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Fe, Pb, Mn, and Cd Concentrations in Edible Mushrooms (*Agaricus campestris*) Grown in Abakaliki, Ebonyi State, Nigeria

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Abstract**—**The health and environmental risk of eating mushrooms grown in Abakaliki were evaluated in terms of heavy metals accumulation. Mushroom samples were collected from four different farms located at Izzi, Amajim, Amana and Amudo and analyzed for (iron, lead, manganese and cadmium) using Bulk Scientific Atomic Absorption Spectrophotometer 205. Results indicates mean range of concentrations of the trace metals in the mushrooms were Fe (0.22-152. 03), Mn (0.74-9.76), Pb (0.01.0.80), Cd (0.61-0.82) mg/L respectively. Accumulation of Cd on the four locations under investigation was higher than the UK Government Food Science Surveillance and World Health Organization maximum recommended levels in mushroom for human consumption. The Fe and Mn contaminants of Amudo were significant and show the impact of anthropogenic/atmospheric pollution. The potential sources of the heavy metals in the mushrooms were from urban waste, dust from mining and quarrying activities, natural geochemistry of the area, and use of inorganic fertilizers

Keywords**—***Agaricus campestris,* edible, health implication heavy metal, mushroom.

I. Introduction

THE environmental and health effects of metal ion pollution are important and pose complex problems. Some metals like cobalt, iron and copper are known to be vital for life while lead, arsenic, cadmium and mercury, etc are toxic [1], [2].

Concentrations of some of these trace elements may increase to toxic levels through natural processes or through the perturbation of the environment by human activities, e.g. mining, quarrying, industrial processing and other numerous applications [3], [4].

The beneficial and detrimental effects ascribed to a metal are caused by specific chemical compounds containing this metal e.g. Cr (VI) is toxic while Cr (III) is not. The nature of these compounds, the modes of transport and transformations, the mechanisms of toxicity and proper method of preventing toxicity are not well known [5]. Volatile heavy metals like mercury and arsenic can be released into the atmosphere where they combine with the air [6]. These metals are also present in foodstuffs either in greater or lesser amounts depending on various factors such as soil and underlying rock types [7]. These are of interest to nutritionists, chemists and toxicologists [8]. Toxic elements are destructive to human life and can retard growth and development. They can remain bound in top soil or air particles from where plants absorb them, or leach into ground water during precipitation [9]. They can also be taken into the body by inhalation, ingestion and skin absorption. Heavy metals may enter and accumulate in the body tissues faster than the body's detoxification pathways can dispose of them leading to a gradual build up which will result to poisonous effect.

Toxicity of lead is enhanced by its ability to bio-concentrate and bio-transform in the tissues [10], [11]. Lead ions produce physiological poisoning by becoming attached or absorbed on the cellular enzymes, causing inhibition of enzymatic control of respiration, photosynthesis and poor respiration [9]-[11]. Lead is primarily absorbed via respiration and ingestion, circulated through the bloodstream and thereafter enters all tissues of the body [9]. Lead can displace calcium from the bones causing them to be brittle [8], [10]. Bioaccumulation in the liver or kidney can occur leading to liver or kidney malfunction, disruption of the central nervous system (encephalopathy) resulting in uncoordinated muscular control and poor eyesight has also been reported [11], [12]. However, these symptoms do not become noticeable until the level in a particular organism exceeds its tolerance limit for the metal. Every organism has a different limit for each toxic substance for example lead can be tolerated in plants at a higher concentration than mercury [11]. Iron is essential to all known organisms. Ingestion of large amounts of iron can lead to excessive levels of iron in the blood [11]. High iron level damages the cells of gastrointestinal tract, preventing them from regulating iron absorption. High blood concentration of iron can damage cells in the heart and liver, and can cause serious problems including long-term organ damage and even death [11].

Manganese occurs in rocks and soils and is widespread in the environment. As a micronutrient, plants require it for photosynthesis and animals for neural development [13]. In excess concentrations, it may be toxic to fish and humans and may impair drinking water quality at concentrations above 0.05 mg/l according to the WHO [14].

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Cadmium is highly dispersed by human activities and as a result, its background concentration in soils is difficult to evaluate. Low concentrations of the metal are present in uncontaminated soils and based on the fairly low Clark's value of sedimentary rocks ($CaCO_3$ 0.035mg/kg) [15], [16]. Toxicity is enhanced by its absorption and bioaccumulation in the tissues which increases with age and may cause renal damage. It has high affinity for sulphur of thiolate [17].

