African Journal of Agriculture and Food Science
ISSN: 2689-5331
Volume 3, Issue 4, 2020 (pp. 103-121)
www.abjournals.org

BIOACCUMULATION OF TOXIC METALS FROM TRACE METALS POLLUTED SOILS USING FIVE SPECIES OF MUSHROOM FOUND IN ANAMBRA STATE, NIGERIA

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ABSTRACT: *The aim of the study was to provide base-line data on five mushroom species and determine their potential for remediation of metal polluted soil in Anambra State, Nigeria. Five species of Mushroom namely Termitomyces robustus, Agaricus bisporus, Pleurotus tuber-regium, Amanita phalaoides and Amanita verosa were collected from eleven locations in Uke, Abatete, Ideani, Nnobi, Nnewi (Okpuno-egbu), Nnewi (Umudim) and Ozubulu between 2012 and 2016 in Anambra State, Nigeria. They were kept in clean collection bags and identified by a taxonomist. Some of the mushroom samples were later oven dried at 75 0 C for 4 h and kept for chemical analysis while some were used for cultivation. During cultivation, seeds from matured mushrooms were scrapped from their veils into already compounded substrates/soil from their natural habitats and refuse dump soil. The seeds were allowed to germinate within 4-5 days, the fruiting bodies/spawns were watered once daily for 14 days. The matured mushrooms were harvested, cleaned and oven dried at 75 0 C for 4 h. The dried mushroom samples (wild and cultivated respectively) were homogenized into a fine powder using a blender with titanium blade and stored in pre-cleaned bottles for chemical analysis. These samples were subjected to various chemical analyses using standard methods by the Association of Official Analytical Chemist (AOAC). The data were subjected to one-way analysis of variance (ANOVA) at 95 % level using Statistical Package for Social Scientists (SPSS) version 16.0. Bioaccumulation factors ranged as follows: Cd (0.14 -1.78), Co (0.06 – 3.01), Cr (BDL), Cu (0.01 – 0.35), Fe (1.17 -2.22), Mn (0.38 – 13.53), Ni (0.08 – 1.95), Pb (0.08 – 1.50) and Zn (0.22 – 10.13). The obtained values were above the acceptable limit in food.*

KEYWORDS: Mushrooms, Toxic Metal, Pollution, Anambra State, South-East, Nigeria.

INTRODUCTION

Mushrooms are a special group of fungi which are saprophytic in nature due to lack of chlorophyll. They grow in dark, damp places and produce a wide range of enzymes which progressively breakdown complex substances into simpler inorganic matter (Agrahar *et al* 2005).

In many parts of the world, such as China, United State of America (USA), Canada, India, Italy, Mexico and Turkey, mushrooms are highly priced and in massive production for local consumption and export.

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www.abjournals.org

In USA, the gross domestic products (GDP) for mushroom was about seven million tonnes in 2005, amounting to 30 million dollars annually (Agrahar *et al* 2005). In Nigeria, mushrooms are grossly under exploited as only a few types are considered edible. There is no evidence of mushroom cultivation as a commercial venture in Nigeria, but this could be used as a means of poverty alleviation due to its short cropping cycle, cheap planting inputs, less land requirement, high profit and quick returns on investment.

Till date, mushrooms collection in Nigeria is mainly from the wild and this practice is fraught with fear of mistaking those regarded as poisonous and non-edible for those regarded as edible (Emuh 2009). This has been occasionally attributed to many deaths after mushroom meals (Emuh 2009). Industrialization, urbanization and indiscriminate refuse disposal have impacted negatively on the environment, thereby posing problems of contamination with pesticides, petroleum hydrocarbons, heavy metals and other potential pollutants. Mushrooms have been reported to be good accumulator of trace metals in polluted environment (Emuh 2009, Agrahar, *et al* 2005).

In many countries of the world including Nigeria, edible mushrooms have been priced as delicacies. Apart from their medicinal values, they constitute an important food source in the world. Mushrooms have been reported to be rich in protein, glycogen, vitamins, crude fibres and essential mineral compounds. In fact, Agrahar, *et al* 2005 and Hammam, 2004 reported the rich nutrient contents of mushrooms compared to those of meat and vegetables. Mushrooms such as *Flammulina velutipes Lentinus edodes, Agaricus bisporus, Pleurotus oestratus, Volvariella volvacea and Agaricus campestris* among others, have been cultivated for food in several countries of the world especially in America, Europe and Asia (Hitivani et al 2003).

Health risk from mushroom consumption has been difficult, due to very limited knowledge in chemical compositions of the metals and their bioavailability in man (Isildak *et al* 2004). Some countries have established statutory limits for the metals in edible mushrooms. It was reported that mushroom breaks down toxic pollutants into non-toxic substances (Isildak *et al* 2004). Also reported was the removal of heavy metals and other harmful contaminants from environment by Shiitake mushroom (Jonathan, 2002). The scavenging of metals from polluted sites by mushrooms was due to remediation and purifying abilities of mushrooms. Mushrooms grow in the presence of heavy metals, secretes enzymes and detoxify such contaminants (Juhasz, *et al* 2002). It was reported that mushroom channels heavy metals from land to fruity bodies for removal from the soil/environment (Kalac, *et al* 2000). This is first by denaturing the toxins and finally absorbing such heavy metals. Mushrooms were reported to be hyper accumulators of heavy metals and radioactive metals that are toxic when consumed and thus has the ability to eliminate them from the environment (Sasek, 2003). Similarly reported was the use of Turkey tail mushroom and Phoenix oyster mushroom mycelia to eliminate 97 % mercury ion from water (Schliephake, *et al* 2003).

