PHYSICO-CHEMICAL AND TOXICOLOGICAL PROFILES OF FIVE SPECIES OF MUSHROOM IN ANAMBRA STATE, NIGERIA AND THEIR POTENTIAL FOR BIOREMEDIATION OF TRACE METAL POLLUTED SOIL

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APPROVAL PAGE

This research project has been approved for Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, University of Nigeria, Nsukka.

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CERTIFICATION

Okaroh, Bridget Chinweude, a postgraduate student in the Department of Pure and Industrial Chemistry with registration number PG/Ph.D/06/40981 has satisfactorily completed the requirement for the award of the Degree of Doctor of Philosophy in Analytical Chemistry. The work embodied in this thesis is original and has not been submitted in part or full for any other diploma or degree of this or any University.

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DEDICATION

This work is dedicated to Almighty God who has been my strength. Also, to my lovely children Okaroh, Mmesoma Precious, Okaroh, Ifenna ThankGod and Okaroh, Chinaemeze Godswill.

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ABSTRACT

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 2009 and 2012 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 hours 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 (Table 3.2). 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 oven dried at 75 ^{0} C for 4 hours. The dried mushroom samples (wild and cultivated respectively) were homogenized into fine powder using 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 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. The moisture content (MC) ranged from 81.79 % to 97.84 %, the highest value was from *Amanita phalaoides* and the least value was from *Agaricus bisporus*. Dry matter (DM) ranged from 2.63 % to 18.36 % showed an indication of high roughages content of the mushrooms. Crude protein (CP) ranged from 8.16 % to 24.67 % which compared favourably with values of seeds and legumes. Ash contents ranged from 3.26 % to 14.33 % were indications of high mineral elements present in the studied mushroom species. Low values of Lipid (fat/oil) ranged from 1.00 % to 6.68 % gave acceptance of the mushrooms as excellent dietary food for diabetic and coronary heart disease patients. Crude fibre (CF) ranged from 2.62 % to 15.37 %. There were no significant differences at $p>0.05$ between values of wild and cultivated mushrooms. Carbohydrates contents of 32.00-35.40 %, implied that mushrooms can function effectively in low fat diet such as those required by patients with cardiovascular diseases and diabetes. The vitamin C were detectable at levels ranging from 0.01-0.37 mg/100 g, values determined showed that the studied mushrooms were not good sources of vitamin C, although they could make important contribution to diet. The mean anti-nutritional factors of phytic acid 0.26 mg/100 g, cyanide 0.16 mg/100 g, tannins 0.31 mg/100 g were low when compared with 1.00 mg/100 g found in WHO guideline levels for these toxicants in food. Essential metals concentrations (mg/kg) for wild mushroom samples ranged as follows: Na (152.36 ï 777.42), K (166.88 ï 933.81), Ca (83.64 -545.00), Mg (476.57ï 1191.00), Fe (154.68ï 684.74) while values

for cultivated mushroom samples ranged as follows: Na (332.77 ï 1061.12), K (196.36 ï 844.23), Ca (219.69 ï 1841.08), Mg (549.66 ï 1566.11) and Fe (401.66 ï 777.18) mg/kg. The essential metal concentrations were within WHO guideline values for food. Trace/toxic metal concentrations (mg/kg) for wild mushroom samples ranged as follows: Cd (3.88 ï 6.68), Co (0.48 ï 1.52), Cr (BDL), Cu (0.12 ï 0.72), Mn (8.25 ï 24.42), Ni (1.40 ï 16.85, Pb (3.60 ï 5.03) and Zn (25.00 ï 61.17). Values (mg/kg) for cultivated mushroom samples ranged as follows: Cd (4.40 ï 9.88), Co (0.49 ï 6.04), Cr (BDL), Cu (0.01 ï 0.36), Mn (11.60 ï 21.72), Ni (2.19 ï 22.05), Pb (3.68 ï 6.33) and Zn (9.33 - 68.56). There were significant differences at ($p < 0.05$) between mean trace metal concentrations of wild and cultivated mushrooms. All the soil/substrates samples used for cultivation were polluted with Cd and Cr while 40 % were polluted with Pb. However, Pb showed elevated values in other metals. There were elevated values of Ni in all the mushroom samples. The rest of the metals were below WHO guideline levels. Bioaccumulation factors ranged as follows: Cd (0.14 - 1.78), Co (0.06 ï 3.01), Cr (BDL), Cu (0.01 ï 0.35), Fe (1.17 -2.22), Mn (0.38 ï 13.53), Ni (0.08 ï 1.95), Pb (0.08 ï 1.50) and Zn (0.22 ï 10.13). These values were above acceptable limit in food.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Mushrooms

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[1]. In many parts of the world, such as China, United State of America (U.S.A), Canada, India, Italy, Mexico and Turkey, mushrooms are highly priced and in massive production for local consumption and export. In U.S.A, the gross domestic products (GDP) for mushroom was seven million tonnes in 2005, amounting to 30 million dollars annually[1]. In Nigeria, mushrooms are grossly under exploited as only a few types are considered edible. There is little 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[2]. 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[2]. This has been occasionally attributed to many deaths after mushroom meals[2]. 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[1, 2].

In many countries of the world including Nigeria, edible mushrooms have been priced as delicacies[1, 2]. 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[3]. In fact, Agrahar and Hammam[1, 3] 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[4].

Health risk from mushroom consumption has been difficult, due to very limited knowledge in chemical compositions of the metals and their bioavailability in man[5]. 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[4, 5]. Also reported was the removal of heavy metals and other harmful contaminants from environment by Shiitake mushroom[5, 6]. 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[7]. It was reported that mushroom channels heavy metals from land to fruity bodies for removal from the soil/environment[8]. 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[9]. Similarly reported was the use of Turkey tail mushroom and Phoenix oyster mushroom mycelia to eliminate 97 % mercury ion from water[10].

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[11]. 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[12]. 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[13]. 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[14].

1.1.1 Characteristics of Mushrooms

Mushrooms (Fig.2.2) grow in dark, damp places and feed on decaying matter. Mature mushroom develops spores in the gills located on the under-side of the cap. New mushrooms grow from these spores which are of microscopic sizes[15]. These are the fungal equivalent of seeds[16].When the spores are ripped, millions of them, like fine powder, are dispersed as they drift away in the wind. If they land in a dark, damp place with adequate decaying matter, they germinate and grow into new mushrooms. They develop a thread-like structure called hyphae. Lots of hyphae grow together and form the mycelium. Mushrooms often grow in a ring. The fruiting body starts to grow above the surface like a little button protected by a cap which covers the veil. The veil splits and falls down around the stalk (stem) and form the annulus. The life cycle of mushroom is between two to three weeks[16, 17.]

Mushroom is a fungus, which feeds by secreting enzymes and digests food externally and absorb the nutrients in net like chain called hypha. The net like chain (hypha) is exposed to stimuli in their ecological niche and act as a conscious intellect and respond to stimuli. Dense and regular branching of hypha endows fungi with potentials to pervade any substrate thoroughly[18]. The higher the mycelium thickness,

the higher the rate of mechanical penetration and breaking down of substrate. This culminates the higher rate of digestion of substrate through the secretion of extra-cellular enzymes. This shows the potentials of bioremediation capabilities of mushroom[19]. This hypha/mycelium penetrates contaminated soils, thus placing a mat on them; it is the process of breaking down and adsorption of toxic products or pollutants. Generally, the bonds in hydrocarbon and petroleum products are similar to bonds that hold the plant materials together. The enzymes produced by mushroom which are lignin peroxidase, manganese peroxidase and laccase penetrate, break and digest or mineralizes these hydrocarbon, petroleum products and pesticides to primary non-solid products and are liberated in form of water and carbon dioxide[20]. These enzymes act singularly or collectively in aiding mycelium to break down natural or human made resistant materials[21]. Similarly, it was reported that the mycelium of Shiitake mushroom exposed to heavy metals of cadmium, copper, lead, mercury and zinc increased the production of enzymes laccase, decolorized them and subsequently absorbed the heavy metals[22].

1.1.2 Economic Importance of Mushrooms

Mushrooms can be of much economic value in tropical rain forest ecology and can play important role in food security programme[22, 23]. In Australia, mushrooms are their second cash crop after potatoes. In China, United States of America, Russia and Hungary, mushrooms are highly priced and are even exported[23]. Globally, countries like United States of America, Mexico, Canada, Italy, India, China, South Africa and Turkey have engaged in massive production of edible mushrooms for export and its food values[24]. In Nigeria, mushrooms are consumed mainly in rural areas, and only a negligible quantity gets to the cities[25]. Moreover, mushrooms are available only from the wild (that is not cultivated) and collection of wild mushrooms

had been and still is a source of income in rural communities. Nigeria being in the tropics, mushroom production as a commercial venture could be an effective means of poverty alleviation. This is due to its short cropping cycle and low cost of planting input[26]. These features of mushrooms can be harnessed in several ways in Nigeria as is already being done in some African countries such as Kenya, Uganda and Zimbabwe. In the 1990ôs, the world production of cultivated edible mushroom was estimated at 7 million tonnes, in the same period the total market value for medicinal and edible mushrooms was estimated to be in excess of USD.30 billion[27].

1.1.3 Edible, Medicinal and Poisonous Mushrooms in South-East, Nigeria

The most popular edible mushroom in Nigeria is the *Pleurotus tuberregium* (Fr.) Singer and it is eaten as food or used as food supplement[28]. It was reported to be a good substitute for meat in many rural villages especially in southï Eastern States. In the same vein, *Chlorophyllum molybditis* also featured amongst edible mushrooms in this area and have been analyzed for its nutrient contents and considered safe for consumption[29]. *Amanita* and *Chlorophyllum* species earlier reported as poisonous but are consumed in Nigeria is not totally understood but it is believed that the controversy may stem from any one or combination of factors relating to environment, genetic and physiological differences which were determinants of tolerance level to toxins amongst racially, geographically and traditional varied people. The Yoruba and Hausa tribes have been associated with mushrooms more than the other tribal groups. The Yoruba tribes are however reported to have identified and used much greater number of mushrooms for food and medicine than the Hausa tribes[30].

In Nigeria, a variety of edible and medicinal mushrooms are sourced from the wild due to unorganised mushroom farming culture. This practice (mushroom

scouting/hunting) existed for generations and mostly embarked upon by children and women. About twenty-five edible mushroom species of good repute whose knowledge were handed down generational lines via oral communication have been identified in Nigeria[31]. These edible mushrooms, collected from various farmlands, forests and plantations may be sold or cooked fresh, after treatment with warm salt water, with the addition of essential ingredients like pulped pepper, tomatoes, onions, salt and oil or smoked and/or sun-dried for later use.

Traditionally, mushrooms are used for nutritional, medicinal and mythological benefits in Nigeria. The uses of mushroom genetic resources are not only of high interest in agronomy, agriculture, human food and animal feed but also for the discovery, production and development of molecules or components with high added value in industries such as chemical and pharmaceutical industries[32]. This emphasizes the relative significance of field study and effective documentations as the bedrock for efficient mushroom exploitation. The nutrients and toxicological profile of edible wild mushrooms in Nigeria have been studied[29, 33]. There is however dearth of information on the anti-oxidant property of edible and medicinal mushrooms indigenous to Nigeria. The level of mushroom nutriceuticals on a global scale confirmed that mushrooms are good health food and reports abound in Nigeria on their use for the treatment of malnutrition in infants, diabetes, obesity or hyperlipidemea, sterility, anaemia, mumps, fever and protein deficiency. Recently, *Ganoderma* species have been successfully tested in poultry farming for the improvement of egg-laying and disease resistant capacity of birds in Nigeria. At the 2nd African Conference on edible and medicinal mushrooms in 2009, it was reported that beta-glucan based dietary supplements of mushroom origin are effective for the treatment of Buruli ulcer caused

by *Mycobacterium ulcerans* in Ghana while *Ganoderma lucidum* (Leyss.) *Karst* and *Eimeria tenella* can be used for the treatment of infected broiler chickens[31, 34].

1.1.4 Statement of Problems

In many parts of the world, such as China, United State of American (USA), Canada, India, Italy, Mexico and Turkey, mushrooms are highly priced and in massive production for local consumption and export. In U.S.A, the gross domestic products (GDP) for mushroom were about seven million tonnes in 2005, amounting to 30 million dollars annually[48].

In Nigeria, mushrooms are grossly underexploited as only a few types are considered edible. There is insignificant mushroom cultivation as a commercial venture in Nigeria, but this could massively 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[49]. 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. This has been occasionally attributed to deaths after mushroom meals[50].

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[51]. Mushroom are hyper accumulators of heavy metals and radioactive metals that are toxic to consume and are thus eliminated from the environment[52].

Edible mushrooms are fungi, which belong to the class Basidiomycetes. They have been found to provide rich addition to diet in the form of carbohydrate, proteins, fibre, ashes, minerals, valuable salts, vitamins and enzymes. The economic trends in

the country has demanded looking inwards for solutions to the national food problems. Protein deficiency in Nigerian infants was highlighted by many authors[1, 23, 27.] Nutritionists have proposed increasing the consumption of vegetable and plant proteins. It has been reported that if the quantity of straw burnt in the field could be utilized to grow mushrooms, millions of tonnes of mushrooms for table use could be produced annually[53].

Unsuccessful attempts to grow mushrooms have been made at Obasanjoôs Farms at Ota, Ogun State, Nigeria. Consumers depend on either imported mushrooms whose price are not within the reach of many Nigerians or on seasonal flushes which are not dependable, both in quality and quantity.Several factors contribute to the relative neglect of mushroom growing in developing countries. The major limiting factor in Africa, Latin America and the world is lack of the technical skills and theoretical knowledge necessary for spawn production. Other important problems include the identification of local edible species suitable for commercialization. The advantage of local species over imported temperate ones is the possibility without air conditioned environment. Information on the biology and characteristics of local mushroom species are scarce. There is scarcity of information on the studies of mushroom species in Anambra State, Nigeria.

1.1.5 Aim and Objectives of the Study

The aim of this 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.

The specific objectives were to:

i. determine the physicochemical and toxicological profiles of five wild and cultivated mushrooms in the study area
ii. determine their nutritional and anti-nutrional properties and
iii. determine their ability to bioremediate metal polluted soil.

1.1.6 Scope of Study

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 2009 and 2012 in Anambra State, Nigeria. Some of the samples were cultivated on soils and substrates, harvested and both (wild and cultivated) samples were subjected to chemical analyses using standard methods by Association of Official Analytical Chemists (AOAC). Atomic absorption spectrophotometer was used in determining the concentrations of the heavy metals in the mushroom samples while some mineral elements were determined using flame photometer. It was expected that the analyses would give an overview of the physic-chemical and toxicological profiles of the five species of mushroom in Anambra State, Nigeria. Also their possible use in biotechnology for bioremediation of trace metal polluted soil was investigated. The mushroom samples were selected using a validated food frequency questionnaire which was crried out within Anambra State to ascertain their status and edibility.

1.1.7 Significance of Study

The country has entered economic recession as a result of non-diversifiation of the economy, the fall in oil price has put the country into serious financial crisis. Presently the gross domestic product (GDP) from non-oil sector is at 20 % while in

countries like China, Canada U.S.A and Australia the gross domestic products (GDP) from mushroom alone is about 40 %. Mushrooms as vegetative food will ensure food security (Fig.2.1), reduce some health risks associated with consumption of high cholesterol and carbohydrate foods. Nigerian unemployment rate is at increase, with mushroom cultivation, more youth will be engaged and their social life will be enhanced. The nation is confronted with various ecological and environmental challenges such as climate change, deforestation, aggressive soil erosion, coastal erosion, water pollution, oil pollution, flooding and urbanization, many of the indigenous mushroom species are disapearing very fast. This study will provide a platform that may draw the attention of the government to urgently rescure our fast disappearing natural resources including mushrooms. Successes have been recorded in Kenya, Zimbabwe, Swizerland, Malawi, Tanzania, Namibia and others through mushroom farming, it is expected that in future, Nigeria will have similar success story. The fear of food poisoning after mushroom meals will be eliminated. The study will provide a platform for better mushroom management and production in the country.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mushroom Resources and Uses in Nigeria

Nigerians have used mushrooms only for food and folk medicine for many decades. Only of recent, *Auricularia auricular* Judae (Bull) Quél, *Lentinus squarrosulus* Mont., *Pleurotus tuberregium* (Fr.) Singer and *Volvariella volvacea* (Bull.) Singer, some of the common edible mushrooms have been successfully cultivated on small-scale basis[53]. Thus mushroom resources in Nigeria remain grossly under-studied and their attractive potentials under-exploited for addressing economic and industrial development. Resourceful biotechnological approach in the application of mushrooms in agriculture, medicine, industry and environment is informal and uncommon in the country[54.]

Mushrooms are wide spread in nature and they remained the earliest form of fungi known to mankind. In Nigeria, many people in urban and rural areas are familiar with mushroom forming fungi growing around them some of which they exploit for food and medicinal purposes. Among the Nigerian tribes, Yoruba speaking people have used mushrooms for various purposes more than the rest. There are varieties of mushrooms including lichenized and mycorrhizal found in Nigeria. They are potentially resourceful tools in economic and technological developments. It is saddening to see that people from all works of life associate mushrooms with negative events in Nigeria and most African countries[54].

In African countries, mushroom resource exploration and exploitation is fraught with lack of infrastructure and technical support from national and international agencies, scarcity of mushroom scientists, including mushroom taxonomists, culminating in poor knowledge of mushroom biodiversity. African

nations are seldom listed among the producers and exporters of edible mushrooms and mushroom products[55]. The flux of unprecedented global reports on mushroom potentials, explorations and exploitations show that Nigeria and in fact Africa is yet to be aware of the huge potentials of mushroom production.

2.2 Cultivation of Mushrooms

A close look at the traditional farming system reveals that there has been a place for mushroom production in Nigeria. In some parts of the country, the local population produce mushrooms by taking proper note of the site of occurrence of a mushroom in the rainy season[55]. The site is protected either by fencing or ring weeding such that it is isolated from other parts of the farm or the forest. The site is regularly visited for mushroom harvesting. Mushroom collection from the wild had been in the past and still is a source of income for rural dwellers in Nigerian communities. Therefore, mushroom production as a commercial venture will be an effective means for poverty alleviation. This is due essentially, to its short cropping cycle, low cost production, high profit and quick return. These features of mushrooms can be harnessed in several ways in Nigeria as is being done in other countries such as India, Kenya, Uganda and Zimbabwe for economic empowerment of our people[56].Mushroom cultivation is a big-time multimillion dollar business world over.

About two decades ago, the world production of cultivated edible mushroom was estimated to be about 7 million tonnes[56]. The combined total market value for medicinal and edible mushrooms for the same period was estimated to be in excess of U.S. $30 billion. In China for example, the production of mushrooms increased steadily over time (Table 2.1). It is being anticipated that in the nearest future, Nigeria will be able to have similar data for comparison.

Table 2.1: Production of Cultivated Mushrooms in China during the period 1978– 2000.

Year	Production(x 1000MT)	Increase (%)
1978	60	-
1986	585	875.0
1994	2640	351.3
1997	3918	48.4
2000	6630	69.2

(Source: Chang and Miles, 2004)[43]

In Africa, it has been reported that even if the production cost for mushroom is doubled it would still remain more profitable than that of either maize wheat or soyabean. Paradoxically, however, the production of mushrooms can even be integrated into the already existing agro-system such as maize, wheat and soyabean programmes[55].

The reliance on naturally growing mushrooms has greatly undermined the development of mushroom cultivation to a commercial scale despite available substrate materials in some African nations. Despite the fact that about 20 % of the world population were reported starving[29], African nations are still lacking in mushroom utilization. Tapping into the benefits of commercial mushroom production in Nigeria will reduce the countryôs unemployment rate, increase her food security and revenue base. The number of cultivable edible mushrooms worldwide is over hundred species with an annual production of over 4.5 million tonnes[57]. The provision of safe sustainable access to edible and medicinal mushrooms in Nigeria can be achieved in a number of ways which may include (i) promoting opportunities for co-operation between stakeholders such as the mushroom farmers, researchers/ mycologists, politicians and other mushroom prospectors (marketer, non governemtal

organizations (NGOs) and government agencies on agriculture, youths and women etc.) in the country; (ii) through the creation of public enlightenment initiatives via talk shows on the positive potentials of mushrooms and mushroom products on radio and television programs, monthly newsletter, seminars and workshops. This will remove the negative publicity associated with mushrooms, increase market sources of edible mushrooms, limit the dangers associated with mushroom hunting from the wild and improve awareness on both the nutrient quality and benefits of mushroom consumption; (iii) developing a model that allows for spawn availability to farmers and steady flow and/or exchange of proprietary culture (mother cultures and pure-lines). This is in addition to the cross fertilization of cultivation technologies between developing and industrial nations, and creation of recognized indigenous mushroom growers association. The establishment of sustainable regional mushroom germplasm banks and research centers to maintain mushroom genetic stability, quality control of mushroom culture collections and spawn, and preservation of cultures of extant and extinct mycoresources can also enhance the overall uses of mushrooms in the country. One cannot but add that the elevation of mushrooms to a cash-crop status in Nigeria requires improved political will and availability of infrastructure such as steady electricity, flowing water and buildings.

Despite the high level of progress made through the global network on mushroom research and development under the aegis of Food and Agricultural Organisation (F.A.O) and the advancement of mushroom cultivation industries in many developed nations, growing mushrooms in homes or even on a commercial scale is still uncommon in Nigeria. Researchers are therefore challenged to reduce dependence on naturally occurring mushrooms, the incidences of mushroom poisoning and expand the nation's edible and medicinal mushroom base. Many

indigenous edible mushrooms heritage and knowledge may have escaped recognition and documentation and/or completely lost over the years. Although, few works such as have been reported[58] a long-term study on the ethnomycological, taxonomic and myco-diversity profile of indigenous mushroom resources on a national scale will form the inertia for mushroom prospecting initiatives and successful exploitation in developmental economic issues in Nigeria.

2.2.1 Mushroom Production Using Agro-Industrial Wastes

One of the new technological advances in mushroom production is the use of agro-industrial and other wastes of diverse origin as substrates. Nigeria by virtue of her population, generates several tons of agricultural, industrial, municipal and domestic wastes that overwhelm the nation's waste disposal machinery[58]. Most of these wastes are degradable by mushrooms[59, 60]. These wastes are used as substrates or to supplement substrates or as manures for compost in mushroom cultivation. Such potential degradable wastes include; industrial effluents and many lignocellulosic wastes such as waste papers, banana and plantain leaves, and/or peelings, sawdust of different tree origin, oil palm fruit fibres, bunches and cakes. There are huge potential socio-economic benefits associated with the effective and efficient bioconversion of agro-industrial wastes to valued edible sporocarps. The growth of mushroom production industries and the use of agro-industrial based substrates as the major raw material may provide a partial solution to the nation's waste management problems and pollution challenges, as well as poverty and rising youth unemployment. The potential use of spent substrates in crop farming as soil conditioner and mycorrhization practices have also been emphasized[61]. Mushroom spore has been successfully adopted in Congo and South Africa in *Pinus* agroforestry with *Pisolithus tinctorius*[61]. Documented account in forest and/or agro-forest management in Nigeria

is dearth despite high incidence of mycorrhiza mushrooms[61]. To fully tap into the vast mycorrization potentials of mushrooms, it is significant to improve the nation's research on mycorrhizae diversity and mycorrhizian status of many indigenous Nigerian trees[62].

2.2.2 Yields of Mushrooms

A mushroom is the fleshy, spore bearing fruiting body of a fungus, typically produced above ground on soil or on its food source[63]. The need for large scale mushroom production abound since mushroom is a vegetable food source consumed all over the world (China, Germany, Russia, Hungary, Italy, Asia. Australia, Canada, North America, India, Kaysei, Turkey) where mushrooms are happily priced for export and they have international market (Tables 2.2 and 2.3). Mushroom grows easily in tropical rain forest ecology[64]. The protein content of edible mushrooms can be compared with legumes such as soyabean and groundnut and animal products as milk[64].

In Australia, the Australian Mushroom Growers Association statistics document domestic *Agaricus* production at 46 tonnes between (1999ï 2000)[64]. Mushroom is vegetable crop in Australia after potatoes. Seven companies produce 50 % of all *Agaricus* production in Australia and the top 20 companies account for 75 % total production is spread between 116 commercial growers employing about 2,5000 peoples. It was further reported that annual per capital consumption of mushroom increased from 0.65 kg in 1974 to 2.7 kg in 1999 ï 2000 (*Agaricus* 2.41 %) 95 % of domestic production is consumed as fresh products[65].

Human king has utilized mushrooms from earliest days. The 5300 year old ice man discovered in the European Alps in 1991 carried three types of polypore mushrooms with them. Caesar declared who could enjoy their unique flavour and the

Greeks write of mushroom delicacies and export trade in mushroom in the ancient world. Chinese cultivation of shiitake began as early as the twelfth century and commercial cultivation of Agaricus began in Europe around the 1600s[66].

Approximately 14, 000 described species of the 1.5 million fungi estimated in the world produce fruiting bodies that are large enough to be considered as mushroom. It is estimated that these are at least 10,000 species of mushroom in North America alone. About 250 North America species are edible although similar numbers are poisonous. Most of the common, conspicuous, attractive mushrooms are known to be either edible or toxic. Globally, edible mushrooms from North America, Ontario, Eastern Canada, Pennsylvania, mid-Atlantic, Italy, kaysei and Turkey have their chemical composition as water, carbohydrates, fats, and oils, proteins, fibre, ashes, minerals, vitamins, anti-nutritional substances, hallucinogenic substances. The world market for the mushroom industry in 2005 was valued at over 45 billion USA dollar[67]. Yields of mushroom differ widely depending on cultural methods and quality of substances. In Vietnam, one meter of bed produced a maximum yield of about 1 kg of mushrooms in 2 weeks[67].

In the Philippines, average yield are 2.95 kg/ meter of rice straw bed with daily average harvest of 0.35 kg for the 1st and 0.08 kg for the succeeding flushes. Replacing dry rice straw with cheap waste material like cotton residue, average mushroom yields amounted to 85.7 % of dry straw weight. Fresh oil palm waste yielded an average of 1.54 to 2.6 kg mushroom per 100 kg of rice straw[67]. It was observed that with good management, yield on these cheap wastes can even surpass those on rice straw[67]. The addition of 1 g urea and rice bran to 200 g of waste gave the fastest mycelium growth. It was reported that up to 2/3 fresh sisal tow would be used

to produce mushroom yield of 3 % of sisal weight. Yield obtained on banana leaves use over 10 % dry leaf weights[68].

In modern mushroom yields more than 5 kg/m of bed are considered to be uneconomical. In the Netherlands average yields mushroom species, *Volvoriella volyacea* showed the highest mycelium yield and protein content. World animal production of mushrooms has continued to grow. The introduction of the very productive hybrid strains developed at the Horst U (Varieties) now cultivated worldwide has led to further increase in production[68]. Apart from using productive strains in mushroom cultivation improved technique of culture have also helped in the increased production observed over the years.

In addition mushroom growers have always shown themselves to be very co-operative minded. The main exporters of mushrooms are South Korea, France and Netherlands. Main importers are West Germany and the USA[68]. As of now, it is difficult to ascertain how much mushrooms that are produced in Nigeria; as there is no organized commercial production, the mushrooms are collected from the wild (forests, bushes, farm lands, rotten wood etc). It is difficult to estimate the quantity of mushroom produced annually in Nigeria. Yields of mushroom differ widely depending on cultural methods and quality of substrate[69, 70]. In Vietnam, one meter of bed produced a maximum yield of about 1 kg of mushroom in 2 weeks[72].

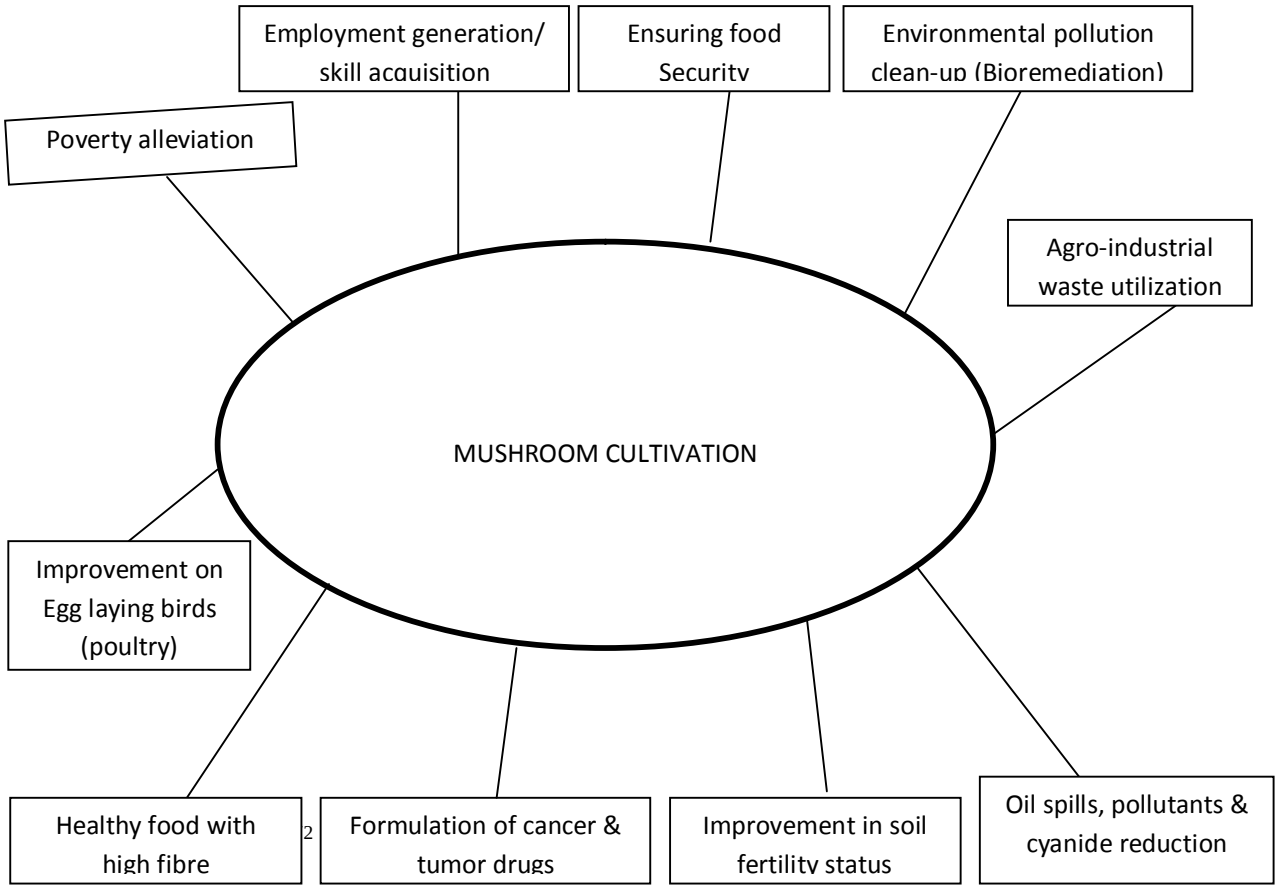


Fig 2.1: Economic Importane of Mushroom Cultivation to National Development

Fig.2.1 shows the huge potential in mushroom cultivation as reported by Aymans[72] when he investigated some Nigerian mushrooms. Ten research findings were stated as follows:

- Oil spills, pollutants and cyanide reduction.
- Improvement on soil fertility status.
- Formulation of cancer and tumor drugs.
- Healthy food with high fibre.
- Improvement on egg laying birds (poultry).
- Poverty alleviation.
- Employment generation/skill acquisition.
- Ensuring food security.
- Environmental pollution clean-up bioremediation.
- Agro-industrial waste utilization.

Table 2.2: **World Production of Mushroom (tonnes, kg, %)**

Mushroom species	1986		1997	
Agricus bisporus	1,227	56.2 %	1,956	32.8 %
Lentinula edodes	314	14.4 %	1.564	25.4 %
Pleurotus spp	169	7.7 %	876	14.2 %
Auricularia	119	5.5 %	485	7.9 %
Volvanella volvacea	178	8.2 %	181	3.0 %
Grifola frondosa	__	__	33	0.5 %
Others	175	8.0 %	1.063	17.2 %
Total	**2.182**	**100.00 %**	**6.158**	**100.00 %**

Source: Zadrazil[63]

Table 2.3: **Composition of Substrate Materials for Mushroom Cultivation**

Substrate	Weight in grammes	C %	N %	C: N	Ph	ASH%
Beech sawdust	30.6	48	0.09	510	5.0	0.52
Reed stems	20.1	42	0.84	50	4.0	8.71
Rape straw	22.7	48	0.63	70	6.0	5.77
Sun flower stalks	25.1	43	0.73	60	5.8	8.32
Rice husks	29.0	35	0.50	70	5.9	19.00

Source: Zadrazil[63]

Tables 2.2 and 2.3 contain the mushroom yields and substrates as reported by Zadrazil[63] when he cultivated mushrooms in Phillipine, he formulated the substrates in Table 2.3 and the yield was stated in Table 2.2.

2.3 Structural Characteristics of Mushrooms

Mushrooms (Fig.2.2) are the fruiting bodies of macroscopic, filamentous and epigeal fungi made up of hyphae which form interwoven web of tissue known as mycelium in the substrate upon which the fungus feeds. Most often their mycelia are buried in the soil around the root of trees, beneath leaf litters, in the tissue of a tree

trunk or in other nourishing substrate[64, 73]. Mushrooms belong to the class basidomycetes in the order *Agaricus* whose fleshy fruit bodies and hyphae are borne on gills. Their fruit bodies are ephemeral structures whereas their mycelia, living on dead organic matter in the soil may last for years. The fruiting bodies are commonly called mushroom or toadstool. Ordinarily the edible species are called mushrooms while the poisonous species are referred to as toadstools. Morphologically, the two groups are indistinguishable but often differing in chemical composition.

Mushrooms due to their morphological characteristics belong to the *Thallophyta* (the lower plants). In its simplest form the life cycle of a mushroom may be traced from a spore which under favourable conditions germinates to form a mass of branched hyphae of mycelia which colonies a competitive substrate[69,74]. This represents the vegetative stage of growth. When a substrate is fully colonized with mycelium, the vegetative growth ceases. Typically, some of the hyphae form primordial or filaments which are the beginning of the reproductive stage. These develop further to form the stripe (stalk), the pileus (cap) of the fruit, body, which when mature exposes the gill tissue or generative tissue on the under-side, from which spores are liberated, so that the life cycle is perpetuated. Although a mushroom fruit is a macrostructure and clearly visible, the spore and hyphae are microscopic and dominates the reproductive time. Mushrooms are the fruiting bodies of a larger organisms. They grow out of a mass of mycelium, a web of thread-like hyphae that grow underground or colonize a substrate such as dead wood or cardboard. The mycelium excretes enzymes which breakdown complex substances into simpler molecules. They can uptake heavy metals into their fruiting bodies[75].

