to such an extent where most of microbes, enzymes and moulds will be inactive and will not grow due to reduced moisture and water activity <sup>61</sup>. In general, <sup>61</sup> reported that moisture content of 15-20% is aimed but, water activity levels below 0.6 lead to complete restriction for microbial growth. Variation in moisture content among fish species is greatly dependent upon the quantity of fat in the body which could also be related to the feed availability of the fish <sup>62</sup>. Moisture content plays a significant role in the flow and other mechanical properties of the foods however, <sup>63</sup> is of the opinion that it all depends largely on the method, extent of drying and the humidity in the surrounding atmosphere. The trend of % moisture content in Table 1 is that moisture is higher in all aquacultred catfish, tilapia and croaker fish than their counterpart wild fish (Fig. 1) therefore, shelf life (4 – 6 months) of the wild catfish, tilapia and croaker fish would be higher than those of the aquaculture.

# 3.2 Ash content

Table 2 shows ash content of wild and aquacultured cat, tilapia and croaker fish.

### Table 2. Ash content of sampled fish

| Samples | Ash content (%) |  |
|---------|-----------------|--|
| Wcf     | 1.750±0.354     |  |
| Acf     | 1.860±0.354     |  |
| Wtf     | 1.640±0.231     |  |
| Atf     | 2.620±0.141     |  |
| Wcrf    | 1.480±0.213     |  |
| Acrf    | 1.950±0.141     |  |

Table 3. Concentration (mg/ Kg) of Cd, Cr, Cu and Pb in sampled fishes

The data in Table 2 are graphically presented in Fig. 2.



### Figure 2. Bar chart for % ash content of the sampled fish

Result for ash content presented Table 2 and Fig. 2 shows that among the wild fish, catfish has the highest ash content  $(1.750\pm0.354\%)$  followed by tilapia fish  $(1.640\pm0.231\%)$  and the croaker fish  $(1.480\pm0.213\%)$  on the other hand, aquacultured croaker fish has the highest ash content:  $1.950\pm0.141\%$  then catfish:  $1.860\pm0.354\%$  and the lowest in the tilapia fish:  $2.620\pm0.141$ . According to Uzma *et al.* (2018) the variation in the body ash content of these fish species may be attributed with the health condition and the availability of food in their respective feeding environment.

# 3.3 Concentration (mg/Kg) of Cd, Cr and Cu in wild and aquacultured sampled fish

Tables 3 shows concentration (mg/Kg) of Cd, Cr and Cu in wild and aquacultured cat, tilapia and croaker fish respectively.

| Sample | Results              | Cd   | Cr                            | Cu   |
|--------|----------------------|--|-------------------------------|--|
| Wcf    | Min.<br>Max.<br>Mean | $\begin{array}{c} 0.040 \\ 0.070 \\ 0.055 {\pm} 0.021 \end{array}$ | 0.430<br>0.470<br>0.450±0.028 | N1.450<br>2.500<br>1.975± 0.742                                    |
| Acf    | Min.                 | 0.083  | 4.790                         | 2.610  |
|        | Max.                 | 0.120  | 5.630                         | 3.420  |
|        | Mean                 | 0.102±0.026  | 5.210±0.594                   | 3.015±0.573  |
| Wtf    | Min.<br>Max.<br>Mean | 0.050<br>0.060<br>0.055±0.007                                      | 0.250<br>0.640<br>0.445±0.276 | $\begin{array}{r} 2.230 \\ 2.310 \\ 2.270 \pm \ 0.057 \end{array}$ |
| Atf    | Min.                 | 0.072  | 3.120                         | 2.410  |
|        | Max.                 | 0.810  | 4.450                         | 3.550  |
|        | Mean                 | 0.441±0.522  | 3.785±0.940                   | 2.980±0.806  |
| Werf   | Min.                 | 0.040  | 0.150                         | 2.300  |
|        | Max.                 | 0.060  | 0.540                         | 2.340  |
|        | Mean                 | 0.050±0.014  | 0.345±0.276                   | 2.320±0.028  |
| Acrf   | Min.                 | 0.092  | 4.830                         | 3.110  |
|        | Max.                 | 0.120  | 5.720                         | 3.820  |
|        | Mean                 | 0.106±0.019  | 5.275±0.629                   | 3.465±0.502  |
| WHO/FA | AO (2007)            | 0.02   | 5.0                           | 2.0 - 3.0  |

The data in Table 3 are graphically presented in Fig. 3.