The use of mushrooms as food and medicines must have dated from ancient Greek, Egyptians, Romans Chinese, Mexicans and even Africans. There are also evidence of uses in religious and tradomedicinal practices (Akpaja et al 2003). Reports show that some species of mushrooms are poisonous and have claimed the lives of historic figures, such as Pope Clement VII, King Charles VI of France and Czar Alexis of Russia (Wasser, *et al* 2003). The most celebrated casualty was that of Roman Emperor Claudius Ceasar. There was however the belief that the mushrooms that killed him were deliberately poisoned before being introduced into his meal by political enemies (Stamets, 2005). In Nigeria, daily newspaper reported in 1986, the death

of a whole family in Okpokhumi-Emai in Owan East local government area of Edo State after consuming soup prepared with mushrooms. There were many such reports in the literature all over the world (Okhuoya, *et al* 2010). On the strength of these the study aims to determine the physicochemical and toxicological profiles of five wild and cultivated mushrooms, as well their nutritional and anti-nutritional properties and their ability to bio-remediate metal polluted soil in Anambra Stat of Nigeria.

Uptake of Trace Metals by Mushrooms

Rapid industrial development has led to an increased discharge of industrial wastes, which may contain metal salts in concentrations well beyond their natural levels in the environment (Kalac et al 2000). The metal pollutants include lead, chromium, mercury, uranium, selenium, zinc, arsenic, manganese, cadmium, gold, silver, copper, nickel, etc(Kalac,2010). The main cause of concern is their toxicity with some being carcinogenic and mutagenic. These toxic metals may be derived from mining operations, refining of ores, sludge disposal, fly ash from incinerators, the processing of radioactive materials, metal plating, or the manufacture of electrical equipment, paints, alloys, batteries, textile dyeing, leather tanning, pesticides or preservatives.

Mushrooms (macromycetes or macrofungi) are vegetative organisms with the ability to accumulate heavy metals. This ability is explained by the presence of a rich network of hyphase which occurs in a considerable volume in the upper layer of soil. This allows mushrooms to collect required water and minerals from the soil for production of a fruiting body (Chang et al 2004). The large-surface created by mycelium, which is in contact with the substrate, make mushrooms more predisposed to absorb heavy metals present in soil than the majority of other soil organisms (Onuoha et al 2009). Every species of mushrooms has a specific capacity, genetically controlled, for absorption of one or another heavy metal from the soil (Al-Masri et al 2010). Heavy metal concentration in the fruiting body reflects the heavy metal content available to the mycelium in the substrate, as well as the capacity of the mycelium of each species to uptake heavy metals from the substrate (Al-Masri et al 2010). Consequently, mushrooms can be appreciated as bioaccumulators[46] which can be successfully utilized in mycoremediation technologies, where their features concerning the uptake of heavy metals are beneficial. The capacity of mushrooms to extract heavy metals from soil was tested with *Agaricus* macrosporus which effectively extracted Cd, Hg and Cu (Jumpponen et al 2004, Labarere et al 2000).

Bioavailability and Distribution of Chemical Pollutants

Plants and animals absorb these elements from soil, sediments and water by contact with their external surfaces, through ingestion and also from inhalation of air borne particles and vaporized metals (Alemawor, *et al* 2009, Okoye, 1989, Lepp, 1981). The assimilation of an element (i.e the bioavalability fraction) depends on a number of chemical and physicochemical factors such as chemical speciation, solubility in organic medium and pH.

In soils, metal and metalloids can occur in both solid and aqueous (soil solution) phases. In solution, these elements can exist, either as free ions or as various complexes associated with organic or inorganic ligands or as suspended colloidal particles. In the solid phase, they can be adsorbed or absorbed on organic and inorganic soil components, exists as minerals or precipitated with other minerals. In general, ions in solution are more available for plant and animal uptake, immediately entering the food chain (Alemawor et al 2009). However, metal

ions present in the solid phase may be available under certain biological and physico-chemical conditions such as exudation of special chelators, desorption, redox and pH changes etc. Significant contamination of seeds plants and plant products with toxic elements due to contaminated soil and water has been observed as a result of release of these toxicants into the sea, rivers, lakes or even irrigation channels (Okoye,2000). The consumption of contaminated vegetation constitutes an important route of animal exposure to heavy metals. Animals are exposed to these toxicants through a number of other routes. The most important among these are respiratory mostly for gases and particulate matters; dermal contact with chemicals able to cross the skin barrier, and from various food sources.

Absorption of metals and metal compounds inhaled as particles are influenced by several processes that include deposition, mucociliary and alveolar clearance, solubilization and chemical binding. After entering the body, the metal deposited in nasopharygeal, tracheobronchial, or pulmonary compartments may be transported by mucociliary action to the gastrointestinal tract. Food is the most important route for accumulating most chemical elements (essential and toxic metals).

Chemical Toxicity

Transition metals readily form stable covalent complexes and usually interact as part of macromolecules (protein, enzymes, hormones, etc.) according to their chemical characteristics including oxidation state (Okoye, 2002). This tendency ensures that in vivo, these metals are complexed with particular biological groups, such as sulphydry1 (-SH), amino (-NH), hydroxy1 (-OH), disulphide (-SS), and carboxylic (-COOH) groups of amino acids, peptides, proteins, phospholipids, citrate, ascorbate, and other tissue constituents(Okoye,2002). These groups are also found in important biomolecules with catalytic, structural or transport fuctions. Each transition metal possesses its affinity for organic binding. Elevated values of equilibrium constants are observed for biomolecules rich in –SH groups, towards which metals such as Pb, As, and Hg show particular reactivity (Li et al 2001). Proteins such as metallothioneins, ferritin, transferring lactoferrin, melanotransferrin, hemosiderin, ceruloplasmin, and amino acids (glutathione (GSH), cysteine, histidine and others) are examples of biomolecules able to bind toxic metals in biological matrixes. The reactivity for a wide range of biological ligands is the basis of the damaging actions of many metal ions at molecular level, and determines the characteristic toxicity of the absorbed metal. The knowledge of mechanisms of action is relevant for identifying possible targets and possible related biomarkers effects. Health effects induced by toxic metals vary greatly; from irritant and acute or chronic systemic toxic effects to teratogenic, mutagenic and carcinogenic effects (Sharma et al 2008). The reaction elements occurring in food, mostly as organic complexes or associated with fibers often have a low solubility within the intestinal lumen and are frequently poorly absorbed. Additionally, the effect of other micronutrients on metal absorption/toxicity is also important. Micronutrients can interact with toxic metals in several ways in the body. These include, absorption and excretion of toxic metal; transport of metals in the body; binding to target proteins; metabolism and sequestration of toxic metals; and finally, in secondary mechanisms such as oxidative stress. Therefore, a diet poor in micronutrient can have an important influence on the toxicity of non-essential metals such as cadmium, lead, mercury, arsenic (Okoye, 2001).