They are eukaryotic (they have discrete membrane nucleus). They have rigid cell walls, many of which contain chitin. They absorb nutrients through their walls and

secrete extracellular enzymes to degrade polymers which cannot be absorbed. They produced spores by means of sexual and asexual reproduction. They lack chlorophyll which means they depend on food previously elaborated by other organisms or primary producers. Their cell walls are made up of chitin or cellulose or mixture of both. Their reproductive units are mainly spores and myceliar fragments. Their ancestral stock is called Edmyceptosis. The cultivation of edible mushrooms is one of the few examples of microbial culture in which the micro-organism cultivated is used directly as a human food. Mushrooms are unable to synthesize their food such as carbohydrates because they cannot utilize the energy in sunlight. They can grow well in complete darkness although sunlight does not harm the growth. However, there are mushrooms that need light for fructification and good shape and colour of the fruiting-bodies. The mushrooms breath by taking in oxygen and exhaling carbon dioxide, thus following exactly the opposite process from ordinary plant life, though it reproduces itself in exactly the same way as plants from seed[50, 76].

The mushrooms growing in a field, passes from the fully opened fruit in 4 to 5 days gradually bursting through the annulus or from veil which is formed between the cap and stalk on the under- side; until the cap eventually becomes flat with the gills exposed. Those gills produce spores in the same way as a plant produces seeds, and if an open mushroom is placed gill down on a sheet of white paper and allowed to remain in that position for 48 h, the exact form of the gills will be reproduced by minute spores which have fallen from them. In the field, these spores fall amongst grass and decayed leaves and are eaten by horses, to pass out in the droppings and appear again eventually as the fruiting body, the mushroom.

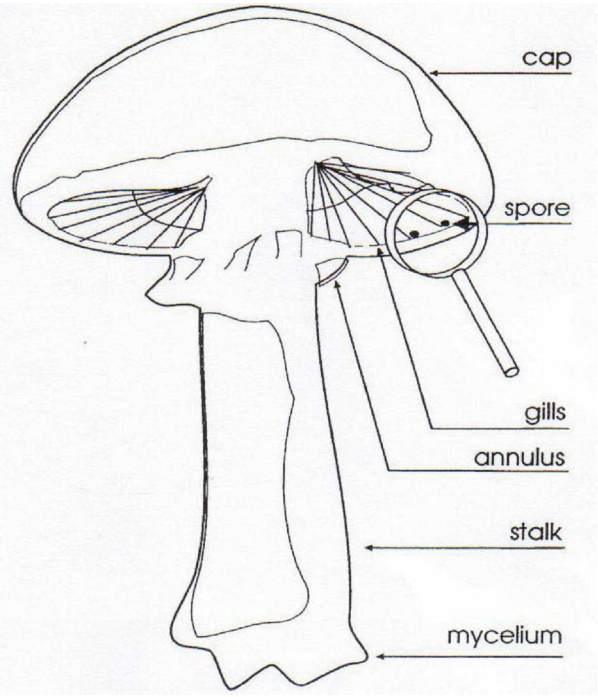


Fig 2.2: **A Typical Structure of Mushroom**

2.4 Physico-Chemical Profiles of Mushrooms

Several authors like Jonathan and Fasidi have shown in their works that edible mushrooms are rich in ascorbic acid, amino acids, protein, minerals, flavor and medicinal values[66]. They are considered to be healthy foods because of their high and qualitatively good protein content, low fat and cholesterol content, minerals and vitamins[66]. They are among the few natural sources of vitamin D**,** which is essential for healthy bones and teeth. Mushrooms are also good sources of the B-vitamins, riboflavin (B_2), niacin (B_3) and pantothenic acid (B_5). These vitamins help break down proteins, fats and carbohydrates so they can be used for energy. Mushrooms can be an important source of B-vitamins for people who donôt eat meat. Compositional analyses of the main cultivated varieties have revealed that, on a dry weight basis, mushrooms normally contain 19 to 35 % protein[69]. The low total fatty acid content, and high proportion of polyunsaturated fatty acids (72 to 85 %) relative to total fatty acids, is considered a significant contributor to the health benefits of mushrooms[69].

Fresh mushrooms (Table 2.6) contain relatively large amounts of carbohydrate, ranging from 51 to 88 % and fibre (4 to 20 %) (dry weight). Mushrooms also appear to be good sources of other vitamins, including thiamine, riboflavin, niacin, biotin and minerals. Most fresh mushrooms contain up to 90 % water[69]. Generally, mushrooms are rich in protein, very low in carbohydrates, rich in high molecular weight complex carbohydrates (polysaccharides), high in antioxidants, and very low in fat. They also contain enzymes such as lipase, hydrolyzing and oxidizing enzymes and tyrosinase which was associated with albinism[69]. They also contain anti-bacterial, hormones, saponins, tannins, phytate, cyanic acid, cardiac glycosides, volatile and non-volatile oil constituents, hemicelluloses, $B_{12,}$ phenols and amino acids. They lack cholesterol, vitamin A, and C.

2.5 Survey of Mushroom Species in South-Eastern, Nigeria.

Macro-fungi such as mushrooms, puffballs and morel are important dietary components in many countries of the world. In South-Eastern Nigeria, edible mushrooms(Tables 2.4 and 2.5) are used for medicinal purposes and as important food source. They are usually collected during rainy season, few available mushroom farms are unable to meet market demand. In the southern part of the country, edible fungi are considered as luxury food and important table delicacy especially among the rural dwellers. Mushrooms such as *T.robustus, T.globules, Vvolvacea, L.subnudus* and young sporophores of *P.tuber-regium* are usually served as alternative to meat[69]. This is because people in the villages are exposed to natural vegetation (tropical rain forest) in which mushrooms grow. During the rainy season, different species of both edible and non edible species usually grow on various natural substrates such as garden soil, decaying wood, termite nest, palm litters, under the shade provided by cocoa, leak, coffee and rubber plantations. People in the villages (mushroom hunters)

usually wake up early in the morning to look for wild edible mushrooms. The mushroom hunting activities are always interesting, competitive, rewarding and profitable venture especially among the women and children. The collected edible species are usually sorted out, cooked or sold in the local markets. Alternatively, some special species may be hawked along the local and major roads to attract the attention of buyers. Mushrooms growing in the wild have been found to be nutritious and important for medicinal purposes. Mushrooms have been considered as rich food because they contain protein, sugars, glycogen, lipids, vitamins, amino acids and crude fibres[72]. They also contain important mineral nutrients which are required for normal functioning of the body. In fact Chang indicated that the food value of mushrooms lies between meat and vegetables[70].

Table 2.4: List of South-Eastern Mushrooms, Their Habitat, Colour and Edibility[29]

S/N	Mushroom types	Habitat	Colour	Edibility
1	*Lentinus subnudus*	Dead wood	White	Edible
2	*Chlorophyllum molybditis*	Fallen logs	White	Edible
3	*Marasmus species*	On forest soil	Light brown	Edible
4	*Pleurotus tuber regium*	On top of African ebony tree	Light brown	Edible
5	*Auriculana polytricha*	Dead wood	Ochraccons	Edible
6	*Lycoperdon pusilum*	Damped soil under shade	Light brow	Edible
7	*Lycoperdon gigateum*	Soil among fallen leaves	Brown	Edible
8	*Pleurotus florida*	Base of rotten farm waste	Hyalie	Edible
9	*Psathyrella atroumbonata*	Solid base of dead tree	White	Edible
10	*Schizophyllum commune*	Dead wood	White	Edible
11	*Termitomyces microcarpius*	Soil on Termite nest	Bright yellow	Edible
12	*Termitomyces Robustus*	Soil onTermite nest aku-mkpu	Bright white	Edible
13	*Termitomyces globulus*	Termite nest	Hyalie	Edible
14	*Tricholoma lobayensis*	Soil on hidden plant debris	Hyalie	Edible
15	*Volvanetta esulenta*	Palm waste	Pink	Edible
16	*Amanita Muscaria*	Refuge dump	Reddish	non-edible
17	*Agaricus bisporus*	On live or dead wood	White	Edible
18	*Tall mushroom*	Dead Rubber tree	Brown	Edible
19	*Kworokworo*	Refuge dump	Very brownish	Edible
20	*Achi mushroom*	Rotten Achi	Very dark	Edible
21	*Ero ata*	In forests	White	Edible
22	*Ero nchakili*	On top of dead Barnbo tree	Yellowish	Edible
23	*Breadfruit mushroom*	Dead Bread fruit tree	White	Edible
24	*Amanita phalaoides*	Rotten palm bunck	Yellow	Non-edible
25	Amanita verosa	In mixed woods or forest	Dark red	Non-edible
26	*Caprinus comatus*	In forests	Gray	Edible
27	*Voluariella volvacea*	In forests	White	Edible
28	*Pleurotus nebrodensis*	In forests	White	Edible
29	*Hypsizigus marmoreus*	In forests	Brown	Edible
30	*Hericum erinaceus*	In mixed woods	Brown	Edible
31	*Agrocybe aegerita*	O trees	White	Edible
32	*Lentinus edodes*	On dead wood	White	Edible
33	*Collybia velutipes*	On dead wood	White	Edible
34	*Rusula albida*	In forests	Brown	Edible
35	*Clitocybe congloata*	On soil in forests	White	Edible
36	*Pleurotus eryngii*	On dead wood	White	Edible
37	*Lepista Sordida*	On trees	White	Edible
38	*Pleurotus Ostreatus*	In mixed woods	Brown	Edible

Table 2.4 contains the list of South-Eastern mushrooms, their habitat, colour and edibility as provided by people through a questionnaire.

2.6 Survey of Mushroom Types, Habtat, Colour, Edibility and State of Origin in Nigeria

Table 2.5: Survey of Mushroom Types, Habitat and Colour from Other States in Nigeria[29]

S/N	Mushroom types	State of collection	Habitat	Colour
1.	*Auriculana polytricha*	Akwa Ibom,Oyo Rivers	Dead wood	Ochraceons
2.	*Lentinus Subnudus*	Bayelsa, Ondo Osun	Dead wood	White
3.	*Lycoperdon Pusilum*	Oyo, Ogun, Edo	Damped soil under shade	Light brown
4.	*Lycoperdon giganteum*	Oyo, Ogun, Osun	Soil among fallen leaves	Brown
5.	*Pleurotus tuber-regium*	Ondo, Edo, Rivers	Wood	White
6.	*Pleurotus florida*	Osun, Rivers, Oyo	Base of rotten farm waste	Hyalie
7.	*Psathyrella atroumbonata*	Edo, Ogun	Solid base of dead tree	White
8.	*Schizophyllum commune*	Delta, Oyo, Osun, Edo	Dead wood	White
9	*Termitomycesmicro carpus*	Ogun Oyo Osun	Termite nest	Bright yellow
10.	*Termitomyces globalus*	Osun, Oyo Edo	Termite nest	Hyalie
11.	*Tricholoma lobayensis*	Rivers, Oyo, Edo	Soil hidden plant dedris	Hyaline
12.	*Volvariella esculenta*	Ogun, Osun, Oyo	ON palm waste	Pink

Source: Global J. Biotech and Biochem, 1(1): 16-21, 2006[29]

Table 2.5 contains the mushroom types, their habitat and colour from other States in Nigeria.

2.7 Nutritional Profiles of Some Nigerian Mushrooms.

Table 2.6: Nutritional Compositions of Some Nigerian Mushrooms %/100 g

Mushroom sample	Moisture content	Dry matter content	Soluble sugars	Total Lipids	Glycogen content	Protein Content	Crude Fibres content	Ash content
A. polytricha young	97.1	2.9	4.8	4.9	5.7	9.3	3.4	4.7
A. polytricha Matured	95.6	4.4	5.9	5.2	7.3	8.5	3.5	5.2
L.subnudus Young	92.8	7.2	11.4	3.6	15.8	6.5	4.3	5.9
L. Subnudus Matued	90.3	9.7	8.9	4.5	10.7	5.1	6.5	7.1
L.Pusilum Young	95.0	5.6	13.7	7.2	16.7	24.3	3.5	4.5
L.Pusilum Matured	98.5	1.5	15.7	7.9	20.1	23.7	5.1	8.6
L. giganteum Young	96.9	3.6	16.9	10.5	22.4	25.3	4.5	6.39
L. giganteum Matured	95.7	4.3	17.5	10.0	25.8	23.3	5.57	15.5
P. tuber-regium Matured	88.6	11.4	7.7	1.7	10.7	16.3	15.6	9.2
P. florida Young	94.7	5.8	9.4	0.9	12.5	15.3	3.5	9.7
P.Florida Matured	91.8	7.6	8.5	1.2	11.5	14.9	5.3	11.5
P.atroum bonata Young	90.4	9.6	7.9	5.2	12.5	18.5	10.7	10.3
P.atroum bonata Matured	89.9	10.1	7.8	6.3	13.1	16.3	12.6	9.0
S.Commune Young	87.3	12.9	9.2	5.2	10.7	10.5	6.5	10.1
S. Commune Matured	85.4	14.6	8.3	5.8	11.8	10.1	10.1	13.1
T.microcarpus Young	90.3	9.7	14.7	8.1	19.5	28.1	12.1	11.7
T.microcarpus Matured	88.2	11.18	13.6	9.4	36.2	27.3	13.7	14.1
T.globulus Young	92.3	7.7	15.4	7.1	19.8	34..1	9.6	17.3
T. globules Matued	90.5	9.5	13.9	6.3	18.4	31.5	11.1	14.3
T. lobayensis Young	93.9	6.1	7.6	3.8	10.7	13.9	7.2	9.3
T. Lobayensis Matured	91.0	9.0	7.9	4.6	10.1	13.1	9.8	9.5
V. esculenta Young	95.7	4.9	10.8	11.1	14.7	26.7	5.7	14.5
v. esculenta Matured	94.5	5.5	8.9	10.8	10.6	25.4	8.3	10.8

Source: Global J. Biotech and Biochem, 1(1): 16-21, 2006[29]

2.8 Survey of Mineral Elements of Some Nigerian Mushrooms.

Table 2.7: Mineral Element Contents of Some Wild Nigerian Mushrooms (mg/kg)

Mushroom Samples	Ca	Mg	K	Na	P	Mn	Fe	Cu	Zn
A.polytricha Young	0.6	1.7	35.1	0.3	20.0	0.3	0.65	0.15	0.07
A.polytricha Matured	0.9	1.2	40.3	0.4	19.7	0.29	0.70	0.10	0.06
L.subnudus Young	1.6	2.9	23.3	2.3	2.7	0.08	0.50	0.10	2.05
L.subnudus Matured	1.9	2.0	21.0	2.0	2.3	0.07	0.55	0.15	1.90
L.pusilum Young	0.9	3.7	30.1	1.7	15.7	1.01	0.70	0.17	1.2
L.pusilum Matured	1.4	4.1	27.5	2.3	13.3	0.80	0.75	0.16	1.40
L.giganteum Young	3.7	3.3	47.1	3.3	20.0	0.90	0.40	0.07	0.90
L.gigantcum Matured	4.9	2.9	41.3	3.7	21.0	1.00	0.50	0.06	0.70
P.tuber-regium Young	1.7	0.9	7.8	1.3	3.6	0.10	0.35	0.00	1.80
P.tuber-regium Matured	2.1	0.7	10.51	1.7	4.6	0.10	0.30	0.02	2.03
P.florida Young	0.3	1.3	13.1	0.2	13.0	0.20	0.09	0.05	0.50
P.florida Matured	0.5	1.78	14.6	0.3	13.7	0.70	0.079	0.05	0.50
P.atroumbonata Young	3.1	4.1	45.1	6.3	15.7	0.50	0.20	0.07	0.75
P.atreumbonata Matured	0.5	5.0	4.16	6.7	14.3	0.40	0.20	0.07	0.80
S.commune Young	3.1	0.4	16.5	0.5	8.3	0.30	0.10	0.08	1.10
S.commune Matured	4.7	0.7	17.1	0.8	7.9	0.30	0.10	0.08	1.30
T.microcarpus Young	3.1	3.1	60.1	2.3	25.1	0.70	0.70	0.07	2.85
T.microcarpus Matured	3.9	4.2	57.4	1.7	23.3	0,75	0.70	0.07	2.85
T.globulus Young	5.7	5.3	50.3	1.5	32.4	0.80	0.90	0.08	3.05
T.globulus Matured	4.9	6.7	46.5	1.9	29.8	0.90	0.80	0.09	3.15
T.lobayensis Young	1.3	0.4	15.5	2.2	9.7	0.30	0.30	0.09	0.80
T.lobayensis Matured	1.7	0.7	21.7	2.0	10.3	0.29	0.35	0.16	0.90
T.esculenta Young	0.6	2.7	53.4	6.1	18.6	0.45	0.45	0.18	2.30
T.esculenta Matured	1.0	2.2	49.5	5.7	18.9	0.43	0.40	0.04	2.70

Source: Global J.biochem, 1(1),16-12,2006[29]

2.9 Survey of Health Properties of Some Species of Mushrooms.

Table 2.8: Health Properties of Some Mushrooms[29]

Hed Khon mushroom	*Termitomyce sp*	Good for brain & Memory
Hed Fang mushroom	*Volvaniella vot vaceae*	Heals wounds
Hed Muerk mushroom	*Coprinus sp*	Help the digest and decrease phlegm
Hed Aunoo mushroom	*Auncularia sp*	Clean Lungs
Hed Kradum mushroom	*Agaricus sp*	Increase motherôs milk
Hed Hua-ling mushroom	*Hericuium Erinacius*	Heal wounds in intestine
Hed Nangram mushroom	*Pleurotus sp*	Decrease muscle malpighia
Hed Hom mushroom	*Lentinula edodes*	Good for liver
Hed Yanag mushroomi	*Flammulina velutipes*	Good for kidney and urine
Hed Kraeng	*Agrocybe Cylindra ceae*	Decrease leucorrhea
Hed Ranghael Skirt Mushrrom	*Schizophy llum Commune*	Cure dysentery and decrease Rotting
Hed Hu-noo khao mushroom	*Dictiophara sp*	Good for sperm, semen and Kidney
Hed Bod mushroom	*Trimella Fuciformis Lentinus sp*	Control the whole body system.

2.10 Survey of Heavy Metals in Wild Mushrooms

Numerous data on metals concentrations in (Table 2.7) the fungal fruiting bodies were published[78]. Because the macro fungi are integral part of the forest ecosystems, sometimes the soil-to-mycelium transfer of metals depends on relationship between mycelium and symbiotic plants species affecting element absorption and translocation[79]. In addition, the metals are distributed unevenly within the fruiting body, the highest concentrations have been observed in the spore-forming part, but not in the spore, a lower content in the rest of the cap and the lowest level in the stipe. High level of metals concentration was observed near metals polluted area and metals smelter[80.]

Some reports[81, 82] observed, that cadmium was accumulated by the edible *Amanita rubescens*, the toxic *Amanita muscaria*, and the edible *Boletus edulis*, and mercury, lead, and copper by *Lepista ruda* and *Lepiota rhacodes*. Many edible mushrooms including the most popular *Boletus edulis* accumulate selenium.Average selenium concentration in nine samples of *Boletus edulis* was 13 mg/kg. The consumption of wild edible mushrooms is increasing, even in the developed world, due to a good content of proteins as well as a higher content of trace minerals[83]. These functional characteristics are mainly due to their chemical composition[84]. Lead, cadmium, iron, copper, manganese, zinc, cobalt, chromium, iron, copper, magnesium, aluminum, tin, and arsenic were chosen as representative trace metals whose levels in the environment represent a reliable index of environmental pollution. Metals such as iron, copper, zinc, and manganese are essential metals since they play an important role in biological systems, whereas aluminum and lead are non-essential metals as they are toxic even in traces[85]. The essential metals can also produce toxic effects when the metal intake is excessively elevated. Heavy metal concentrations in mushroom are considerably higher than those in agricultural crop plants, vegetables, and fruit. This suggests that mushrooms possess a very effective mechanism that enables them readily to take up some heavy metals from the ecosystem. Many wild edible mushroom species have been known to accumulate great concentrations of heavy metals such as lead, cadmium, iron, copper, manganese, zinc, chromium, nickel, aluminum, and mercury[86]. The accumulation of heavy metals in macrofungi has been found to be affected by environmental and fungal factors. Environmental factors, such as organic matter, pH, and metal concentrations in soil, and fungal factors, such as species of mushroom, morphological part of fruiting body,

development stages, age of mycelium, and biochemical composition, affect metal accumulation in macrofungi.

The metabolism of fungi namely macromycetes is substantially different from that of green vascular plants. These heterotrophic organisms pick up nutrients from substance through the whole surface of mycelium. Nutrients are obtained by decomposition of organic matter utilizing a variety of enzymes including metallocomplexes. The system of enzymes also takes part in the formation of organic compound of their body in which many elements are bound as chelates or in the SH group of amino acids. As a consequence of these processes, many essential and toxic elements accumulate in the fruit bodies of macromycetes, the accumulation being species dependant and also strongly influenced by the composition of the substrate[87]. These macro nutrient elements enter human bodies through the food chain and FAO / WHO has set a limit for heavy metal intakes based on body weight. For an average adult (60 kg body weight), the provisional tolerable daily intake for copper, zinc, iron and lead are 3 mg, 60 mg 48 mg and 214 µg/g, respectively[88.] The uptake of metal iron in mushrooms is in many respects different from plants. For this reason, the concentration variations of metals depend on mushroom species and their ecosystems[89]. In general, levels of zinc, cadmium and lead in some mushroom were found to be higher than legal limits. The heavy metals levels of mild edible mushrooms should be assessed often to evaluate the possible danger to human health.

2.11 Environmental Pollution Issues in Nigeria

The toxicity of heavy metals depends on a number of factors[101]. Specific symptomatology varies according to the metal in question, the total dose absorbed, and whether the exposure was acute or chronic. The age of the person can also influence toxicity. For example, young children are more susceptible to the effects of

lead exposure because they absorb several times the percent ingested compared with adults and because their brains are more plastic such that even brief exposures may influence developmental processes[102]. The route of exposure is also important. Elemental mercury is relatively inert in the gastrointestinal tract and also poorly absorbed through intact skin, yet inhaled or injected elemental mercury may have disastrous effects. Exposure to metals may occur through the diet, medications, from the environment, or in the course of work or play. Where heavy metal toxicity is suspected, time is taken to study dietary, occupational, and recreational history since identification and removal of the source of exposure is frequently the only therapy required. A full dietary and lifestyle history may reveal hidden source of metal exposure. Metals may be contaminants in dietary supplements, or they may leach into food and drink stored in metal containers like lead decanters. Persons intentionally taking colloidal metal for their purported health benefits may ultimately develop toxicity. Metal toxicity may complicate some forms of drug abuse, for example, beer drinkerôs cardiomyopathy was diagnosed in alcoholics in Quebec, and later Minnesota, during a brief period in the 1970s when cobalt was added to beer on tap to stabilize the head[102]. More recently, a parkinsonian syndrome among Latvian injection drug users of methcathinone has been linked to manganese toxicity[103].

2.11.1 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[41]. The metal pollutants include lead, chromium, mercury, uranium, selenium, zinc, arsenic, manganese, cadmium, gold, silver, copper, nickel, etc[42]. 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[43]. 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[44]. Every species of mushrooms has a specific capacity, genetically controlled, for absorption of one or another heavy metal from the soil[45]. 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[45]. 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[46, 47].

2.11.2 Bioavailability and Distribution of Chemical Pollutants

The uptake of chemical elements by living organisms is a dynamic process (Fig.2.3)[113]. 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[104, 105, 113]. 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[105]. 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[106]. 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. Metals can also be phagocyted by macrophages.

Food is the most important route for accumulating most chemical elements (essential and toxic). Certain elements, such as mercury, present in organisms of lower trophic levels can be efficiently transferred to higher levels organisms, becoming more concentrated at the top of the food chain, a phenomenon known as biomagnification[105, 106].

2.11.3 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[107]. 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. These groups are also found in important biomolecules with catalytic, structural or transport fuctions[107].Each transition metal possesses its affinity for organic binding. In general, elevated values of equilibrium constants are observed for biomolecules rich in ï SH groups, towards which metals such as Pb, As, and Hg show particular reactivity[108.] 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 of effects. Health effects induced by toxic metals vary greatly; from irritant and acute or chronic systemic toxic effects to

teratogenic, mutagenic and carcinogenic effects[109]. 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 nonessential metals such as cadmium, lead, mercury, arsenic[110].

2.11.4 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[35]. 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, were from vehicles that spew lethal smoke, unrestricted noise making and in most cases, lack of specification for industrial waste[36] still bedevils the country.

The African Centre for Environmental Protection (ACEP) describes or defines environment as the totality of surrounding conditions and its features[36]. 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[37]. 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[38]. 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[38, 39] 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[39]. These methods currently undermine bioremediation and pose varying degrees of environmental problems. However, prospects for use mushrooms in bioremediation of metal pollution have been reported[40].

2.12 The Need for Environmental Monitoring and Control

During the last decades of the 20th century, rapid development of new technologies, increasing populations and urbanization, led to alarming increase in environmental problems[111]. In almost every country, air, water and soil pollution, decrease in arable land, danger of radiation, accumulation of solid wastes, depletion

of energy carriers and of mineral resources, deaths of parts of plants and animal kingdom have become dominant problems. The problems arising from increasing urbanization include the obsolescence of the infrastructure, the pollution of the city atmosphere, the lack and bad quality of drinking water, overcrowding and traffic difficulties, the decrease in the areas of greenery, parks and generally, the so called damage done by civilization[112].

The importance of environmental protection and conservation measures has been increasingly recognized in the past three decades. It is now generally accepted that environmental protection will not be sustainable without developmental controls. Thus, environmental protection and development are two sides of the same coin. Wise management of the environment requires an ability to forecast, monitor, measure and analyse environmental trends and assess the capabilities of air, land and water at different levels to detect environmental trace elements[112].

Interest in the role of trace elements in biological processes has resulted in many studies in recent years. The influence of trace elements on various clinical disorders in humans and animals is currently considered of great importance. This has led to awareness that trace element deficiency and/or excess occurs in many diseases that affect physical and mental health. The number of elements classified as essential for health has increased as a result of scientific research. A deficiency of essential elements can be caused by poor diet or physical malfunction, and such a deficiency can cause pathological disoreders. For many elements, there is a normal range of concentration necessary to maintain a healthy state.

The growing load of environmental pollution leads to an increase in the number of so called civilization damage that menace the whole of mankind, as often different chemicals get into the human organism. In Nigeria, the growing rate of

industrialization is gradually leading to contamination and deterioration of the environment. Thus industrialization and heavy metal pollution are positively correlated. Most studies on heavy metal pollution have been performed in the Southern part of Nigeria, apparently are more industrialized than the Northern part[106]. The accumulation of persistent organic substances means another source of danger for the environment. Apart from this, the quality of biologically undegradable debris such as construction rubble, plastics, glass, tins and car wrecks is also increasing[113].

In addition to automotive and industrial sources, cottage industries and the burning of paper products, discarded rubber, battery casings and painted woods for cooking and heating represent additional hazards to individual households. At present, the impact of these pollutions is confined mostly to the urban centres with large populations, high traffic density and consumer oriented industries. Natural sources of pollution include weathering of mineral deposits, bush burning and wind blow dust.

Among the heavy metals, the most serious is presently associated with lead emission[108]. Environmental exposure to lead is a continuing source of mortality and morbidity throughout the world. Chronic low dose exposure in the domestic environment from house paint, portable water and dust is becoming an important public health hazard (particularly with the proposed discontinuation of leaded petrol worldwide)[109]. This type of exposure is very important to children since their exposure to leaded petrol is limited because they are not yet members of the work force and therefore spend most of their time in the domestic environment. Even though both adults and children can suffer from chronic lead exposure, the effect especially on the neurobehavioral system, is more marked in children[114].

Developing countries currently pay little attention to environmental health problems including chronic lead exposure partly on account of lack of awareness and

due to competing needs, such as the problem of infectious diseases including HIV/AIDS[115]. Yet environmental hazards account for more morbidity, disability and mortality in developing countries than in the developed countries. The critical issues however are that the preparations are not being made towards the protection of the environment. Many developing countries are currently making efforts to reduce exposure to lead from leaded petrol but these attempts are so very little to address exposure in the domestic enviormnemnt[114] or to increase the awareness of their population about the hazards associated with chronic low dose exposure in the domestic environment[115].

These countries should learn from the mistakes of the developed nations and recognize that rapid deterioration of the environment can occur. Although most countries in the sub region of West African recognize the need to combat pollution, environmental controls are either non-existent or inadequate[116]. Most industries discharge their effluents into the environment without any prior treatment and the manufacture of pollution intensive products are being shifted to the developing countries where strict controls do not exist. Lead paint, lead solders and lead cosmetics are unregulated in some countries. Even where controls exist on paper, there are difficulties and inadequate resources to enforce them. The establishment of comprehensive monitoring systems on information gathering should be given priority by governments of the developing countries in the sub-region with support and encouragement from the international agencies[115, 116].

2.13 Concept of Environmental Pollution

Industrialization and technological development processes have led to the introduction of hazardous chemicals into the environment ð water, air and land. These have increased the number and level of environmental pollutants such as heavy

metals, agrochemicals (herbicides, pesticides, halogenated polycyclic hydrocarbons), sewage wastes, food additives and other allied contaminants, thereby, exposing man and animals to health risks. The dangers and health hazards caused by the above pollutants can be reduced by the use of microbial degrading enzymes and natural plant products proved to have cytoprotective properties against the free radicals generated by the harmful pollutants[116].

2.13.1 Pollution

Pollution is the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to the living resources and ecological systems, damage to structures or amenity or interference with legitimate uses of the environment. Pollution had always been misused for contamination which can be defined as the presence of elevated concentrations of a substance in the air, water, soil or any other such thing not necessarily resulting in a deleterious effect. Water pollution, therefore, is the direct or indirect human introduction of substances into the water environment such as to harm living resources, affect human health by various cytotoxic and infilterative disorders and impair water environment quality[116].

2.13.2 Classes of Pollution

Pollution can be classified based on transport media as air, water and soil.

2.13.3 Air Pollution

This can be defined as any gaseous or particulate matter in the air that is not normal constituent of the air or is not normally present in such a high concentration. Air pollution is one of the most difficult to control because it poses international and national threat. The pollution in air can be caused by burning fossil fuels e.g. oil, natural gas and coal as well as those released everyday by vehicle exhaust such as Carbon monoxide, oxides of nitrogen and hydrocarbons such as ethane and methane

(CO, NO_2, NO_3, C_2H_4, C_2H_6, respectively). Two other types of pollution have been classified. The first is characterized by SO_2 and smoke from incomplete combustion of coal and by conditions of fog and cool temperatures. This is termed the reducing type of pollution because of its chemical nature. The second is characterized by hydrocarbons, oxides of nitrogen and phytochemical oxidants and because of the meterological inversion layer, it is described as an oxidizing type of pollution or phytochemical air pollution.

2.13.4 Land/ Soil Pollution

Land pollution does with the terrestrial environment which extends from the top of the growing vegetation to the capillary fringe of groundwater which is the primary home of most living things on earth. Land pollution is the introduction of harmful chemicals into the environment to grow and increase food supply, to protect man and his crops from pests and diseases and dispose off wastes. Unintended entry also occurs through transport accidents, inaccurate or inappropriate application procedure and leaking sewage facilities. Because the chemicals and other living things share the environment, this can lead to significant exposure to the chemicals and therefore, resulting in detrimental health impact. The rains carry non ï degradable pollutants and wastes into groundwater, nearby streams and the soils too.The wastes in the landfill can also decompose under heat and pressure of the soil above releasing harmful gases like methane (CH_4).

2.13.5 Water Pollution

Water pollution becomes most obvious when it involves poisoning of drinking water or causes the death of a large number of fish or other aquatic population. This could be caused by sewage. Disposal of sewage wastes into a large volume of water could reduce the biological oxygen demand to such a great level that the entire

oxygen may be removed. This would cause the death of all aerobic species ï fish/mushrooms.

Some toxic chemicals released into the rivers and seas such as Pb, Cu, Zn, Hg, CN will cause the death of fish, algae and lesions in human beings even at very low concentrations. These are related to occupational hazards and constitute elements of environmental pollution especially with respect to pollution by heavy metals. Such incidents had been reported in the past. For example, the Minimata and Niigata epidemics in Japan in 1950s and 1960s respectively.

Three forms of mercury pollute the environment:

1. Metallic mercury ï this is excreted in urine mainly in the inorganic form.
2. Inorganic mercurous or mercuric ions ï Hg forms very stable complexes with the free ï SH (thiol) groups of proteins and consequently inhibits enzymes with ï SH at their reactive sites irreversibly.
3. Organo mercury (Hg is part of an organic compound) and remains in organic form in the tissues e.g. hair. The rate of removal of mercury from the blood is very slow. Hg is removed by prolonged treatment of patients with an appropriate mercury ion chelator like N ï acetylpenicillamine.

The second case of Hg poisoning was the epidemic of consumption of fish from polluted Kalu River in the Thana district of Mumbia, India. The major symptom was paralysis. Another major epidemic of water pollution was the Itai ï itai disease which affected a Japanese population that consumed fish and other foods harvested from coastal waters into which cadmium ï containing industrial effluent was being discharged. The disease was characterized by brittleness of bones, muscular weakness and loss of appetite.

Lead poisoning is another serious source of environmental pollution which causes water pollution. Lead poisoning is characterized by central nervous system (CNS) damage, anaemia and deposition of Pb in bones and teeth. The major sources of this pollutant are paint manufacturing industries/factories, lead smelting works; petrol engines discharged inorganic Pb salts, metallic Pb and organic Pb respectively. Pb $(C_2H_4)_4$ ï is used as an anti knock in petrol engines and is a pollutant. The anaemia caused by Pb is due to inhibition of haem biosynthesis. Inorganic Pb inhibits aminolaevulinic acid dehydratase and ferrochetalase (haem synthetase) which catalyses the formation of the pyrrole porphobilinogen and incorporation of Fe^{2+} into protoporphyrin IX respectively. Pb forms very stable complexes with ï SH groups of enzymes. The above is by no means exhaustive. In 1996, there were cases of chromium and cadmium pollution in sediments of Suzhou Creek while cases of metal poisoning were earlier recorded in USA and Britain between 1974ï 1975. The susceptibility of any individual within a group of people equally exposed to a metal toxin varies with age, sex and social habits, such as smoking and alcohol usage.