**Figure 3a.** Bar chart for concentration (mg/Kg) cadmium (Cd), chromium (Cr) and copper (Cu) in wild and aquacultured cat, tilapia and croaker fish.



**Figure 3b.** Bar chart for concentration (mg/Kg) cadmium (Cd), chromium (Cr) and copper (Cu) in wild and aquacultured cat, tilapia and croaker fish.

Table 3 shows concentrations (mg/ Kg) of Cd, Cr and Cu (Fig. 3a and b) in sampled wild fish where catfish and tilapia fish are of higher concentration of Cd, 0.055±0.021 and 0.055±0.007 respectively while croaker fish contained 0.050±0.014 all of which are about 0.03 mg/Kg above the WHO/FAO (2007) permissible level (0.02 mg/Kg) and, in the sampled aquacultured fish species, concentration of Cd far outweighed the threshold of WHO/FAO (2007), 0.02 mg/Kg in the order: Atf (0.441±0.522 mg/Kg) > Acrf (0.106±0.019 mg/Kg) > Acf (0.102±0.026 mg/Kg). These might pose health risk to consumers. No known beneficial function of cadnium in the human body (Genchi et al., 2020; Sinicropi et al., 2010; Friberg et al., 2019) however, it is a cumulative toxin (Chen et al., 2014; Lane et al., 2015; Oladipo et al., 2016). Concentration (mg/Kg) of Cr in the wild fish species range from 0.345±0.276 - 0.450±0.028 in the order of Wcf (0.450±0.028 mg/Kg) > Wtf (0.445±0.276 mg/Kg) > Wcrf (0.345±0.276 mg/Kg) all of which are below the WHO/FAO (2007) permissible level (5.0 mg/Kg) but Cr is quit high in the aquacultured fish species ranging from 3.785±0.940 - 5.275±0.629 mg/Kg in the order of Acrf (5.275±0.629 mg/Kg) > Acf (5.210±0.594 mg/Kg) > Atf (3.785±0.940 mg/Kg) all except in Atf are above the WHO/FAO (2007) threshold. Wilbur et al. (2012); Liu et al. (2018); Sharma et al. (2011) reported that chromium is an essential nutrient required for normal energy metabolism nevertheless, human health is adversely affected due to the exposure of chromium (Guertin, 2004) and certain effects of Cr like mouth ulcers, indigestion, acute tubular necrosis, vomiting, abdominal pain, kidney failure and even death have been reported by Beaumont et al., 2008). However, on Cu in wild fish species, it was higher in croaker fish, 2.320±0.028 mg/Kg followed by tilapia, 2.270±0.057 mg/Kg and low in cat fish, 1.975±0.742 mg/Kg relative to the WHO/ FAO (2007) permissible specification, 2 - 3 mag/Kg in contrast, Cu is relatively high in aquacultured catfish and croaker fish: 3.015±0.573 mag/Kg and 3.465±0.502 mag/Kg respectively but within the threshold in aquacultured tilapia fish, 2.980±0.806 mag/Kg. Importance of copper to humans have been widely reported among which are a study carried out by Bonham et al. (2002); Uriu-Adams and Keen (2005) whose findings are that Cu appears to have many important functional roles in body that apparently relate, among others, to the maintenance of immune function, bone health and haemostasis also, Bost et al. (2016); Pham et al. (2013) have it that copper is a vital micronutrient for humans, but an excess of it in the body, particularly in cells, can lead to cytotoxicity emphasizing that the free hydrated form (such as  $Cu^{2+}$ ) has the potential to be toxic by altering membrane permeability and protein synthesis, as well as various enzymatic activities and, Royer and Sharman (2023) added that copper metabolism plays an important role in physiologic homeostasis however, its toxicity induces several pathologic processes that are detrimental to human health.