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Land Pollution and Control Measures in Nigeria

The Federal Government established the Federal Environmental Protection Agency (FEPA) by Decree 58 of 1988 and mandated it among others, to establish environmental guidelines or regulations and standards for the abatement and control of all forms of pollution. In 1999, the Agency published the National Interim Guidelines and Standards for Industrial effluents, gaseous emissions and hazardous Waste Management in Nigeria (Khan et al 2006). The document provided the first significant move in Nigeria towards environmental/public health protection. Unfortunately, till date the problems of mounting refuse that litters our environment, from vehicles that spew lethal smoke, unrestricted noise making and in most cases, lack of specification for industrial waste (Zapotoczny et al 2006) still bedevils the country.

The African Centre for Environmental Protection (ACEP) describes or defines environment as the totality of surrounding conditions and its features (Zapotoezny et al 2006). Scientifically, it is described as the combination of physical, chemical, biological and social factors in which a living organism exists, that affects the organism, community and influences its development or existence (Ibekwe et al 2008). Environment can simply be considered as the surroundings in which we live, work and enjoy leisure, which consists of air, soil, surface and ground water, providing habitat for mankind and other animals, plant species and serving as a source for food, water, fuel, raw materials and breathing air (Kwu et al 2012). Natural disaster and the activities of man in the quest to meet his needs have contributed greatly to global environmental issues. Our fragile ecosystem is under attack on various sides as a result of infrastructural development, human/animal wastes, natural disasters and so on.

Most human activities have been known to impact negatively on arable lands contaminating them with pesticides, petroleum hydrocarbons, heavy metals and waste engine oil pollutants, and consequently causing arable land shortage and other environmental challenges. A survey of land use practice in Nigeria revealed that bush fallowing is more popular in addressing the problems of low-yield agricultural lands. This practice according to reports (Maduka,2002,Grarcia et al 2005) allows for the slow process of natural restoration or remediation. However, strategies reportedly used in recovering contaminated or polluted farmlands are capital and labour intensive and these include excavation followed by incineration and/or secured land-filling (Maduka, 2002). These methods currently undermine bioremediation and pose varying degrees of environmental problems. However, prospects for using mushrooms in bioremediation of metal pollution have been reported (Garcia et al 2005).

EXPERIMENTAL

Sampling

The study was carried out by laboratory experiment. Five species of wild mushroom namely: *Termitomyces robustus, Agaricus bisporus, Pleurotus tuber-regium , Amanita phalaoides* and *Amanita verosa* were collected from eleven locations in Uke, Abatete, Ideani, Nnobi, Nnewi (Opkuno-egbu), Nnewi (Umudim) and Ozubulu (see Fig. 2) between 2009 and 2012 all in Anambra State, Nigeria.

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The samples were kept in clean collection bags and identified by a taxonomist. Some of the mushroom samples were later oven dried at 75 ^{0}C for 4 h and kept for chemical analysis while some were used for cultivation.

