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# Contamination and Dietary Intake Risks Assessment of Heavy Metals in Some Species of Wild Edible Mushrooms Grown in Southern Nigeria

**Uche G. Nwokeke**[1] and **Christian Ebere Enyoh**[2]

Department of Chemistry, Faculty of Physical Sciences, Imo State University, Owerri, Nigeria

[1,2]E-mail address: nwokekeug@gmail.com , cenyoh@gmail.com

**ABSTRACT**

In this study, wild edible mushroom collected from Rivers and Imo state Nigeria were evaluated for heavy metals and dietary intake risks assessment. Four species of edible mushrooms were collected each in Imo (*Lentinus squarrosulus*, *Pleurotus ostreatus*, *Lenziles betulina* and *Grifola polypilus frondosa*) and River state (*Lentinus squarrosulus*, *Pleurotus tuber-regium*, *Lenziles betulina*, *Auricularia aurucula*) and metals extracted using $HNO_3$ before analysis using flame atomic absorption spectrometry (F-AAS) in Varian AA 240 FS apparatus. Results showed that all metals showed concentrations lower than the permissible limits of World Health Organization (WHO) and Food and Agricultural Organization (FAO) while Pb was not detected in all samples analyzed and Cd in samples collected from Rivers state. However, Cd was only detected (0.003 mg/kg) in one sample (*Lenziles betulina)* collected from Imo state. Fe exceeded recommended values in *Lentinus squarrosulus*, *Pleurotus ostreatus* and *Lenziles betulina* in Imo State. Bioaccumulation factors for all mushrooms were generally low (< 3). However, the potential tolerable weekly intake and target hazard quotient values for the four mushroom species were usually extremely low except for Fe (2.24) in *Lenziles betulina*. Consumption of mushrooms from these areas might pose no potential risk in terms of heavy metals except for Fe content in *Lenziles betulina* from Rivers state. However, in general, it can be concluded that the consumption of the studied mushroom species from all sites does not present any health risk arising from their regular consumption.

***Keywords*****:** Bioaccumulation, Hazard qoutient, Mineralization, Mushroom fruiting body Nigeria, Trace elements

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## 1. INTRODUCTION

Heavy metals are pervasive environmental components that may be attributed to either natural or human sources of origin. It is becoming more apparent that heavy metal pollution is a major issue in contemporary civilization, and it is currently considered to be one of the most severe challenges facing modern society. It is because of their significant persistence, toxicity, and propensity to bioaccumulate into environmental components and, as a result, into the food chain that they are considered to be hazardous (Burges et al., 2015; Isiuku and Enyoh, 2020).

Long-term industrialisation and subsequent fast urbanization increase xenobiotics and therefore heavy metals in the urban environment, endangering the global ecosystems and human health (Záhorcová et. al., 2016).

Certain heavy metals (Hg, Cd, Cr, Ni, Pb), arsenic, and critical trace elements (Cu, Zn) represent a major danger to the environment's quality, which has an effect on human health (Isiuku and Enyoh, 2020). They introduced into the environment through natural (volcanic, weathering, etc.) and human processes (e. g. extraction and processing of minerals, combustion of fossil fuels and waste, etc.).

Cadmium and lead are non-essential trace elements that are hazardous to plants, animals, and humans at low quantities. They enter the body mostly via inhalation and/or resorption, causing harm to certain organ systems (Timoracká et al., 2011). But large levels of heavy metals may enter the body via diet. Zinc and copper are important trace metals, yet excessive quantities may be harmful to humans. They regulate inflammatory and oxidative processes, among others (Mocchegiani et al., 2012). For example, high copper concentrations impair CNS function and some physiological processes (Záhorcová et. al., 2016).

Ecosystems depend on edible wild mushrooms to cycle elements and organic matter (Petkovek and Pokorny, 2013; Nnorom et. al., 2020). They may, together with microorganisms, biodegrade substrate and therefore use agricultural and/or human waste (Záhorcová et. al., 2016). Many nations, particularly those in Africa, consider certain mushroom species delicacies. Mushroom fruiting bodies are loved for their texture, taste, and nutritional value (Nnorom et. al., 2020). They are low in calories and rich in vital physiologically important elements, -glucans, and antioxidants (Keles et. al., 2011; Kalac, 2013). Humans get fiber, essential nutrients including thiamin, riboflavin, vitamin D and calcium from them (Wang et al., 2014; Shasho, 2020; Okigbo et al., 2021).