Higher levels of Cd, Cr and Cu in the analysed aquacultured fish species is probably due to either their high levels in the raw materials (the corns or grains) used for producing/ formulating their feeds or as a result of fortification of feeds with these metal for medicinal and nutritional reasons and the ponds source of water might be polluted or contaminated with the metals. In general, the results indicate that the levels of Cd, Cr, and Cu in the sampled fish species vary significantly. Aquacultured fish tend to have higher concentrations of these metals compared to their aquacultured counterparts. This disparity is attributed to differences in habitat, diet, and exposure to industrial pollutants. The bioaccumulation of these metals poses a risk to consumers, as prolonged intake can lead to adverse health effects. The potential health risks were assessed using established guidelines and parameters, revealing that some levels exceed safe consumption thresholds, particularly for vulnerable populations such as children and pregnant women.

### CONCLUSION

The significance of monitoring heavy metal levels in wild and aquacultured fish is emphasized by this research. The results indicate that although wildcaught fish may present a safer option in terms of heavy metal exposure compared to aquacultured fish, it is important to remain vigilant to ensure that contamination levels stay within safe boundaries. The potential health dangers of consuming fish tainted with Cd, Cr, and Cu underscore the necessity for stricter regulatory actions and public awareness initiatives to reduce possible health risks

# ACKNOWLEDGEMENT

The Gwagwalada Area Council, Federal Capital Territory, Nigeria, is much appreciated by the authors for their collaboration and assistance during this study. We are especially grateful to the local fish markets in Dobi, Gwagwalada, and Zuba for allowing us to use their samples for our study. Additionally, we are grateful for the appropriate institutions' technical support and lab space, which were crucial to the accomplishment of this work. Finally, we would like to express our gratitude for the advice and support we received from our mentors and colleagues, whose knowledge and perspective were invaluable in ensuring the caliber of this study.

#### REFERENCES

- 1. L. Järup, Hazards of heavy metal contamination. Br Med Bull, vol. 68, no. 1, pp. 167–182. (2003). [PubMed][Google Scholar]
- A. Hazrat, K. Ezzat, what are heavy metals? Long standing controversy over the scientific use of the term 'heavy metals' - proposal of a comprehensive definition. Toxicological & EnvironmentalChemistry. DOI:10.1080/02772248.2017.1413652.https://doi.org/10.1080/02772248.20 17.14136. (2017).
- M. A. Barakat, New Trends in Removing Heavy Metals from Industrial Wastewater. Arabian Journal of Chemistry, vo. 4 no.4, pp. 361-377. doi: 10.1016/j.arabjc.2010.07.019. (2011).
- C. H. Walker, R. M. Sibly, S. P. Hopkin, D. B. Peakall, Principles of Ecotoxicology', 4th ed. Boca Raton: CRC Press. (2012).
- P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, D. J. Sutton, Heavy metal toxicity and the environment. Experientia supplementum, vol.101, no. 1, pp. 133 - 164. <u>https://doi.org/10.1007/978-3-7643-8340-4\_6</u>. (2012).
- K. J. Appenroth, Definition of "heavy metals" and their role in biological systems. Journal of Soil Heavy Metals. pp.19 - 29. (2010).
- E. M. Dallatu, E. E. Jude, A. U. Atumeyi, O. O. Ernest, E. Ibrahim, Determination and Health Risk Assessment of Cd, Cr AND Cu in Wild *Clarias gariepinus* (Catfish), *Oreochromis niloticus* (Tilapia fish) and *Micropogonias undulatus* (Croaker Fish) Sold in Gwagwalada Area Council, FCT Nigeria. (2024).
- S. Morais, F. G. Costa, P. M. de Lourdes, Heavy Metals and Human Health. In Environmental Health - Emerging Issues and Practice', In Tech. <u>https://doi.org/10.5772/29869.(2012).</u>