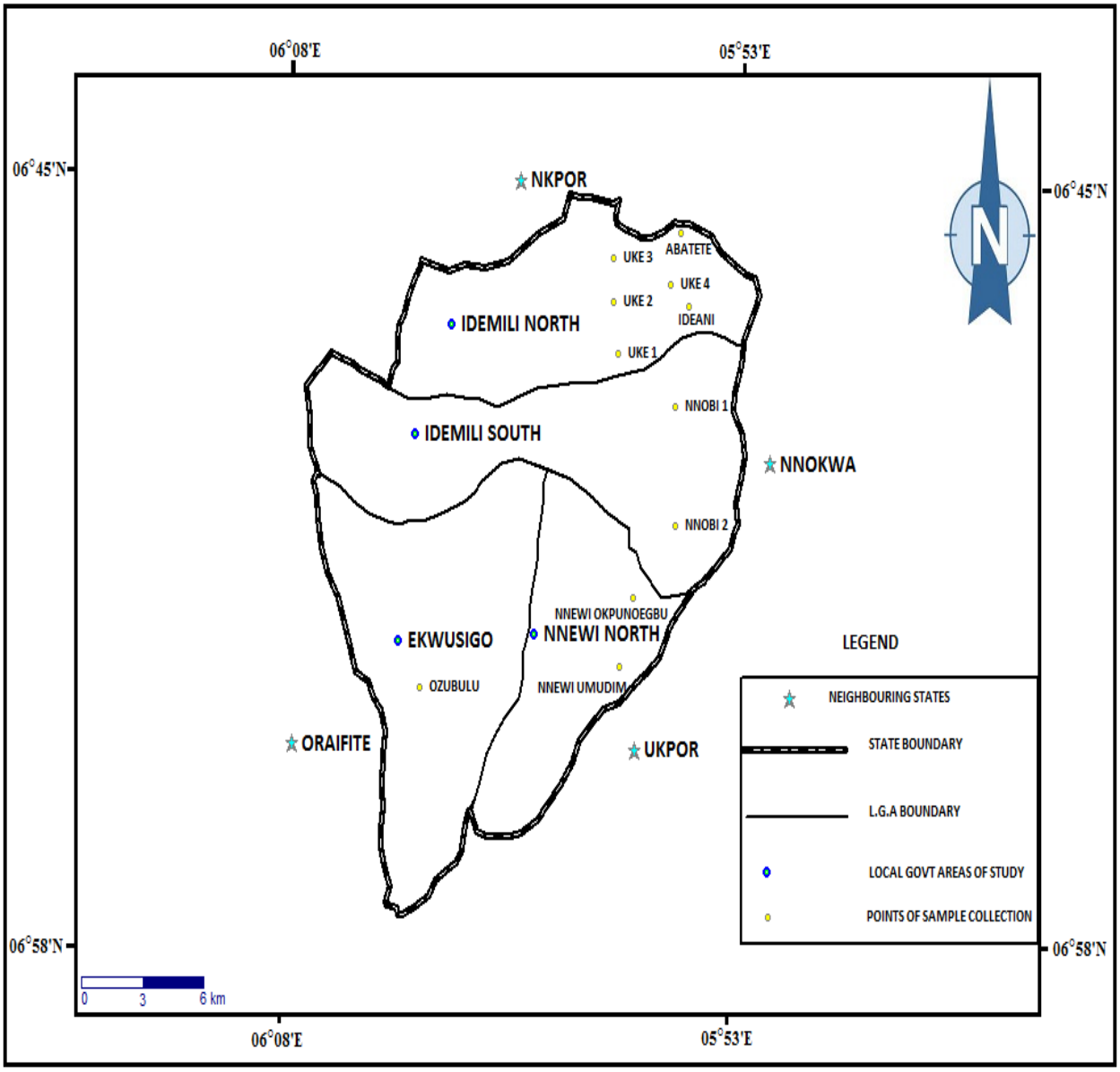

Fig 2: Map of Idemili North & South, Nnewi North & Ekwusigo L.G.A. Showing Sampling Sites

Source: Ministry of Land and Survey Anambra State Modified from Field Trip 2012

Table 1 shows the specific sites, environment or substrate, habitat, mushroom species and their local names.

Table 1: Sampling Sites, Habitat, Mushroom Species, their Local Names and Edibility

S/No	Site	Habitat	Specie of Mushroom	Local Name	Edibility
1	Uke	Termite nest behind family house	*Termitomyces robustus*	ERO-MKPU	Edible
2	Uke	Dead bread fruit tree by road side	*Agaricus bisporus*	ERO-OSISI	Edible
3	Uke	Rotten wood on farm land	*Agaricus bisporus*	ERO-OSISI	Edible
4	Uke	Soil in farm land	*Amanita verosa*	ERO-OHIA	Non- edible
5	Abatete	Rotten plant leaves inside bush	*AmanitaPhalaoides*	ERO-AGBUGBO	Non-edible
6	Ideani	Dead wood inside bush	*Pleurotus Tuber-regium*	ERO-OSU	Edible
7	Nnobi	Dead wood in farm land	*Agaricus bisporus*	ERO-OSISI	Edible
8	Nnobi	Forest soil	*Amanita verosa*	ERO-OSISI	Edible
9	Nnewi Okpuno	Refuse dump	*Agaricus bisporus*	ERO-OSISI	Edible
10	Nnewi Umudim	On soil inside bush near disused battery in the bush	*Amanita verosa*	ERO-OHIA	Non-edible
11	Ozubulu	Near termite nest on farmland	*Termitomyces robustus*	ERO-MKPU	Edible

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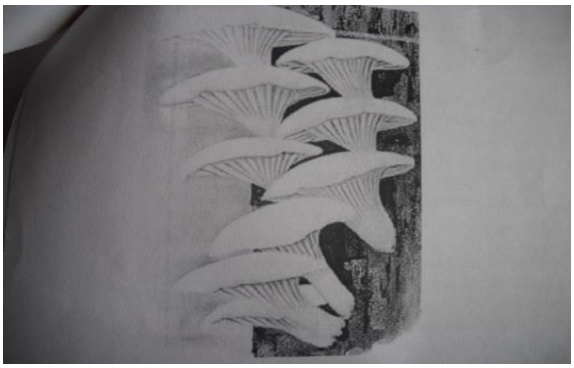
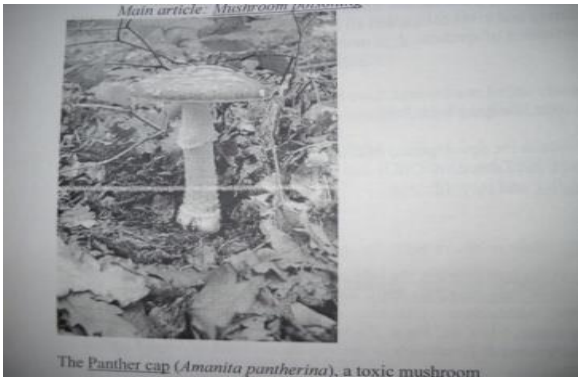


3a: *Agaricus bisporus*

3b: *Termitomyces robustus*



3c: *Amanita phalaiodes*

3d*: Amanita verosa*

3e: *Pleurotus tuber-regium*

Fig. 3: Photographs of the Collected Mushrooms

Cultivation/Bioremediation Process

During cultivation, seeds from matured mushrooms were scrapped from their veils into already compounded substrates/soils from their natural habitats and refuse dump soil using Zadrail methods (see Table 2).

The seeds were allowed to germinate within 4 - 5 days, the fruiting bodies/spawns were watered once daily for 14 days. The matured mushrooms were harvested, cleaned and also oven dried at 75 0C for 4 h.