Heavy metal pollutants available in the environment are of major concern because of their impacts on man's source of livelihood, which finally puts his health at risk e.g. plants growing in a heavy metal polluted environment can accumulate the toxic metal at high concentration causing serious health risk to human health when consumed [18].

This study sought to assess the health and environmental risks associated with eating mushrooms grown within the suburbs of Abakaliki Ebonyi State. The primary focus was to evaluate the level of Fe, Pb, Mn, and Cd in the mushrooms grown in these areas and its health and/or environmental implications.

II. Materials and Methods

A. Design of the Study

In order to build up a comprehensive picture of what might have been happening in terms of heavy metal accumulation in edible mushrooms, the following strategies were developed and followed:

- Four case study locations were selected and samples of mushrooms were collected and analyzed for selected heavy metal (Fe, Pb, Mn and Cd) concentrations.
- These four toxic metals were selected for different reasons. Lead and Cadmium can pose a health risk to humans via direct ingestion or through plant uptake and also via food chain contamination [19]. Iron and Manganese are of interest because they are essential plant micronutrients, but can also pose toxicity risk [20].

B. Area Description

Ebonyi State, Nigeria has an area of approximately 5,935 square kilometers and lies between latitude $7^0 30^l$ and longitude 5^0 50^l E and 6^0 45^l E. Large deposits of salt and other solid minerals are abundant in the State. The vegetation of the State is semi-savanna with seasonal variations of hot, mild, cold weather and mixed grid vegetation with agrarian, forestry and swamp ideal for agriculture especially for rice cultivation. It has a mean temperature of 30°C during the hottest period (February – April) and 21°C during the coldest period (December – January) respectively. The mean annual rainfall is between 1,500mm and 1,800mm. The climate is a tropical hot humid type characterized by heavy rainfall, high temperature and sunshine with two marked seasons: the rainy and dry seasons. The rainy season commences from April to October, while the dry season begins from November to March [21]. The distribution of soils in Ebonyi State ranges from humus, loamy, sandy to clay and sometimes a mixture of both types of soil. Chernozem-like soils (dark black soils rich in humus and carbonates, red tropical soils and laterietes (leached iron-bearing soils) characterize most soils in the State.

The four different locations were all in Ebonyi State, three of them (Izzi, Amana and Amajim) sited in the suburbs of Abakaliki, the Ebonyi State capital while Amudo is a rural community about 35km south of Abakaliki. Izzi is located on the northern outskirts of Abakaliki town; Amana is on the eastern outskirts of the town while Amajim is on the western outskirts.

C. Sample Collection

Mushroom is the umbrella-shaped fruiting body (sporophore) of certain fungi, typically of the order *Agaricales* in the class *Basidiomycetes*. Mushroom indicates the common edible fungus of fields (*Agaricus compestris*). Under good nourishment and suitable temperature and moisture, a mycelium will produce a new crop of sporophores each year during its fruiting season [22].

Edible mushroom samples were detected by throwing them to fowls and if taken implies that they were edible. The edible mushroom samples were collected from their staple (the primary food materials of which the mushroom was grown). These consisted of dead wood, grassland and gardens from four different locations in the suburb of Abakaliki and one rural area of the State. Random sampling method was adopted for this work [10]. In this method, six different mushroom kinds from the same species were selected and used for analysis.

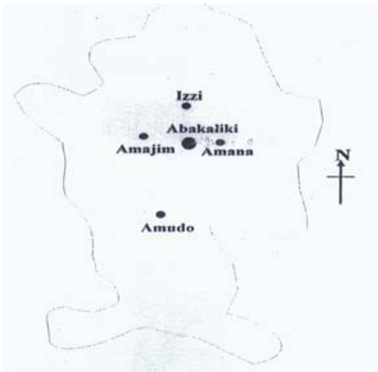

Fig. 1 Map of Ebonyi State showing the sampling locations

D. Duration of Sampling

The samples were collected in the month of November 2012 with a locally made basket sealed with polythene bag, to prevent contamination for 18 hours before use. Samples were first rinsed with de-ionized water, dried in an ambient environment condition from dust and later homogenized.

E. Sample Analysis

3g of the homogenized mushroom samples were ashed at 550^OC for 30 minutes and allowed to cool. $10cm^3$ of *aqua regia* was added to the ash obtained and heated at 60^OC for one hour on a hot plate until the sample was completely digested and the acid mixture dried [23].