- M. Jaishankar, T. Tseten, N. Anbalagan, B. B. Mathew, K. N. Beeregowda, Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary toxicology, vol. 7, no. 2, pp. 60 - 72. <u>https://doi.org/10.2478/intox-2014-0009</u>. (2014).
- K. H. Hama Aziz, F. S. Mustafa, K. M. Omer, S. Hama, R. F. Hamarawf, K.O. Rahman, Heavy metal pollution in the aquatic environment: efficient and low - cost removal approaches to eliminate their toxicity: a review. RSC advances, vol. 13, no. 26, pp. 17595 - 17610. https://doi.org/10.1039/d3ra00723e.(2023).
- 11. M. Zaynab, R. Al-Yahyai, R, A. Ameen, Y. Sharif, L. Ali, M. Fatima, K. A. Khan, S. J. Li, King Saud Univ. Sci. 34:101653. [Google Scholar]. (2022).
- A. Singh, A. Sharma, K. Verma, R. L. Chopade, R. P. Pandit, P. Nagar, M. Sankhla, Heavy Metal Contamination of Water and Their Toxic Effect on Living Organisms. IntechOpen, doi: 10.5772/intechopen.105075 (2022).
- K. Nath, S. Shyam, D. Singh, Y. K. Shanna, Effect of chromium and tannery effluent toxicity on metabolism and growth in cowpea (Vigna sinensis L. Saviex Hassk) seedling. Res Environ Life Sci., vol.1, no.1, pp. 9 - 94. [Google Scholar]. (2008).
- 14. M. F. Hughes, B. D. Beck, Y. Chen, A. S. Lewis, D. J. Thomas, Arsenic exposure and toxicology: a historical perspective. *Toxicological sciences: an* official journal of the Society of Toxicology, 123(2), 305–332. https://doi.org/10.1093/toxsci/kfr184. (2011).
- K. E. Nachman, P. A. Baron, G. Raber, K. A. Francesconi, A. Navas-Acien, D. C. Love, Roxarsone, inorganic arsenic, and other arsenic species in chicken: a U.S.-based market basket sample. Environmental health perspectives, vol. 121, no. 7, pp. 818 - 824. <u>https://doi.org/10.1289/ehp.1206245</u>. (2013).
- 16. X. Chen, K. Wang, Z. Wang, C. Gan, P. He, T. Liang, T. Jin, G. Zhu, Effects of lead and cadmium co-exposure on bone mineral density in a Chinese population Bone. vol. 63, nol. 1, pp. 76-80, <u>10.1016/j.bone.2014.02.017</u>. (2014).
- A. Ghani, Effect of chromium toxicity on growth, chlorophyll and some mineral nutrients of *Brassica juncea* L. Egyptian Acad J Biol Sci, vol. 2, no. 1, pp. 9 - 15. [Google Scholar]. (2011).