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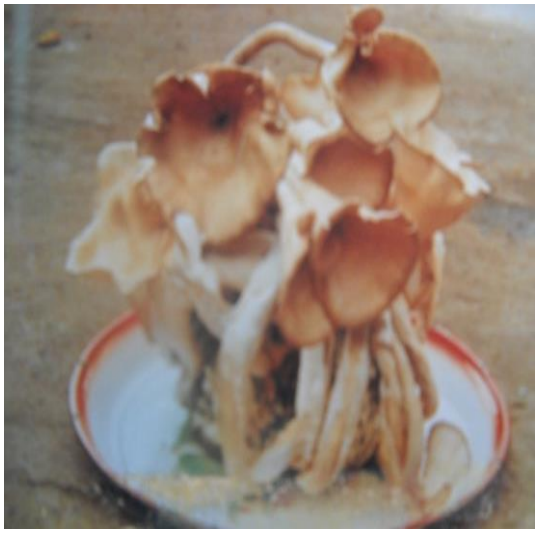
Table 2: Compounded Substrates for Spawn Production (Zadrazil 1980)

S/No.	Substrates	Quantity (g)	Mushroom SP
1	Elephant grass	400	*Agaricus bisporus*
	Chicken manure	120	
	Rice bran	20	
	Brewer's waste	22	
	Urea	6	
	Soya bean meal	5	
2	Elephant grass	500	*Amanita verosa*
	Cassava leaves	500	
	Chicken manure	400	
	Spent grain	72	
	Urea	14.5	
3	Saw dust	800	*Amanita phalaiodes*
	Urea	20	
4	Rice straw	300	*Termitomyces robustus*
	Chicken manure	150	
	Wheat bran	12.5	
5	Soil from sites 1,5,8,10 and 11		*Amanita verosa, amanita phalaiodes, termitomyces robustus*

The spawn production and fruiting bodies of pleurotus tuber-regium (matured) are shown below in Figs. 4a and 4b.

4a: Spawn production of Pleurotus Tuber-regium



4b: Fruiting bodies of Pleurotus tuber-regium (matured)

Fig 4: The Spawn production and Fruiting bodies of Pleurotus Tuber-regium

Table 3 contains the characteristic resonance lines of metals determined.

Table 3: Characteristic (Resonance) lines of Metals determined by Atomic Absorption Spectrophotometer (AAS) (AOAC 2005)

Elements	Wavelength (nm)
Cd	228.8
Co	240.7
Cr	357.9
Cu	324.7
Fe	248.3
Pb	217.0
Zn	213.9
Ni	232.0
Mn	279.1

Preparation of Samples for Metals Determinations

Ashing of Mushroom Samples (Morris, 1999, David, 1976, Gorsuch, 1976)

Porcelain crucibles with covers were cleaned and dried at 450 °C for 30 min and kept inside the muffle furnace. The dishes were allowed to cool and weighed. This was repeated until constant weights were achieved.

2.0 g of dried ground mushroom samples were accurately weighed into the crucibles and 1 mL of concentrated HNO_3 was added and left over-night. The sample was charred (carbonized) over a Bunsen burner flame for escape of gases and transferred into the muffle furnace at 450 °C to ash for 4 h with periodical check for complete ashing (when a whitish residue appeared). The furnace was switched off and the residue allowed to cool. The ashed samples were later removed and put in a dessicator.

Solution of the Mushroom Samples

5 mL of 10 % HCl solution was added to the ash and heated in water bath for complete dissolution. 5 mL of 10 % nitric acid were also added and boiled in water bath for complete disolution. The sample solution was transferred quantitatively using a stirring rod and through a funnel with acid treated filter paper, into a clean dry 50 mL standard flask and made up the volume with de-ionized water, after rinsing both crucible and filter paper. The resulting solution was used for flame photometer or atomic absorption spectrophotometer.

Digestion of Substrates and Soil Samples

1.0 g of dried substrate was digested in a 500 mL flask with a mixture of concentrated nitric acid and perchloric acid in the ratio of 4:1 for 1 h on an electric hot plate. The resulting residue was re-dissolved in 0.1 M HNO_3 and filtered using 0.1M HCl pre-washed filter paper. The filtrate was made up to 50 mL mark in a volumetric flask with de-ionized water.

Preparation of Stock Solutions of other Trace Metals (Okoye, 2005).

Stock metal solutions were prepared as follows:

Cobalt: 4.76 g of cobalt sulphate was dissolved with de-ionized water and made up to 1litre to get 1000 mg/L stock solution.

Iron: 2.78 g of ferrous sulphate($FeSO_4$) (analar grade) was dissolved in de-ionized water containing 50 mL 0.1M sulphuric acid. The solution was standardized by titrating with 20 % potassium dichromate solution using N-phenlanthanic acid as indicator.

Manganese: 3.08 g manganese sulphate ($MnSO_4$) was dissolved with 200 mL deionized water. To the solution 1.5 mL of conc.nitric acid was added and made up to one litre. The solution was found to contain 1000 mg/L of manganese.

Zinc: 4.40 g of zinc sulphate ($ZnSO_4$) was dissolved in de-ionized water and made up in a 1litre flask. The solution was standardized with 0.1M EDTA using eriochrome black-T as indicator. The solution was found to contain 1000 mg/L Zn^{2+}.

NB: At least five serially diluted standard solutions of each metal were prepared by diluting the stock solution with 0.1 M HCl.

Chromium stock solution: 1000 µgCr/mL: Dissolve 0.1923 g CrO_3 in mixture of 10 mL de-ionized water and 1 mL HNO_3. Dilute to 100 mL with de-ionized water.

Nickel stock solution: (1000µg Ni/mL): Dissolve 0.100 g Ni powder in 5 mL HNO_3 by heating at (75 -80) °C, cool to room temperature and dilute to100 mL mark with de-ionized water.

Copper stock solution: (1000 µg Cu/mL): Pickle Cu metal in (1+9)HNO_3 solution to 0.100 g. Dissolve in 5 mL (1+1) HNO_3 Solution by heating at (75 -80) °C, cool to room temperature and dilute to 100 mL mark with de-ionized water.