Organic pollutants contain hydrogen, carbon and oxygen e.g. petroleum products (gasoline, oil, pesticides) solvents, cleansing agents, polychlorinated biphenyls (once used in electrical transformers), human and animal wastes. These also constitute serious sources of water pollution. Some of these pollutants are found to cause cancer and other health effects in humans. They are toxic to fresh water and salt water organisms. Hazardous wastes are potential dangers due to their nature or quantities. They have the characteristics of toxicity, flammability, corrosivity with a high tendency to remain/persist in the body e.g. phenols, arsenic, mercury, lead and a host of others.

2.13.6 Immediate Consequences

Crops grown on soils polluted by industrial chemicals accumulate varying concentrations of these chemicals. In the course of absorbing water and nutrients from the soil, plants take up the chemicals. The agrochemicals cannot be degraded because they lack the enzymic machinery to degrade and excrete them. They are deposited in tissues and cellular structures including those of edible parts that are not active in metabolism[116].

1. Aquatic foods harvested from coastal waters, rivers and waterways into which industrial effluents have been deposited contain high levels of these harmful chemicals which induce cytotoxicity in cells.
2. Meat and dairy products from livestock grazing on polluted pastures, drinking polluted water e.g. Hg, Cd, Pb, radioactive materials, complex organic compounds e.g. polycyclic aromatic hydrocarbons and halogenated aromatic compounds. The human body cannot handle these pollutants.

2.13.7 Water Pollution

One is at a loss considering the enormity of hazards caused by water pollutants and their toxicological consequences on man's health. Xenobiotic oxidants are discharged into water with the result that oxidative stress is imposed on aquatic organisms (fish, crab, prawn etc). Free radicals are generated such as those of organomercury, Pb, Cd and the other heavy metals resulting in the peroxidative deterioration of membrane lipids.Lipids yielding breakdown products like hydroperoxides, peroxyl radicals, endo-peroxides, carbonyls and aldehydes, malonaldehyde, mahendialdehyde and other TBA ï reactive species, low molecular weight hydrocarbon gases (ethane and pentane) which are all toxic to cells. Oncogenesis/Carcinogenesis could develop. Cytotoxic enzyme markers such as

gamma glutamyl transferase, alkaline phosphatase, acid phosphatase, serum amino transferases, all show-elevated activities. Physiological Parameters ï PCV, HB, RBC status are compromised while WBC proliferates with attendant increased lymphocyte maturation.Lesions ï fatty degenerations, necrosis, cirrhosis, destruction of blood vessels and connective tissues occur.

All these are as a result of the free radicals generated from consumption of water pollutants which cause lipid peroxidation of the membranes. If the source of pollution in the water is microbial, the following diseases may ensure:

These compounds are structurally equipped to take part in photosensitivity reactions because they are highly light absorbing. They act as complementary pathways to enzymic pathways, which quench free radicals in the system .Natural plant products like Sacoglottis gabonensis stem bark extract, a Nigerian palm wine beverage additive are to be included in formulating dietary regiments [115,116].

2.13.8 Role of Microbial Enzymes in the Degradation of Pollutants

Oxidoreductases, hydrolases and transferases degrade wastes. Phosphatases, glucosidases, xylosidases, chitinases and sulphatases are induced in biochemical nutrient cycles. In water saturated peat lands, few enzymes are able to degrade aromatic phenolic rings to render the carbon skeleton available to other hydrolytic enzymes. Phenolic oxidaseï glucosidaseï endo ï 1 ï 4 ï gluconase, cellobiohydrolase degrade structural cellulose to energy rich glucose molecules. Soil mineral content also helps action of glucosidase. Hydrolases degrade pesticides - organophosphorus, pyrethroid and carbamate insecticides, dithiocarbamate dinitrophenol insecticides. Benzenoid, polycyclic and some heterocyclic substances form epoxides which are highly damaging to the cells. These enzymes degrade the epoxides which can act as mutagens and carcinogens. All these pollutants are usually discharged into streams,

rivers and seas. Most of them are non- biodegradable in nature for example dieldrin and endrin.

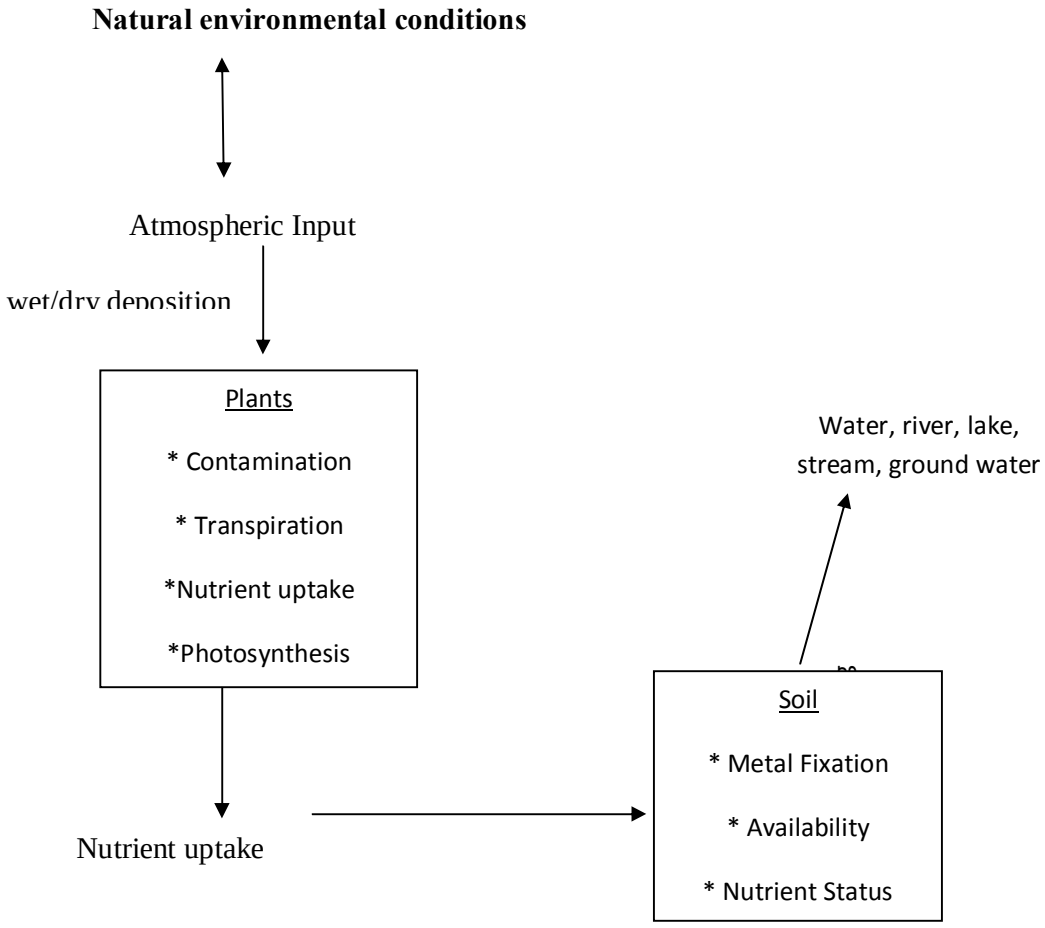


Fig.2.3: Natural Processes
Source: Lepp 1981[113]

2.14 Effects of Heavy Metals Pollution on Environment

2.14.1 Lead Pollution

In humanôs exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure[117]. Various effects occur over a broad range of doses, with the developing fetus and infant being more sensitive than the adult. High levels of exposure may result in toxic biochemical effects in humans which in turn cause problems in the synthesis of hemoglobin, effects on the kidneys, gastrointestinal tract, joints and reproductive system, and acute chronic damage to the nervous system. Lead poisoning, which is so serves as to cause evident illness, is now very rare indeed. At intermediate concentration however, there is persuasive evidence that lead can have small, subtle, subclinical effects, particularly a neuropsychological developments in children[116, 117].

2.14.2 Cadmium

Cadmium derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and humans. Cadmium is biopersistent and once absorbed by an organism, remains resident for many years (over decades for humans) although it is eventually excreted. In humans, long term exposure is associated with renal malfunction. High exposure can lead to obstructive lung disease and has been linked to lung cancer, although data concerning the later are difficult to interpret due to compounding factors (Table 2.8). (Cadmium may also produce bone defects (Ostemalacia, osteoporosis) in humans and animals. In addition, the metal can be linked to increase blood pressure and affects the myocardium in animals, although most human data do not support, but these findings[116, 117].

The average daily intake is estimated as 0.15 mg from air and 1mg from water. Smoking a packet of 20 cigarettes can lead to the inhalation of around 2-4 mg of cadmium, but levels may very widely.

2.14.3 Chromium.

Chromium is used in metal alloys and pigments for paints, cement, paper, rubber, and other materials. Low-level exposure can irritate the skin and cause ulceration long-term exposure can cause kidney and liver damage, and damage to circulatory and nerves tissues. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium[117].

2.14.4 Copper

People with wilsonôs disease are at greater risks for health effects from over exposure to copper. Copper normally occurs in drinking water from copper pipes, as well as from additive designed to control algal growth[118].

2.14.5 Nickel

Small amount of nickel is needed by human body to produce red blood cells, however, in excessive amounts, can become mildly toxic short-term over exposure to nickel is not known to cause any problems, but long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation. The EPA does not currently regulate nickel levels in drinking water. Nickel can accumulate in aquatic life, but its presence is not magnified along food chains[119, 120].

2.15 Concept of Bioremediation

On the planet, about 1-2 million soils species of fungi exist; about 150,000 species are classified as mushrooms while 50,000 of which 14,000 have been identified with specie name[124]. The genetic diversity of fungi is vast by design and apparently crucial for life to continue. These silent mycelia tsunamis affect all

biological system upon which they are dependent. Fungi can selectively raise or lower soil pH, increasing the usability of existing soils without the need for additional adjustment prior to use.

Bioremediation of areas degraded by pollutants or otherwise impacted through mismanagement of ecosystem is a form of biotechnological approach aimed at rehabilitating the area for positive uses. Most of the restoration of Polycyclic Aromatic Hydrocarbons (PAHs) contaminated sites depends on the activities of bacteria (microbial biodegradation) like *Pseudomonas aeruginosa*, or use of plants (Phytoremediation) or by the use of fungi (Mycoremediation)[159]. Crude oil contains significantly high amount of Polycyclic Aromatic Hydrocarbon compounds. These are chemical compounds that consist of fused aromatic rings and do not contain hetero-atoms or carry substituents[128]. As pollutants, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic. Polycyclic aromatic hydrocarbons are lipophilic, meaning they mix more easily with oil than water. The larger compounds are less water-soluble and less volatile thus, prone to evaporation. Because of these properties, in their studies on the environmental implications of oil exploration and production activities observed that oil spillage constitute by far the most significant source of pollution. These spills often occur from pipelines, flow lines, delivery line failure, or through accidental discharge at flow stations, refineries and export terminals. Oil pollution has damaging effect on the entire ecosystem and makes the soil condition unfavourable for plant growth due to reduction in the level of available plant nutrient or rise in the toxic levels of certain elements. The effect on plants ranges from chlorosis, bleaching, spotting of leaves, necrosis, malformations to epidermal cells and mesophyll layers, yield reduction and impaired fecundity, also reported that root stress reduces leaf

growth through stomata conductance described plant growing in oil polluted soils as generally retarded and chlorotic on their leaves, coupled with dehydration of the plants indicating water deficiency. This justifies the need for bioremediation which is the use of microorganisms in the biological processes to break down the organic pollutants. Low molecular weight PAHs are usually readily degraded, while high molecular weight PAHs resists extensive bacteria degradation in soil and sediment media[125] White rot fungi would be expected to have greater access to poor bio-available substrates, since they secrete extracellular enzymes involved in the oxidation of complex aromatic compounds like lignin. It is an edible rot fungus, (i.e *Pleurotus pulmonarius*) known for its ability to degrade crude oil. *Glomus mosseae*, an arbuscular mycorrhiza fungus (AM fungus), helps plants in the uptake of nutrients such as phosphorus and micro nutrients from the soil. It has been observed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonization of land by plants and in the evolution of the vascular plants. There are several vegetal species that are capable of growing in soils polluted with hydrocarbons[125].

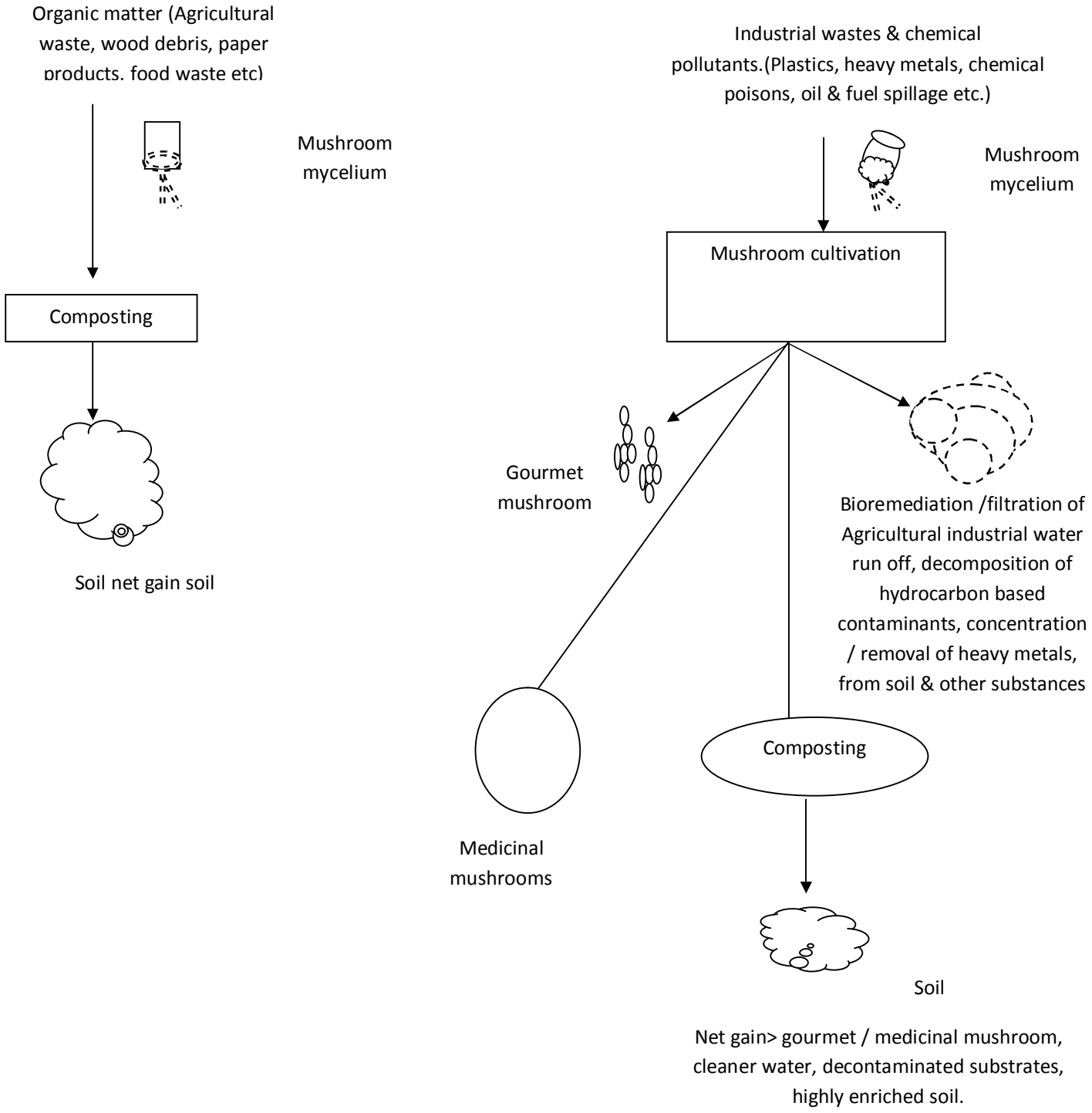


Fig. 2.4: Reduction of organic matter and chemical pollutant through Mushroom cultivation

Source: Journal of Environmental pollution, Cont and Mgt[159].

2.15.1 Mushrooms and Bioremediation

Bioremediation can be defined as any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition[121]. Bioremediation may be employed to attack specific soil contaminants, such as degradation of chlorinated hydrocarbons by bacteria. An example of a more general approach is the clean-up of oil spills by the addition of nitrate and/or sulfatefertilisers to facilitate the decomposition of crude oil by indigenous or exogenous bacteria.

Naturally occurring bioremediation and phytoremediation have been used for centuries. For example, desalination of agricultural land by phytoextraction has a long tradition. Bioremediation technology using microorganisms was reportedly invented by George M. Robinson[122]. He was the assistant county petroleum engineer for Santa Maria, California. During the 1960s, he spent his spare time experimenting with dirty jars and various mixes of microbes.

Bioremediation technologies can be generally classified as *in situ* or *ex situ*[158]. *In situ* bioremediation involves treating the contaminated material at the site while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation technologies are bioventing, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants (bioaugmentation) [159].

Not all contaminants, however, are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by organisms. The assimilation of metals such as mercury into the food chain may worsen matters. Phytoremediation is useful in these circumstances, because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts, which are then harvested for removal[122]. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use.

The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds and they will certainly accelerate the development of bioremediation technologies and biotransformation processes[122].

Mycoremediation is a form of bioremediation in which fungi are used to decontaminate the area[122]. The term mycoremediation was coined by Paul Stamets and refers specifically to the use of fungal mycelia in bioremediation.

One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant. Certain strains have been reported to successfully degrade the nerve gases and sarin.

In an experiment conducted in conjunction with Thomas[162] a major contributor in the bioremediation industry, a plot of soil contaminated with diesel oil was

inoculated with mycelia of oyster mushrooms; traditional bioremediation techniques (bacteria) were used on control plots. After four weeks, more than 95 % of many of the PAH (polycyclic aromatic hydrocarbons) had been reduced to non-toxic components in the mycelial-inoculated plots[159]. It appears that the natural microbial community participates with the fungi to break down contaminants, eventually into carbon dioxide and water. Wood-degrading fungi are particularly effective in breaking down aromatic pollutants (toxic components of petroleum), as well as chlorinated compounds (certain persistent pesticides) Mycofiltration is a similar or same process, using fungal mycelia to filter toxic waste and microorganisms from water in soil[158]. Mycelium produces extracellular enzymes and acids that break down recalcitrant molecules such as lignin and cellulose, the two primay components of woody plants. Lignin oxidase dismantle the long chains of hydrogen and carbon, converting wood into simpler forms, on the path to decomposition. Also the same enzymes are excellent at breaking base structure common to oils, petroleum products, pesticides, and other pollutants[123].

2.15.2 Monitoring the Process of Bioremediation

The process of bioremediation can be monitored indirectly by measuring the Oxidation Reduction Potential or redox in soil and groundwater, together with pH, temperature, oxygen content, electron acceptor/donor concentrations, and concentration of breakdown products (e.g. carbon dioxide). Table 2.9 below shows the (decreasing) biological breakdown rate as function of the redox potential.

Table 2.9: Decressing Biological Breakdown Rate [124]

Process	Reaction	Redox potential Watts
aerobic:	$O_2 + 4\ e^{\bar{1}} + 4\ H^+$ Ÿ $2H_2O$	600 ~ 400
Anaerobic:		
Denitrification	$2NO_3^{\bar{1}} + 10\ e^{\bar{1}} + 12\ H^+$ Ÿ $N_2 + 6H_2O$	500 ~ 200
Manganese IV reduction	$MnO_2 + 2\ e^{\bar{1}} + 4\ H^+$ Ÿ $Mn^{2+} + 2H_2O$	400 ~ 200
Iron III reduction	$Fe(OH)_3 + e^{\bar{1}} + 3\ H^+$ Ÿ $Fe^{2+} + 3H_2O$	300 ~ 100
Sulfate reduction	$SO_4^{2\bar{1}} + 8\ e^{\bar{1}} + 10\ H^+$ Ÿ $H_2S + 4H_2O$	0 ~ ī 150
Fermentation	$2CH_2O$ Ÿ $CO_2 + CH_4$	ī 150 ~ ī 220

Source: Dilna [159]

Chemical analysis is also required to determine when the levels of contaminants and their breakdown products have been reduced to below regulatory limits[124].

As the diagrams above illustrate, the processing of organic materials (Agricultural waters, industrial wastes) into composted soil through cultivation is a superior use of resources when compared with composting alone. Mushroom cultivation offers many benefits to farmers, food processing, breweries, pharmaceutical industries, forest based industries and any one whose operations result in the generation of organic waste. Mathematically growing mushroom= creating fertile soils.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sampling

Questionnaire and the response to the questionnaire were used to select the mushroom species (Appendix I and Table 4.1) and they were collected from different towns and villages located in Idemili North, Idemili South, Nnewi North, and Ekwusigo local government areas (L.G.As) in Anambra State, South-East Nigeria, as shown in Fig.3.1. Anambra State is located between longitude 6 ^{0} 37ôE and latitude 6 ^{0} 48ôE and longitude 7 ^{0} 27ôE and latitude 6^{0} 40ôN[126, 127]. Five species of Wild mushrooms namely:-*Termitomyces robustus, Agaricus bisporus, Pleurotus tuber-regium, Amanita phalaoides* and *Amanita verosa* were collected from Uke, Abatete, Ideani, Nnobi, Nnewi, and Ozubulu during the season of availability (July-October), between 2009 and 2012. Table 3.1 shows the specific sites, environment or substrate, habitat, mushroom species and their local names.

The mushroom samples were identified by a taxonomist, Mr Alfred Ozioko of Centre for Ethnomedicine and Drug Development, Bioresources Development and Conservation Programme, Nsukka, Enugu State. The herbarium/voucher numbers are Tr-INTERCEDD/1591, Ptr-INTERCEDD/1592, Ab-INTERCEDD/1593, Av-INTERCEDD/1594, Aph-INTERCEDD/1595. The wild mushroom samples were collected together with the soil/substrates on which they grew. The mushrooms were immediately taken to the laboratory and stored in refrigerator at 4 ˚C to avoid dehydration and possible spoilage. Photographs of the mushrooms are shown in plate 3.1 (a-e).

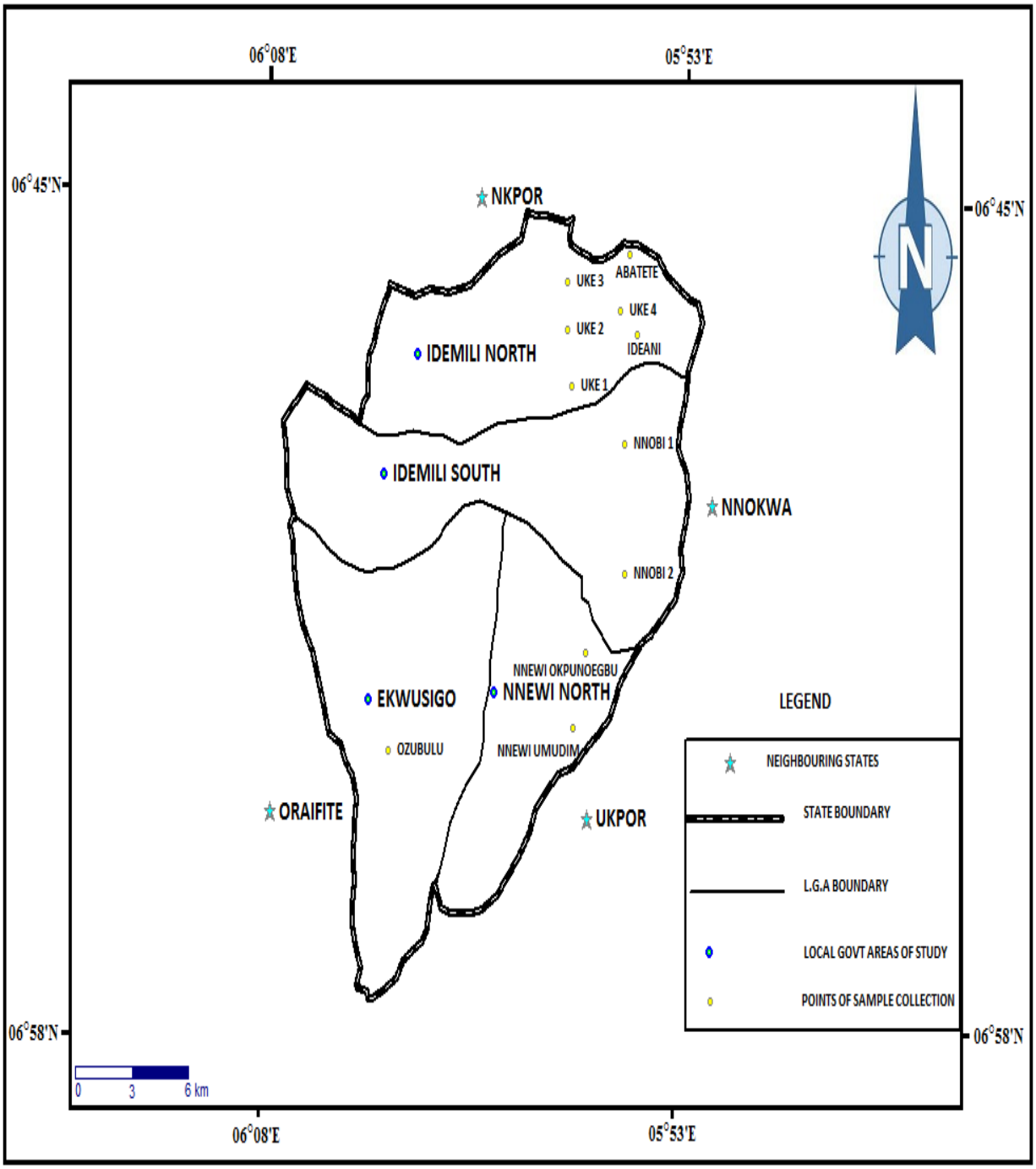


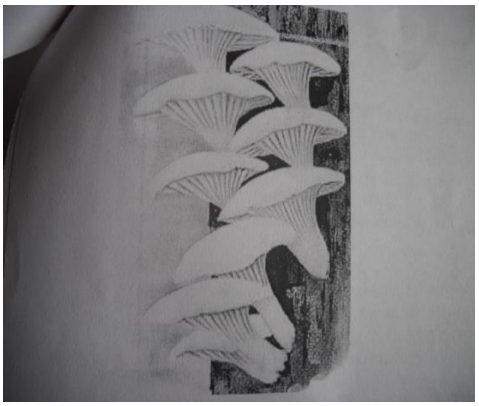
FIG 3.1: MAP OF IDEMILI NORTH & SOUTH, NNEWI NORTH & EKWUSIGO L.G.A SHOWING SAMPLING SITE.

SOURCE: MINISTRY OF LAND AND SURVEY ANAMBRA STATE MODIFIED FROM FIELD TRIP 2012

Table 3.1: 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	*Amanita phalaoides*	ERO-AGBUGBO	*N*on-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

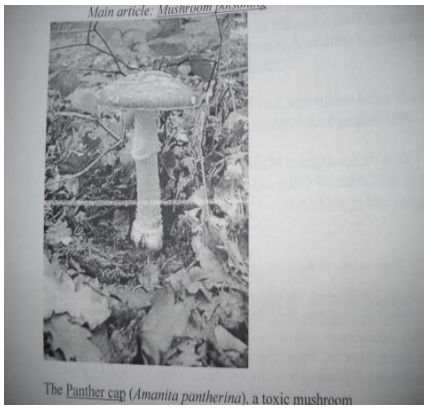
*Peopleôs Speculations.



a: *Agaricus bisporus*

b: *Termitomyces robustus*

c: *Amanita phalaiodes*



D: *Amanita verosa*

e: *Pleurotus tuber-regium*

Plate 3.1: Photographs (a-e) of the Studied Mushrooms

3.2 Cultivation of Mushrooms

3.2.1 Preparation of Substrates for Spawn Production

Nutrients preparation followed the same methods described in literature (see Tables 2.2 and 2.3)[63]. Four substrates were prepared as shown in Table 3.2. The fifth method was soil collected from the sites. The substrates were ground into fine powder and put inside wide mouthed bottles covered with cotton wool and aluminium foil. The bottles were auto-claved at 125 °C at 15 psi pressure for 1 h, allowed to cool to room temperature and spread on petri dishes for spawn production.

3.2.2 Production of Spawn

Mycellia were scrapped from the top of mature mushrooms and allowed to fall on the surface of the substrates in petri dishes. The spawns which sprouted were allowed to develop into mycelia cultures within 4-5 days before planting the fruiting bodies.

3.2.3 Planting of Fruiting Bodies of Mushrooms

Autoclaved substrates were put in clean colourless wide-mouthed bottles and allowed to cool to room temperature. The cultured mycelia were transferred by carrying about 2 cm^2 of substrate and mycelia and placing it on the cooled substrate surface to develop into fruiting bodies (mature mushrooms),which occurs within 21 days. The spawn bottles were visually inspected everyday for increase in length, appearance of cap, presence of off sectoring, discolouration, infection and disappearance of smaller mushrooms as the big ones mature/appear.

Sectoring is described as any mycelia growth which differed in appearance, growth rate and colour from the typical appearance of the mycelia obtained.

Table 3.2: Compounded Substrates for Spawn Cultivation

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

3.2.4. Production of Fruiting Bodies Using the Sclerotium or Under Ground Tuber of *Pleurotus tuber-regium* (Ero-Osu)

The sclerotium or underground tuber of *Pleurotus tuber-regium* was conditioned by steeping in water until the moisture content of the tuber increased by 65 %. It was then wrapped in polyethylene bag and kept until pin heads -like structures appeared. Then the polyethylene bag was unwrapped and left open, the tuber was scantily watered once a day until fruiting bodies develop[55, 63].

f: Production of Spawn of *Pleurotus tuber-regium*

g: Production of Fruiting Bodies of *Pleurotus tuber-regium*

Plate 3.2: Photographs (f-g) of Production of Spawn and Fruiting Bodies of *Pleurotus tuber-regium*

3.3 Bioremedition Process

Soil samples from Uke (site 1), Abatete (site 5) Nnobi (site 8), Nnewi umudim (site 10) and ozubulu (site 11) as shown in Table 3.1 were used. Compounded substrates as formulated were also used for each specie of mushroom as shown in Table 3.2. The soil samples were mixed with stipulated substrate and put into a wide mouthed glass bottles wth a cover. They were auto claved at 125 °C for 2 h at 15 psi pressure. The glass bottles were cooled to room temperature and seeds from mature mushroom were scrapped into the glass bottles and allowed to germinate within 4-5 days (Fig.2.4). The germinated seeds (mycelia) were transferred by cutting about 2 cm^2 of soil/substrates/mycelia and placing it on the cooled substrate surface to develop into fruiting bodies which matured wihin 14 days. The soil/substrates and mushroom samples were subjected to chemical aanlysis. The bioaccumulation factors were calculated as follows:

$$\text{Bioaccumulation Factor} = \frac{Metal\ Concentration\ in\ Mushroom}{Metal\ Concentration\ in\ \ Soil / Substrates}$$

3.4 Diagrams of Working Flame Photometer and Atomic Absorption Spectrophotometer

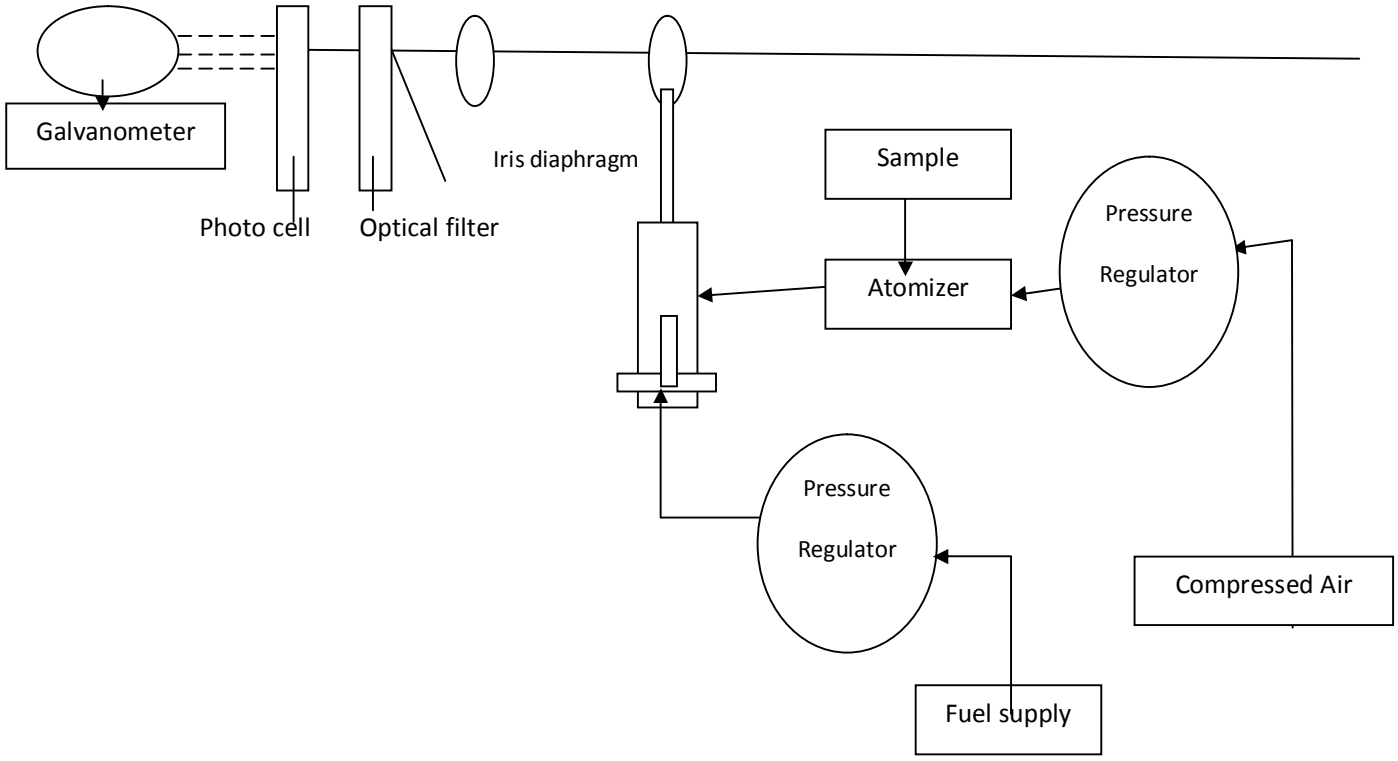


Fig.3.2: Layout of a Simple Flame Photometer

Sourced: (AOAC)[128]

Flow Chart of Flame Photometer

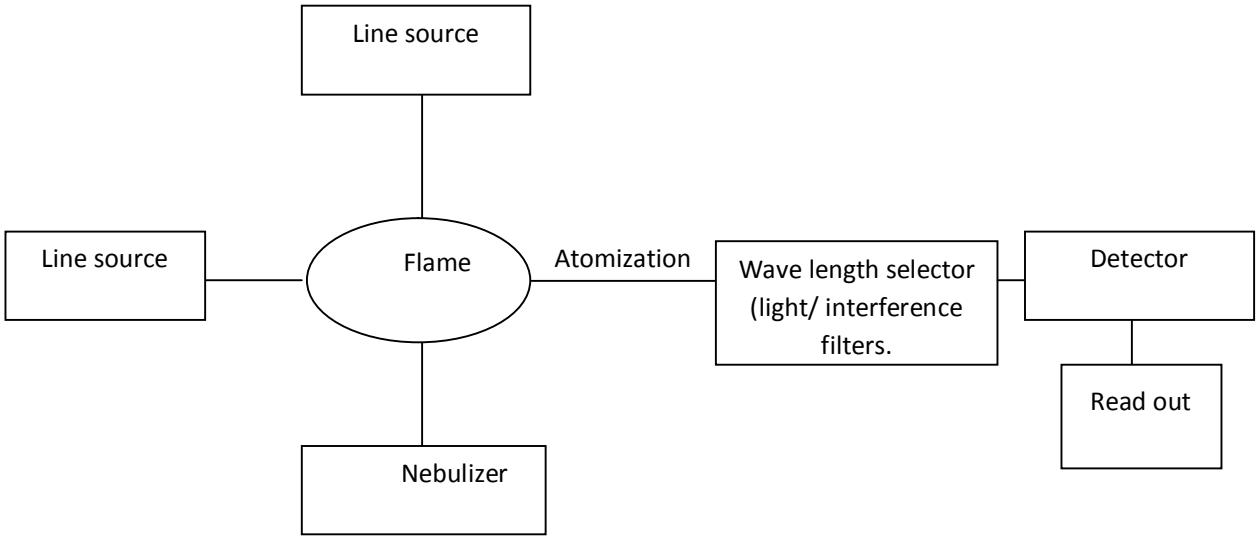


Fig. 3.3**: Layout of a Flame Photometer**

Sourced: (AOAC) [128]

3.4.1 Operation of Flame Photometer

The blank and standard solutions were separately sucked into the nebulizer by compressed air which is also the oxidant gas. The fine mist formed mixes with the fuel gas (propane or butane) and get into the flame where the sample mist decomposes into atomic vapour. The atoms get excited by heat and emit radiations which pass through the lens, the Iris diaphragm and the optical filter, which allows only the characteristic band to pass through to the detector (photocell). The detector signal goes to the readout device which is a galvanometer (detector). The regions of the spectrum suitable for the determination of any metal is selected by changing the optical filter as each metal has a filter made to select its characteristic band. Average value of three readings were taken. A plot of calibration graph completes the calibration of the calibration of the instrument for analysis. Sample solutions are aspirated hereafter and concentrations are determined from the calibration graph. Note

that flame photometer is used for analysis of sodium and potassium and may also be used for lithium and calcium.