Mushrooms have long been recognized to collect significant quantities of heavy metals, making them suitable for biomonitoring environmental pollution, especially forest ecosystem contamination (Záhorcová et. al., 2016; Nnorom et. al., 2020). Metals in mushrooms are influenced by several variables including temperature, ambient circumstances, and macromolecule content in the cell wall of each species (Ostos et al., 2015). As opposed to higher plants, mushrooms have many times the bioaccumulation potential of xenobiotics - heavy metals from the soil (intake from the atmosphere is minimal) (Saba et al., 2015).

Even though the soil contaminant level is modest, some edible mushroom species may contain significant levels of heavy metals. Alonso et al. (2003) discovered that the hymenophore has the greatest amounts of trace elements, followed by the spores and then the stem. The aim of the paper is to determine the level and health risks of studied heavy metals (Fe, Cu, Zn, Pb and Cd) from the substrate into the aboveground parts of edible macroscopic mushrooms collected in Southern Nigeria.

## 2. MATERIALS AND METHOD

### 2. 1. Description of sampling area

Imo and Rivers are two of the regions researched in the Niger Delta, which is located in the southern portion of Nigeria. Geographically, these locations are located in latitude 4.8156° N, longitude 7.0498° E, and latitude 5.4891° N, longitude 7.0176° E, respectively. These two towns are among the most populous in Nigeria's Niger Delta region, which is renowned for its petroleum sector and its attendant environmental effects. The research site has a sub-equatorial environment with two distinct seasons: dry and rainy. April marks the start of the wet season, which peaks in June, July, and August and concludes in October. The dry season lasts from October to March. However, owing to uncertainty in global climate events, the time of these seasons is changing today. High rainfall events are common in the region, with mean values ranging from 2,200 to 4,000 mm and a net recharge of approximately 2,270 mm (Ologunorisa and Ogobonaye 1999; Muze et al. 2020). Approximately 87 percent of these rainstorms occur between May and October (Okoro et al. 2014). Because of the steep slopes in the catchment region, the frequency and severity of the rainfalls result in increased surface overflow. Heavy rainstorms are often linked with forceful rainstorms, resulting in severe flooding and significant sheet erosion. This scenario leads to pollutant penetration into water aquifers as well as surface runoff contamination of streams and rivers (Ibe et al. 2019).

### 2. 2. Sample collection, preparation and pre-analytical procedure

Four species of edible mushrooms were collected each in Imo (*Lentinus squarrosulus*, *Pleurotus ostreatus*, *Lenziles betulina* and *Grifola polypilus frondosa*) and River state (*Lentinus squarrosulus*, *Pleurotus tuber-regium*, *Lenziles betulina*, *Auricularia aurucula*). After collecting the mushroom samples, organic and inorganic debris was physically removed using a ceramic knife, and the cap (hymenophore) was separated from the remainder of the fruit body. The samples were then cut and dried at 45 °C to weight. The dry samples were homogenized in a porcelain mortar and bagged. 5 $cm^3$ concentrated $HNO_3$ and the same volume deionized water mineralized 1 g dry mushroom samples. The sample was then filtered via filter paper and deionized water filled to 50 $cm^3$.

### 2. 3. Analytical procedure

Quantitative determination of the concentration of the studied trace elements (Fe, Cu, Zn, Pb and Cd) was carried out in the mineralized samples by flame atomic absorption spectrometry (F-AAS) in Varian AA 240 FS apparatus (Varian, Australia).

### 2. 4. Statistical analysis and risk assessment

All data on the concentration of the studied contaminants in the samples were processed by descriptive statistical analysis using mean values and standard deviation in Microsoft Excel 2013 (Microsoft, USA).

#### 2. 4. 1. Evaluation of mushrooms' mineral biocontamination potential

The ability of mushrooms to absorb and accumulate the elements detected in their flesh was determined using the bio-contamination factor (BCF), which is the ratio of the element concentration in mushroom fruiting bodies to that of a standard:

$$BCF = \frac{\text{Concentration of element in mushroom}}{\text{Concentration of element in Reference}} \quad (1)$$

For BCF values < 1 are low contamination, 1≤BCF<3 are moderately contaminated, 3≤ BCF ≤ 6 are considerably contaminated and BCF ≥ 6 very highly contaminated.
The pollution load index (pPLI) can then be computed as the nth root of the product of individual BCf, presented in equation (2). When PLI > 1, the mushroom is polluted by metal and not polluted when PLI < 1.