The digested ash sample was dissolved in 30cm^3 of de-ionized water, stirred and filtered. The clear filtrate obtained contained solutions of nitrates, chlorates and the heavy metals were stored in clean plastic bottles for analysis using the Buck Scientific Atomic Absorption Spectrophotometer 205.

F. Statistical Analysis

The purpose of the mushroom sampling strategy was to assess whether the differences between locations were the result of intrinsic soil characteristics or mining effect or both. To do this, a two-way analysis of variance (ANOVA) test was applied to the samples for every location [24]. In this context, the test was used to distinguish between locations on the basis of intrinsic soil chemical characteristics by averaging across location samples and thereafter used for assessing whether the heavy metals were evenly distributed across locations (status effect). Thus, the 'status' effect revealed whether there were any overall differences between locations or not.

III. Results and Discussion

Table I shows the range, mean values and standard deviation for the total concentration of Mn, Pb, Cd and Fe in every location sample tested. All locations consists of 3 replicates tests. These results can be contrasted with the applied standard range in mushroom [25]. In all cases, except for Mn and Fe (Amudo) and Cd (all 4 locations), the values from the case study locations were within the normal range (see Table I and Fig. 2). Fe and Mn values were far above the standard range given for adequate levels, however, it is among the essential micro-nutrients. Therefore it can be said that the Amudo mushrooms were polluted with Fe and Mn.

In this study the mean value and range of lead in Amana (0.80 ppm; 0.70-0.90 ppm), Amajim (0.63 ppm; 0.50-0.80 ppm), and Amudo (0.63 ppm; 0.53-0.73 ppm) respectively when compared with the permissible concentration of 0.012-6 ppm, were within the normal range and could be considered safe for consumption.

In all the locations, the Cd concentrations exceeded the draft minimum and maximum required standard in mushroom for human consumption. Cadmium and lead are of additional concern because of their toxicity to human and animals. Cadmium is a particular problem, because it can adversely affect human health and also causes phytotoxicity in plant tissues [26].

Table I also shows that some of the level of the four heavy metals studied appears to fall within the normal range for mushrooms in some locations. Although some of these concentrations in the four locations investigated were not very high to presume imminent serious pollution problems except in Amudo that contained extremely high Fe and Mn level. Further researches are currently ongoing in these areas to ascertain potential sources. However, the soil in this area is clayey which could be the source of iron.

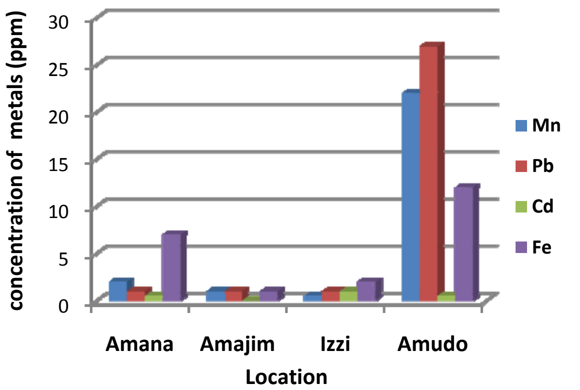

Fig. 2 Comparison of the mean concentration of the metals based on location

The statistical results in Table II reveal that the small mushroom samples precluded any formal attempt at correlating the concentration of heavy metals found in the mushroom with the locations. According to Table II, the ANOVA analysis revealed that Cd was evenly distributed in all the four locations while Mn, Pb, and Fe were not. The magnitude and distribution of trace heavy metals will vary depending on the natural geochemistry of the area and human activities. Thus, in the mushroom samples of Abakaliki town, the major sources of pollution were likely from urban wastes (commercial and domestic activities), since there were little or no industrial activities in this area. Furthermore, another probable source of contamination was air borne dust.

TABLE I
Heavy Metal Concentrations (ppm) in Mushroom Samples from Selected Locations in Ebonyi State

	Manganese (Mn)			Lead (Pb)			Cadmium (Cd)			Iron (Fe)		
Location	Range	Mean	SD	RANGE	MEAN	SD	Range	Mean	SD	Range	Mean	SD
Amana	1.4-1.6	1.50	0.09	0.7-0.9	0.80	0.10	0.5-0.7	0.61	0.10	22-23	22.11	0.30
Amajim	0.7-0.9	0.79	2.00	0.5-0.8	0.63	0.15	0.7-0.9	0.82	0.00	28-29.	28.85	0.23
Izzi	0.1-0.2	0.14	0.02	6-7 ($\times10^{-3}$)	0.01	1 $\times10^{-3}$	0.5-0.7	0.62	0.12	0.2-0.24	0.22	0.03
Amudo	9.5-10.0	9.76	0.23	0.5-0.7	0.63	0.10	0.6-0.9	0.73	0.11	150-155	152	2.62
WHO*	0.01-2.6			0.012-60			0.012-60			0.12-35		