- A. Mutlu, B. K. Lee, G. H. Park, B. G. Yu, C. H. Lee, Long-term concentrations of airborne cadmium in metropolitan cities in Korea and potential health risks. Atmos Environ, vol. 47, no. 1, pp. 164 - 173. [Google Scholar]. (2012).
- S. Kumar, Occupational and Environmental Exposure to Lead and Reproductive Health Impairment: An Overview. Indian journal of occupational and environmental medicine, vol. 22, no. 3, pp. 128 - 137. <u>https://doi.org/10.4103/ijoem.IJOEM12618</u>. (2018).
- 20. M. T. Hayat, M. Nauman, N. Nazir, S. Ali, N. Bangash, Environmental hazards of cadmium: Past, present, and future. In Cadmium Toxicity and Tolerance in Plants. Elsevier: Amsterdam, The Netherlands, pp. 163 - 183. [Google Scholar]. (2019).
- 21. M. Wang, Z. Chen, W. Song, D. Hong, L. Huang, Y. Li, A review on cadmium exposure in the population and intervention strategies against cadmium toxicity. Bull. Environ. Contam. Toxicol. 106, 65–74. [Google Scholar] [CrossRef] [PubMed]. (2021).
- 22. T. S. Nawrot, J. A. Staessen, H. A. Roels, E. Munters, A. Cuypers, T. Richart, A. Ruttens, K. Smeets, H. Clijsters, J. Vangronsveld, Cadmium exposure in the population: From health risks to strategies of prevention. Biometals, vol. 23, no. 1, pp. 769 - 782. [Google Scholar] [CrossRef][PubMed]. (2010).
- 23. R. M. Rafati, S. Kazemi, A. A. Moghadamnia, Cadmium toxicity and treatment: An update. *Caspian journal of internal medicine*, 8(3), 135-145. <u>https://doi.org/10.22088/cjim.8.3.135</u>. (2017).
- 24. D. Krewski, R. A. Yokel, E. Nieboer, D. Borchelt, J. Cohen, J. Harry, V. Rondeau, Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. JJ Toxicol Environ Health B Crit Rev, vol. 10, no. 1, pp. 1 269. [PMC free article] [PubMed] [Google Scholar]. (2009).
- WHO, Aluminium; Geneva: World Health Organization, International Programme on Chemical Safety Environmental Health Criteria, 194. [Google Scholar]. (1997).
- 26. J. Matschullat, Arsenic in the geosphere a review. Sci Total Environ, vol. 249, no. 1 - 3, pp. 297 - 312. [PubMed] [Google Scholar. (2000).
- A. H. Smith, E. O. Lingas, M. Rahman, Contamination of drinking water by arsenic in Bangladesh: a public health emergency. Bull World Health Organ, vol. 78, no. 9, pp. 1093 - 1103. [PMC freearticle] [PubMed] [Google Scholar]. (2000).