Diagram of a Working Atomic Absorption Spectrophotometer

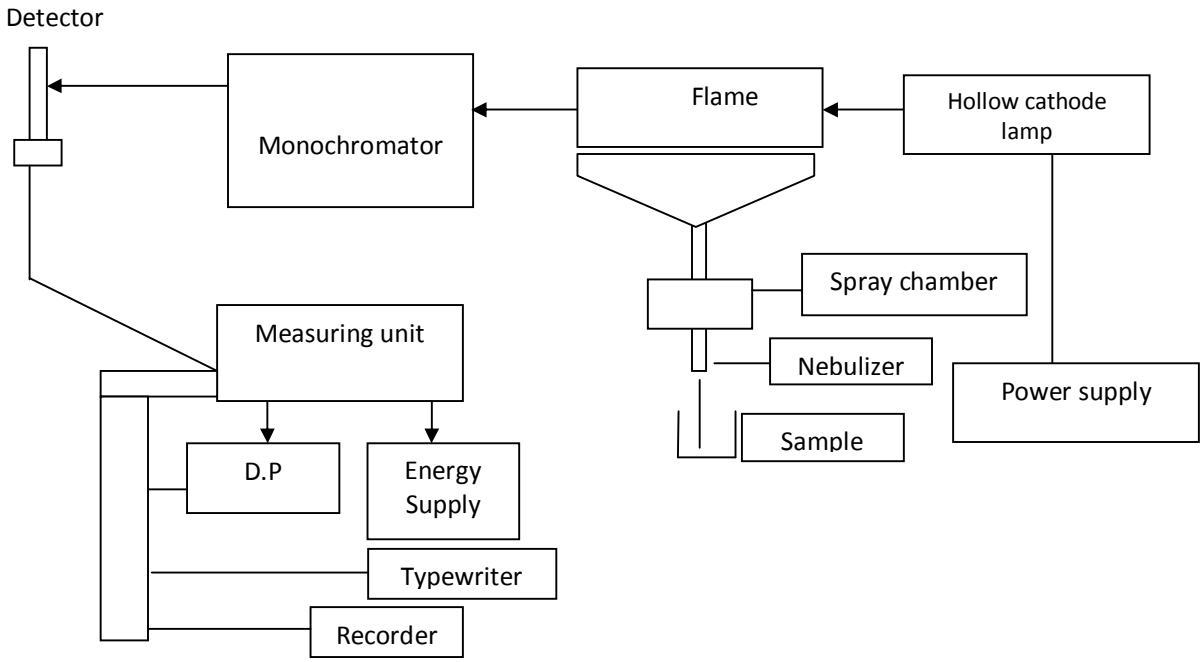


Fig.3.4: **A Flow Chart of Atomic Absorption Spectrophotometer**

Diagram of a Hollow Cathode Lamp

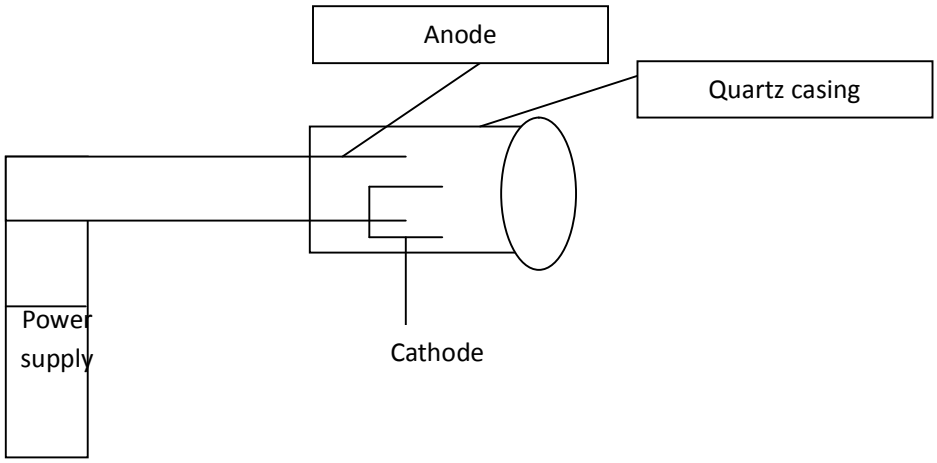


Fig 3.5: A Hollow Cathode Lamp

Sourced: (AOAC) [128]

3.4.2 Operation of Atomic Aborption Spectrophotometer.

AAS, Agilat Technologies 200 seriesAA model 240 FSAA, United Kingdom, Atomic Absorption Spectrophotometer was used. The hollow cathode lamp of the desired metal was installed in the instrument. The slit width was set according to the manufacturerôs guide for the element to be measured (see Table 3.3). The instrument was turned on and the current was set according to the manufacturerôs guide. The instrument was allowed to warm up until the energy source stabilized. The process took 10 to 20 min. The current was re-adjusted after warming up. The burner head was then installed and lighted using air-acetylene mixture. The atomizer was rinsed by aspirating the blank (de-ionized water), and the instrument was zeroed. Each metal standards and sample solutions were atomized using air-acetylene flame. During atomization, the background was corrected using a hydrogen continuum lamp in the range 185-350 nm. This range was extended slightly by the use of a deuterium arc lamp for the range 185-430 nm. The two continuum lamps have similar source diameters to hollow cathode lamps and the light beams can be easily superimposed for accurate correction. Stock standard solutions (1000 mg/L) from MBH Analytical LTD (Holland) for each metal were diluted accordingly to prepare working standard solutions used to calibrate the instrument. The atomic absorption spectrophotometer was adjusted in such a way that absorption and concentration of each sample was read three times and mean values were given as the observed values on the digital scale of the AAS. The detection limit for each element was determined using the lowest possible dilution and are included in Table 3.3.

The absorbance were recorded and printed- out.

Table 3.3: Standard Analytical Condition for Flame Photometer or AAS[128]

Element	Wavelenght (nm)	Lamp current(mA)	Slit width(nm)	Slit height	Sensitivity (µg/mL)	Detection limit(µg/mL)
K	7440.1	9	1.0	Normal	0.04	0.0005
Ca	422.7	8	1.0	Normal	0.05	0.009
Cd	228.8	3	0.5	Normal	0.06	0.0004
Co	240.7	3	0.5	Normal	0.025	0.0005
Cr	359.3	6	0.2	Normal	0.09	0.003
Cu	324.7	6	0.5	Normal	0.025	0.001
Fe	248.3	7	0.2	Normal	0.05	0.005
Na	589.0	7	0.2	Normal	0.025	0.009
Pb	217.0	5	1.0	Normal	0.06	0.0005
Zn	213.9	5	0.5	Normal	0.008	0.0005
Ni	232.0	4	0.2	Normal	0.04	0.009
Mg	766.5	9	1.0	Normal	0.05	0.005
Mn	279.1	6	0.2	Normal	0.09	0.001

Sourced: (AOAC)[131]

3.5 Proximate Analyses of Studied Mushroom Samples

3.5.1 Sample Preparation

Mushroom samples were oven dried at 75 °C, ground and kept in polythene bottles. Soil/substrate samples were oven dried at 105 °C until constant weights were obtained. The dried samples were pulverized and kept in treated polyethylene containers.

3.5.2 Determination of Moisture Content and Dry Matter[128]

Three dried porcelain crucibles were weighed and kept in a desiccator. 5.0 g of ground mushroom samples were weighed in the porcelain crucibles and put into the oven (Gen. Lab JENNY WAY, MODEL MINO/30 England) and dried at 75 °C for 6

h. Further drying and weighing were conducted until constant weights were obtained[128]. The weight obtained after further drying for 6 h at the same temperature (75 °C) represented the dry matter, also expressed in percentage[128].

3.5.3 Determination of Ash Content[128]

5.0 g ovenï dried mushroom samples were weighed in a previously dried and weighed porcelain crucibles. The crucibles and their contents were placed in a muffle furnace (Vecstar F EF3, Jenny way, Chesterfield, U.K) and ashed at 450 °C for 4 h. The furnace was switched off and the crucibles were left to cool, before being transferred into a dessicator and weighed later after attaining room temperature. The loss on ignition and the % ash contents were calculated.

3.5.4 Determination of Crude Protein Content[128]

Crude protein was determined by the micro Kjeldahl method[128]. 5.0 g of dried finely ground mushroom samples were weighed into 500 mL round bottom Kjeldahl flask. 2.0 g of digestion catalyst (K_2SO_4) mixed with H_2O were added followed by 20 mL of concentrated H_2SO_4. The flask was gently heated until frothing subsided .The heat was increased until a colourless or pale green digest was obtained. The digest was allowed to cool and then diluted to 25 mL with distilled water. 20 mL of the diluted digest was placed in a distillation flask with addition of 25 mL of 4 M NaOH solution followed by distillation. The distillate was collected in a receiver containing 10 mL of 0.1M boric acid solution. After distillation, 0.1M HCl solution was used to titrate excess boric acid to violet end point (just before the solution goes back to pink). Amount of NH_3 liberated was obtained by difference. The crude protein was calculated using a conversion factor of 6.25.

3.5.5 Determination of Total Lipids[128]

5.0 g of dried and ground mushroom sample was weighed in a 10 mm thimble, and put in a soxhlet extractor having a 60 mL flask. A clean dry 25 mL round ï bottom flask containing black beads to serve as boiling chips to ensure smooth boiling was accurately weighed. 20 mL of ether was added to the flask which was connected to the extractor and extraction was carried out for 4-6 h[147]. At the end of extraction, the flask was removed and put in the oven at 40 °C for 30 min. Later cooled in a desiccator and reweighed. The total lipid was then calculated and expressed in %.

3.5.6 Determintion of Crude Fibre Content[129]

5.0 g of powdered mushroom sample was weighed into a porcelain crucible and ashed in a muffle furnace at 450 °C for 4 h. 2.0 g of the ashed sample was weighed into a beaker and 100 mL of 1.25 % H_2SO_4 was added. The beaker was covered with a watch glass and gently heated on a hot plate for 30 min. The supernatant was decanted under suction through a pre-weighed sintered glass crucible and the residue and the supernatant were washed three times with about 50 mL of boiling water. The content was washed into the sintered glass crucible and dried in an oven for 3 h at 105 °C. It was cooled and weighed to constant weight. The difference divided by the sample weight expressed in percentage gives the % fibre content.

3.5.7 Determination of Ethanol Soluble Sugar[129]

5.0 g of dried powdered mushroom samples were extracted with 50 mL of 80 % ethyl alcohol in a soxhlet extractor for 6 h. The crude extract was diluted to 100 mL with 80 % ethyl alcohol. The quantity of soluble sugar in the extract was titrated using the phenol sulphonic acid method[129].

3.5.8 Determintion of Vitamin C Content[129]

10 mL of 1 M ascorbic acid solution was pipetted into 50 mL conical flask and titrated with 0.1 M dichlorophenol indophenol (DCPIP) dye. A faint pink colour that persists for at least 5 s indicated the end point. Therefore, titre value is 1 mL of 0.1 M DCPIP= w (mg) ascorbic acid=0.002 mg ascorbic acid standard. 5.0 g of ground mushroom sample was weighed in a 50 mL conical flask. 5.0 mL of water was added followed by 5 mL of 10 % ascorbic acid. The flask was carefully shaken for 30 min. The sample solution was then titrated with 0.1 M DCPIP dye solution. The equation mg Vitamin C = titration value x 0.002 mg (ascorbic acid factor) was used to calculate vitamin C content of the mushroom.

3.6 Determination of Anti-Nutritional (Non-Metallic Toxicants) Factors in the Mushrooms Samples

3.6.1 Phytate Content[130]

5.0 g of finely ground mushroom samples were extracted with 100 mL of 0.5 M HCI for 2 h. 25 mL of the extract was neutralized with 0.5 M NaOH and later made slightly acidic with 0.17 M HCI. The solution was diluted to 50 mL with distilled water and 10 mL of the solution was heated, cooled and centrifuged. 25 mL of distilled water was added to the residue and heated for 3 min at 100 °C. The residue was digested with 1 mL of 60 % HCI, 5 mL 10 % HNO_3 and 0.5 mL 10 % H_2SO_4 for 15 min. The digest was cooled and diluted to 50 mL with distilled water. Stock solution of potassium hydrogen phosphate was prepared by dissolving 0.4393 g KH_2P0_4, dried at 105 °C, in de-ionized water and diluted to 1 litre. 25 mL of the stock solution was diluted to 500 mL to get working solution. Aliquots of standard solution containing 0.5, 1.0 and 1.5 mg/L were prepared. 2.0 g of $(NH_4)_6.MO_7O_{24}H_2O$ was dissolved with 50 mL de-ionized water. 28.5 mL of concentrated H_2SO_4 was added cooled and diluted to 1 litre with de-ionized water. This was used to set the

absorbance at 827 nm, plotted calibration curve from the standards prepared and used that to determine mg of phosphorus in the sample aliquot.

3.6.2 Cyanide Content[130]

5.0 g of ground mushroom samples were dissolved with 200 mL of distilled water and diluted slightly with 10 % sulphuric acid and heated softly. The vapour was allowed to enter into a flask containing few drops of 0.1 M nitric acid. Phosphorus and phosphine were oxidized to phosphoric acid which was precipitated as the yellow molybdophosphate on the addition of ammonium molybdate solution. It was Filtered and titrated against 0.2 M silver nitrate solution until a faint but permanent turbidity was obtained. The obtained value after filtration minus value after titration expressed in % = the cyanide content of the sample.

3.6.3 Tannin Content[130]

5.0 g of finely ground mushroom sample was suspended in 50 mL distilled water and extracted by shaking continuously for 60 seconds. The sample was washed and rinsed 3 times with 50 mL distilled water. The final volume of filtrate was made up to 60 mL, 3 mL of O.1 M $FeCl_3$ was added to the filtrate, followed by 3 mL of 0.008 M $K_3Fe(CN)$. The solution was allowed to stand at ambient temperature for 10 min and then filtered. The absorbance of the distilled water which is the blank and test sample were read at 720 nm using a UV/VIS spectrophotometer JENWAY (MODEL 6715, Staffordshire, UK. The concentration of the test sample was read and expressed as mg/g of dry sample.

3.7 Preparation of Samples for Metals Determinations

3.7.1 Ashing of Mushroom Samples[129, 130]

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.

3.7.2 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.

3.7.3 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.

3.8 Determination of Soduim and Potassium.

3.8.1 Standard Solutions of Sodium and Potassium[128, 130].

2.54 g NaCl and 1.91 g KCl were each weighed out and dissolved in de-ionized water in 1 litre volumetric flask and made up to mark with de-ionized water to serve as stock solutions. The solution contained 1000 mg/L Na or K. The working sodium and potassium standard solutions were prepared by diluting 0.1 mL, 0.3 mL and 0.5 mL to 100 mL to get 1, 3 and 5 mg/L solutions respectively.

3.9 Preparation of Stock Solutions of other Trace Metals[128, 130, 132].

Stock metal solutions were prepared as follows:-

3.9.1 Calcium:- 2.49 g of calcium carbonate (analar grade) was added 10 % HCl to effect complete dissolution in a 100 ml beaker. The solution was diluted to mark in 1 litre volumetric flask with de-ionized water. This solution was 1000 mg/L calcium concentration.

3.9.2 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..

3.9.3 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.

3.9.4 Magnesium:- 10.01 g magnesium sulphate ($MgSO_4$) was dissolved in 200 mL de-ionized water. To this was added 1.5 mL concentrated nitric acid and the solution was made up to 1L mark. The solution was standardized by titrating with 0.1 M

EDTA solution using eriochrome black-T as indicator. The solution was found to contain 1000 mg/L of magnesium ions.

3.9.5 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.

3.9.6 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.

3.9.7 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.

3.9.8 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.

3.9.9 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.

3.9.10 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.

3.9.11 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.

3.10 Determination of Trace Metals by AAS[128, 130, 131, 132].

3.10.1 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.

3.11 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 Duncanôs multiple range test[133, 134, 136]

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

The result of the questionnaire distributed was presented in Table 4.1. There were high preferences for *Termitomyces robustus*, *Pleurotus tuber-regium* and *Agaricus bisporus* as shown in the Table 4.1. 100 % of the respondents were willing to buy cultivated mushrooms in the study area.

Table 4.1 Response to the Questionnaire Distributed

Name of Major Types of Mushrooms Consumed in your Area Rank them in Order of Preference	**Ist Termitomyces sp**	**2nd Pleurotus Tuber-Regium "Osu" Ero "osu"**	**3rd Agaricus Bisporus Ero osisi**
How do you prepare your mushrooms?	With vegetable 80 80 %	Ground 5 5 %	Fried gravies 15 15 %
How do you preserve mushrooms?	Drying 80 80 %	Deep freezing 5 5 %	Canning 15 15 %
How often do you eat mushrooms?	Often 5 5 %		Occasionally 95 95 %
How do you get your supply of mushrooms	Local market 60 60 %		Farms 40 40 %
What time of the year is mushroom most abundant.	Rainy season 100 100 %	Dry season	Early rains
How do you know that a mushroom is poisonous	Wholesomeness Aroma, colour 89 89 %		Parts eaten off by files 11 11 %
Why do you eat mushroom occasionally	Not available 90 90 %		Cannot afford it 10 10%
Have you heard of mushroom cultivation	Yes 10 10 %		No 90 90 %
If people start growing mushroom, will you buy	100 100 %		

The proximate Compositions, vitamin C and anti-nutritional profiles of studied wild mushrooms are shown in Table 4.2 and those of cultivated mushrooms are presented in Table 4.3. The moisture content (MC) ranged from 81.79 % to 97.84 %, the highest value was from *Amanita Phalaoides* and the least value was from *Agaricus bisporus*. These values were similar to values recorded by Jonathan (2006)[29] in Table 2.6, when he evaluated the nutritive values of some wild Nigerian mushrooms. Dry matter (DM) ranged from 2.63 % to 18.36 % was an indication of high roughages contained by mushrooms. Crude protein (CP) ranged from 8.16 % to 24.67 % which compared well with the following seeds and legumes; (cowpea19.06 %, *Treculia Abrilana* 19.31 %, Bean seed 20.80 %, *colocy cilrullus* 22.00 % and ground nut 23.05 %)[20]. Ash contents 3.26 % to 14.33 % were indications of high mineral contents of mushrooms. Also these values agreed with some literature reports[20, 28, 29]. Lipid (fat/oil) 1.00 % to 6.68 % were indication that mushrooms are excellent dietary food for diabetic and coronary heart disease patients[27, 29]. Crude fibre (CF) of wild mushrooms ranged from 2.62 % to 11.37 % while the values of cultivated mushrooms ranged from 8.31% to 15.37 %. These values were close to data obtained by Kalac (2009)[27]in some European mushrooms. Other values obtained for Ethanol soluble sugar (ESS), carbohydrate $(CH_2O)n$, vitamin C were found similar, showing no significant differences ($p > 0.05$) between the wild and cultivated mushrooms. The values obtained for anti-nutritional factors (mg/100 g); Cyanide (0.02- 0.21), Tannins (0.12-0.55) and phytic acid (0.01- 0.70) for wild mushrooms while values for cultivated mushrooms ranged as follows; cyanide (0.04- 0.31), Tannins (0.01-0.67) and Phytic acid (0.01-0.70). These values were lower than 1.00 mg/100 g found in (WHO 1995)[137] guidelines for these toxicants in food.

Table 4.2: Mean Proximate Compositions, Vitamin C and Anti-nutritional Profiles of Wild Mushrooms (%/100 g)

Mushroom Species	Moisture Content	Dry Mattter Content	Crude Protein	Crude Fibre	Ash	Lipid	Ethanol Soluble Sugar	Carbohydrate	Vitamin C	Cyanide	Tannins	Phytic acid
Termitomyces robustus	88.85 ±0.04	7.55 ±0.03	23.95 ±0.01	7.95 ±0.08	13.30 ±0.04	5.45 ±0.14	11.93 ±0.38	38.45 ±0.37	0.15± 0.01	0.04 ± 0.01	0.33 ± 0.02	0.01 ± 0.01
Agaricus bisporus	81.23 ±0.27	18.78 ±0.17	24.18 ±0.60	9.05 ± 0.09	11.00 ±0.13	0.70 ±0.14	11.93 ±0.16	22.38±0.22	0.15 ±0.01	0.21 ± 0.02	0.33 ± 0.01	0.35 ± 0.14
Pleuotus tuber-regium	89.00 ±0.01	11.00 ±0.11	15.60 ±0.60	10.80 ±0.71	14.60 ±0.39	2.90 ±0.06	10.50 ±0.01	21.70±0.40	0.10 ±0.01	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
Amanita phalaoides	97.10 ±0.10	2.90 ±0.09	8.50 ±0.22	2.20 ±0.03	3.80 ±0.13	2.90 ±0.04	10.50 ±0.01	69.90 ±0.10	0.00 ±0.00	0.04 ± 0.02	0.55 ± 0.14	0.70 ± 0.20
Amanta verosa	84.09 ±0.06	15.93 ±0.47	10.38 ±0.18	11.43 ±0.59	13.80 ±0.70	2.20 ±0.70	7.80 ±0.23	35.40 ±0.09	0.12±0.002	0.04 ± 0.01	0.12 ± 0.06	0.15 ± 0.04
Range	81.23 - 97.10	2.90 - 18.78	8.50 ï 24.18	2.20 ï 11.43	3.80 - 14.60	0.70 - 5.95	9.80 ï 11.93	21.70 ï 69.90	0.10 - 0.15	0.02- 0.21	0.12- 0.55	0.01 ï 0.70

Table 4.3: Mean Proximate Compostions, Vitamin C and Anti-nutritional Profiles of Cultivated Mushrooms (%/100 g)

Mushroom Species	Moisture Content	Dry Mattter Content	Crude Protein	Crude Fibre	Ash	Lipid	Ethanol Soluble Sugar	Carbohydrate	Vitamin C	Cyanide	Tannins	Phytic acid
**Termitomyces robustus*	-	-	-	-	-	-	-	-	-	-	-	-
Agaricus bisporus	86.80 ±0.10	13.00 ±0.14	31.03 ±0.50	13.57 ± 0.32	13.30 ±0.04	3.60 ±0.11	8.85 ±0.10	18.00±0.09	0.37 ±0.02	0.14 ± 0.01	0.67 ± 0.20	0.04 ± 0.01
Pleuotus tuber-regium	83.00 ±0.02	17.00 ±0.13	12.43 ±0.22	11.22 ±0.44	11.00 ±0.13	0.70 ±0.40	0.60 ±0.10	20.00±0.10	0.16 ±0.001	0.04 ± 0.02	0.01 ± 0.01	0.30 ± 0.03
Amanita phalaoides	94.00 ±0.02	6.00 ±0.22	18.00 ±0.81	14.60 ±0.32	14.60 ±0.39	1.33 ±0.31	9.10 ±0.70	45.00±0.70	0.17±0.20	0.31 ± 0.06	0.55 ± 0.14	0.70 ± 0.21
Amanta verosa	94.67 ±0.10	5.33 ±0.31	13.23 ±0.11	8.90 ±0.11	15.00 ±0.00 4	2.63 ±0.02	6.10 ±0.11	32.00 ±0.89	0.22±0.00 1	0.14 ± 0.04	0.01 ± 0.01	0.01 ± 0.01
Range	83.00-94.67	5.33 - 17.00	12 ï 31.03	8.90 ï 14.60	11.00 - 15.00	0.70 ±3.60	0.60 ï 9.10	18.00 ï 45.00	0.16 -0.37	0.04-0.31	0.01-0.67	0.01 ï 0.70

*Not planted.

Table 4.4 is the mean concentrations of essential metals in wild and cultivated mushrooms. These data for wild and cultivated mushrooms are close at $p>0.05$ and comparable with values reported for fruits/vegetables by Okoye (2001)[110] and mushrooms by Okaroh (2005)[55], however higher values were reported in some edible mushrooms in South Eastern, Nigeria[29]. Mean values obtained for Na, Ca and K were comparable with WHO 2004 guideline levels while values for Mg and Fe showed higher values in mushrooms.

Table 4.4: Mean Concentrations (mg/kg) of Essential Metals in Wild and Cultivated Mushrooms

Mushroom species	Wild					Cultivated				
	Na	K	Ca	Mg	Fe	Na	K	Ca	Mg	Fe
Tr	370.99 ± 0.27	886.32 ±0.14	83.64 ±0.15	686.76 ±0.04	213.09 ±0.19	-		-	-	-
Ab	474.64 ±0.33	714.88 ±2.15	252.67 ±0.80	476.57 ± 0.97	268.11 ±0.19	668.49 ±0.33	844.23 ± 4.33	588.61 ±2.20	549.66 ±1.11	446.19 ±0.72
Ptr	152.36 ±0.14	678.39 ± 0.27	399.76 ±0.84	601.83 ±0.44	154.68 ±0.15	332.77 ±1.59	698.17 ± 0.63	801.19 ±1.20	899.10 ±3.11	401.66 ±0.92
Aph	777.42 ± 6.27	933.81 ±2.14	545.00 ±0.64	1,191.00 ±2.04	404.32 ±1.19	1,061.12 ±7.33	701.99 ±0.28	1,841.00 ±0.14	1,566.11±0.81	772.01 ±3.12
Av	669.59 ±0.84	166.88 ±0.16	111.40 ±0.77	1,178.00 ±1.85	684.74 ±0.55	698.60 ±0.89	196.39 ± 0.93	219.69 ±1.20	1,279.00 ±2.11	777.18 ±1.12
Range	152.36 - 777.42	66.88-933.81	83.64-545.00	476.57-1,191.00	154.68 - 684.74	332.77-1,061.12	196.39-844.23	219.67-1841.08	549.66-1,566.11	401.66-777.18
WHO 2004	1500	4700	1300	420	18	1500	4700	1300	420	18

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

Table 4.5: **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	BDL	-	-	-	-	-	-	-	-
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	0.95	0.90	0.29	0.63	11	0.59	0.50	2.3	0.95
CODEX 1995	3	0.01-0.1	0.05	17	0.1	0.4	0.13-0.26	0.1	3	0.01-0.1	0.05	17	0.1	0.4	0.13-0.26	0.1

*Not Ccultivated., *Tr-Termitomyces robustus,Ab-Agaricus bisporus,Ptr-Pleurotus tuber-regium,Aph-Amanita phalaoides,Av-Amanita verosa*

Chromium was not detectable in any of the mushroom samples, while copper in all mushroom samples was below the WHO guideline concentrations in food. The rest of the metals showed concentrations above WHO 2004 guideline levels in all analyzed samples (wild and cultivated mushroom samples).

Table 4.6: Mean Concentrations (mg/kg) (n=3) of Trace Metals in Veils and Stems of wild and cultivated mushroom Samples.

Mush type	WILD								CULTIVATED							
	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Trs	0.44 ±0.01	0.98 ±0.11	BDL	0.66 ±0.11	0.28 ±0.12	0.33 ±0.22	1.22 ±0.01	0.77 ±0.02	--	--	--	--	--	--	--	--
Trv	1.02 ±0.19	1.08 ±0.10	BDL	0.98 ±0.03	0.66 ±0.01	1.29 ±0.02	2.33 ±0.03	8.77 ±0.15	--	--	--	--	--	--	--	--
Abs	0.55 ±0.11	0.11 ±0.15	BDL	0.72 ±0.12	0.14 ±0.10	0.99 ±0.06	1.26 ±0.07	0.19 ±0.01	0.33 ±0.01	0.44 ±0.04	BDL	0.32 ±0.19	1.66 ±0.22	0.12 ±0.10	0.33 ±0.11	6.12 ±0.92
Abv	0.75 ±0.01	0.39 ±0.18	BDL	0.98 ±0.10	1.33 ±0.06	1.67 ±0.09	1.73 ±0.11	1.19 ±0.04	0.66 ±0.16	0.72 ±0.10	BDL	0.73 ±0.03	2.73 ±0.89	0.19 ±0.04	0.64 ±0.03	8.41 ±0.88
Aps	1.82 ±0.81	0.33 ±0.42	BDL	0.88 ±0.11	0.74 ±0.05	0.88 ±0.22	0.77 ±0.16	0.79 ±0.09	1.22 ±0.77	0.99 ±0.44	BDL	0.33 ±0.18	1.39 ±0.78	0.10 ±0.01	0.67 ±0.03	6.44 ±0.97
Apv	3.18 ±1.01	0.99 ±0.78	BDL	0.92 ±0.16	0.88 ±0.18	1.06 ±0.66	1.11 ±0.19	1.03 ±0.67	1.32 ±0.19	1.01 ±0.02	BDL	0.44 ±0.02	3.12 ±0.88	0.97 ±0.44	0.97 ±0.19	6.96 ±0.96
Avs	1.44 ±0.55	0.67 ±0.01	BDL	0.99 ±0.04	0.08 ±0.01	0.88 ±0.10	0.17 ±0.03	1.04 ±0.66	0.76 ±0.43	0.86 ±0.19	BDL	0.58 ±0.02	0.12 ±0.01	0.09 ±0.02	0.75 ±0.16	7.54 ±1.22
Avv	1.88 ±0.66	0.68 ±0.19	BDL	0.99 ±0.03	0.28 ±0.11	0.89 ±0.02	0.26 ±0.01	2.90 ±0.99	0.89 ±0.11	1.01 ±0.55	BDL	0.87 ±0.44	0.68 ±0.22	0.19 ±0.01	0.98 ±0.34	8.11 ±2.44
Pts	0.77 ±0.10	0.39 ±0.11	BDL	0.77 ±0.22	2.44 ±1.02	2.19 ±1.22	2.16 ±1.04	0.66 ±0.33	0.67 ±0.22	0.22 ±0.01	BDL	1.33 ±0.96	2.33 ±1.55	0.98 ±0.44	0.65 ±0.29	9.44 ±3.11
Ptv	1.56 ±0.77	0.77 ±0.21	BDL	0.78 ±0.22	2.99 ±1.50	4.43 ±1.29	4.66 ±2.09	0.70 ±0.16	0.68 ±0.22	0.29 ±0.01	BDL	1.55 ±0.98	3.56 ±1.39	1.34 ±0.77	0.97 ±0.21	9.77 ±2.79
WHO 2004	0.90	0.29	0.63	11	0.59	0.50	2.3	0.95	0.90	0.29	0.63	11	0.59	0.50	2.3	0.95
Codex 1995	3	0.01-0.1	0.05	17	0.1	0.4	0.13-0.26	0.1	3	0.01-0.1	0.05	17	0.1	0.4	0.13-0.26	0.1

V-Veil, S-Stem, Tr-Termitomyces robustus, Ab-Agaricus bisporus, Ap-Amanita phalaiodes, Av-Amanita verosa, Pt-Ppleurotus tuber-regium.

There were no significant differences between metal concentrations in wild and cultivated mushrooms. However, significant differences ($p<0.05$) exist with higher values in veil than in stems. Chromium was not detectable in all the studied mushroom samples.