$$PLI = (BCF_1 \times BCF_2 \times BCF_3 \ldots \ldots BCF_n)^{1/n} \quad (2)$$

#### 2. 4. 2. Assessment of potential health risk from mushroom consumption

To evaluate the non-carcinogenic health risk associated with trace metal consumption from mushrooms, target hazard quotients (THQ) were calculated. The THQ is often used to determine the risk of metal poisoning from consuming contaminated food. The United States Environmental Protection Agency (USEPA) recommended THQ (2002). THQ greater than 1 indicates that the user has been exposed to a risk that may have a negative outcome. This study evaluated the non-cancerous risk of trace metal exposure from mushroom consumption using THQ (a ratio of the estimated dose of contaminant metal and the reference dose below which there will not be any appreciable risk). The equation below summarizes the methodology used to assess THQ (Isiuku and Enyoh, 2020).

$$THQ = \frac{\text{Mc} \times \text{IR} \times \text{EF} \times \text{ED} \times 0.001}{\text{RfD} \times \text{BW} \times \text{ATn}} \quad (3)$$

where THQ is for non-carcinogenic risk, *M*C is the metal concentration in mushroom (mg/kg dry weight). IR is the mushroom ingestion rate (g/person/day), assuming 0.5 g for an average level consumer (Nnorom et. al., 2020). EF is the exposure frequency (365/days/year). ED is the exposure duration (70 years), equivalent to the average lifetime. RfD is the reference dose of individual metal: Cd (1.00), Fe (7.00), Zn (3.00), Cu (4.00), Pb (0.00035) (mg/kg/day). BW is an average body weight of 60 kg. ATn is the average exposure time for non-carcinogens (365 days/year ×ED). An index more than 1 is considered not safe (USEPA, 2002) indicating a likely negative impact on the consumers' health. The higher the THQ, the higher the chances of risk to the consuming population.

#### 2. 4. 3. Estimated weekly intake (EWI)

The estimated weekly intake (EWI) was calculated to estimate the potential hazard from the consumption of a meal of mushrooms using the formula:

$$EWI = \frac{\text{Mc} \times \text{Consumption rate}}{\text{BW}} \quad (4)$$

The weekly intake of metals via mushroom eating was estimated by multiplying the average weekly consumption of 210 g by the average concentration of the target analyte (metal) in the mushroom and then dividing by the average body weight of 60 kg (Nnorom et. al., 2020).

The findings were compared to the FAO/WHO provisional tolerable weekly intake (PTWI) for metals.

## 3. RESULTS AND DISCUSSION

### 3. 1. Distribution of heavy metals

The concentrations of heavy metals in the wild edible mushrooms are presented in Table 1. The concentrations were compared to permissible limits for foods provided by World Health Organization (WHO) and Food and Agricultural Organization (FAO) (WHO/FAO, 2001; 2004; 2007). Generally, all metals showed concentrations lower than the permissible limits while Pb was not detected in all samples analyzed and Cd in samples collected from Rivers state. However, Cd was only detected (0.003 mg/kg) in one sample (*Lenziles betulina)* collected from Imo state which was lower than WHO/FAO limits of 0.02 mg/kg (Table 1).

Iron has a role in a number of metabolic activities, including oxygen transport, DNA synthesis, and electron transport, in virtually all living species. It is well recognized that getting enough iron in your diet is critical for preventing anemia (Zhu et. al., 2010). The concentration of Fe ranged from 1.235 mg/kg in *Grifola polypilus frondosa* to 5.827 mg/kg in *Lentinus squarrosulus* collected in Imo State and from not detected in both *Lentinus squarrosulus* and *Pleurotus tuber-regium* to 1.235 mg/kg in *Auricularia aurucula* collected from Rivers state. The recommended limit for Fe is given as 3 mg/kg (Table 1). All samples did not exceed this limit, indicating the mushrooms are not contaminated by Fe from the farms. In other studies, very high Fe concentrations which ranged from 86.0 ± 6.5 mg/kg to 843 ± 43 mg/kg were reported in different species of mushroom in China (Zhu et. al., 2010). In Turkey, concentrations reported ranged from 31.3 mg/kg to 3,904 mg/kg (Sesli and Tüzen 1999; Sesli et al. 2008; Türkekul et al. 2004; Mendil et al. 2005) and more recently Keskin et. al., (2021) reported Fe concentrations which ranged from 13.3 to 507.4 mg/kg from Belgrad forest in Istanbul, Turkey. These values were higher than the current study. However, results were comparable with the minimum value (1.05 mg/kg) reported for some mushroom samples collected from forests of south eastern Nigeria (Nnorom et. al., 2020).