- M. A. Hoque, W. G. Burgess, M. Shamsudduha, K. M. Ahmed, Delineating low-arsenic groundwater environments in the Bengal Aquifer System, Bangladesh. Appl Geochem, vol. 26, no. 4, pp. 614 - 623. [Google Scholar]. (2011).
- M. C. Henson, P. J. Chedrese, Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. Exp Biol Med (Maywood) 2004;229(5):383-392. [PubMed] [Google Scholar]. (2018).
- 30. S. Chakraborty, A. R. Dutta, S. Sural, D. Gupta, S. Sen, Ailling bones and failing kidneys: a case of chronic cadmium toxicity. Ann Clin Biochem, vol. 50, no. 5, pp. 492 - 495. [PubMed] [Google Scholar]. (2013).
- 31. National Toxicology Program, 15th Report on Carcinogens [Internet]. Research Triangle Park (NC): National Toxicology Program. Chromium Hexavalent Compounds: CAS No. 18540-29-9. Available from: <u>https://www.ncbi.nlm.nih.gov/books/NBK590757/</u>. (2021).
- 32. J. Emerit, C. Beaumont, F. Trivin, Iron metabolism, free radicals, and oxidative injury. Biomed Pharmacother, vol. 55, no. 6, pp. 333 - 339. doi: 10.1016/s0753-3322(01)00068-3. PMID: 11478586. (2001).
- J. Albretsen, The toxicity of iron, an essential element. Veterinary medicine. pp. 82–90. [Google Scholar]. (2006).
- 34. M. Markowitz, Lead Poisoning. Pediatr Rev, vol. 21, no. 10, pp. 327 335. [PubMed] [Google Scholar]. (2000).
- 35. ATSDR, Toxicological Profile for Chromium (Final Report). NTIS Accession No. PB2000-108022. Atlanta, GA: Agency for Toxic Substances and Disease Registry. 455 pp. (2000).
- 36. FAO/WHO, Guidelines for drinking-water quality. Sixty-first meeting, 10– 19 June 2003, Rome. 2004, Joint FAO/WHO Expert Committee on Food Additives. Available from <u>http://ftp.fao.org/es/esn/jecfa/jecfa61sc.pdf</u>) [Google Scholar]. (2007).
- European Commission, Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (OJ L 364, 20.12.2006, p. 5). (2018).
- K. <u>Avery</u>, Ranked: The World's Fastest Growing Cities. Retrieved 5.06, August, 2024, from <u>https://www.visualcapitalist.com/ranked-the-worlds-fastest-growing-cities/</u>. (2021).
- 39. Wikipedia contributors, Gwagwalada In Wikipedia, The Free Encyclopedia', Retrieved 07:51, July 12, 2024, from <u>https://en.wikipedia.org/w/index.php?title=Gwagwalada&oldid=120823502</u> <u>3</u>. (2024).
- 40. S. B. Zarith, Y. I. Moh, Determination of Heavy Metal Accumulation in Fish Species in Galas River, Kelantan and Beranang Mining Pool, Selangor. International Conference of Environmental Forensics, Procedia Environmental Sciences, vol. 30, no. 1, pp. 320 – 325. (2015).
- N. <u>Cevat, T. Goknur</u>, Determination of Heavy Metal Levels in Fish Samples Collected from the Middle Black Sea. Kafkas Universitesi Veteriner Fakultesi Dergisi, vol. 16, no. 1, pp. 119-125. (2010).
- 42. M. O. <u>Yazkan</u>, Z. <u>Feramuz</u>, G. <u>Muharrem</u>, Cu, Zn, Pb and Cd content in some fish species caught in the Gulf of Antalya. Turkish Journal of Veterinary and Animal Sciences, vol. 26, no. 6, pp. 1309 - 1313. (2002).
- 43. G. I. Onwuka, Food Analysis and Instrumentation: Theory and Practice. Naphthali Print, Lagos, pp. 133 - 137. (2005).
- 44. A. H. Boyd, Principles and Methods of Moisture Measurement. Seed Technology Papers, p.132. https://scholarsjunction.msstate.edu/seedtechpapers/132. (2021).
- 45. J. Y. Ahn, D. Y. Kil, C. Kong, B. G. Kim, Comparison of Oven-drying Methods for Determination of Moisture Content in Feed Ingredients. Asian-Australasian journal of animal sciences, vol. 27, no. 11, pp. 1615-1622. https://doi.org/10.5713/ajas.2014.14305. (2014).
- 46. C. J. Ibeabuchi, E. Bede, N. O. <u>Kabuo</u>, O. Chigozie, Proximate composition, functional properties and oil characterization of 'Kpaakpa' (Hildegardia barteri) seed. vol. 5, no. 1, pp. 16 - 29. DOI: <u>10.31248/RJFSN2019.079</u>. (2020).
- 47. O. A. Olopade, I.O. Taiwo, A. A. Lamidi, O. A. Awonaike, Proximate Composition of Nile Tilapia (*Oreochromis niloticus*) (*Linnaeus, 1758*) and Tilapia Hybrid (Red Tilapia) from Oyan Lake, Nigeria. Bulletin UASVM Food Science and Technology, vol. 73, no. 1, ISSN-L 2344-2344; Print ISSN 2344-2344; Electronic ISSN 2344-5300. DOI: 10.15835/buasvmcnfst:11973. (2016).
- 48. A. O. Abimbola, O.Y. Kolade, A.O. Ibrahim, C. E. Oramadike, P. A. Ozor, Proximate and Anatomical Weight Composition of Wild Brackish Tilapia