Lead standard solutions: Stock solution-1 mg/L: Dissolve 1.000 g Pb powder in 20 mL HNO_3 (1+1) in 1 L volumetric flask and dilute to volume with de-ionized water. (2) Working solution: 5 µg/mL. Pipet 1 mL stock solution into 200 mL volumetric flask and dilute to volume with de-ionized water.

Cadmium standard solutions: Stock solution-1 mg/L: Dissolve 1.000 g Cd powder in 20 mL HNO_3 (1+1) in 1 L volumetric flask, and dilute to volume with water. (2) Working solution-1 µg/mL. Pipet 10 mL stock solution into 100 mL volumetric flask, and dilute to volume with de-ionized water. Pipet 2 mL of diluted solution into 100 mL volumetric flask and dilute to volume with de-ionized water.

Determination of Trace Metals by AAS (Okoye,2005).

Preparation of Mushroom Sample Solutions

2.0 g of dried and ground mushroom samples were ashed in glazed crucible after pre-burning(charring) over a Bunsen flame in a fume chamber. The ashing was done at 450 °C for 4 h in a muffle furnace. After cooling, the ash was transferred into a 50 mL beaker by dissolving in 10 mL concentrated HNO_3 and rinsing with 10mL conc. HCl. The solution was covered with

watch glass and warmed gently for 10 min, the solution was then cooled, decanted into 100 mL volumetric flask, and made up to the mark with de-ionized water.

Bioaccumulation factors of trace metals of wild and cultivated mushroom samples were calculated as follows:

$$\text{Bioaccumulation Factor} = \frac{\text{Trace metal concentration in mushrooms}}{\text{Trace metal concentration in soil/substrates}}$$

Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) using Statistical Package for Social Scientists (SPSS) version 16.0. Significantly different means were determined using Duncan's multiple range test(Okoye,2005,Yusuf,2004,AOAC,1990)

RESULT AND DISCUSSION

Results

Table 4 contains mean Concentrations of Toxic Metals in wild and cultivated mushrooms.

Table 4: Mean Concentrations (mg/kg)of Trace Metals in Wild and Cultivated Mushrooms.

Mush room species	Wild								Cultivated							
	Cu	Co	Pb	Zn	Cd	Ni	Mn	Cr	Cu	Co	Pb	Zn	Cd	Ni	Mn	Cr
Tr	0.12 ±0.01	0.59 ±0.01	5.03 ±0.01	50.88 ±0.01	4.48 ±0.01	16.85 ±0.01	24.42 ±0.01	--	--	--	--	--	--	--	--	--
Ab	0.39 ±0.01	1.21 ±0.01	4.56 ±0.01	28.72 ±0.01	4.41 ±0.01	1.40 ±0.01	15.20 ±0.01	-BDL	0.09 ±0.01	2.03 ±0.01	6.33 ±0.01	68.56 ±0.01	9.15 ±0.01	2.19 ±0.01	21.72 ±0.01	BDL
Ptr	0.22 ±0.01	0.48 ±0.61	3.60 ±1.01	25.00 ±6.01	4.30 ±0.91	3.04 ±1.01	13.60 ±3.01	BDL	0.01 ±0.01	0.49 ±0.01	3.68 ±1.21	45.48 ±0.01	4.40 ±1.31	3.08 ±1.01	13.60 ±2.01	BDL
Aph	0.72 ±0.41	1.52 ±0.21	4.62 ±0.01	34.02 ±5.01	3.88 ±1.01	4.77 ±1.61	16.60 ±2.01	-BDL	0.22 ±0.01	6.04 ±0.81	5.55 ±1.01	48.33 ±7.01	4.99 ±1.01	6.89 ±0.91	18.80 ±0.71	BDL
Av	0.66 ±0.02	0.63 ±0.01	3.83 ±0.04	61.17 ±0.08	6.68 ±1.01	15.07 ±3.01	8.25 ±1.01	-BDL	0.36 ±0.01	4.83 ±0.71	4.89 ±0.88	9.33 ±0.81	9.88 ±1.01	22.05 ±2.01	11.60 ±0.98	BDL
Range	0.12-0.72	0.48-1.52	3.60-5.03	25-61.1	3.88-6.68	1.40-16.8	8.25-24.4	-BDL	0.01-0.36	0.49-6.04	3.68-6.33	9.33-68.5	4.99-9.88	2.19-22.0	11.60-21.7	BDL
WHO 2004	0.90	0.29	0.63	11	0.59	0.50	2.3	BDL	0.90	0.29	0.63	11	0.59	0.50	2.3	BDL
CODEX 1995	3	0.01-0.1	0.05	17	0.1	0.4	0.13-0.26	BDL	3	0.01-0.1	0.05	17	0.1	0.4	0.13-0.26	BDL

Tr-Termitomyces robustus,Ab-Agaricus bisporus,Ptr-Pleurotus tuber-regium,Aph-Amanita phalaoides,Av-Amanita verosa

Chromium was below detectable level, while copper in all mushroom samples was below the WHO guideline concentrations in food. Other trace metals were higher than WHO 2004 guideline levels in all anlyzed samples(wild and cultivated).

African Journal of Agriculture and Food Science
ISSN: 2689-5331
Volume 3, Issue 4, 2020 (pp. 103-121)
www.abjournals.org

Table 5 contains mean concentrations of trace metals in soils and substrates from where mushroom samples were collected.