Table 4.7 contains mean concentrations of trace metals in soils and substrates from where wild mushroom samples were collected.

Table 4.7: Mean Concentrations (mg/kg) (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	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	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	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	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	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
WHO agric soil	0.05-0.10	2-5.60	0.10	20.00	400-777	50-200	20.00	20.00	100
WHO $_{\text{Polluted soil}}$	3-5.00	5-10.00	4-10.00	50-140	400-780	50-200	75-200	50-250	300-700

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

Table 4.8: Bioaccumulation 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

*- Could not be planted.

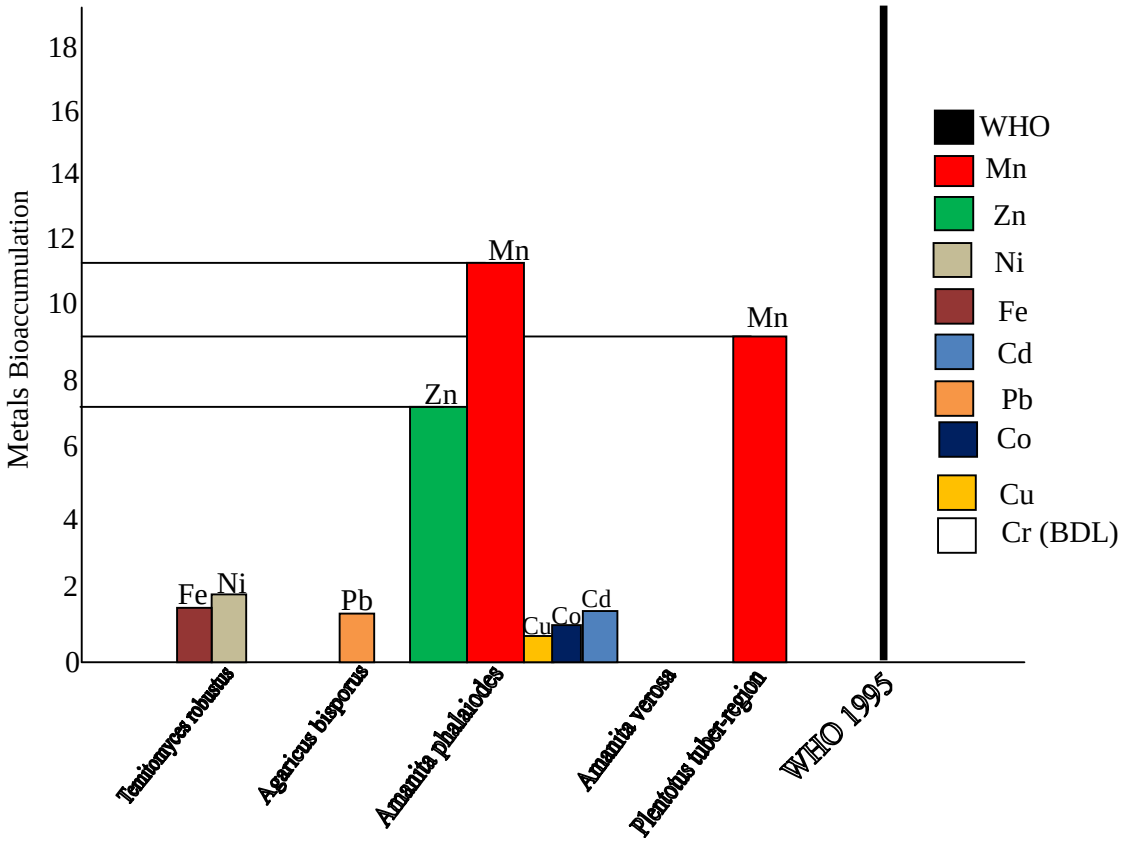


Fig. 4.8a: Bioaccumulation Factors in Wild Mushroom Samples

Chromium was below detectable limit in all species of mushrooms for wild and cultivated. Generally, bioaccumulation factors were very low in spite of the fact that many of the mushroom species have levels of metals higher than WHO guideline values in food. Only Mn and Zn showed substantial bioaccumulation (see Figs. 4.8a and 4.8b).

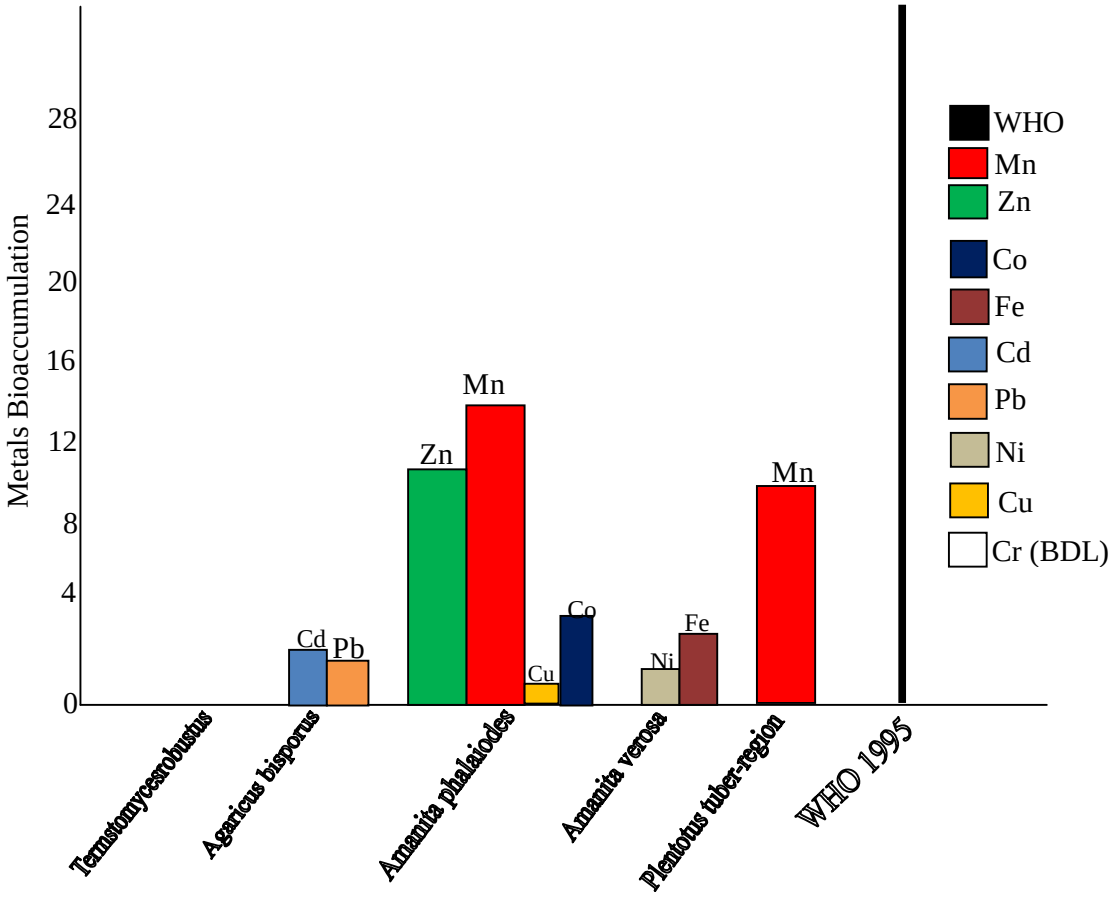


Fig. 4.8b: Bioaccumulation Factors of Trace Metals in Cultivated Mushrooms.

Table 4.9a and b contain trace metal concentrations in studied mushrooms and Romanian mushrooms.

Table 4.9a: Trace Metal Concentrations (mg/kg) from Mushroom Samples Used.

Studied Mushrooms	Metal Concentrations (mg/kg)								
	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Termitomyces robustus	4.48	0.59	BDL	0.12	213.09	24.42	16.85	5.03	50.88
Agaricus bisporus	4.41	1.21	BDL	0.39	268.11	15.20	1.40	4.56	28.72
Amanita phalaoides	3.88	1.52	BDL	0.72	404.32	16.60	4.77	4.62	34.02
Amanita verosa	6.68	0.63	BDL	0.66	684.74	8.25	15.07	3.83	61.17
Pleurotus tuber-regium	4.30	0.48	BDL	0.22	154.68	13.60	3.04	3.60	25.00

Table 4.9b: Trace Metal Concentrations in Some Romanian Mushroom Samples

Wild Romanian mushrooms	Metal Concentrations (mg/kg)								
	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Amanita vaginata	0.03	0.02	0.18	7.11	101.60	0.70	1.12	1.93	112.10
Amarinta phalaoides	0.30	0.01	0.52	10.2	421.9	0.89	0.64	3.03	137.40
Armillariella mella	0.11	nd	1.10	10.43	543.80	3.06	1.02	2.36	124.00
Agaricus compestris	0.06	0.04	0.03	10.30	391.90	1.90	1.06	1.32	135.70
Amanita rubescus	0.08	0.01	0.55	12.90	308.90	0.82	0.97	0.68	115.20

The values of trace metals determined in the studied mushroom samples are compared with values found in literature for some Romanian mushroom samples in Tables 4.9a and b, clearly, higher values were obtained in the present study than in the Romanian mushrooms.

Tables 4.10a and b contain the bioaccumulation factors of trace metals from studied mushrooms and Romania mushrooms.

The studied mushrooms have higher bioaccumulation factors than Romania mushrooms as reported in literature[162]. Chromium was below detectable levels in studied mushrooms.

Table 4.10a: Bioaccumulation factors of Trace Metals from Mushroom Samples Used.

Present study	Bioaccumulation factors of Trace Metals								
	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Termitomyces robustus	0.74	0.54	BDL	0.01	1.79	9.25	1.95	0.08	2.70
Agaricus bisporus	0.86	0.28	BDL	0.04	1.30	6.38	0.08	1.08	1.76
Amanita phalaoides	1.15	0.76	BDL	0.35	1.17	11.94	0.58	0.97	7.13
Amanita verosa	0.63	0.06	BDL	0.06	1.45	0.78	0.65	0.63	0.76
Pleurotus tuber-regium	0.14	0.26	BDL	0.12	1.54	9.78	0.46	0.21	2.49

Table 4.10b: Bioaccumulation factors of Trace Metals from Romanian mushroom samples

Ramanian Mushroom species	Bioaccumulation factors of Trace Metals								
	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Amanita vaginata	*0.10*	0.12	0.15	0.62	0.12	0.51	0.46	0.24	1.49
Amanita rubesceus	*0.19*	0.02	0.41	0.82	0.36	0.31	0.46	0.13	1.41
Amarinta phalaoides	*0.46*	0.36	0.28	0.49	0.55	0.32	0.32	0.32	0.52
Armillariella mella	*0.45*	nd	0.32	0.31	0.49	0.49	0.13	0.52	1.32
Agaricus compestris	0.06	0.05	0.03	0.45	0.47	0.33	0.39	0.32	1.37

Also, comparing the BCF(s), of the two studies (Tables 4.10a and b) the same trend is observed, that is higher values in the presently studied mushrooms than for the Romanian samples[162]. This is an indication that the soil from where the presently studied mushrooms were collected were more polluted than the environment from where the Romanian mushrooms were collected. So, showing clearly that BCF(s), partly depend on the level of pollution. This means that safe levels of trace metals in mushrooms could be achieved when mushrooms are harvested from unpolluted soil or substrate.

4.2 Discussion

4.2.1 Samples Collection and Preparation

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[148]. 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[110, 130, 148]. Distilled water was used for preparation of solutions for proximate analysis while de-ionized water was used for preparation of solutions for metal analysis.

4.2.2: Proximate Compositions, Vitamin C and Anti-nutritional factors of Mushroom Samples.

Proximate composition, vitamin C and mineral content are indices of nutritional qualities. The values obtained from studied mushrooms clearly indicate that these mushrooms have high nutritional qualities, however with low anti-nutritional factors which were below WHO permissible limits in food (Tables 4.1 and 4.2). Crude protein (CP) of (8.50-24.18) % are fairly comparable with those of legumes (Cowpea 19.06 %[152], Beans 20.80 %[151,] Groundnut 23.05 %) [154] and meat muscle (22.00 %[154]). Ash contents of 3.26 %-14.33 % are indications of high mineral contents of mushrooms. Low lipid (fat/oil) of 1.00 % -6.68 % are indications that mushrooms are excellent dietary food for diabetic and coronary heart disease patients[139]. From the analytical data obtained, it is conceivable that studied mushroom species namely *Termitomyces robustus, Agaricus bisporus, Amanita phalaoides, Amanita verosa and Pleurotus tuber – regium,* hold tremendous promise in narrowing the protein supply deficiency prevalent in several developing countries of Africa including Nigeria[154].

Low carbohydrates contents of 32.00-35.40 % explained why some mushroom species are used locally as binder, bulking agent (in melon cake-a local snack), thickener in soups or infant formular. This implies that mushrooms can function effectively in low fat diet such as those required by patients with cardiovascular diseases, obesity and diabetes.

The vitamin C were detectable at levels ranging from 0.01 to 0.37 mg/100 g. However, the values of vitamin C determined shows that the mushroom species are not reliable sources of Vitamin C, although they could make important contribution to diet.

Low anti-nutritional factors which also shows that the toxic effect of these substances may not be experienced by the consumers, since there is further destruction of these substances during cooking. Similar values 0.36 mg/100 g were reported for phytates in some mushrooms and food[145] and cowpea (0.36 mg/100 g) [150]. The mean phytic acid contents 0.26 mg/100 g were comparable or similar to wild and cultivated mushroom1.00 mg/100 g (WHO 1995) guideline levels. Phytates chelate mineral elements such as Ca, Mg, Fe and Zn which renders the elements unavailable for absorption[149]. Phytates also inhibit the activities of digestive enzymes: amylase, pepsin and pancrease[150, 154]. The mean cyanide contents 0.16 mg/100 g of these mushroom species were low when compared with 0.36 mg/100 g reported in literature[80] and also reported in WHO guideline levels. This factor can be eliminated through blanching and cooking prior to consumption[55]. Mean value of 0.31 mg/100 g for tannins is slightly below the WHO1995 maximum permissible levels as such may contribute to ill health associated with mushroom consumption at large quantities. Tannins comprised of a heterogeneous group of plant polyphenols which are capable of interacting with proteins. Tannins are high molecular weight compounds (mol. Wt. 500-5000) containing sufficient phenolic hydroxyl groups to permit the formation of stable cross-links with proteins[29].

These data were close at ($p>0.05$) and comparable with values reported for fruits/vegetables[55, 110] however higher values were reported in some edible mushrooms in South Eastern, Nigeria[29].

4.2.3. Concentrations of Essential Metals in Studied Mushroom Samples.

The concentrations of essential metals in Table 4.3 also indicated that these mushroom species were good sources of these elements and could provide up to 50 % of the recommended daily allowances for these elements[152]. The mean values

obtained in studied mushroom were also higher than those reported in literature[80]. For example, mean sodium concentration of studied mushrooms was 777.42 mg/kg, cowpea 277.33 mg/kg and vegetables 252.67 mg/kg. Concentration of sodium varies in species parts which was similar to what was reported in literature[21, 154]. Sodium and potassium are systemic electrolyte and essential in co-regulating ATP. ATP (Adenosine triphosphate) is a nucleotide, $C_{10}H_{16}N_5O_{13}P_3$, that is composed of adenosine and three phosphate groups and releases when hydrolyzed to ADP. It is present in all cells, where it is used to store and transport energy needed for biochemical reactions. It is found in mitochondria of all plant and animal cells. It is major source of energy for cellular reaction, energy is released during the ATP conversion to ADP [154, 155].

Calcium was dominant in the veil part of all the five species showing the attractiveness and the physical support of the veil structure. Also, adequate calcium has contributed immensely to the succulent nature of the veil. These values were higher than values reported for vegetables[142]. Its nutritional benefits are as follows: needed for muscle development, heart and digestive system, builds bones and for the functioning of blood cells. Fe is required for many proteins and enzymes synthesis notably hemoglobin to prevent anemia. From the data obtained, the studied mushrooms could be considered as good sources of iron and magnesium. Magnesium is required for effective functioning of ATP and for bones developemt[156].

4.2.4: 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.

4.2.5: 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[113, 152].

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.

4.2.6: Potentials of the Studied Mushrooms for 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[159]. 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.

CHAPTER FIVE

5.0 SUMMARY OF THE RESEARCH FINDINGS

- The study revealed that Termitomyces robustus has a lot of ecological complications during cultivation which probably made its planting unsuccessful in this study.
- Pleurotus tuber-regium, Amanita verosa, Agaricus bisporous and Amanita philadiodes could be grown easily on artificial substrates or soil.
- Edibility of mushrooms depends on pollution status of the soil/substrates with respect to metals levels in the soil. In order words, planting of mushrooms on safe soil will not likely pose danger to human health.
- It was also observed that Amanita species accumulate heavy metals more than other species of mushroom studied.
- This work has successfully provided a platform to rescue mushrooms, through mushroom cultivation for better mushroom production and management
- Bioremediation of soils polluted with Mn and Zn were achieved.

5.1 CONCLUSION

This research is meant to stand as a guide to other researchers, nutritionists and agriculturists who seek information on the level of metals in mushrooms grown in the study area and also give anyone who has the opportunity of laying hands on this research the base line data of the studied mushroom species. 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;-

1. The studied mushrooms were found to be all edible with high nutritional compositions and low anti-nutritional factors below WHO permissible limits in food and vegetables.
2. They are good sources of essential metals namely- Na, K, Ca, Mg and Fe.
3. They are accumulators of toxic metals namely- Cd, Co, Mn, Ni, Pb and Zn.
4. All the accumulated toxic metals were above WHO guideline values which could impair their edibility.
5. 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 as could be deducted from their accumulation potential of these metals.
6. 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.
7. The cultivation of Amanita phalaoides, Pleurotus tuber-regium, Amanita verosa and Agaricus bisporus were achieved.

5.2 CONTRIBUTIONS TO KNOWLEDGE

- The present work has made the following contributions to knowledge.
- Harbarium/Voucher numbers of these mushrooms were established.
- Physico-chemical and toxicological profiles of the studied mushrooms were determined.
- All the studied mushrooms were found to be edible and could only be impaired by toxic metal if planted or harvested from a polluted soil.
- The work has shown that when compared with international standards, the studied area were polluted with Mn, Zn, Cd and Pb.

- 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 soils.
- Safe levels of Mn and Zn could be achieved in polluted soils by taking advantage of mushrooms short cropping cycle and planting them many times in a season.
- The cultivation of *Amanita phalaoides*, *Pleurotus tuber-regium*, *Amanita verosa* and *Agaricus bisporus* were established.

5.3 RECOMMENDATIONS

- With environmental changes due to climatic change, deforestation, aggressive soil erosion, many of the indigenous mushroom species are disappearing very fast. The attention of government has been drawn to urgently rescue our fast disappearing natural resources including mushrooms
- The government, as a matter of priority, should sponsor a national documentation of Nigerian mushrooms in the six geopolitical zones. This must be followed by having national gene banks as our heritage.
- Universities and other research institutions (IRs) which are actively involved in mushroom research should be specially funded for the establishment of laboratories with the facility for training more mushroom scientists.
- There is a very wide gap between the mushroom scientists and growers. This is certainly not a healthy situation for the general development of the mushroom production in the country.

For the mushroom industry to grow and contribute meaningfully to the countryôs gross domestic products (GDP) of the country, the following recommendations were suggested:

1. There should be a meeting point for both the scientists and the growers. Growers should attend symposia, workshops, seminars and conferences on mushroom cultivation.
2. The mushroom scientist should make concerted efforts to conduct studies that will have direct applications in the mushroom production sector.
3. Research laboratories should be the sole producers of mushroom seed for growers. They should be the best strains for the growers as well.
4. It is notable from the present work and the vast literature on mushroom that they are rich in proteins and should be popularized to contribute to the dietary needs of the people of this country.
5. Government and the private sector should be involved in popularizing these crops by investing funds in research of local edible species whose technology of production will be indigenous. Dependence on foreign technology and exotic strains may not be sustainable.
6. Soft loans should be given to interested students with training in mushroom science to establish small scale farms. Mushroom poisoning can only be eliminated in our country through the commercial production of edible mushrooms on safe soil, so that people will no longer depend on mushrooms from the wild. The market is always available for commercially produced mushrooms and the country could even earn foreign exchange from such venture.

7. Mushroom growers association should be formed to bring farmers together. Such a forum will provide new methods and new strains for cultivation.
8. The country through Nigerian Standard Organization (SON) should begin to establish mushroom Standards for the country in collaboration with Nationl Agency for Drug, Administration and Control (NAFDAC) and mushroom scientists.

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APPENDIX 1

DEPARTMENT OF PURE & INDUSTRIAL CHEMISTRY
SCHOOL OF POST GRADUATE STUDIES
UNIVERSITY OF NIGERIA, NSUKKA.

Dear Respondent,

QUESTIONNAIRE

The questionnaire is designed for adult who consume mushrooms and is aimed at obtaining information on the local edible and non-edible mushrooms in Anambra State, Nigeria.

Instruction:

Tick ☐ where applicable or fill in the answers in the spaces provided

1. Do you know what mushrooms are?

   Yes ☐

   No ☐

2. Do you like mushroom meals?

   Yes ☐

   No ☐

3. How do you prepare your mushrooms?

   With vegetable

   Fried gravies

   Ground for soup and flavouring

4. How do you preserve the mushrooms?

   Drying ☐ Deep freezing ☐

   Canning ☐ Do not preserve at all ☐

5. How often do you eat mushrooms?

   ☐ Once a week

☐ Twice a week

☐ Occasionally

6. Name the major type of mushrooms you consume in your area (use local names)

   1. .é .
   2. é
   3. é
   4. é
   5. é

7. Which of these mushrooms do you like best?

   1. é
   2. é
   3. é

8. How do you get your mushrooms?

   ☐ From the forest or woods

   ☐ Buy from the local markets

   ☐ Buy canned mushrooms

9. What time of the year is the mushroom most abundant?

   é ..

10. Have you heard of mushrooms poisoning?

    Yes ☐

    No ☐

11. Are there ways of knowing which mushrooms are poisonous?

    Yes ☐

    No ☐

12. If 10 above is yes, how?.

.....

.....é é é é é é é é é é ...é é é é é

é é

13. Do you know of anybody that died of mushrooms poisoning?

Yes ☐

No ☐

14. Have you heard that mushrooms can be cultivated?

Yes ☐

No ☐

15. If people start growing mushrooms in their farms, will you buy?

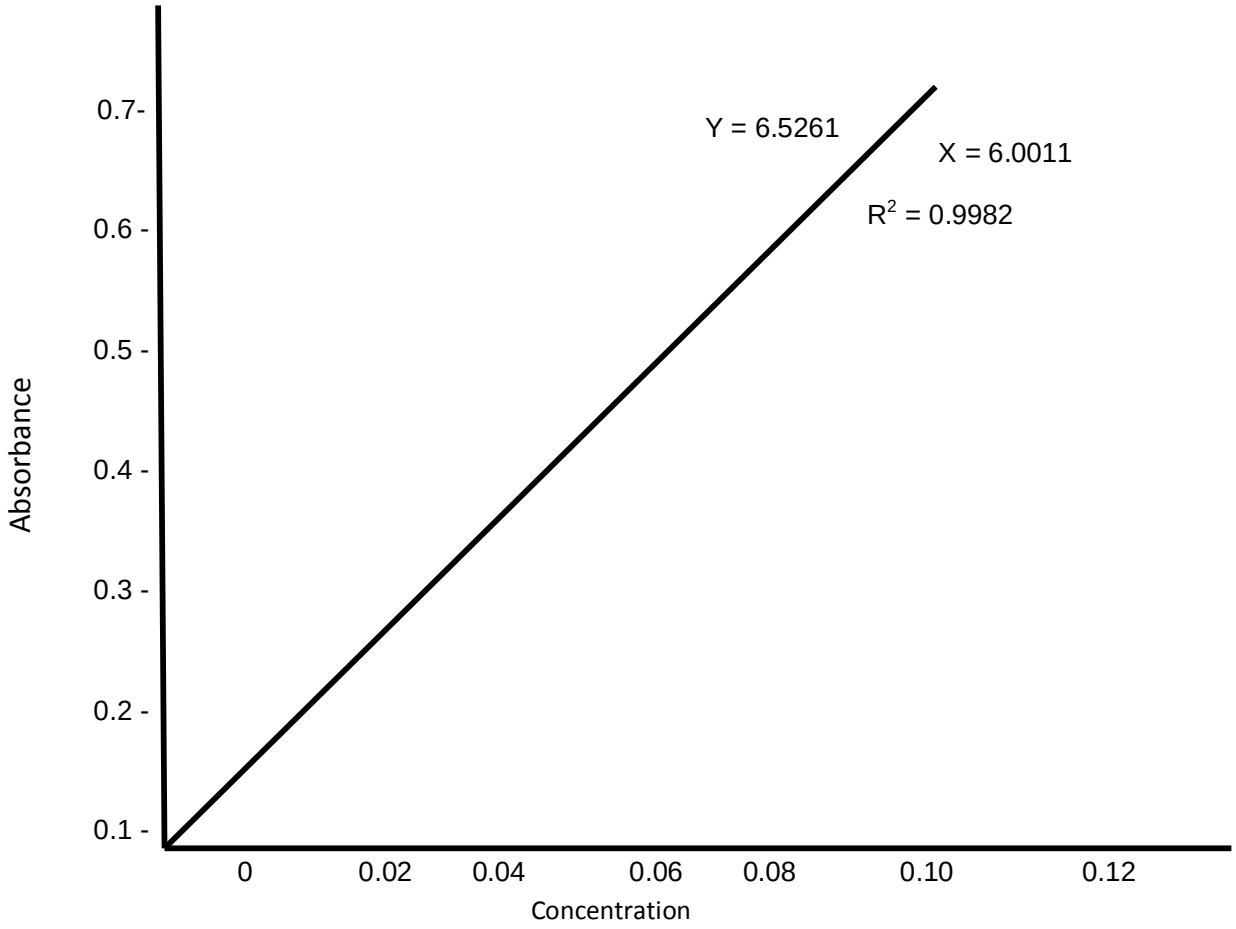
Yes ☐ No ☐

OKAROH, B.C (MRS).

APPENDIX II

Absorbances of Standard Solutions of Cyanide at 490 nm

S/N	Concentration in mg/L	Absorbance at 490nm
1	0.02	0.120
2	0.04	0.273
3	0.06	0.400
4	0.08	0.520
5	0.10	0.650



Graph of absorbance versus Conc for cyanide at 490 nm

APPENDIX III

T-TEST
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/MISSING=ANALYSIS

/VARIABLES=Cyanide Tannin Phytate

/CRITERIA=CI(.95).

T-Test

[DataSet1] C:\Users\ADMIN\Desktop\HOD STAT\hod data.sav

Comparing both wild and cultivated samples

One-Sample Statistics

	N	Mean	Standard Deviation	Standard Error Mean
Cyanide	9	.1089	.09968	.03323
Tannin	9	.2222	.22326	.07442
Phytate	9	.2567	.27982	.09327

One-Sample Test

	Test Value = 0					
	T	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Cyanide	3.277	8	.011	.10889	.0323	.1855
Tannin	2.986	8	.017	.22222	.0506	.3938
Phytate	2.752	8	.025	.25667	.0416	.4718

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T-TEST

/TESTVAL=0

/MISSING=ANALYSIS

/VARIABLES=Cyanide Tannin Phytate

/CRITERIA=CI(.95).

T-Test

[DataSet2] C:\Users\ADMIN\Desktop\HOD STAT\hod cultivated.sav

Comparing between cultivated samples alone

One-Sample Statistics

	N	Mean	Standard Deviation	Standard Error Mean
Cyanide	4	.1575	.11206	.05603
Tannin	4	.1600	.26153	.13077
Phytate	4	.2625	.31941	.15971

One-Sample Test

	Test Value = 0					
	T	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Cyanide	2.811	3	.067	.15750	-.0208	.3358
Tannin	1.224	3	.308	.16000	-.2562	.5762
Phytate	1.644	3	.199	.26250	-.2458	.7708

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/SHEET=name 'Sheet1'

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/READNAMES=on

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EXECUTE.

DATASET NAME DataSet4 WINDOW=FRONT.

T-TEST

/TESTVAL=0

/MISSING=ANALYSIS

/VARIABLES=Cyanide Tannin Phytate

/CRITERIA=CI(.95).

T-Test

[DataSet4]

Comparing wild samples alone

One-Sample Statistics

	N	Mean	Standard Deviation	Standard Error Mean
Cyanide	5	.0700	.07874	.03521
Tannin	5	.2720	.20352	.09102
Phytate	5	.2520	.28288	.12651

One-Sample Test

	Test Value = 0					
	T	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Cyanide	1.988	4	.118	.07000	-.0278	.1678
Tannin	2.988	4	.040	.27200	.0193	.5247
Phytate	1.992	4	.117	.25200	-.0992	.6032

APPENDIX IV

PREPARATION OF STANDARDS FOR ANALYTICAL PROCEDURES

i) Calcium

2.497 g of oven dried $CaCO_3$ was dissolved and diluted to contained 1000 ppm Ca^{2+} ions. From this stock solution, calcium standard solutions of concentrations 0.0, 3.0, 6.0, 9.0, parts per million (ppm) were prepared.

ii) Magnesium

1.0 g magnesium ribbon was accurately weighed and dissolved in 10 mL concentrated HCl. The solution was boiled and evaporated almost to dryness of a water bath. Dionized distilled water was added and the solution was transferred into a 1000 mL volumetric flask and made up to mark with distilled water. From the stock solution containing 1000 mg/L of Mg^{2+} ions, four standard solutions of concentrations 0.0, 0.5, 1.0 and 1.5 ppm were prepared.

To each of the calcium and magnesium solutions, strontium chloride solution was added such that there was 1500 mg /L of strontium ions in the final solution.

iii) Zinc

A stock solution containing 100 mg/L of Zn^{2+} was prepared by dissolving 1.0 g of Zn ribbon in 10 mL of conc. HCl. The solution was evaporated almost to dryness and the salt was redissolved in 100 mL of deionized water. Standard solutions of concentration 0.0, 0.5, 1.0 and 1.5 ppm were prepared from the stock solution.

iv) Iron

A stock solution containing 1000 mg/L Fe^{3+} ions was prepared from 1.0 g of pure iron wire. This was dissolved in 10 mL concentrated HNO_3 boiled on a water bath and diluted to 100 mL with deionized water.

v) A stock solution containing 1000 mg/L of Cu^{2+} ions was prepared by dissolving 2.682 g of $CuCl_2$ $2H_20$ distilled water and finally diluting to 1000 mL standard solutions of concentration 0.0, 0.5, 1.0, 2.0 ppm were prepared from this stock.

vi) Calibration Curves

Calibration curves were prepared for each element using the respective standard solutions. The appropriate lamps and correct wave length for each element were specified in the instruction manual of the instrument.

Determination of Sodium by flame photometry

Apparatus

Flame photometer

Sodium filter

Volumetric flask

Air pump.

Reagents

a) Stock sodium solution

1000 ppm:- 2.542 g dry NaCl was dissolved in deionized water and diluted to 1 litre.

b) Working standards: suitable dilutions were made of the stock to give Na 10 ppm, 5 ppm, 2.5 ppm and 1.0 ppm.

Procedure

1. Standard and stock samples were run in the instrument with the correct filters in position.
2. Duplicate determination consisting of two aliquots were taken from a single sample digest. Sodium concentrations were determined on a calibration curve obtained from the standard solutions.

Potassium

A stock solution containing 1000 mg/L of K^+ ions was prepared by dissolving 1.909 g of analar KCl in deionized distilled water. The solution was made to 100 mL mark with deionized distilled water. From the stock solution, standard solutions of concentration 0.0, 2.0, 4.0 and 6.0 ppm were prepared. A total solution of 1 % lithium-chloride was prepared and added to potassium standard solution such that the concentration in the final solution was 0.1 %

Apparatus

Vortex mixer

Test tubes

Screw-Cap tube (16 ×125 nm)

Spectrophotometer

Reagents

Absolute ethanol

Heptane

PREPARATION OF WORKING SOLUTIONS OF SODUIM AND POTASSIUM

To prepare dilute working solutions, calculate the volumes of the stock solution needed for dilution to the required concentration as follows:

$C_1V_1 = C_2V_2$

Where C_1 and V_1 are the concentration and volume of the stock solution while C_2 and V_2 are the expected concentration and volume of the dilute working solution. Thus, if 100 mL standard flask was used to prepare 10 ppm solution, the volume of the stock solution needed, V_1 is given by

$1000 \times V_1 = 100 \times 10$

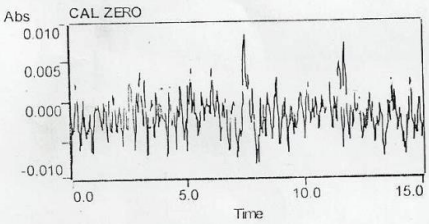
$V_1 = \frac{100 \times 10}{1000} = 1.0$ mL

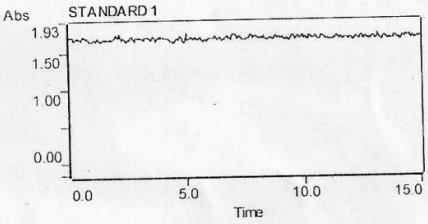
1 mL solution was taken using a graduated pipette into 100 mL standard flask and diluted to the mark with de-ionized water. This gives 10 mg/L solution.The 10 mg/L solution was then diluted further to obtain much more dilute solutions with high accuracy using the same formula. Thus to prepare serially diluted working solutions of concentrations 1,2,3,4 and 5 mg/L, in 10 mL standard flask, 1,2,3,4 and 5 mL of the 10 mg/L solutions were pipetted into separate 10 mL standard flasks and diluted to the marks with de-ionized water.