Copper is a necessary mineral that functions as a cofactor for many enzymes involved in energy generation, iron metabolism, neuropeptide activation, connective tissue formation, and neurotransmitter synthesis (Collins, 2014). Copper has a role in a variety of physiologic processes, including angiogenesis, neurohormone balance, gene expression control, brain development, pigmentation, and immune system function (Collins, 2014). Cu was not detected in 50 % of samples collected from Imo state while ranging from 0.002 mg/kg in *Pleurotus ostreatus* to 0.003 mg/kg in *Lentinus squarrosulus*. However, Cu was detected only in *Lenziles betulina* (0.545 mg/kg). The concentrations were lower than the permissible limits of 40 mg/kg stipulated by WHO/FAO, indicating that they are not yet contaminated.

The concentrations of Cu obtained in the current study were very low compared to other related studies. Záhorcová et. al., (2016) found Cu which ranged from 18.1 ±9.91 mg/kg to 27.0 ±11.9 mg/kg in edible wild mushroom species [*M. procera* (Scop.) Singer, *B. recitulatus* Schaeff., *C. cibarius* Fr., *S. grevillei* (Klotzsch) Singer, A. campestris L., R. xerampelina (Schaeff.) Fr., L. salmonicolor R. Heim & Leclair, *C. gibba* (Pers. Ex Fr.) Kumm., *X. chrysenteron* (Bull.) Quél., *M. oreades* (Bolton)] growing in a former mining area in Slovakia.

Zhu et. al. (2010) reported Cu concentration which ranged from 6.8 to 31.9 mg/kg in Turkey and also the ones reported from south eastern Nigeria (Nnorom et. al., 2020).

**Table 1.** Concentrations of heavy metals in the Wild Edible Mushrooms.

| Sample | Fe | Cu | Zn | Pb | Cd |
|---|---|---|---|---|---|
| **Imo State** | | | | | |
| *Lentinus squarrosulus* | 5.827 | 0.003 | 1.037 | ND | ND |
| *Pleurotus ostreatus* | 5.338 | 0.002 | 0.851 | ND | ND |
| *Lenziles betulina* | 3.209 | ND | 0.511 | ND | 0.003 |
| *Grifola polypilus frondosa* | 1.235 | ND | 0.710 | ND | ND |
| Mean | 3.261 | 0.001 | 0.777 | ND | 0.001 |
| SDV | 2.052 | 0.002 | 0.222 | ND | 0.002 |
| **Rivers State** | | | | | |
| *Lentinus squarrosulus* | ND | ND | ND | ND | ND |
| *Pleurotus tuber-regium* | ND | ND | ND | ND | ND |
| *Lenziles betulina* | 0.269 | 0.545 | 0.111 | ND | ND |
| *Auricularia aurucula* | 1.235 | ND | 1.444 | ND | ND |
| Mean | 0.376 | 0.136 | 0.389 | ND | ND |
| SDV | 0.587 | 0.273 | 0.705 | ND | ND |
| **WHO/FAO (2001; 2004; 2007)** | 3.000 | 40 | 60 | 0.300 | 0.020 |

## 3. 2. Biocontamination factors (BCF) and pollution load index

Assessing the capacity of mushrooms to absorb metals from their environments is done by determining the biocontamination factor (BCF), which is defined as the ratio of the metal content in the fruiting body to the metal content in the reference material (in dry matter). All BCF for all mushrooms were generally low (< 3) (Table 2). The overall accumulation load of metals indicates that the mushrooms are not polluted by metals, which is similar to other related study (Nnorom et. al., 2020).

**Table 2.** Biocontamination factors of heavy metals in the Wild Edible Mushrooms.

| Sample | $BCF_{Fe}$ | $BCF_{Cu}$ | $BCF_{Zn}$ | $BCF_{Pb}$ | $BCF_{Cd}$ | PLI |
|---|---|---|---|---|---|---|
| **Imo State** | | | | | | |
| *Lentinus squarrosulus* | 1.941 | 7.5E-4 | 0.017 | - | - | 0.029 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Pleurotus ostreatus* | 1.779 | 5E-5 | 0.014 | - | - | 0.011 |
| *Lenziles betulina* | 1.070 | - | 0.009 | - | 0.15 | 0.038 |
| *Grifola polypilus frondosa* | 0.412 | - | 0.011 | - | - | 0.067 |
| **Rivers State** | | | | | | |
| *Lentinus squarrosulus* | - | - | - | - | - | - |
| *Pleurotus tuber-regium* | - | - | - | - | - | - |
| *Lenziles betulina* | 0.090 | 0.014 | 0.002 | - | - | 0.014 |
| *Auricularia aurucula* | 0.412 | - | 0.024 | - | - | 0.099 |

### 3. 3. Health risks assessment

The results for the provisional tolerable weekly intake (PTWI) for the heavy metals are presented in Table 3. In the United States, provisional tolerated weekly intake (PTWI) is defined as the maximum amount of pollutants that a consumer weighing 60–70 kg may eat in a given week (Nnorom et. al., 2020). When compared to the recommended PTWI values (Table 4), only Fe in *Lentinus squarrosulus* (20.39) and *Pleurotus ostreatus* (18.68) were higher than the limits, all other metals from eating of the investigated mushrooms is lower than the PTWI. In most cases, the predicted intakes are lower than the PTWI estimates (Árvay et. al., 2019).