Table 5: Mean Concentrations (n=3) of Trace Metals in soils and substrates from where Wild Mushroom Samples were collected

Soil/sub	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Garden soil	6.04 ±0.44	1.1066 ±0.04	2.09 ±0.10	10.62 ±1.02	119.33 ±1.22	2.64 ±0.66	8.66 ±0.62	66.06 ±0.40	18.83 ±0.10
Decayed wood$_1$	5.15 ±0.44	BDL -	30.10 ±0.78	10.18 ±1.71	205.78 ±2.78	40.44 ±0.99	18.45 ±0.40	4.22 ±0.33	16.35 ±0.72
Farmland soil$_1$	10.55 ±2.33	10.91 ±1.86	8.89 ±0.79	10.77 ±0.97	106.39 ±2.67	10.54 ±0.88	23.19 ±0.79	6.06 ±0.69	80.83 ±2.55
Farmland soil$_2$	3.38 ±0.98	2.01 ±0.12	0.19 ±0.01	2.08 ±0.77	344.57 ±2.96	1.39 ±0.49	8.18 ±0.89	4.77 ±0.68	4.77 ±0.70
Decayed wood$_2$	30.30 ±2.86	1.88 ±0.48	0.04 ±0.01	1.39 ±0.22	444.58 ±0.70	1.39 ±0.10	6.67 ±0.69	16.90 ±0.80	10.04 ±0.25
Farmland soil$_3$	6.04 ±1.29	1.10 ±0.62	2.09 ±0.16	10.62 ±1.66	205.78 ±0.96	2.64 ±0.66	8.66 ±1.01	66.06 ±2.91	18.83 ±1.22
Refuse soil	17.65 ±1.59	4.44 ±0.01	6.44 ±0.98	15.79 ±1.77	601.33 ±6.98	7.89 ±0.57	18.89 ±1.59	108.04 ±4.08	22.44 ±1.33
Sub(leaf litters)	3.18 ±0.19	2.01 ±0.67	0.03 ±0.01	2.08 ±0.72	106.39 ±2.16	1.39 ±0.14	2.18 ±0.60	1.77 ±0.80	1.89 ±0.44
WHOagric soil	0.05-0.10	2-5.60	0.10	20.00	400-777	50-200	20.00	20.00	100
WHO $_{\text{Polluted soi}}$l	3-5.00	5-10.00	4-10.00	50-140	400-780	50-200	75-200	50-250	300-700

Tr-*Termitimyces robustus,Ab-Agaricus bisporus,Av-Amanita verosa,Aph-Amanita phalaiodes*

All the soil samples were polluted with Cd and Cr while 40 % are polluted with Pb which also shows elevated values in others. There are also elevated values of Ni in all the samples. The rest of the metals were below WHO guideline levels.

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Table 6 Contains the bioaccumulation of trace metals in wild and cultivated mushrooms.

Table 6: Bioaccumulation Factors of Trace Metals in Wild and Cultivated Mushrooms.

Mush Species	BIOACCUMULATION FACTORS																		
	Wild									Cultivated									
	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Tr*	0.74	0.54	-	0.01	1.79	9.25	1.95	0.08	2.70	-	-	-	-	-	-	-	-	-
Ab	0.86	0.28	-	0.04	1.30	0.38	0.08	1.08	1.76	1.78	0.47	-	0.01	2.16	0.54	0.12	1.50	4.19
Av	0.63	0.06	-	0.06	1.45	0.78	0.65	0.63	0.76	0.94	0.44	-	0.03	2.22	1.10	0.95	0.79	6.12
Aph	1.15	0.76	-	0.35	1.17	11.94	0.58	0.97	7.13	1.48	3.01	-	0.11	1.27	13.53	0.84	1.16	10.13
Ptr	0.14	0.26	-	0.12	1.54	9.78	0.46	0.21	2.49	0.15	0.26	-	0.01	1.77	9.78	0.46	0.22	4.53

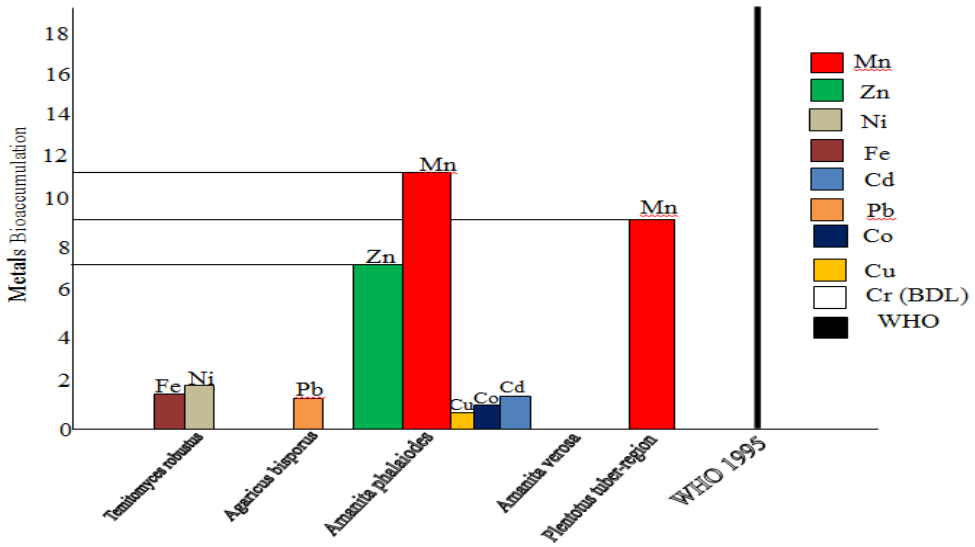

Fig. 5: Bar chart representation of bioaccumulation factors in wild mushroom samples

African Journal of Agriculture and Food Science
ISSN: 2689-5331
Volume 3, Issue 4, 2020 (pp. 103-121)