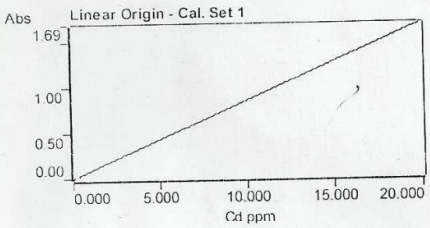
Analyst	emma
Date Started	1:54 PM 3/3/2014
Worksheet	Cd test Okaroh
Comment	
Methods	Cd
Computer name	USER-14FE31A725
Serial Number:	AA1109M002

Method: Cd aqueous (Flame)

Sample ID	Conc ppm	%RSD	Mean Abs	BG Abs
CAL ZERO	0.000	12.1	-0.0018	0.0016
	Readings			
	-0.0020	-0.0016	-0.0018	
STANDARD 1	20.000	0.2	1.6871	0.0031
	Readings			
	1.6850	1.6911	1.6851	







Str 1	0.149	4.5	0.0125	0.0057
	Readings			
	0.0130	0.0128	0.0119	
Utr_2	0.049	7.2	0.0042	0.0058
	Readings			
	0.0043	0.0043	0.0038	
Str 3	0.142	5.0	0.0120	0.0058
	Readings			
	0.0113	0.0122	0.0124	
Str_2	0.130	2.3	0.0109	0.0060
	Readings			
	0.0111	0.0110	0.0107	
utr 2	0.079	8.5	0.0067	0.0050
	Readings			

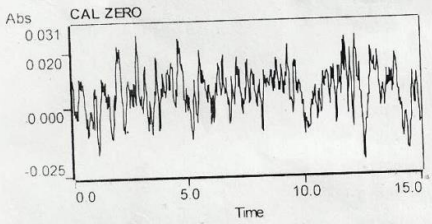
Mrs Okaro

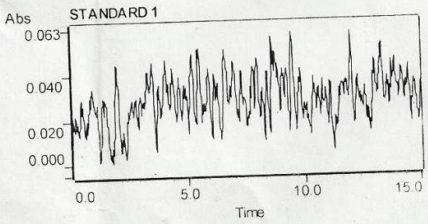
SpectrAA Report. 3:49 PM 10/30/2013

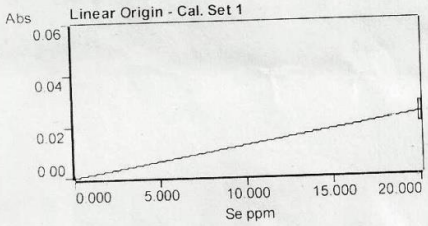
Analyst	emma
Date Started	3:38 PM 10/30/2013
Worksheet	Se test Mrs okaro 4
Comment	
Methods	Se
Computer name	USER-14FE31A725
Serial Number:	AA1109M002

Method: Se aqueous (Flame)

Sample ID	Conc ppm	%RSD	Mean Abs
CAL ZERO	0.000	26.7	0.0044
	Readings		
	0.0037	0.0058	0.0038
STANDARD 1	20.000	17.3	0.0235
	Readings		
	0.0189	0.0267	0.0250







ss01	18.333	3.4	0.0216
	Readings		
	0.0208	0.0217	0.0222
ss02	15.083	13.4	0.0177
	Readings		
	0.0193	0.0150	0.0189
ss03	17.686	12.0	0.0208
	Readings		
	0.0228	0.0217	0.0180
us01	17.640	6.4	0.0208
	Readings		
	0.0193	0.0209	0.0220
us02	16.154	7.6	0.0190
	Readings		

	0.0201	0.0196	0.0174
us03	16.188	15.8	0.0190
	Readings		
	0.0225	0.0170	0.0176
str1	14.719	6.8	0.0173
	Readings		
	0.0176	0.0160	0.0183
str2	16.857	12.3	0.0198
	Readings		
	0.0226	0.0187	0.0181
str3	13.367	11.8	0.0157
	Readings		
	0.0138	0.0160	0.0174
utr1	15.199	20.3	0.0179
	Readings		
	0.0137	0.0194	0.0205
utr2	10.289	19.7	0.0121
	Readings		
	0.0098	0.0119	0.0146
utr3	14.408	29.6	0.0170
	Readings		
	0.0155	0.0128	0.0225
cynthia	17.110	5.8	0.0201
	Readings		
	0.0204	0.0212	0.0188

SpringBoard Research Laboratories

@ Road 3, House 1, Udoka Housing Estate, Awka. Anambra State.
08100382601
046844587
08022087994
E-mail: Springboardlab@yahoo.com

* Analysis of Heavy Metal * Micronutrients * Phytochemistry
* Soil Analysis * Water Analysis * Toxicology * Consultancy

Name of Customer: Mrs. Okaroh
Sample: Mushroom
Test Required: Analysis of Metals
Date Received: 3rd Feb, 2014.

Chromium level in pass per million (ppm). Detectable level = 0.001ppm						
	1st Conc	2nd Conc	3rd Conc	Mean Conc	%RSD	
Utr1	1.049	0.650	0.020	0.567	90.2	
Utri2	0.718	0.718	0.533	0.657	17.1	
Utri3	0.775	0.398	0.880	0.671	36.5	
Stri1	0.643	1.058	1.286	0.996	32.5	
Stri2	1.046	0.669	0.690	0.795	26.5	
Stri3	0.819	0.941	0.798	0.860	9.2	
Cadmium level in pass per million (ppm). Detectable level = 0.001ppm						
	1st Conc	2nd Conc	3rd Conc	Mean Conc	%RSD	
Str1	0.154	0.152	0.141	0.149	4.5	
Utr2	0.050	0.050	0.044	0.049	7.2	
Str3	0.133	0.144	0.146	0.142	5.0	
Str2	0.132	0.131	0.127	0.130	2.3	
Utr1	0.074	0.086	0.075	0.079	8.5	
Utr3	0.054	0.053	0.065	0.058	11.7	
Zinc level in pass per million (ppm). Detectable level = 0.001ppm						
	1st Conc	2nd Conc	3rd Conc	Mean Conc	%RSD	
Stri1	0.419	0.441	0.367	0.410	9.3	
Utr1	0.102	0.039	0.060	0.068	47.7	
Str3	0.101	0.143	0.142	0.129	18.6	
Stri2	0.125	0.140	0.148	0.138	8.5	
Utri2	0.100	0.053	0.037	0.064	51.2	
Utr3	0.080	0.049	0.093	0.075	30.2	
Copper level in pass per million (ppm). Detectable level = 0.001ppm						
	1st Conc	2nd Conc	3rd Conc	Mean Conc	%RSD	
Stri1	0.115	0.115	0.130	0.121	7.4	
Utr1	0.071	0.068	0.094	0.079	18.9	
Utr2	0.126	0.126	0.106	0.119	10.6	
Str1	0.139	0.134	0.137	0.137	2.3	
Str2	0.166	0.158	0.155	0.161	3.6	
Utr3	0.135	0.105	0.110	0.118	14.3	
Manganese level in pass per million (ppm). Detectable level = 0.001ppm						
	1st Conc	2nd Conc	3rd Conc	Mean Conc	%RSD	
Str1	0.285	0.386	0.220	0.301	27.6	
Str2	0.302	0.045	0.493	0.282	80.1	
Utr1	0.162	0.182	0.00	0.074	>100	
Utr3	0.345	0.213	0.162	0.239	39.1	
Utr2	0.076	0.275	0.250	0.199	53.1	
Utr3	0.253	0.396	0.126	0.259	51.7	

Lead level in pass per million (ppm). Detectable level = 0.001ppm						
	1st Conc	2nd Conc	3rd Conc	Mean Conc	%RSD	
Utr1	0.00	0.068	0.090	0.053	>100	
Str2	0.086	0.007	0.086	0.063	73.8	
Utr2	0.00	0.090	0.065	0.041	>100	
Str1	0.071	0.039	0.094	0.071	36.3	
Str3	0.033	0.092	0.059	0.059	43.6	
Utr3	0.00	0.00	0.00	0.00	47.3	
Cobalt level in pass per million (ppm). Detectable level = 0.001ppm						
	1st Conc	2nd Conc	3rd Conc	Mean Conc	%RSD	
Utr2	0.00	0.00	0.00	0.00	>100	
Str2	0.976	1.350	0.699	1.013	32.6	
Utr1	0.00	0.182	0.365	0.146	>100	
Str3	0.349	0.623	0.635	0.540	30.2	
Str1	1.341	1.087	1.329	1.257	11.6	
Utr3	0.170	0.450	0.960	0.523	76.3	
Nickel level in pass per million (ppm). Detectable level = 0.001ppm						
	1st Conc	2nd Conc	3rd Conc	Mean Conc	%RSD	
Str1	0.00	0.00	0.212	0.029	>100	
Str2	0.147	0.00	0.183	0.049	>100	
Utr1	0.00	0.00	0.00	0.00	57.2	
Str3	0.072	0.00	0.00	0.009	>100	
Utr2	0.00	0.00	0.00	0.00	>100	
Utr3	0.00	0.00	0.00	0.00	>100	

Okeke David Okechukwu

Tested by

ANTI- NUTRIENT FACTORS DETERMINATION

PHYTIC ACID

APPARATUS

Volumetric flasks (1000 mL, 500 mL, 250 mL, 100 mL, 50 mL)

Beakers: Pipettes (1 mL, 5 mL, 10 mL, 2mL)

Glass stoppered bottles

Flask shaker

Kjeldahl digestion flasks (30 mL capacity)

Reagents

1. 0.05 N HCl, 5 mL Concentrated HCl diluted to 100 mL with distilled water, 0.17 N HCl; 1.0 N HCl
2. Concentrated H_2SO_4
3. 2 % (W/V) NaOH; 2 g of NaOH was made up to 100 mL with distilled water
4. 40 % (W/V) NaOH; 40 g of NaOH was made up to 100 mL with distilled water
5. 65 % of perchloric acid $HC1O_4$
6. Ferric chloride acid solution containing 0.5 mg of ferric ions per mL: - 2.4134 g of $FeCl_3$ were dissolved in 1000 mL of 1N HCl.
7. Standard phosphate solution containing 0.2 mg/L of phosphorus. 0.879 g of potassium dihydrogen phosphate was dissolved in distilled water and made up to 1000 mL
8. Molybdate solution: To prepare this, 25 g of ammonium molybdate were dissolved in 300 mL of the distilled water. This solution was added to 200 mL of water containing 75 ml of Conc. H_2SO_4

9. Hydroquinone solution: To prepare this, 0.5 g of hydroquinone was dissolved in 100ml of distilled water and a drop of concentrated H_2SO_4 was added to retard oxidation. 10.20 % (W/V) sodium sulphite solution: 20 g of anhydrous sodium sulphite were weighed and dissolved in 100 mL of distilled water. The solution was prepared before use.

APPLICATION

1. 10 g of sample was shaken in 150 mL conical flask with 100mL of 0.5N HCl for 2 hours to extract the phytic acid.
2. 30 mL of the diltered extract were neutralized with NaOH and then rendered slightly acidic with HCl and made up to 50 mL.
3. Duplicate 20 mL aliquots were treated in 50 mL centrifuge tubes, with 4 mL $FeCl_2$ solution.
4. The tubes were heated in a boiling water bath for 20 minutes to flocculate the precipitate of ferric phytate, cooled, centrifuged and the supernatant liquid poured off. The precipitate was washed with 5 mL N HCl, centrifuged again and the acid decanted.
5. The precipitate was then stirred up with 2 mL distilled water and heated in a boiling water bath for a few minutes. 2 mL of 2 % NaOH were then added and the heating continued for a further 15 minutes.
6. The solution containing the phytin as sodium phytate, was filtered into a kjeldahl flask, the precipitate ferric hydroxide was well washed with hot distilled water and the washings were added to the filtrate in the flask.
7. 1.0ml of conc. H_2SO_4 and 1.0 mL of 65 % $HClO_4$ were added to the filtrate and the mixture was digested slowly and gently until digestion was complete.

The digest was further heated strongly for 1 hour to remove any residual $HClO_4$.

8. When cooled, about 20 mL of distilled water were added and the contents of the flask neutralized to phenolphalein with 40 % Na0H and made up to 100 mL.
9. An aliquot of 5 mL was taken and the volume made up of 10 mL in each case. A blank containing 1 mL concentrated H_2SO_4 almost neutralized with 40 % NaOH and made up to 100 mL was used in the dilutions.
10. Standard solutions containing 0.025, 0.05, 0.10 and 0.20 mg of p were prepared by diluting 0.25, 0.5, 1.0 and 2.0 mL of the standard solution (containing 0.1 mg/L) to 10 mL with the blank solution.
11. 5 mL of the diluted aliquot were pipetted into a test tube, 2 mL of molybdate solution, 1 mL Na_2SO_3 solution and 1 mL hydroquinone solution were added and the solution diluted to 10 mL with distilled water.
12. It was allowed to stand for 30 minutes for the development of blue colour, and the absorbance read at 620 nm using the corning colorimeter.
13. Triplicate determinations were made for each sample

HYDROCYANIC ACID (A0AC, 2005)

Reagents

1. 0.02 N $AgNO_3$: 1.699 g of $AgNO_3$ was dissolved in 500 mL of distilled water.
2. 2.5 % (W/V) sodium hydroxide solution.
3. 5 % (W/V) Potassium iodide
4. 6 N Ammonium hydroxide: 99.3 mL concentrated NH_4OH were made up to 25 mL with distilled water in a volumetric flask.

TANNINS (Burns, 1971)

Reagents

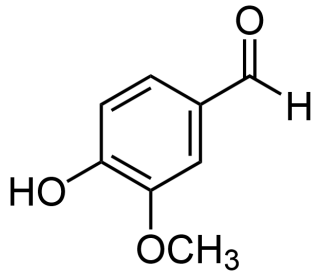
(a) 8 % (W/V) HCl in methanol.

(b) 4 % (W/V) vanillin in absolute methanol. Dissolve 4.0 g vanillin in absolute methanol and make up to 100 mL.

(c) Methanol (analar) CH_3OH

(d) Standard solution of catechin in methanol.

(e) Vanillin ï $C_8H_8O_3$



Structure of Vanillin

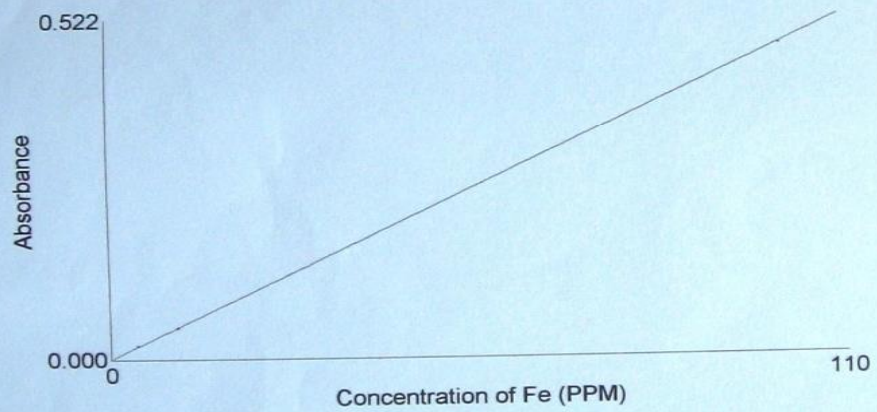
Equal volumes of 8 % (W/V) alkali and 4 % vanillin in methanol were mixed together. The reagent was prepared freshly before use.

APPENDIX V

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Fe.250311.res
Analysis Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Fe, Food Spices
Date	Fri Mar 25 13:13:19 2011
Full Calibration Calibration Mode	Linear LS Through Zero Max Error : 0.3752 R^2 : 1.0000 R : 1.00 Conc = 209.8690 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	-0.0011
Standard 1	4.000	-----	0.0205
Standard 2	10.000	-----	0.0480
Standard 3	100.000*	-----	0.4747

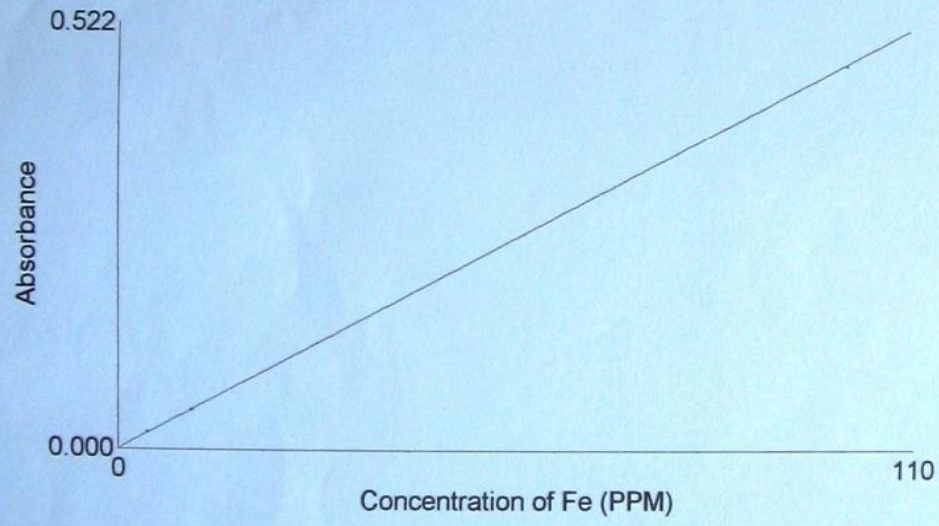


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	11.912	-----	0.0568
APhW2	34.247	-----	0.1632
ABWC3	27.747	-----	0.1322
AVW4	40.970	-----	0.1952
PTRWn5	9.319	-----	0.0444
ABWn6	2.560	-----	0.0122
PTRWn7	4.837	-----	0.0230

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Fe.250311.res
Analysis	
Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Fe, Food Spices
Date	Fri Mar 25 13:13:19 2011
Full Calibration	
Calibration Mode	Linear LS Through Zero Max Error : 0.3752 R^2 : 1.0000 R : 1.00
	Conc = 209.8690 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	-0.0011
Standard 1	4.000	-----	0.0205
Standard 2	10.000	-----	0.0480
Standard 3	100.000*	-----	0.4747

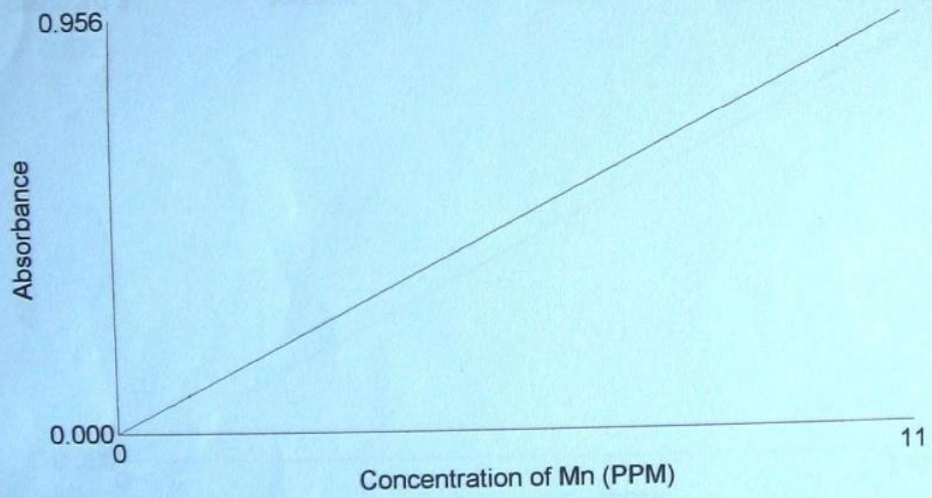


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	11.912	-----	0.0568
APhW2	34.247	-----	0.1632
ABWC3	27.747	-----	0.1322
AVW4	40.970	-----	0.1952
PTRWn5	9.319	-----	0.0444
ABWn6	2.560	-----	0.0122
PTRWn7	4.837	-----	0.0230

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Mn.250311.res
Analysis Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Mn, Food Spices
Date	Fri Mar 25 13:18:06 2011
Full Calibration	
Calibration Mode	Linear LS Through Zero Max Error : 0.0013 R^2 : 1.0000 R : 1.00
	Conc = 11.5086 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	-0.0016
Standard 1	1.000*	-----	0.0870
Standard 2	10.000	-----	0.8688

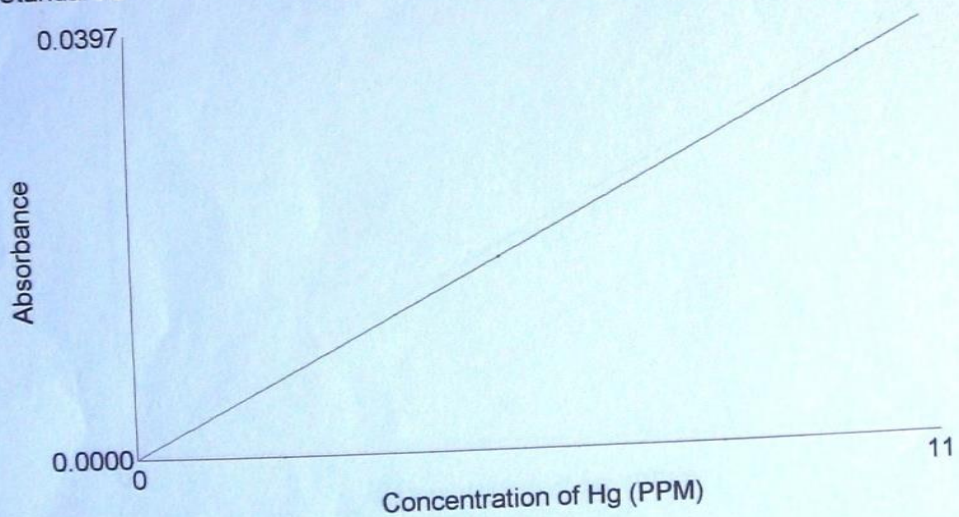


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	1.198	-----	0.1041
APhW2	0.840	-----	0.0730
ABWC3	0.723	-----	0.0628
AVW4	0.618	-----	0.0537
PTRWn5	0.885	-----	0.0769
ABWn6	0.711	-----	0.0618
PTRWn7	0.680	-----	0.0590
PTWn8	0.755	-----	0.0656

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Hg.250311.res
Analysis Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Hg, Food Spices
Date	Fri Mar 25 12:31:41 2011
Full Calibration Calibration Mode	Linear LS Through Zero Max Error : 0.0045 R^2 : 1.0000 R : Conc = 277.5294 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	0.0026
Standard 1	5.000*	-----	0.0180
Standard 2	10.000	-----	0.0360

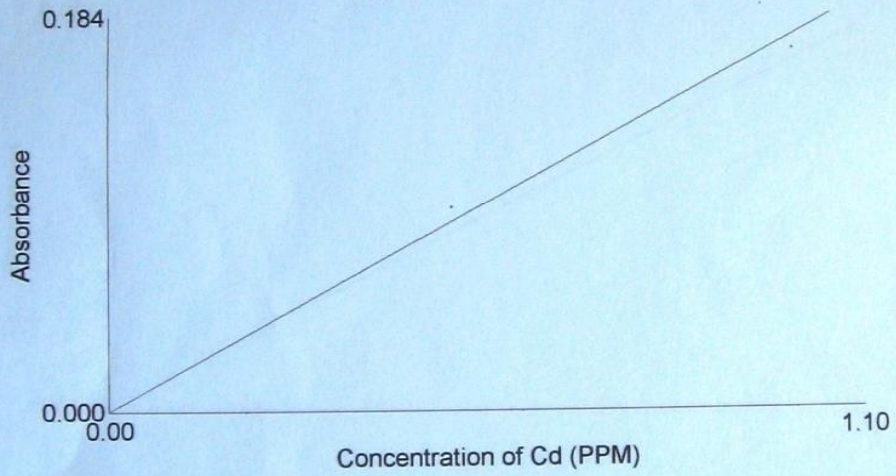


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	ND	-----	-0.1008
APhW2	ND	-----	-0.1253
ABWC3	ND	-----	-0.1179
AVW4	ND	-----	-0.0705
PTRWn5	ND	-----	-0.0025
ABWn6	ND	-----	-0.0028
PTRWn7	ND	-----	-0.0034
PTWn8	ND	-----	-0.0079

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Cd.250311.res
Analysis Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Cd, Food Spices
Date	Fri Mar 25 12:47:54 2011
Full Calibration Calibration Mode	Linear LS Through Zero Max Error : 0.0381 R^2 : 1.0000 R : 1.00 Conc = 5.7459 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	0.0022
Standard 1	0.500	-----	0.0937
Standard 2	1.000	-----	0.1674

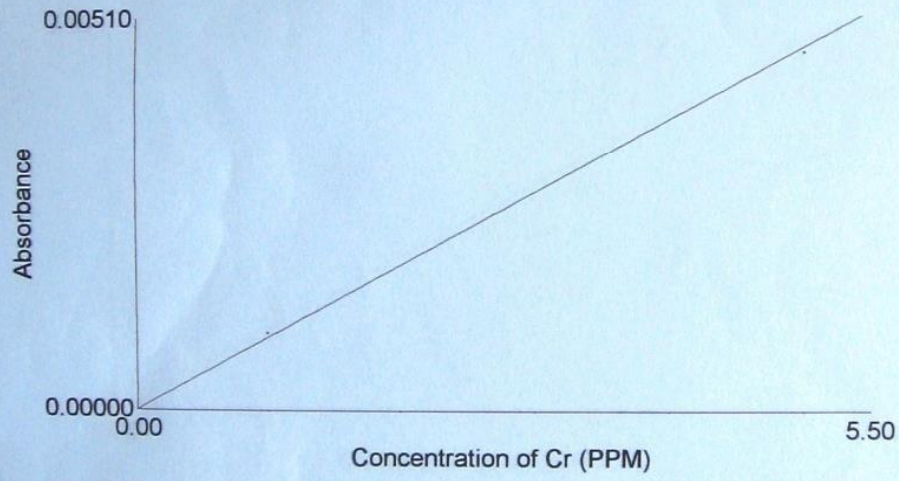


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	0.224	-----	0.0389
APhW2	0.194	-----	0.0338
ABWC3	0.201	-----	0.0350
AVW4	0.233	-----	0.0405
PTRWn5	0.256	-----	0.0446
ABWn6	0.224	-----	0.0390
PTRWn7	0.215	-----	0.0374
PTWn8	0.207	-----	0.0360

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Cr.250311.res
Analysis Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Cr, Food Spices
Date	Fri Mar 25 12:55:41 2011
Full Calibration Calibration Mode	Linear LS Through Zero Max Error : 0.0644 R^2 : 1.0000 R : 1.00 Conc = 1064.3540 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	0.0002
Standard 1	1.000*	-----	0.0010
Standard 2	5.000	-----	0.0046

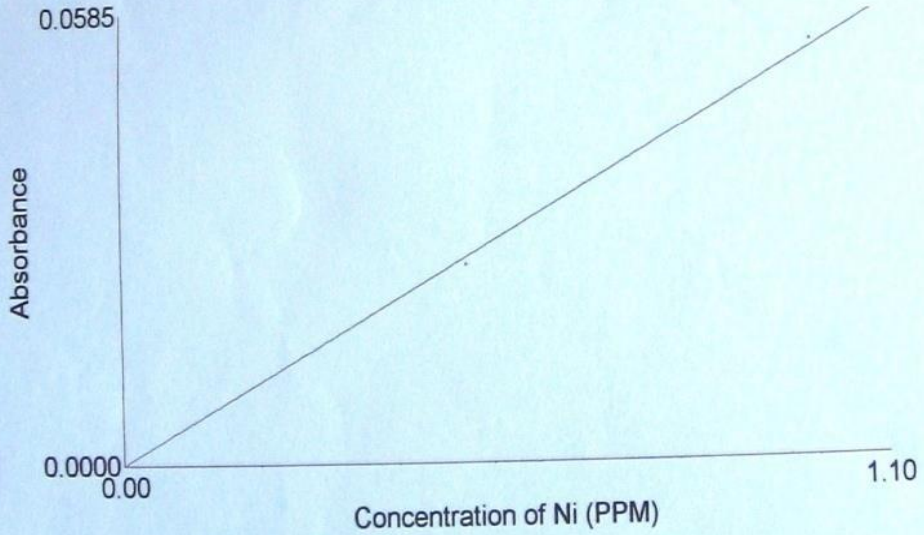


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	ND	-----	-0.0013
APhW2	ND	-----	-0.0013
ABWC3	ND	-----	-0.0006
AVW4	ND	-----	-0.0018
PTRWn5	ND	-----	-0.0019
ABWn6	ND	-----	-0.0004
PTRWn7	ND	-----	-0.0009
PTWn8	ND	-----	-0.0024

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Ni.250311.res
Analysis Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Ni, Food Spices
Date	Fri Mar 25 12:34:59 2011
Full Calibration	
Calibration Mode	Linear LS Through Zero Max Error : 0.0177 R^2 : 1.0000 R : 1.0(
	Conc = 19.1314 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	0.0012
Standard 1	0.500	-----	0.0252
Standard 2	1.000	-----	0.0532

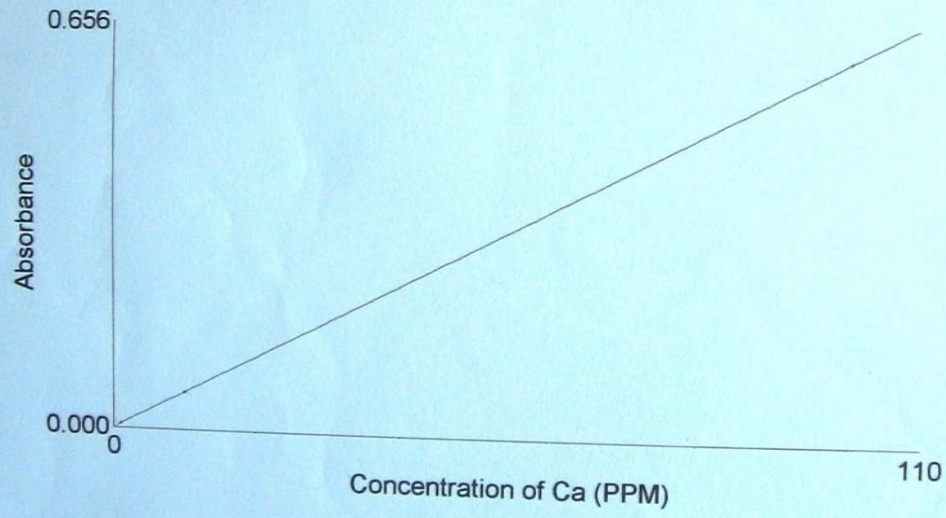


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	0.780	-----	0.0408
APhW2	0.332	-----	0.0173
ABWC3	0.112	-----	0.0058
AVW4	0.229	-----	0.0120
PTRWn5	-0.007	-----	-0.0004
	0.000*	-----	0.0000
ABWn6	0.055	-----	0.0029
PTRWn7	0.152	-----	0.0079

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Ca.250311.res
Analysis Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Ca, Food Spices
Date	Fri Mar 25 13:22:42 2011
Full Calibration	
Calibration Mode	Linear LS Through Zero Max Error : 0.0821 R^2 : 1.0000
	Conc = 167.6656 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	-0.0018
Standard 1	1.000*	-----	0.0059
Standard 2	10.000	-----	0.0601
Standard 3	100.000*	-----	0.5960

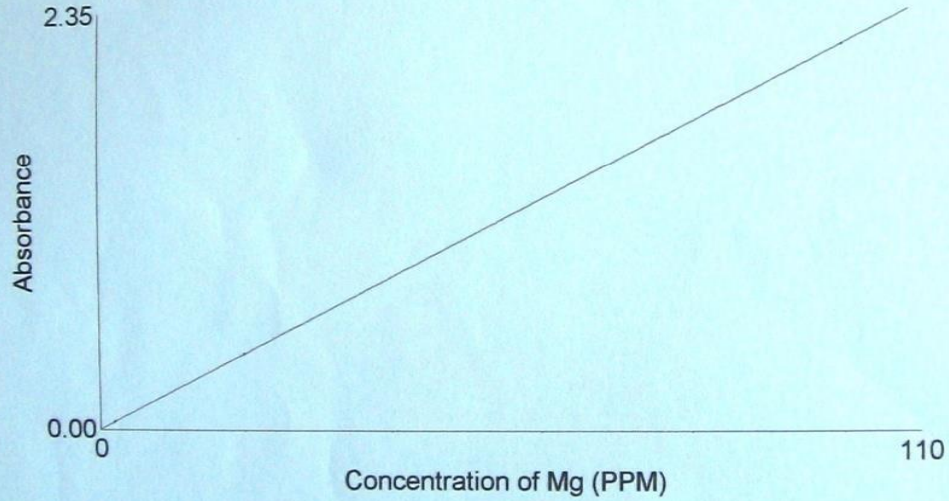


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	3.009	-----	0.0179
APhW2	1.364	-----	0.0081
ABWC3	6.344	-----	0.0378
AVW4	0.318	-----	0.0019
PTRWn5	8.469	-----	0.0505
ABWn6	29.027	-----	0.1731
PTRWn7	1.236	-----	0.0074

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Mg.250311.res
Analysis	
Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Mg, Food Spices
Date	Fri Mar 25 13:27:34 2011
Full Calibration	
Calibration Mode	Linear LS Through Zero Max Error : 0.0160 R^2 : 1.0000 R :
	Conc = 46.8371 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	-0.0042
Standard 1	2.000*	-----	0.0429
Standard 2	20.000	-----	0.4272
Standard 3	100.000*	-----	2.1347

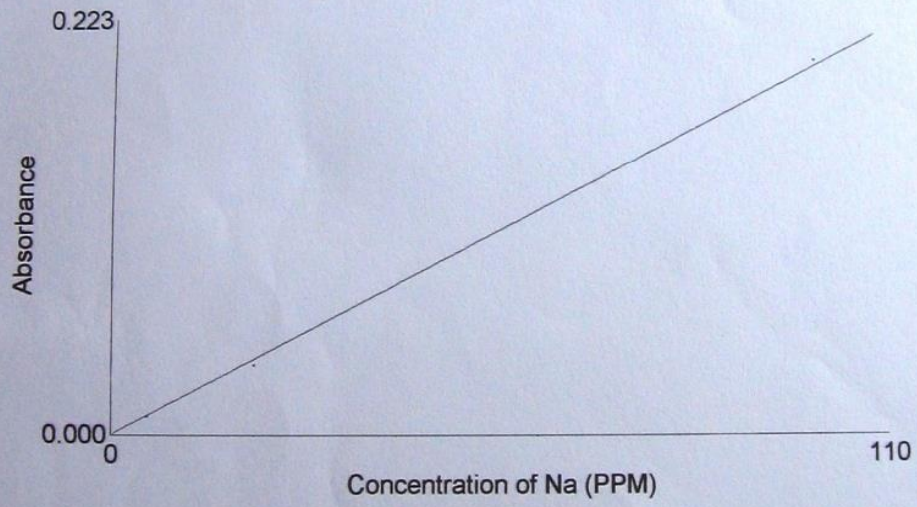


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	39.646	-----	0.8465
APhW2	47.203	-----	1.0078
	0.000*	-----	0.0000
ABWC3	45.029	-----	0.9614
AVW4	11.011	-----	0.2351
	0.000*	-----	0.0000
PTRWn5	59.550	-----	1.2714

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Na.250311.res
Analysis	
Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Na, Food Spices
Date	Fri Mar 25 13:32:36 2011
Full Calibration	
Calibration Mode	Linear LS Through Zero Max Error : 1.7281 R^2 : 0.9997 R : 0.9
	Conc = 500.7766 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	0.0032
Standard 1	5.000*	-----	0.0096
Standard 2	20.000	-----	0.0369
Standard 3	100.000	-----	0.2031

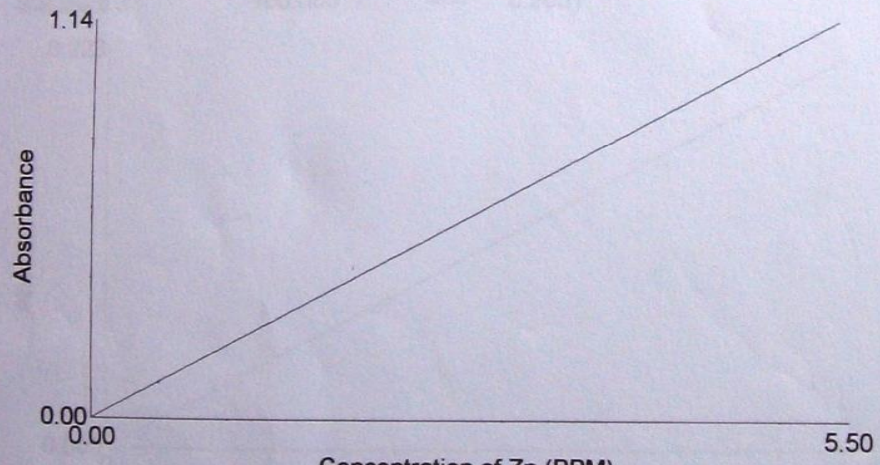


Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	19.695	-----	0.0393
APhW2	13.981	-----	0.0279
ABWC3	15.201	-----	0.0304
AVW4	40.247	-----	0.0804
PTRWn5	38.431	-----	0.0767
ABWn6	5.364	-----	0.0107
PTRWn7	7.618	-----	0.0152

ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File	C:\GENERAL\Zn.250311.res
Analysis Filename	C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element	Zn, Food Spices
Date	Fri Mar 25 13:04:29 2011
Full Calibration Calibration Mode	Linear LS Through Zero Max Error : 0.0046 R^2 : 1.0000 R : 1.00 Conc = 4.8135 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	-0.0054
Standard 1	0.500	-----	0.1029
Standard 2	5.000*	-----	1.0397



Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	2.543	-----	0.5283
APhW2	1.746	-----	0.3628
ABWC3	1.336	-----	0.2776
AVW4	2.416	-----	0.5019
PTRWn5	2.179	-----	0.4527
ABWn6	0.892	-----	0.1853
PTRWn7	1.253	-----	0.2602
PTWn8	2.081	-----	0.4324

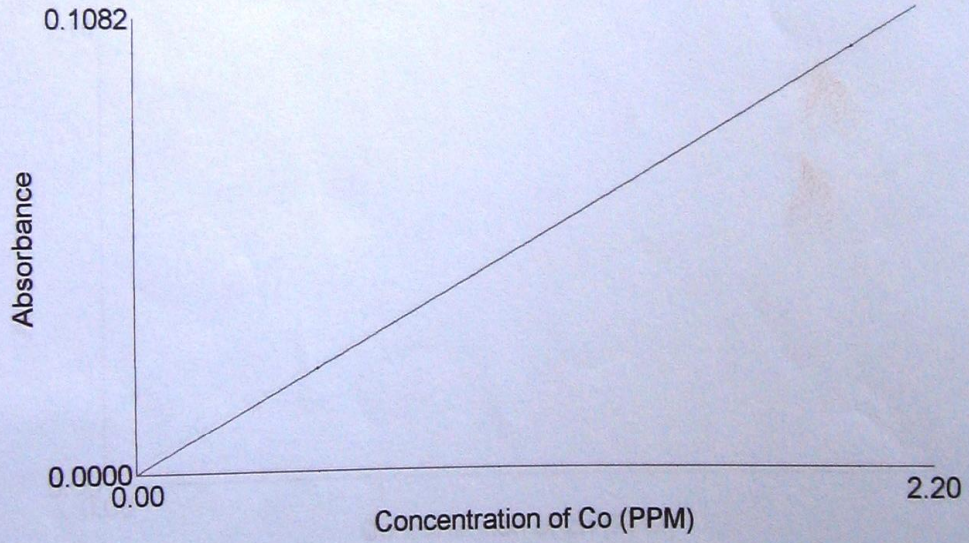
ANAL CONCEPT LTD, 18 OROIGWE RD,OROIGWE,P/H.