[a] *WHO* = Normal range of elements in mushrooms according to WHO (Peter et al., 1985 [18]).*

TABLE II
TWO-WAY ANOVA TEST FOR SAMPLES OBTAINED FROM FOUR DIFFERENT LOCATIONS WITHIN ABAKALIKI METROPOLIS, EBONYI STATE

Source of Variation	Sum of squares (ss)	Degree of freedom (df)	Mean square (ms)	Means square estimate (F-values)
		Mn(ppm)		
Between groups (factors effect)	182.9635	3	60.9878	3080.1919
Within groups (error)	0.1581	8	0.0198	
Total		**11**		
		Pb(ppm)		
Between groups (factors effect)	1.10103	3	0.3671	33.3727
Within groups (error)	0.0878	8	0.0110	
Total		**11**		
		Cd (ppm)		
Between groups (factors effect)		3	0.0299	2.67
Within groups (error)		8	0.112	
Total		**11**		
		Fe (ppm)		
Between groups (factors effect)		3	14110.7679	8077.14
Within groups (error)		8	1.7470	
Total		**11**		

Various studies have shown that unusually large concentration of heavy metals in plant can be caused by contamination with anthropogenic emission and not soil composition [27], but rather through foliar uptake [26]. Therefore, it was necessary to investigate the levels of trace metals in the dumps (refuse) and the ashes from the urban waste since some of the local farmers use them as manures to ascertain their exact health and environmental risks, so as to identify a strategy for utilization of the urban wastes generated in the metropolis, because of the significance of 'farm status interaction' effect and fertilization practice [18].

Also, the possibility that the concentration of Mn, Pb, Cd, and Fe found in the mushrooms were caused by atmospheric pollution as a result of mining and quarrying of rocks needs to be explored further. Also wastes from domestic activities (ash) are used as farm manure and with increasing urbanization and consumerism on the part of the town's population; this waste can probably be a source of heavy metals. Therefore, the possibility that the concentration of Mn, Pb, Cd, and Fe found in the mushrooms were caused by atmospheric pollution as a result of mining and quarrying of rocks needs to be explored further. Also wastes from domestic activities (ash) are used as farm manure and with increasing urbanization and consumerism on the part of the town's population; this waste can probably be a source of heavy metals. Therefore, it was necessary to investigate the levels of trace metals in the dumps (refuse) and the ashes from the urban waste since some of the local farmers use them as manures to ascertain their exact health and environmental risks, so as to identify a strategy for utilization of the urban wastes generated in the metropolis, because of the significance of 'farm status interaction' effect and fertilization practice [18].

The practice is using urban ashes (wastes) as manures could be significant, although it has associated environmental problems like the problem of waste disposal [28]. Urban centers produce most of the world's waste and between a third and half of this goes uncollected [28]. It contributes to urban pollution and health risks, yet it has great potential because it can be very rich in nutrient. By disposing of urban waste on city plots, farmers can obtain a cheap supply of nutrients while alleviating the waste disposal problem at the same time. There are many examples of waste utilization in the developing world e.g. use of composted waste as practiced in Calcutta [29]; untreated and unsorted waste, wood and household waste as practiced in Nigeria [30] and waste water as practiced in Senegal, Burkina Faso and Mauretania [31].

IV. CONCLUSION

The finding of the study suggests that the mushroom concentrations of the four heavy metals listed above were within typical mushroom levels and that there should not be any problems of either toxicities or deficiencies for mushroom growth. Only one location Amudo showed abnormally high concentration for Mn and Fe levels. The four locations had high levels of Cadmium, which could be linked to agricultural, anthropogenic and domestic activities since there were little or no industrial activities in the areas. Mushroom samples were collected from each location and although none of the metals was present in phytotoxic concentrations (except in Amudo location), Mn and Fe, Cd were present in concentrations that were above the required standard in mushroom for human consumption.

However, the high level of Cd evenly distributed among the location suggests that mushroom may be selectively accumulating Cd. Further studies are required to establish the source of the Cd and the source of the Amudo's high Mn and Fe concentrations which should include water quality throughout the farming season. One potential source that requires investigation is atmospheric pollution due to quarry activities, as mushroom may be accumulating high level of heavy metals through foliar contamination. Studies on soil, water and atmospheric deposition is ongoing in these areas.

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