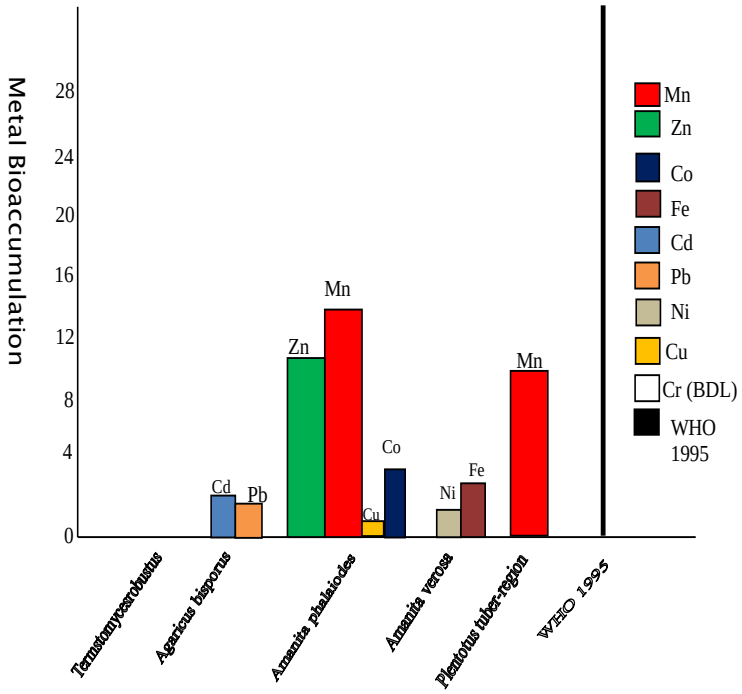

Fig 6: Bar Chart of Bioaccumulation Factors of Trace Metals in Cultivated Mushrooms.

In table 6 and figs 5&6 bioaccumulation factors were low in spite of the fact that many of the mushroom species have levels of metals higher than WHO guideline values for food. Only Mn and Zn showed substantial bioaccumulation. Chromium was below detectable limit in all the species of mushroom for wild and cultivated.

DISCUSSION

Randomized sample collection is key to obtaining reliable and precise data. Due to the fact that mushrooms are seasonal and substrate specific, samples were collected during the period of availability (rainy season Table, 4.1). In other words, there were no pre-designated sites. However, sampling was carried for 3 years in order to randomize as much as possible. The samples were leached by placing it in watch glass and allowing tap water to run on it gently to avoid crumbling (Hurrell,2001). Food samples are normally dried at 105 °C to constant weight to ensure accurate weights and maintain integrity of the sample.

Preparation of mushroom samples for analyses include: drying, grinding, ashing or digestion. Each of these steps was a potential source of contamination: thus in preparing the samples, great attention was paid to preserve the original chemical constituents of the mushroom samples. This was done by using clean laboratory wares.

Ashing aid (HNO_3) was applied to ensure non-volatilization of volatile metals during ashing, and to ensure solubility of the ash, since all nitrates are soluble. Addition of HNO_3 as ashing aid has been used extensively with excellent recoveries (Okoye, 2001, Hurrell, 2001, David,

1976). Distilled water was used for preparation of solutions for proximate analysis while de-ionized water was used for preparation of solutions for metal analysis**.**

Trace Metal Concentrations in Soils and Substrates.

From Table 9, it is evident that the soils and substrates from where the studied mushrooms were collected showed concentrations of Cd, Cr, Ni and Pb higher than WHO guideline values while the rest of the metals were below acceptable levels.

Concentrations and Bioaccumulation of

Trace Metals.

Among the trace metals determined, copper was the only metal with concentration below WHO guideline levels in food. Other trace metals showed concentrations higher than WHO guideline values in food. This observation supported the claim that under natural conditions, heavy metals concentrations of some species of mushrooms could be high even if the degree of soil pollution is low (Onyeka, 2013, Lepp, 1981, David, 1976). Moreover, the short cropping cycle of 10-14 days indicates that these species of mushroom have high rate of trace metal uptake and accumulation which could be exploited in bioremediation of trace metal polluted soil.

Potentials of the Studied Mushrooms forr Trace Metals Removal from Polluted Soils.\

Bioaccumulation factors are indices of the levels of acquisition or retention of persistent contaminants by living organisms relative to the concentrations of such contaminants in the ecosystem. The bioaccumulation factors calculated in the present study are largely low, although the concentrations of the metals except Cu and Cr in the analyzed samples exceeded WHO recommended levels. Comparatively, Mn and Zn showed considerable BCF levels. Mn has BCF of 11.94 -13.53 in *Amanita phalaoides*; 9.78 in *Pleurotus tuber-regium* and 9.25 in *Termitomyces robustus; while* Zn has 7.13-10.13 in *Amanita phalaoides* and 6.12 in *Amanita verosa*.

The studied mushrooms, can effectively remove Mn and Zn from metal polluted soil. Mushrooms have several advantages over other bioremediation agents: they have shorter life span, higher accumulation capacity and ease of removal of biomass (Dilna et al 2011). The short cropping cycle of mushrooms, 10-14 days is advantageous in that cropping could be carried on many times in a season. In this way, *Amanita phalaoides* shows great potential for removal of Mn and Zn, just, *Pleurotus tuber-regium* and *Termitomyces robustus* for Mn and *Amanita verosa* for Zn.

CONCLUSION

Mushrooms being a popular food delicacy of modern world have gained increasing attention in bioremediation and biotechnology. The following conclusions were drawn from the studied mushrooms. They are:

- They are accumulators of toxic metals namely;- Cd, Co, Fe, Mn, Ni, Pb, and Zn.
- All the accumulated toxic metals are above WHO guideline values which could impair their edibility.

- For purposes of bioremediation, *Amanita phalaoides* has great potential for removal of Mn and Zn, *Termitomyces robustus and Pleurotus tuber-regium* for Mn and *Amanita verosa* for Zn from trace metal polluted soil

- Safe levels of Mn and Zn could be achieved in polluted soils by taking advantage of their short cropping cycle and planting many times in a season.

- The cultivation of *Amanita phalaoides*, *Pleurotus tuber*-regium, *Amanita verosa* and *Agaricus bisporus* were achieved.

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