Results File C:\GENERAL\Co.250311.res

Analysis Filename C:\Program Files\GBC Avanta Ver 2.02\Analysis1.anl
Element Co, Food Spices
Date Fri Mar 25 13:00:04 2011

Full Calibration
Calibration Mode Linear LS Through Zero Max Error : 0.0009 R^2 : 1.0000 R
Conc = 20.3199 * Abs

Sample Label	Conc. (PPM)	%RSD	Mean Abs.
Cal Blank	-----	-----	-0.0010
Standard 1	0.500*	-----	0.0247
Standard 2	2.000	-----	0.0984



Sample Label	Conc. (PPM)	%RSD	Mean Abs.
TrWn1	0.020*	-----	0.0010
APhW2	0.076	-----	0.0037
ABWC3	0.087	-----	0.0043
AVW4	0.037	-----	0.0018
PTRWn5	0.025	-----	0.0012
ABWn6	0.044	-----	0.0022
PTRWn7	0.024	-----	0.0012
PTWn8	0.022	-----	0.0011

ANAL CONCEPT LTD. RC. 306699
Environmental/Analytical Services Consultants
HEAD OFFICE: Flat 6, Royal Estate Plot 18, Oroigwe Road by Pipeline, Elimbgu, Port Harcourt. E-mail: analconcepts@yahoo.com
POSTAL ADDRESS: P.O.Box:398, Woji Obio / Akpor L.G.A. Port Harcourt. GSM: 08033090265, 08023290108, 08032765181

10th May, 2011

ATTN: Mrs. Okarah Bridget

Nnewi Technology Incubation Center.

TABLE OF RESULT

S/N	Parameter (s)	TrWn 1	APhW2	ABWC 3	AvW 4	PTRWn 5	ABWn6	PTRWn7	PTWn 8
1.	Ca (mg/kg)	60.18	27.28	126.88	6.36	169.38	580.54	24.72	399.76
2.	Co (mg/kg)	0.40	1.52	1.74	0.74	0.50	0.88	0.48	0.44
3.	Pb (mg/kg)	5.58	4.62	6.94	5.46	0.38	0.72	3.60	1.70
4.	Mg (mg/kg)	792.92	944.06	90.58	220.22	1,191.00	1,033.68	999.60	1,183.96
5.	Zn (mg/kg)	50.86	34.92	26.72	48.32	43.58	17.84	25.06	41.62
6.	Cr (mg/kg)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
7.	Fe (mg/kg)	238.24	684.94	554.94	819.40	186.38	51.20	96.74	154.68
8.	Cd (mg/kg)	4.48	3.88	4.02	4.66	5.12	4.48	4.30	4.14
9.	Se (mg/kg)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10.	Ni (mg/kg)	15.60	6.64	2.24	4.58	1.10	<0.001	3.04	1.10
11.	Hg (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
12.	Na (mg/kg)	393.90	279.62	304.02	804.94	768.62	107.28	152.36	777.42
13.	Mn (mg/kg)	23.96	16.80	14.46	12.36	17.70	14.22	13.60	15.10

NOTE: **BDL- BELOW DETECTION LIMIT**

Aigberua Ayobami
For: **ANAL CONCEPT LTD**

ANAL CONCEPT LTD. DATE: 10/05/11 SIGN:

APPENDIX VI

STATISTICAL DATA OF PROXIMATE RESULTS OF WILD MUSHROOM SAMPLES

Descriptives

		N	Mean	Standard Deviation	Standard Error	95 % Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Moisture	TR1	5	87.2000	3.70135	1.65529	82.6042	91.7958	82.00	91.00
	TR II	5	90.7800	4.17337	1.86639	85.5981	95.9619	84.00	94.50
	AB 2	5	71.4000	8.33097	3.72572	61.0557	81.7443	60.10	80.40
	AB3	5	78.3800	5.10363	2.28241	72.0430	84.7170	72.70	86.00
	AB7	5	74.2000	5.63028	2.51794	67.2091	81.1909	65.00	80.00
	AB9	5	80.2200	11.76741	5.26255	65.6088	94.8312	60.60	90.40
	PTR 6	5	66.8000	14.44645	6.46065	48.8624	84.7376	50.00	89.00
	AV 4	5	91.9000	6.86404	3.06969	83.3772	100.4228	80.00	97.10
	APH 5	5	81.6000	2.07364	.92736	79.0252	84.1748	80.00	85.00
	AV8	5	75.4000	4.21900	1.88680	70.1614	80.6386	70.00	80.00
	AV10	5	76.0340	3.11059	1.39110	72.1717	79.8963	72.00	80.00
	Total	55	79.4467	10.08170	1.35942	76.7213	82.1722	50.00	97.10
Drymatter	TR1	5	15.3600	16.58578	7.41738	-5.2340	35.9540	7.00	45.00
	TR II	4	7.5250	2.01722	1.00861	4.3152	10.7348	5.50	9.50
	AB 2	5	5.1800	1.60062	.71582	3.1926	7.1674	3.40	7.60
	AB3	5	6.7200	1.58019	.70668	4.7579	8.6821	4.90	8.60
	AB7	4	8.0750	1.15866	.57933	6.2313	9.9187	6.40	8.90
	AB9	5	7.5400	1.84472	.82498	5.2495	9.8305	5.50	10.50
	PTR 6	5	5.0200	1.99299	.89129	2.5454	7.4946	2.80	8.20
	AV 4	5	8.2400	1.14586	.51245	6.8172	9.6628	6.50	9.50
	APH 5	5	7.1800	.68337	.30561	6.3315	8.0285	6.40	7.90
	AV8	5	8.3040	1.65924	.74204	6.2438	10.3642	6.80	10.90
	AV10	5	8.1720	1.33386	.59652	6.5158	9.8282	6.85	10.11
	Total	53	7.9430	5.47871	.75256	6.4329	9.4531	2.80	45.00
Protein	**TR1**	**4**	**21.2250**	**10.87731**	**5.43865**	**3.9168**	**38.5332**	**10.70**	**34.10**
	TR II	4	20.9750	8.98939	4.49470	6.6709	35.2791	13.10	31.50

	AB 2	5	15.1200	2.79321	1.24916	11.6518	18.5882	11.10	18.10
	AB3	5	21.2000	2.77399	1.24056	17.7556	24.6444	18.70	25.40
	AB7	4	24.3750	5.12144	2.56072	16.2256	32.5244	18.50	30.00
	AB9	5	20.4200	4.00400	1.79064	15.4484	25.3916	15.10	26.00
	PTR 6	4	14.6000	1.08012	.54006	12.8813	16.3187	13.10	15.60
	AV 4	4	6.6000	1.34907	.67454	4.4533	8.7467	5.50	8.50
	APH 5	4	12.4250	1.13248	.56624	10.6230	14.2270	11.00	13.60
	AV8	4	11.9875	4.12803	2.06401	5.4189	18.5561	8.70	18.00
	AV1 0	4	11.8200	3.13756	1.56878	6.8274	16.8126	8.70	15.90
	Total	47	16.5900	6.83898	.99757	14.5820	18.5980	5.50	34.10
Lipid	TR1	4	5.0000	2.03142	1.01571	1.7676	8.2324	2.80	7.10
	TR II	4	5.9000	2.25536	1.12768	2.3112	9.4888	3.80	8.90
	AB 2	5	3.3000	2.21359	.98995	.5515	6.0485	.00	5.60
	AB3	5	5.8400	4.14825	1.85515	.6893	10.9907	1.40	10.60
	AB7	4	4.0250	1.44309	.72154	1.7287	6.3213	2.10	5.40
	AB9	5	1.8200	1.66493	.74458	-.2473	3.8873	.00	4.40
	PTR 6	4	.0500	.10000	.05000	-.1091	.2091	.00	.20
	AV 4	4	1.5250	1.28420	.64210	-.5184	3.5684	.00	2.90
	APH 5	4	.7200	.87955	.43977	-.6796	2.1196	.00	1.90
	AV8	4	1.4500	.71414	.35707	.3136	2.5864	.90	2.50
	AV1 0	4	1.5125	.90035	.45017	.0798	2.9452	.40	2.50
	Total	47	2.8836	2.67183	.38973	2.0991	3.6681	.00	10.60
Ash	TR1	4	10.7250	5.03082	2.51541	2.7198	18.7302	5.20	17.30
	TR II	4	12.1500	4.21149	2.10575	5.4486	18.8514	9.30	18.40
	AB 2	5	10.1800	4.21865	1.88664	4.9419	15.4181	6.80	17.00
	AB3	5	7.7600	4.00849	1.79265	2.7828	12.7372	3.50	14.10
	AB7	4	12.0500	3.44141	1.72071	6.5739	17.5261	9.40	17.10
	AB9	4	12.2250	2.49983	1.24992	8.2472	16.2028	9.80	15.10
	PTR 6	4	12.4050	1.78963	.89481	9.5573	15.2527	10.80	14.60
	AV 4	4	4.6250	.84212	.42106	3.2850	5.9650	3.80	5.80
	APH 5	4	4.0250	1.35493	.67746	1.8690	6.1810	2.50	5.40
	AV8	4	18.5000	2.72029	1.36015	14.1714	22.8286	15.80	22.20

	AV10	4	17.2750	3.61236	1.80618	11.5269	23.0231	13.70	22.00
	Total	46	10.9917	5.20017	.76672	9.4475	12.5360	2.50	22.20
Fibre	TR1	4	10.4500	4.66083	2.33041	3.0336	17.8664	4.20	14.30
	TR II	4	9.1000	1.70685	.85342	6.3840	11.8160	7.20	11.10
	AB 2	5	9.6600	1.99575	.89252	7.1820	12.1380	8.10	13.10
	AB3	5	8.0600	1.26610	.56622	6.4879	9.6321	6.10	9.30
	AB7	4	9.6750	1.67207	.83604	7.0144	12.3356	7.60	11.50
	AB9	4	10.1500	1.07548	.53774	8.4387	11.8613	8.80	11.20
	PTR6	4	9.7250	1.41745	.70873	7.4695	11.9805	7.70	10.80
	AV 4	4	3.8250	2.62599	1.31300	-.3535	8.0035	2.20	7.70
	APH5	4	2.9500	.69522	.34761	1.8437	4.0563	2.00	3.60
	AV8	4	16.5250	2.00728	1.00364	13.3310	19.7190	14.10	18.50
	AV10	4	13.7000	3.73898	1.86949	7.7504	19.6496	8.50	17.00
	Total	46	9.4130	4.15027	.61192	8.1806	10.6455	2.00	18.50
Ethanol soluble sugar	TR1	4	10.2500	3.60971	1.80485	4.5061	15.9939	7.60	15.40
	TR II	4	9.5750	2.93641	1.46820	4.9025	14.2475	7.60	13.90
	AB 2	4	10.6750	3.11595	1.55798	5.7168	15.6332	7.80	15.10
	AB3	4	9.3500	3.31210	1.65605	4.0797	14.6203	4.40	11.40
	AB7	4	9.2000	2.87750	1.43875	4.6213	13.7787	6.50	13.10
	AB9	4	10.8000	3.18538	1.59269	5.7313	15.8687	7.90	15.30
	PTR6	4	4.0500	1.51767	.75884	1.6350	6.4650	2.50	6.10
	AV 4	4	9.4500	2.30145	1.15072	5.7879	13.1121	6.20	11.50
	APH5	4	7.4000	2.83314	1.41657	2.8918	11.9082	4.40	10.20
	AV8	4	7.4250	2.16545	1.08272	3.9793	10.8707	5.50	10.50
	AV10	4	8.2000	2.02320	1.01160	4.9806	11.4194	5.70	10.50
	Total	44	8.7614	3.07633	.46377	7.8261	9.6967	2.50	15.40
Vit. C	TR1	4	.6725	.18392	.09196	.3798	.9652	.44	.86
	TR II	4	.1750	.11121	.05560	-.0020	.3520	.04	.30
	AB 2	4	.3850	.40927	.20463	-.2662	1.0362	.01	.81
	AB3	4	.1850	.27934	.13967	-.2595	.6295	.01	.60
	AB7	4	.3050	.38897	.19449	-.3139	.9239	.04	.88
	AB9	4	.4200	.19647	.09823	.1074	.7326	.20	.65

	PTR 6	4	.2050	.12152	.06076	.0116	.3984	.10	.32
	AV 4	4	.0500	.05774	.02887	-.0419	.1419	.00	.10
	APH 5	4	.3000	.25820	.12910	-.1109	.7109	.00	.60
	AV8	4	.2850	.22708	.11354	-.0763	.6463	.10	.60
	AV1 0	4	.2400	.14765	.07382	.0051	.4749	.10	.40
	Total	44	.2930	.26306	.03966	.2130	.3729	.00	.88

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Moisture	Between Groups	3151.266	10	315.127	5.932	.000
	Within Groups	2337.327	44	53.121		
	Total	5488.593	54			
Dry matter	Between Groups	369.275	10	36.927	1.302	.261
	Within Groups	1191.572	42	28.371		
	Total	1560.847	52			
Protein	Between Groups	1255.854	10	125.585	5.048	.000
	Within Groups	895.641	36	24.879		
	Total	2151.494	46			
Lipid	Between Groups	183.713	10	18.371	4.572	.000
	Within Groups	144.668	36	4.019		
	Total	328.380	46			
Ash	Between Groups	819.415	10	81.942	7.216	.000
	Within Groups	397.466	35	11.356		
	Total	1216.881	45			
Fibre	Between Groups	584.808	10	58.481	10.756	.000
	Within Groups	190.304	35	5.437		
	Total	775.112	45			
Ethanol soluble sugar	Between Groups	151.442	10	15.144	1.956	.072
	Within Groups	255.503	33	7.743		
	Total	406.944	43			
Vit. C	Between Groups	1.056	10	.106	1.816	.096
	Within Groups	1.919	33	.058		
	Total	2.976	43			

POST HOC TESTS
HOMOGENEOUS SUBSET
MOISTURE

Duncan

Species	N	Subset for alpha = 0.05				
		1	2	3	4	5
PTR 6	5	66.8000				
AB 2	5	71.4000	71.4000			
AB7	5	74.2000	74.2000			
AV8	5	75.4000	75.4000			
AV10	5	76.0340	76.0340			
AB3	5		78.3800	78.3800		
AB9	5		80.2200	80.2200		
APH 5	5		81.6000	81.6000	81.6000	
TR1	5			87.2000	87.2000	87.2000
TR II	5				90.7800	90.7800
AV 4	5					91.9000
Sig.		.079	.060	.086	.065	.343

Means for groups in homogeneous subsets are displayed.

Dry matter

Duncan

Species	N	Subset for alpha = 0.05	
		1	2
PTR 6	5	5.0200	
AB 2	5	5.1800	
AB3	5	6.7200	
APH 5	5	7.1800	
TR II	4	7.5250	7.5250
AB9	5	7.5400	7.5400
AB7	4	8.0750	8.0750
AV10	5	8.1720	8.1720
AV 4	5	8.2400	8.2400
AV8	5	8.3040	8.3040
TR1	5		15.3600
Sig.		.427	.053

Means for groups in homogeneous subsets are displayed.

Protein

Duncan

Species	N	Subset for alpha = 0.05			
		1	2	3	4
AV 4	4	6.6000			
AV10	4	11.8200	11.8200		
AV8	4	11.9875	11.9875		
APH 5	4	12.4250	12.4250		
PTR 6	4		14.6000	14.6000	
AB 2	5		15.1200	15.1200	
AB9	5			20.4200	20.4200
TR II	4			20.9750	20.9750
AB3	5			21.2000	21.2000
TR1	4			21.2250	21.2250
AB7	4				24.3750
Sig.		.129	.400	.097	.313

Means for groups in homogeneous subsets are displayed.

Lipid

Duncan

Species	N	Subset for alpha = 0.05			
		1	2	3	4
PTR 6	4	.0500			
APH 5	4	.7200	.7200		
AV8	4	1.4500	1.4500	1.4500	
AV10	4	1.5125	1.5125	1.5125	
AV 4	4	1.5250	1.5250	1.5250	
AB9	5	1.8200	1.8200	1.8200	
AB 2	5		3.3000	3.3000	3.3000
AB7	4			4.0250	4.0250
TR1	4				5.0000
AB3	5				5.8400
TR II	4				5.9000
Sig.		.269	.108	.109	.100

Means for groups in homogeneous subsets are displayed.

Ash

Duncan

Species	N	Subset for alpha = 0.05			
		1	2	3	4
APH 5	4	4.0250			
AV 4	4	4.6250			
AB3	5	7.7600	7.7600		
AB 2	5		10.1800		
TR1	4		10.7250		
AB7	4		12.0500	12.0500	
TR II	4		12.1500	12.1500	
AB9	4		12.2250	12.2250	
PTR 6	4		12.4050	12.4050	
AV10	4			17.2750	17.2750
AV8	4				18.5000
Sig.		.140	.092	.052	.604

Means for groups in homogeneous subsets are displayed.

Fibre

Duncan

Species	N	Subset for alpha = 0.05			
		1	2	3	4
APH 5	4	2.9500			
AV 4	4	3.8250			
AB3	5		8.0600		
TR II	4		9.1000		
AB 2	5		9.6600		
AB7	4		9.6750		
PTR 6	4		9.7250		
AB9	4		10.1500		
TR1	4		10.4500	10.4500	
AV10	4			13.7000	13.7000
AV8	4				16.5250
Sig.		.592	.210	.052	.090

Means for groups in homogeneous subsets are displayed.

Ethanol soluble sugar

Duncan

Species	N	Subset for alpha = 0.05	
		1	2
PTR 6	4	4.0500	
APH 5	4	7.4000	7.4000
AV8	4	7.4250	7.4250
AV10	4	8.2000	8.2000
AB7	4		9.2000
AB3	4		9.3500
AV 4	4		9.4500
TR II	4		9.5750
TR1	4		10.2500
AB 2	4		10.6750
AB9	4		10.8000
Sig.		.061	.152

Means for groups in homogeneous subsets are displayed.

Vit. C

Duncan

Species	N	Subset for alpha = 0.05	
		1	2
AV 4	4	.0500	
TR II	4	.1750	
AB3	4	.1850	
PTR 6	4	.2050	
AV10	4	.2400	
AV8	4	.2850	.2850
APH 5	4	.3000	.3000
AB7	4	.3050	.3050
AB 2	4	.3850	.3850
AB9	4	.4200	.4200
TR1	4		.6725
Sig.		.073	.052

Means for groups in homogeneous subsets are displayed.

APPENDIX VII

One Way Anova of Proximate Results of Cultivated Mushroom Samples

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Moisture	AB2 C	5	82.8000	2.28035	1.01980	79.9686	85.6314	80.00	85.00
	AB 3C	5	82.0400	1.64560	.73593	79.9967	84.0833	80.00	84.20
	AB 7C	5	85.6200	4.05857	1.81505	80.5806	90.6594	80.00	90.00
	AB 9C	5	83.8000	3.03315	1.35647	80.0338	87.5662	80.00	88.00
	PTR6C	5	80.4000	1.14018	.50990	78.9843	81.8157	79.00	82.00
	AV 4 C	5	87.4000	4.87852	2.18174	81.3425	93.4575	80.00	92.00
	AV 8 C	5	92.6000	4.61519	2.06398	86.8695	98.3305	85.00	97.00
	AV 10C	5	91.6000	6.73053	3.00998	83.2429	99.9571	80.00	97.00
	APH 5C	5	92.2000	4.26615	1.90788	86.9029	97.4971	85.00	96.00
	Total	45	86.4956	5.74219	.85599	84.7704	88.2207	79.00	97.00
Dry matter	AB2 C	5	9.2600	1.45877	.65238	7.4487	11.0713	7.10	10.50
	AB 3C	5	7.8380	1.15552	.51676	6.4032	9.2728	6.30	9.40
	AB 7C	5	9.0400	.92898	.41545	7.8865	10.1935	7.80	10.10
	AB 9C	5	8.5420	1.11392	.49816	7.1589	9.9251	7.20	10.11
	PTR6C	5	9.1600	.61482	.27495	8.3966	9.9234	8.50	10.10
	AV 4 C	5	9.7000	2.21923	.99247	6.9445	12.4555	7.80	12.90
	AV 8 C	5	11.3800	2.67245	1.19516	8.0617	14.6983	7.60	15.00
	AV 10C	5	10.3920	1.72752	.77257	8.2470	12.5370	7.90	12.16
	APH 5C	5	8.9600	1.65167	.73865	6.9092	11.0108	6.50	10.60
	Total	45	9.3636	1.76900	.26371	8.8321	9.8950	6.30	15.00
Protein	AB2 C	4	23.5000	2.38048	1.19024	19.7121	27.2879	21.00	26.00
	AB 3C	4	18.0000	3.74166	1.87083	12.0462	23.9538	13.00	22.00
	AB 7C	4	30.1625	7.87964	3.93982	17.6242	42.7008	22.90	40.40
	AB 9C	4	21.4450	10.44612	5.22306	4.8229	38.0671	12.08	35.70
	PTR6C	5	21.8000	7.01427	3.13688	13.0906	30.5094	15.00	32.00
	AV 4 C	4	11.2800	2.99013	1.49506	6.5220	16.0380	8.11	15.30
	AV 8 C	4	14.5250	2.09026	1.04513	11.1989	17.8511	12.30	17.00
	AV 10C	4	14.5075	4.52573	2.26286	7.3061	21.7089	10.60	21.00

	APH 5C	4	19.3475	4.48990	2.24495	12.2031	26.4919	15.88	25.81
	Total	37	19.4614	7.39676	1.21602	16.9951	21.9276	8.11	40.40
Lipid	AB2 C	4	2.6750	1.35247	.67623	.5229	4.8271	1.20	4.10
	AB 3C	4	3.4750	1.22848	.61424	1.5202	5.4298	1.90	4.50
	AB 7C	4	4.3000	.82057	.41028	2.9943	5.6057	3.50	5.40
	AB 9C	4	2.3250	1.21209	.60605	.3963	4.2537	.80	3.30
	PTR6C	5	1.2200	.82583	.36932	.1946	2.2454	.00	2.20
	AV 4 C	4	4.2750	1.00125	.50062	2.6818	5.8682	2.90	5.30
	AV 8 C	4	2.4000	.88694	.44347	.9887	3.8113	1.30	3.30
	AV 10C	4	1.0950	1.21355	.60677	-.8360	3.0260	.00	2.19
	APH 5C	4	2.2675	1.07196	.53598	.5618	3.9732	1.33	3.45
	Total	37	2.6311	1.46933	.24156	2.1412	3.1210	.00	5.40
Ash	AB2 C	4	17.2500	.95743	.47871	15.7265	18.7735	16.00	18.00
	AB 3C	4	12.6250	2.41851	1.20925	8.7766	16.4734	10.50	15.70
	AB 7C	4	16.2500	1.50000	.75000	13.8632	18.6368	15.00	18.00
	AB 9C	4	13.7500	2.11739	1.05869	10.3808	17.1192	10.80	15.60
	PTR6C	5	15.0000	4.65994	2.08399	9.2139	20.7861	10.40	22.00
	AV 4 C	4	10.2750	2.29256	1.14628	6.6270	13.9230	7.90	13.40
	AV 8 C	4	10.5550	1.66822	.83411	7.9005	13.2095	8.10	11.78
	AV 10C	4	13.9475	.87007	.43504	12.5630	15.3320	12.89	15.00
	APH 5C	4	15.7000	.94516	.47258	14.1960	17.2040	15.00	17.00
	Total	37	13.9570	3.10114	.50982	12.9231	14.9910	7.90	22.00
Fibre	AB2 C	4	15.4400	1.40316	.70158	13.2073	17.6727	13.40	16.59
	AB 3C	4	8.6250	.76322	.38161	7.4106	9.8394	7.90	9.70
	AB 7C	4	14.2250	.95350	.47675	12.7078	15.7422	13.40	15.60
	AB 9C	4	10.5075	2.01632	1.00816	7.2991	13.7159	7.70	12.13
	PTR6C	5	12.6040	4.92955	2.20456	6.4832	18.7248	7.80	20.80
	AV 4 C	4	8.7500	2.72091	1.36045	4.4204	13.0796	6.30	12.60
	AV 8 C	4	8.9925	1.67677	.83839	6.3244	11.6606	6.70	10.70
	AV 10C	4	11.7425	1.60724	.80362	9.1850	14.3000	9.70	13.60
	APH 5C	4	13.2750	1.35984	.67992	11.1112	15.4388	11.70	14.60
	Total	37	11.6014	3.19058	.52453	10.5376	12.6651	6.30	20.80
Ethanolsolub lesuger	AB2 C	4	7.7250	2.05649	1.02825	4.4527	10.9973	5.70	10.50
	AB 3C	4	10.2500	2.71427	1.35714	5.9310	14.5690	6.77	13.40
	AB 7C	4	9.8250	3.46157	1.73079	4.3169	15.3331	6.70	14.50
	AB 9C	4	7.5500	2.01412	1.00706	4.3451	10.7549	4.90	9.80
	PTR6C	5	5.5620	1.33444	.59678	3.9051	7.2189	4.11	7.70
	AV 4 C	4	6.2400	1.44305	.72153	3.9438	8.5362	4.40	7.76
	AV 8 C	4	5.6425	1.63582	.81791	3.0396	8.2454	4.10	7.90

	AV 10C	4	6.8250	2.03204	1.01602	3.5916	10.0584	4.50	9.10
	APH 5C	4	8.2250	1.63072	.81536	5.6302	10.8198	6.19	9.90
	Total	37	7.4849	2.46983	.40604	6.6614	8.3083	4.10	14.50
Vit. C	AB2 C	4	.4250	.17078	.08539	.1532	.6968	.20	.60
	AB 3C	4	.4250	.27538	.13769	-.0132	.8632	.10	.70
	AB 7C	4	.3250	.28723	.14361	-.1320	.7820	.10	.70
	AB 9C	4	.2925	.31542	.15771	-.2094	.7944	.01	.66
	PTR6C	5	.1740	.27673	.12376	-.1696	.5176	.00	.66
	AV 4 C	4	.1775	.17134	.08567	-.0951	.4501	.00	.40
	AV 8 C	4	.1250	.21063	.10532	-.2102	.4602	.00	.44
	AV 10C	4	.1725	.32510	.16255	-.3448	.6898	.00	.66
	APH 5C	4	.5300	.20976	.10488	.1962	.8638	.25	.71
	Total	37	.2908	.26356	.04333	.2029	.3787	.00	.71

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Moisture	Between Groups	876.879	8	109.610	6.875	.000
	Within Groups	573.920	36	15.942		
	Total	1450.799	44			
Drymatter	Between Groups	42.795	8	5.349	2.029	.071
	Within Groups	94.897	36	2.636		
	Total	137.692	44			
Protein	Between Groups	1038.352	8	129.794	3.902	.003
	Within Groups	931.285	28	33.260		
	Total	1969.636	36			
Lipid	Between Groups	45.319	8	5.665	4.895	.001
	Within Groups	32.403	28	1.157		
	Total	77.722	36			
Ash	Between Groups	189.790	8	23.724	4.247	.002
	Within Groups	156.425	28	5.587		
	Total	346.215	36			
Fibre	Between Groups	202.752	8	25.344	4.334	.002
	Within Groups	163.722	28	5.847		
	Total	366.474	36			
Ethanol soluble	Between Groups	94.932	8	11.867	2.665	.026
	Within Groups	124.670	28	4.452		

sugar	Total	219.602	36			
Vit. C	Between Groups	.663	8	.083	1.263	.302
	Within Groups	1.838	28	.066		
	Total	2.501	36			

Post Hoc Tests

Homogeneous Subsets

Moisture

Duncan

		Subset for alpha = 0.05		
Species	**N**	**1**	**2**	**3**
PTR6C	5	80.4000		
AB 3C	5	82.0400	82.0400	
AB2 C	5	82.8000	82.8000	
AB 9C	5	83.8000	83.8000	
AB 7C	5	85.6200	85.6200	
AV 4 C	5		87.4000	87.4000
AV 10C	5			91.6000
APH 5C	5			92.2000
AV 8 C	5			92.6000
Sig.		.071	.064	.066

Means for groups in homogeneous subsets are displayed.

Dry Matter

Duncan

		Subset for alpha = 0.05		
Species	**N**	**1**	**2**	**3**
AB 3C	5	7.8380		
AB 9C	5	8.5420	8.5420	
APH 5C	5	8.9600	8.9600	
AB 7C	5	9.0400	9.0400	9.0400

PTR6C	5	9.1600	9.1600	9.1600
AB2 C	5	9.2600	9.2600	9.2600
AV 4 C	5	9.7000	9.7000	9.7000
AV 10C	5		10.3920	10.3920
AV 8 C	5			11.3800
Sig.		.124	.126	.051

Means for groups in homogeneous subsets are displayed.

Protein

Duncan

		Subset for alpha = 0.05		
Species	N	1	2	3
AV 4 C	4	11.2800		
AV 10C	4	14.5075	14.5075	
AV 8 C	4	14.5250	14.5250	
AB 3C	4	18.0000	18.0000	
APH 5C	4	19.3475	19.3475	
AB 9C	4		21.4450	21.4450
PTR6C	5		21.8000	21.8000
AB2 C	4		23.5000	23.5000

AB 7C	4			30.1625
Sig.		.083	.060	.056

Means for groups in homogeneous subsets are displayed

Lipid

Duncan

Species	N	Subset for alpha = 0.05		
		1	2	3
AV 10C	4	1.0950		
PTR6C	5	1.2200		
APH 5C	4	2.2675	2.2675	
AB 9C	4	2.3250	2.3250	
AV 8 C	4	2.4000	2.4000	
AB2 C	4	2.6750	2.6750	2.6750
AB 3C	4		3.4750	3.4750
AV 4 C	4			4.2750
AB 7C	4			4.3000
Sig.		.073	.162	.056

Means for groups in homogeneous subsets are displayed.