guineensis and Tilapia melanotheron. Journal of Food Safet, vol.12, no. 1, pp. 100-103. (2010).

- 49. S. Ande, L. Leke, I. Eneji, S. Yakubu, Proximate analysis of smoked and unsmoked fish (cat and tilapia) in Ombi River Lafia Nasarawa State Nigeria. Elixir Food Science, vol. 53, no. 1, pp. 11801- 11803. (2012).
- 50. A. A. Ibitoye, Laboratory Manual of Basic Methods in Analytical Chemistry. 1st Edition, Concept + IT and Educational Consult, Akure. (2005).
- 51. B. <u>Nabil</u>, Sample preparation for flame atomic absorption spectroscopy: An overview. RASAYAN J. Chem., vol.4, no.1, pp. 49 55. <u>http://www.rasayanjournal.com</u>. (2011).
- 52. C. H. Unaeze, Y.A. Onmonya, D. Ebi, H. O. Yusuf, Heavy Metal Contamination in Selected Fishes from Ojo, Lagos Nigeria. Environ. Stud. J., vol. 2, no. 1, pp. 1 - 13. <u>https://doi.org/10.36108/esj/3202.20.0110</u>. (2023).
- 53. S. T. Ametepey, S. J. Cobbina, F. J. Akpabey, A. B. <u>Duwiejuah</u>, Z. N. <u>Abuntori</u>, Health risk assessment and heavy metal contamination levels in vegetables from Tamale Metropolis Ghana. <u>InternationalJournal of Food</u> <u>Contamination</u>, vol. 5. <u>https://doi.org/10.1186/s40550-018-0067-0</u>. (2018).
- 54. USEPA IRIS, US Environmental Protection Agency's integrated risk information system. Environmental protection agency region I. Washington DC 20460. US EPA. <u>http://www.epa.gov/iris/</u>. (2011).
- 55. P. Wongsasuluk, S. Chotpantarat, W. Siriwong, M. Robson, Heavy metal contamination and human health risk assessment in drinking water from shallow groundwater wells in an agricultural area in Ubon Ratchathani province, Thailand. Environ Geochem Health, vol. 2014, no. 36, pp. 169 -182. <u>Article CAS PubMed Google Scholar</u>. (2014).
- 56. H. S. Lim, J.S. Lee, H. T. Chon, M. Sager, Heavy metal contamination and health risk assessment in the vicinity of the abandoned Songcheon au-ag mine in Korea. J Geochem Explor. 96:223–30. <u>Article CAS Google</u> <u>Scholar</u>. (2008).
- 57. H. R. Gebeyehu, L. D. Bayissa, Levels of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia PLoS One. vol. 15, no. 1. e0227883. doi: 10.1371/journal.pone.0227883. PMID: 31999756; PMCID: PMC6992214.
- J. K. Nduka, H. I. Kelle, J. O. Amuka, Health Risk Assessment of Cadmium, Chromium and Nickel from Car Paint Dust from Used Automobiles at Auto-Panel Workshops in Nigeria. Toxicol. Rep. (6) 449 - 456. [Google Scholar] [CrossRef]. (2019).
- **59.** United States Environmental Protection Agency (USEPA), Risk-based concentration table. Washington, DC. (2011).
- 60. Health Canada, Federal Contaminated Site Risk Assessment in Canada, Part V: Guidance on Human Health Detailed Quantitative Risk Assessment for Chemicals (DQRACHEM). (2010).
- 61. A. Jeyakumari, L. GirijaBehere, M. Narasimha, S. J. Laly, Preparation of Dried Fishery Products. Mumbai Research Centre of ICAR-Central Institute of Fisheries Technology, Navi Mumbai. <u>https://krishi.icar.gov.in/jspui/bitstream/123456789/70509/1/23.pdf</u>. (2016).
- 62. S. Uzma, A. Imtia, R. Raiees, Estimation of proximate composition (moisture and ash content) of some economically important fishes of the valley. International Journal of Advanced Research in Science and Engineering, vol.7, Special Issue NO. 4. ISSN: 2319 - 8354. p. 2052. <u>www.ijarse.com</u>. (2018).
- 63. W. L. Lawal, E. O. Idega, Analysis of fish marketing in Benue state. A paper presented at 2001 Annual Conference of Association of Agricultural Economists (NAAE) Held at A.B.U Zaria. pp. 3-5. (2004).
- 64. G. Genchi, M. S. Sinicropi, G. Lauria, A. Carocci, A. Catalano, The Effects of Cadmium Toxicity. International journal of environmental research and public health, vol. 17, no. 11, p. 3782. <u>https://doi.org/10.3390/ijerph17113782</u>. (2020).
- 65. M. S. Sinicropi, D. Amantea, A. Caruso, C. Saturnino, Chemical and biological properties of toxic metals and use of chelating agents for the pharmacological treatment of metal poisoning. Arch. Toxicol, vol. 84, no. 1, pp. 501 - 520. doi: 10.1007/s00204-010-0544-6. [Google Scholar]. (2010).
- 66. L. T. Friberge, G. G. Elinder, T. Kjellstrom, G. F. Nordberg, Cadmium and Health: A Toxicological and Epidemiological Appraisal. vol. 2, Effects and Response, vol. 1, CRC Press; Boca Raton, FL, USA. [Google Scholar]. (2019).
- 67. E. A. Lane, M. J. Canty, S. J. More, Cadmium exposure and consequence for the health and productivity of farmed ruminants. Research in veterinary Science, vol. 101, no. 1, pp. 132 - 139. (2015).