**Table 3.** Estimated metal dietary intakes (mg/kg/bw/week) from mushroom consumption.

| Sample | Fe | Cu | Zn | Pb | Cd |
|---|---|---|---|---|---|
| **Imo State** | | | | | |
| *Lentinus squarrosulus* | 20.39* | 0.01 | 3.63 | - | - |
| *Pleurotus ostreatus* | 18.68* | 0.01 | 2.98 | - | - |
| *Lenziles betulina* | 11.23 | - | 1.79 | - | 0.01 |
| *Grifola polypilus frondosa* | 4.32 | - | 2.49 | - | - |
| **Rivers State** | | | | | |
| *Lentinus squarrosulus* | - | - | - | - | - |
| *Pleurotus tuber-regium* | - | - | - | - | - |
| *Lenziles betulina* | 0.94 | 1.91 | 0.39 | - | - |
| *Auricularia aurucula* | 4.32 | - | 5.04 | - | - |
| **PTWI** | 17 | 30 | 70 | 25 | 7 |

*Value higher than recommended limits

For metals involved with foodborne contamination, the Target Hazard Quotient (THQ) has been recognized as a criterion for risk assessment using the Target Hazard Quotient (THQ). There will be no significant danger below the reference dosage, thus it represents the relationship between the estimated dose of a contaminant and the reference dose. To calculate THQ values, the mean concentration of data collected was used to evaluate the intake of mushroom meal by an average level consumer. The THQ values for the four mushroom species were usually extremely low except for *Lenziles betulina* in Fe (2.24) (Table 4).

Adults with adequate intestinal function have a very low risk of iron overload due to dietary iron sources. Acute iron intakes, on the other hand, may cause stomach distress, constipation, nausea, abdominal discomfort, vomiting, and fainting, particularly if food is not consumed concurrently. Due to the fact that most THQ values were less than 1, it can be concluded that the eating of these mushrooms has no health concerns. This is in agreement with the study of Nnorom et. al., (2020) collected from Southeast, Nigeria.

**Table 4.** Target hazard quotient of heavy metals in the Wild Edible Mushrooms.

| Sample | Fe | Cu | Zn | Pb | Cd |
|---|---|---|---|---|---|
| **Imo State** | | | | | |
| *Lentinus squarrosulus* | 4.85E-4 | 3.98E-7 | 1.37E-4 | - | - |
| *Pleurotus ostreatus* | 4.45E-4 | 2.65E-7 | 1.50E-4 | - | - |
| *Lenziles betulina* | 2.67E-4 | - | 9.03E-5 | - | 1.59E-5 |
| *Grifola polypilus frondosa* | 1.02E-4 | - | 1.25E-4 | - | - |
| **Rivers State** | | | | | |
| *Lentinus squarrosulus* | - | - | - | - | - |
| *Pleurotus tuber-regium* | - | - | - | - | - |
| *Lenziles betulina* | 2.24 | 7.22E-5 | 1.96E-5 | - | - |
| *Auricularia aurucula* | 1.03E-4 | - | 2.55E-4 | - | - |

## 4. CONCLUSIONS

The paper focused on monitoring of the contamination level of four species of edible wild-growing mushrooms in Imo (*Lentinus squarrosulus*, *Pleurotus ostreatus*, *Lenziles betulina*, *Grifola polypilus frondosa*) and Rivers (*Lentinus squarrosulus*, *Pleurotus tuber-regium*, *Lenziles betulina*, *Auricularia aurucula*) states with risk elements (Fe, Cu, Zn, Pb and Cd). Fe exceeded recommended values in *Lentinus squarrosulus*, *Pleurotus ostreatus* and *Lenziles betulina* in Imo State. Based on the PTWI and THQ results, the concentrations of Fe were relatively high in some cases. Consumption of mushrooms from these areas might pose no potential risk in terms of heavy metals except for Fe content in *Lenziles betulina* from Rivers state. However, in general, it can be concluded that the consumption of the studied mushroom species from all sites does not present any health risk arising from their regular consumption.

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