- 68. O. O. Oladipo, J. O. Ayo, S. F. Ambali, B. Mohammed, Evaluation of hepatorenal impairments in Wistar rats coexposed to low-dose lead, cadmium and manganese: insights into oxidative stress mechanism. Toxicology mechanisms and methods, vol. 26, no. 9, pp., 674 - 684. (2016).
- 69. S. Wilbur, H. Abadin, M. Fay, Toxicological Profile for Chromium. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US). Relevance to Public Health. Available from: https://www.ncbi.nlm.nih.gov/books/NBK158854/. (2012).
- 70. L. Liu, S. W. Zhang, J. Lu, X. Y. Pang, J. P. Lv, Antidiabetic effect of highchromium yeast against type 2 diabetic KK-Ay mice. Journal of food science, vol. 83, no. 7, pp. 1956 -1963. (2018).
- 71. S. Sharma, R. P. Agrawal, M. Choudhary, S. Jain, S. Goyal, V. Agarwal, Beneficial Effect of Chromium supplementation on glucose, HbA1C and lipid variables in individuals with newly onset type-2 diabetes. Journal of Trace Elements in Medicine and Biology, vol. 25, no. 3, pp. 149 - 153. (2011).
- J. Guertin, Toxicity and Health Effects of Chromium (All Oxidation States). p. 213. (2004).
- 73. J.J. Beaumont, R. M. Sedman, S. D. Reynolds, C. D. Sherman, L. H. Li, R. A. Howd, M. S. Sandy, L. Zeise, G. V. Alexeeff, Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. Epidem, vol. 19, no. 1, pp. 12-23. (2008).
- 74. M. Bonham, J. M. O'Connor, B. M. Hannigan, J. J. Strain, 2002. The immune system as a physiological indicator of marginal copper status. British Journal of Nutrition, vol. 87, nol. 5, pp. 393 - 403. (2008).
- J. Y. Uriu-Adams, C. L. Keen, Copper, oxidative stress, and human health', Molecular aspects of medicine, vol. 26, no. 4-5, pp. 268 - 298. (2005).
- 76. M. Bost, S. Houdart, M. Oberli, E. Kalonji, J. F. Huneau, I. Margaritis, Dietary copper and human health: current evidence and unresolved issues. Journal of Trace Elements in Medicine and Biology, vol. 35, nol. 1, pp. 107– 115. <u>Publisher Site</u> | <u>Google Scholar</u>. (2016).
- 77. A. N. Pham, G. Xing, C. J. Miller, Waite, Fenton-like copper redox chemistry revisited: hydrogen peroxide and superoxide mediation of copper-catalyzed oxidant production. Journal of Catalysis, vol. 301, no. 1, pp. 54 - 64. View at: <u>Publisher Site | Google Scholar</u>. (2013).