Ash

Duncan

Species	N	Subset for alpha = 0.05		
		1	2	3
AV 4 C	4	10.2750		
AV 8 C	4	10.5550		
AB 3C	4	12.6250	12.6250	
AB 9C	4	13.7500	13.7500	13.7500
AV 10C	4	13.9475	13.9475	13.9475
PTR6C	5		15.0000	15.0000
APH 5C	4		15.7000	15.7000
AB 7C	4		16.2500	16.2500
AB2 C	4			17.2500
Sig.		.055	.062	.071

Ash

Duncan

Species	N	Subset for alpha = 0.05		
		1	2	3
AV 4 C	4	10.2750		
AV 8 C	4	10.5550		
AB 3C	4	12.6250	12.6250	
AB 9C	4	13.7500	13.7500	13.7500
AV 10C	4	13.9475	13.9475	13.9475
PTR6C	5		15.0000	15.0000
APH 5C	4		15.7000	15.7000
AB 7C	4		16.2500	16.2500
AB2 C	4			17.2500
Sig.		.055	.062	.071

Means for groups in homogeneous subsets are displayed.

Fibre

Duncan

Species	N	Subset for alpha = 0.05			
		1	2	3	4
AB 3C	4	8.6250			
AV 4 C	4	8.7500			
AV 8 C	4	8.9925	8.9925		
AB 9C	4	10.5075	10.5075	10.5075	
AV 10C	4	11.7425	11.7425	11.7425	11.7425
PTR6C	5		12.6040	12.6040	12.6040
APH 5C	4			13.2750	13.2750
AB 7C	4			14.2250	14.2250
AB2 C	4				15.4400
Sig.		.109	.059	.057	.058

Means for groups in homogeneous subsets are displayed.

Ethanol soluble sugar

Duncan

Species	N	Subset for alpha = 0.05		
		1	2	3
PTR6C	5	5.5620		
AV 8 C	4	5.6425		
AV 4 C	4	6.2400		
AV 10C	4	6.8250	6.8250	
AB 9C	4	7.5500	7.5500	7.5500
AB2 C	4	7.7250	7.7250	7.7250

APH 5C	4	8.2250	8.2250	8.2250
AB 7C	4		9.8250	9.8250
AB 3C	4			10.2500
Sig.		.126	.078	.112

Means for groups in homogeneous subsets are displayed.

Vit. C

Duncan

		Subset for alpha = 0.05
Species	**N**	1
AV 8 C	4	.1250
AV 10C	4	.1725
PTR6C	5	.1740
AV 4 C	4	.1775
AB 9C	4	.2925
AB 7C	4	.3250
AB 3C	4	.4250
AB2 C	4	.4250
APH 5C	4	.5300
Sig.		.061

Means for groups in homogeneous subsets are displayed.

APPENDIX VIII

: ONEWAY ANOVA FOR TRACE METALS DETERMINATION ON WILD MUSHROOMS (mg/kg)

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Ca	TR1	5	135.8020	152.33895	68.12805	-53.3518	324.9558	55.10	407.66
	TR II	5	65.2620	16.57178	7.41112	44.6854	85.8386	50.98	86.39
	AB 2	5	453.8880	321.19747	143.64387	55.0687	852.7073	96.34	788.64
	AB3	5	303.3600	181.58646	81.20794	77.8906	528.8294	126.25	551.96
	AB7	5	223.0680	102.48672	45.83345	95.8139	350.3221	168.38	404.59
	AB9	5	584.2520	76.68637	34.29519	489.0333	679.4707	461.30	661.51
	PTR 6	5	6.7680	.73384	.32818	5.8568	7.6792	6.00	7.94
	AV 4	5	507.8080	400.52385	179.11971	10.4919	1005.1241	27.28	1134.00
	APH 5	5	300.9400	194.96135	87.18937	58.8635	543.0165	29.72	503.96
	AV8	5	122.3920	256.11709	114.53904	-195.6194	440.4034	6.36	580.54
	AV10	5	110.7280	66.06600	29.54561	28.6962	192.7598	60.18	188.91
	Total	55	255.8425	258.75087	34.88996	185.8924	325.7927	6.00	1134.00
Co	TR1	5	1.122	1.2382	.5537	-.415	2.659	.0	3.0
	TR II	5	.806	.3602	.1611	.359	1.253	.5	1.4
	AB 2	5	1.296	.4930	.2205	.684	1.908	.9	1.9
	AB3	5	1.674	.8179	.3658	.659	2.689	.9	2.9
	AB7	5	.822	.5353	.2394	.157	1.487	.4	1.7

	AB9	5	.902	.0687	.0307	.817	.987	.8	1.0
	PTR 6	5	1.322	.7756	.3468	.359	2.285	.7	2.5
	AV 4	5	1.124	1.0485	.4689	-.178	2.426	.4	2.8
	APH 5	5	1.418	1.7210	.7696	-.719	3.555	.4	4.4
	AV8	5	.936	.2431	.1087	.634	1.238	.7	1.3
	AV10	5	.934	.6335	.2833	.147	1.721	.4	1.8
	Total	55	1.123	.8143	.1098	.903	1.343	.0	4.4
Pb	TR1	5	3.4580	4.54141	2.03098	-2.1809	9.0969	.01	10.50
	TR II	5	6.3340	4.93541	2.20718	.2059	12.4621	2.01	14.80
	AB 2	5	2.7800	2.78819	1.24692	-.6820	6.2420	.72	6.94
	AB3	5	6.7560	1.50291	.67212	4.8899	8.6221	4.93	8.98
	AB7	5	.6180	.25203	.11271	.3051	.9309	.38	.96
	AB9	5	.8760	.17813	.07966	.6548	1.0972	.72	1.15
	PTR 6	5	6.9480	2.17384	.97217	4.2488	9.6472	5.46	10.37
	AV 4	5	2.5120	1.50197	.67170	.6471	4.3769	.96	4.62
	APH 5	5	2.5960	1.61155	.72071	.5950	4.5970	.38	4.50
	AV8	5	4.2840	2.05263	.91796	1.7353	6.8327	.72	5.48
	AV10	5	6.0560	2.31675	1.03608	3.1794	8.9326	4.49	10.10
	Total	55	3.9289	3.22844	.43532	3.0561	4.8017	.01	14.80
Mg	TR1	5	648.2860	375.49256	167.92538	182.0504	1114.5216	92.50	1088.01
	TR II	5	1702.1160	1839.62042	822.70326	-582.0744	3986.3064	792.10	4990.01
	AB 2	5	855.9720	810.79141	362.59694	-150.7585	1862.7025	86.52	2034.12
	AB3	5	95.3820	4.36398	1.95163	89.9634	100.8006	90.58	101.66
	AB7	5	1352.8600	250.61514	112.07850	1041.6802	1664.0398	1191.00	1791.30
	AB9	5	1288.7040	356.00675	159.21106	846.6632	1730.7448	1033.60	1791.61

	PTR 6	5	291.6740	63.51403	28.40434	212.8109	370.5371	220.22	344.21
	AV 4	5	5409.4040	5895.96479	2636.75561	-1911.4032	12730.2112	944.06	11935.00
	APH 5	5	1349.7120	479.82550	214.58449	753.9300	1945.4940	999.60	2196.00
	AV8	5	386.7640	361.67035	161.74390	-62.3091	835.8371	220.22	1033.68
	AV10	5	828.7300	74.09052	33.13429	736.7345	920.7255	791.96	960.92
	Total	55	1291.7822	2211.15544	298.15214	694.0231	1889.5412	86.52	11935.00
Zn	TR1	5	65.5520	24.31741	10.87508	35.3579	95.7461	40.80	101.70
	TR II	5	86.4880	51.82385	23.17633	22.1402	150.8358	50.80	170.39
	AB 2	5	22.1460	4.77732	2.13648	16.2142	28.0778	17.84	27.82
	AB3	5	26.8160	2.80882	1.25614	23.3284	30.3036	22.88	30.82
	AB7	5	44.8760	1.71706	.76789	42.7440	47.0080	43.56	47.58
	AB9	5	18.0160	.90966	.40681	16.8865	19.1455	17.00	19.50
	PTR 6	5	50.4900	3.79227	1.69595	45.7813	55.1987	48.32	57.12
	AV 4	5	41.4760	4.72202	2.11175	35.6128	47.3392	34.92	45.98
	APH 5	5	37.6840	8.28007	3.70296	27.4029	47.9651	25.06	44.59
	AV8	5	43.3640	14.36248	6.42310	25.5306	61.1974	17.84	52.33
	AV10	5	52.9520	2.50625	1.12083	49.8401	56.0639	50.81	55.88
	Total	55	44.5327	25.06993	3.38043	37.7554	51.3101	17.00	170.39
Cr	TR1	5	.00820	.004025	.001800	.00320	.01320	.001	.010
	TR II	5	.00280	.004025	.001800	-.00220	.00780	.001	.010
	AB 2	5	.00100	.000000	.000000	.00100	.00100	.001	.001
	AB3	5	.00100	.000000	.000000	.00100	.00100	.001	.001
	AB7	5	.00100	.000000	.000000	.00100	.00100	.001	.001
	AB9	5	.00100	.000000	.000000	.00100	.00100	.001	.001
	PTR 6	5	.00100	.000000	.000000	.00100	.00100	.001	.001

	AV 4	5	.00100	.000000	.000000	.00100	.00100	.001	.001
	APH 5	5	.00100	.000000	.000000	.00100	.00100	.001	.001
	AV8	5	.00100	.000000	.000000	.00100	.00100	.001	.001
	AV10	5	.00100	.000000	.000000	.00100	.00100	.001	.001
	Total	55	.00182	.002611	.000352	.00111	.00252	.001	.010
Fe	TR1	5	263.6020	75.84407	33.91850	169.4291	357.7749	201.33	395.80
	TR II	5	231.6240	20.55017	9.19032	206.1076	257.1404	200.30	255.20
	AB 2	5	307.2480	335.35877	149.97700	-109.1549	723.6509	51.20	774.02
	AB3	5	622.6660	103.35096	46.21996	494.3388	750.9932	511.81	779.81
	AB7	5	187.3700	7.20150	3.22061	178.4282	196.3118	176.40	196.38
	AB9	5	163.9280	14.59557	6.52734	145.8052	182.0508	151.20	180.12
	PTR 6	5	841.9820	31.15787	13.93422	803.2944	880.6696	819.40	884.40
	AV 4	5	598.4180	251.97257	112.68556	285.5527	911.2833	154.68	785.96
	APH 5	5	269.6900	176.21243	78.80460	50.8934	488.4866	96.74	506.33
	AV8	5	668.3800	365.34559	163.38752	214.7435	1122.0165	51.20	960.40
	AV10	5	220.5420	45.39805	20.30262	164.1729	276.9111	139.43	244.40
	Total	55	397.7682	280.61861	37.83861	321.9063	473.6300	51.20	960.40
Cd	TR1	5	3.5800	1.35484	.60590	1.8977	5.2623	1.40	4.48
	TR II	5	8.2340	5.14428	2.30059	1.8465	14.6215	3.33	15.98
	AB 2	5	6.0840	2.38286	1.06565	3.1253	9.0427	4.02	10.01
	AB3	5	5.4520	1.28545	.57487	3.8559	7.0481	4.02	7.11
	AB7	5	5.4780	1.22814	.54924	3.9531	7.0029	4.33	7.12
	AB9	5	5.2360	1.14363	.51145	3.8160	6.6560	4.41	7.11
	PTR 6	5	5.3020	.87978	.39345	4.2096	6.3944	4.66	6.44
	AV 4	5	4.1740	.46301	.20707	3.5991	4.7489	3.88	4.98

	APH 5	5	5.1320	1.54004	.68873	3.2198	7.0442	4.14	7.80
	AV8	5	5.0460	1.02737	.45945	3.7704	6.3216	4.48	6.88
	AV10	5	4.7560	1.27017	.56804	3.1789	6.3331	3.33	6.82
	Total	55	5.3158	2.14031	.28860	4.7372	5.8944	1.40	15.98
Cu	TR1	5	.00	.000	.000	.00	.00	0	0
	TR II	5	.00	.000	.000	.00	.00	0	0
	AB 2	5	.00	.000	.000	.00	.00	0	0
	AB3	5	.00	.000	.000	.00	.00	0	0
	AB7	5	.00	.000	.000	.00	.00	0	0
	AB9	5	.00	.000	.000	.00	.00	0	0
	PTR 6	5	.00	.000	.000	.00	.00	0	0
	AV 4	5	.00	.000	.000	.00	.00	0	0
	APH 5	5	.00	.000	.000	.00	.00	0	0
	AV8	5	.00	.000	.000	.00	.00	0	0
	AV10	5	.00	.000	.000	.00	.00	0	0
	Total	55	.00	.000	.000	.00	.00	0	0

Ni	TR1	5	36.540	38.1115	17.0440	-10.782	83.862	.9	80.1
	TR II	5	24.212	7.4619	3.3371	14.947	33.477	15.4	33.9
	AB 2	5	.739	1.0479	.4686	-.563	2.040	.0	2.2
	AB3	5	2.464	.5235	.2341	1.814	3.114	2.2	3.4
	AB7	5	1.722	1.0457	.4676	.424	3.020	1.0	3.4
	AB9	5	.006	.0049	.0022	.000	.013	.0	.0
	PTR 6	5	4.938	.5257	.2351	4.285	5.591	4.5	5.6
	AV 4	5	5.568	2.5051	1.1203	2.457	8.679	1.1	7.0
	APH 5	5	2.064	.9701	.4338	.859	3.269	1.1	3.0
	AV8	5	4.614	.3394	.1518	4.193	5.035	4.2	5.1
	AV10	5	15.960	1.8636	.8334	13.646	18.274	14.1	19.1
	Total	55	8.984	15.5042	2.0906	4.793	13.176	.0	80.1
Hg	TR1	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	TR II	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AB 2	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AB3	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AB7	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AB9	5	.0010	.00000	.00000	.0010	.0010	.00	.00
	PTR 6	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AV 4	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	APH 5	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AV8	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AV10	5	.0100	.00000	.00000	.0100	.0100	.01	.01

	Total	55	.0092	.00261	.00035	.0085	.0099	.00	.01
Na	TR1	5	445.354	158.6280	70.9406	248.391	642.317	281.1	706.0
	TR II	5	341.646	51.6586	23.1024	277.503	405.789	288.0	395.0
	AB 2	5	301.964	216.1101	96.6474	33.628	570.300	107.3	666.2
	AB3	5	380.816	98.0822	43.8637	259.031	502.601	301.0	533.0
	AB7	5	793.822	90.0370	40.2658	682.026	905.618	668.6	887.1
	AB9	5	106.424	1.9866	.8884	103.957	108.891	104.3	108.4
	PTR 6	5	831.334	42.2592	18.8989	778.862	883.806	802.0	899.9
	AV 4	5	896.718	219.4242	98.1295	624.267	1169.169	745.7	1279.6
	APH 5	5	661.900	286.0120	127.9085	306.769	1017.031	152.4	833.6
	AV8	5	688.612	333.4834	149.1383	274.538	1102.686	107.3	960.9
	AV10	5	400.736	6.9986	3.1299	392.046	409.426	393.4	409.5
	Total	55	531.757	291.6494	39.3260	452.913	610.601	104.3	1279.6

Mn	TR1	5	45.9240	34.41858	15.39246	3.1877	88.6603	21.91	101.77
	TR II	5	25.3480	2.95539	1.32169	21.6784	29.0176	22.90	30.40
	AB 2	5	18.6280	7.18512	3.21328	9.7065	27.5495	13.41	30.39
	AB3	5	18.0420	2.56017	1.14494	14.8631	21.2209	14.41	20.47
	AB7	5	18.4240	.85763	.38354	17.3591	19.4889	17.70	19.76
	AB9	5	14.8340	.78197	.34971	13.8631	15.8049	14.22	16.12
	PTR 6	5	13.5320	.75254	.33655	12.5976	14.4664	12.36	14.36
	AV 4	5	16.5360	1.39324	.62308	14.8061	18.2659	15.07	17.90
	APH 5	5	15.7540	2.33861	1.04586	12.8502	18.6578	13.60	18.70
	AV8	5	12.8800	.82450	.36873	11.8562	13.9038	12.22	14.22
	AV10	5	21.6340	2.54982	1.14031	18.4680	24.8000	17.70	23.96
	Total	55	20.1396	13.17616	1.77667	16.5776	23.7017	12.22	101.77

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Ca	Between Groups	1837816.537	10	183781.654	4.549	.000
	Within Groups	1777592.275	44	40399.824		
	Total	3615408.812	54			
Co	Between Groups	3.854	10	.385	.531	.859
	Within Groups	31.956	44	.726		
	Total	35.810	54			
Pb	Between Groups	265.754	10	26.575	3.936	.001
	Within Groups	297.079	44	6.752		
	Total	562.834	54			
Mg	Between Groups	1.060E8	10	1.060E7	2.951	.006
	Within Groups	1.580E8	44	3591373.699		
	Total	2.640E8	54			
Zn	Between Groups	19421.726	10	1942.173	5.886	.000
	Within Groups	14517.344	44	329.940		
	Total	33939.070	54			
Cr	Between Groups	.000	10	.000	8.100	.000
	Within Groups	.000	44	.000		
	Total	.000	54			
Fe	Between Groups	2809780.865	10	280978.087	8.570	.000
	Within Groups	1442546.583	44	32785.150		
	Total	4252327.448	54			
Cd	Between Groups	69.470	10	6.947	1.718	.107
	Within Groups	177.899	44	4.043		
	Total	247.369	54			
Cu	Between Groups	.000	10	.000	.	.
	Within Groups	.000	44	.000		
	Total	.000	54			
Ni	Between Groups	6893.716	10	689.372	4.983	.000
	Within Groups	6086.839	44	138.337		
	Total	12980.554	54			

Hg	Between Groups	.000	10	.000	6.454E32	.000
	Within Groups	.000	44	.000		
	Total	.000	54			
Na	Between Groups	3252160.043	10	325216.004	10.670	.000
	Within Groups	1341046.427	44	30478.328		
	Total	4593206.470	54			
Mn	Between Groups	4302.775	10	430.278	3.733	.001
	Within Groups	5072.233	44	115.278		
	Total	9375.008	54			

Post Hoc Tests

Homogeneous Subsets

Ca

Duncan

Sample codes	N	Subset for alpha = 0.05			
		1	2	3	4
PTR 6	5	6.7680			
TR II	5	65.2620	65.2620		
AV10	5	1.1073E2	1.1073E2		
AV8	5	1.2239E2	1.2239E2		
TR1	5	1.3580E2	1.3580E2		
AB7	5	2.2307E2	2.2307E2	2.2307E2	
APH 5	5		3.0094E2	3.0094E2	3.0094E2
AB3	5		3.0336E2	3.0336E2	3.0336E2
AB 2	5			4.5389E2	4.5389E2
AV 4	5				5.0781E2
AB9	5				5.8425E2
Sig.		.143	.112	.104	.051

Means for groups in homogeneous subsets are displayed.

Co

Duncan

Sample codes	N	Subset for alpha = 0.05
		1
TR II	5	.806
AB7	5	.822
AB9	5	.902
AV10	5	.934
AV8	5	.936
TR1	5	1.122
AV 4	5	1.124
AB 2	5	1.296
PTR 6	5	1.322
APH 5	5	1.418
AB3	5	1.674
Sig.		.184

Means for groups in homogeneous subsets are displayed.

Pb

Duncan

Sample codes	N	Subset for alpha = 0.05			
		1	2	3	4
AB7	5	.6180			
AB9	5	.8760			
AV 4	5	2.5120	2.5120		
APH 5	5	2.5960	2.5960		
AB 2	5	2.7800	2.7800	2.7800	
TR1	5	3.4580	3.4580	3.4580	3.4580
AV8	5	4.2840	4.2840	4.2840	4.2840
AV10	5		6.0560	6.0560	6.0560
TR II	5			6.3340	6.3340
AB3	5				6.7560
PTR 6	5				6.9480
Sig.		.058	.063	.058	.067

Means for groups in homogeneous subsets are displayed.

Mg

Duncan

Sample codes	N	Subset for alpha = 0.05	
		1	2
AB3	5	95.3820	
PTR 6	5	291.6740	
AV8	5	386.7640	
TR1	5	648.2860	
AV10	5	828.7300	
AB 2	5	855.9720	
AB9	5	1.2887E3	
APH 5	5	1.3497E3	
AB7	5	1.3529E3	
TR II	5	1.7021E3	
AV 4	5		5.4094E3
Sig.		.266	1.000

Means for groups in homogeneous subsets are displayed.

Zn

Duncan

Sample codes	N	Subset for alpha = 0.05				
		1	2	3	4	5
AB9	5	18.0160				
AB 2	5	22.1460	22.1460			
AB3	5	26.8160	26.8160	26.8160		
APH 5	5	37.6840	37.6840	37.6840		
AV 4	5	41.4760	41.4760	41.4760	41.4760	
AV8	5	43.3640	43.3640	43.3640	43.3640	
AB7	5		44.8760	44.8760	44.8760	
PTR 6	5			50.4900	50.4900	
AV10	5			52.9520	52.9520	
TR1	5				65.5520	65.5520
TR II	5					86.4880
Sig.		.057	.088	.053	.071	.075

Means for groups in homogeneous subsets are displayed.

Cr

Duncan

Sample codes	N	Subset for alpha = 0.05	
		1	2
AB 2	5	.00100	
AB3	5	.00100	
AB7	5	.00100	
AB9	5	.00100	
PTR 6	5	.00100	
AV 4	5	.00100	
APH 5	5	.00100	
AV8	5	.00100	
AV10	5	.00100	
TR II	5	.00280	
TR1	5		.00820
Sig.		.169	1.000

Means for groups in homogeneous subsets are displayed.

Fe

Duncan

Sample codes	**N**	**Subset for alpha = 0.05**	
		1	**2**
AB9	5	163.9280	
AB7	5	187.3700	
AV10	5	220.5420	
TR II	5	231.6240	
TR1	5	263.6020	
APH 5	5	269.6900	
AB 2	5	307.2480	
AV 4	5		598.4180
AB3	5		622.6660
AV8	5		668.3800
PTR 6	5		841.9820
Sig.		.288	.057

Means for groups in homogeneous subsets are displayed.

Cd

Duncan

Sample codes	N	Subset for alpha = 0.05	
		1	2
TR1	5	3.5800	
AV 4	5	4.1740	
AV10	5	4.7560	
AV8	5	5.0460	
APH 5	5	5.1320	
AB9	5	5.2360	
PTR 6	5	5.3020	
AB3	5	5.4520	
AB7	5	5.4780	
AB 2	5	6.0840	6.0840
TR II	5		8.2340
Sig.		.103	.098

Means for groups in homogeneous subsets are displayed.

Ni

Duncan

Sample codes	N	Subset for alpha = 0.05		
		1	2	3
AB9	5	.006		
AB 2	5	.739		
AB7	5	1.722		
APH 5	5	2.064		
AB3	5	2.464		
AV8	5	4.614		
PTR 6	5	4.938		
AV 4	5	5.568		
AV10	5	15.960	15.960	
TR II	5		24.212	24.212
TR1	5			36.540
Sig.		.074	.273	.105

Means for groups in homogeneous subsets are displayed.

Hg

Duncan

Sample codes	N	Subset for alpha = 0.05	
		1	2
AB9	5	.0010	
TR1	5		.0100
TR II	5		.0100
AB 2	5		.0100
AB3	5		.0100
AB7	5		.0100
PTR 6	5		.0100
AV 4	5		.0100
APH 5	5		.0100
AV8	5		.0100
AV10	5		.0100
Sig.		1.000	1.000

Means for groups in homogeneous subsets are displayed.

Na

Duncan

Sample codes	N	Subset for alpha = 0.05			
		1	2	3	4
AB9	5	106.424			
AB 2	5	301.964	301.964		
TR II	5		341.646		
AB3	5		380.816		
AV10	5		400.736		
TR1	5		445.354	445.354	
APH 5	5			661.900	661.900
AV8	5				688.612
AB7	5				793.822
PTR 6	5				831.334
AV 4	5				896.718
Sig.		.083	.256	.056	.062

Means for groups in homogeneous subsets are displayed.

Mn

Duncan

Sample codes	N	Subset for alpha = 0.05	
		1	2
AV8	5	12.8800	
PTR 6	5	13.5320	
AB9	5	14.8340	
APH 5	5	15.7540	
AV 4	5	16.5360	
AB3	5	18.0420	
AB7	5	18.4240	
AB 2	5	18.6280	
AV10	5	21.6340	
TR II	5	25.3480	
TR1	5		45.9240
Sig.		.128	1.000

Means for groups in homogeneous subsets are displayed.

APPENDIX IX

ONEWAY ANOVA for TRACE METALS DETERMINATION ON MUSHROOM CULTIVATED SAMPLES mg/kg

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Ca	AB2 C	5	73.8300	17.09708	7.64605	52.6012	95.0588	55.78	98.74
	AB 3C	5	437.7440	519.15095	232.17136	-206.8670	1082.3550	64.25	1188.30
	AB 7C	5	135.2040	151.00502	67.53150	-52.2935	322.7015	55.10	404.67
	AB 9C	5	377.1300	327.45346	146.44164	-29.4572	783.7172	126.19	788.66
	AV 4 C	5	539.8120	119.99447	53.66316	390.8192	688.8048	404.88	696.51
	AV 8 C	5	584.2120	76.68885	34.29629	488.9902	679.4338	461.30	661.51
	AV 10C	5	6.7680	.73384	.32818	5.8568	7.6792	6.00	7.94
	ApH5C	5	479.2140	403.72395	180.55084	-22.0755	980.5035	29.33	1134.00
	PTR6C	5	299.9120	196.65684	87.94761	55.7303	544.0937	24.74	503.80
	Total	45	325.9807	310.96907	46.35653	232.5552	419.4061	6.00	1188.30
Co	AB2 C	5	.6700	.40106	.17936	.1720	1.1680	.33	1.33
	AB 3C	5	.5580	.23285	.10413	.2689	.8471	.40	.96
	AB 7C	5	1.1220	1.23817	.55373	-.4154	2.6594	.01	2.99
	AB 9C	5	2.9340	2.83024	1.26572	-.5802	6.4482	.92	6.94

	AV 4 C	5	11.1420	2.59948	1.16252	7.9143	14.3697	8.90	15.60
	AV 8 C	5	1.3160	.87549	.39153	.2289	2.4031	.88	2.88
	AV 10C	5	1.7220	.90527	.40485	.5980	2.8460	.68	2.74
	ApH5C	5	1.9340	1.73056	.77393	-.2148	4.0828	.41	4.49
	PTR6C	5	1.4180	1.72096	.76964	-.7189	3.5549	.44	4.44
	Total	45	2.5351	3.48088	.51890	1.4893	3.5809	.01	15.60
Pb	AB2 C	5	.7360	.96064	.42961	-.4568	1.9288	.01	2.40
	AB 3C	5	2.6340	2.19146	.98005	-.0871	5.3551	.40	5.50
	AB 7C	5	3.4180	4.57036	2.04393	-2.2569	9.0929	.01	10.50
	AB 9C	5	.5480	.17880	.07996	.3260	.7700	.33	.80
	AV 4 C	5	2.6160	.99696	.44585	1.3781	3.8539	1.77	4.20
	AV 8 C	5	.8760	.17813	.07966	.6548	1.0972	.72	1.15
	AV 10C	5	6.9380	2.18198	.97581	4.2287	9.6473	5.46	10.37
	ApH5C	5	2.7780	1.92068	.85896	.3932	5.1628	.96	5.65
	PTR6C	5	2.5360	1.56987	.70207	.5868	4.4852	.38	4.50
	Total	45	2.5644	2.63368	.39261	1.7732	3.3557	.01	10.50
Mg	AB2 C	5	670.2400	136.48154	61.03640	500.7758	839.7042	440.39	779.82
	AB 3C	5	848.7800	208.04782	93.04181	590.4545	1107.1055	690.12	1196.17
	AB 7C	5	648.9460	375.07121	167.73694	183.2336	1114.6584	92.80	1088.01
	AB 9C	5	86.4840	39.77396	17.78745	37.0981	135.8699	48.44	144.96
	AV 4 C	5	1132.6520	517.91120	231.61693	489.5803	1775.7237	733.98	2022.14
	AV 8 C	5	1300.5780	347.70139	155.49679	868.8497	1732.3063	1038.60	1791.61
	AV 10C	5	291.6740	63.51403	28.40434	212.8109	370.5371	220.22	344.21
	ApH5C	5	1151.1040	88.00955	39.35907	1041.8257	1260.3823	994.06	1199.00
	PTR6C	5	1349.7740	479.96219	214.64562	753.8222	1945.7258	999.60	2196.31

	Total	45	831.1369	506.31523	75.47702	679.0230	983.2508	48.44	2196.31
Zn	AB2 C	5	68.0000	19.81237	8.86036	43.3997	92.6003	44.50	94.40
	AB 3C	5	395.5420	437.38345	195.60383	-147.5413	938.6253	74.10	960.33
	AB 7C	5	317.6320	516.69663	231.07376	-323.9316	959.1956	40.80	1238.24
	AB 9C	5	45.3960	16.08381	7.19290	25.4253	65.3667	18.18	58.33
	AV 4 C	5	16.3260	3.38057	1.51184	12.1285	20.5235	12.44	20.21
	AV 8 C	5	18.0160	.90966	.40681	16.8865	19.1455	17.00	19.50
	AV 10C	5	50.5220	3.86223	1.72724	45.7264	55.3176	48.32	57.28
	ApH5C	5	41.3280	4.52026	2.02152	35.7154	46.9406	34.98	45.90
	PTR6C	5	37.6840	8.28007	3.70296	27.4029	47.9651	25.06	44.59
	Total	45	110.0496	245.07410	36.53349	36.4211	183.6780	12.44	1238.24
Cr	AB2 C	5	.0024	.00428	.00191	-.0029	.0077	.00	.01
	AB 3C	5	.0010	.00000	.00000	.0010	.0010	.00	.00
	AB 7C	5	.0010	.00000	.00000	.0010	.0010	.00	.00
	AB 9C	5	.0010	.00000	.00000	.0010	.0010	.00	.00
	AV 4 C	5	.0010	.00000	.00000	.0010	.0010	.00	.00
	AV 8 C	5	.0008	.00045	.00020	.0002	.0014	.00	.00
	AV 10C	5	.0028	.00402	.00180	-.0022	.0078	.00	.01
	ApH5C	5	.0010	.00000	.00000	.0010	.0010	.00	.00
	PTR6C	5	.0010	.00000	.00000	.0010	.0010	.00	.00
	Total	45	.0013	.00191	.00028	.0008	.0019	.00	.01

Fe	AB2 C	5	128.2520	42.70447	19.09802	75.2274	181.2766	67.25	174.51
	AB 3C	5	61.1960	52.04258	23.27415	-3.4234	125.8154	14.68	132.60
	AB 7C	5	199.1420	69.74931	31.19284	112.5368	285.7472	122.32	288.24
	AB 9C	5	317.0000	247.03586	110.47779	10.2645	623.7355	77.20	654.90
	AV 4 C	5	317.0000	247.03586	110.47779	10.2645	623.7355	77.20	654.90
	AV 8 C	5	163.9280	14.59557	6.52734	145.8052	182.0508	151.20	180.12
	AV 10C	5	1094.3820	752.59365	336.57011	159.9136	2028.8504	82.33	1866.38
	ApH5C	5	598.4180	251.97257	112.68556	285.5527	911.2833	154.68	785.96
	PTR6C	5	424.4960	344.42751	154.03266	-3.1672	852.1592	96.74	960.40
	Total	45	367.0904	414.92343	61.85313	242.4336	491.7472	14.68	1866.38
Cd	AB2 C	5	1.2760	.67151	.30031	.4422	2.1098	.66	2.40
	AB 3C	5	2.1380	1.46987	.65735	.3129	3.9631	.80	4.44
	AB 7C	5	3.5400	1.32575	.59289	1.8939	5.1861	1.40	4.48
	AB 9C	5	3.9160	1.67303	.74820	1.8387	5.9933	1.33	5.49
	AV 4 C	5	3.9160	1.67303	.74820	1.8387	5.9933	1.33	5.49
	AV 8 C	5	5.2360	1.14363	.51145	3.8160	6.6560	4.41	7.11
	AV 10C	5	5.3020	.87978	.39345	4.2096	6.3944	4.66	6.44
	ApH5C	5	4.1740	.46301	.20707	3.5991	4.7489	3.88	4.98
	PTR6C	5	4.9960	1.57108	.70261	3.0453	6.9467	4.14	7.80
	Total	45	3.8327	1.74810	.26059	3.3075	4.3579	.66	7.80

Cu	AB2 C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	AB 3C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	AB 7C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	AB 9C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	AV 4 C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	AV 8 C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	AV 10C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	ApH5C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	PTR6C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	45	.0000	.00000	.00000	.0000	.0000	.00	.00
Ni	AB2 C	5	3.7820	.57482	.25707	3.0683	4.4957	2.99	4.38
	AB 3C	5	3.7820	.57482	.25707	3.0683	4.4957	2.99	4.38
	AB 7C	5	2.1080	4.69127	2.09800	-3.7170	7.9330	.01	10.50
	AB 9C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AV 4 C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AV 8 C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	AV 10C	5	4.9380	.52566	.23508	4.2853	5.5907	4.50	5.58
	ApH5C	5	5.6300	2.54309	1.13730	2.4723	8.7877	1.10	6.99
	PTR6C	5	2.0820	.99278	.44399	.8493	3.3147	1.10	3.09
	Total	45	2.4824	2.66121	.39671	1.6829	3.2820	.00	10.50

Hg	AB2 C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AB 3C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AB 7C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AB 9C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	AV 4 C	5	55.6120	23.01288	10.29167	27.0377	84.1863	42.72	96.44
	AV 8 C	5	.0080	.00447	.00200	.0024	.0136	.00	.01
	AV 10C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	ApH5C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	PTR6C	5	.0100	.00000	.00000	.0100	.0100	.01	.01
	Total	45	6.1878	18.98496	2.83011	.4841	11.8915	.00	96.44
Na	AB2 C	5	182.8980	194.92117	87.17140	-59.1286	424.9246	24.00	404.00
	AB 3C	5	182.8980	194.92117	87.17140	-59.1286	424.9246	24.00	404.00
	AB 7C	5	441.3460	160.60015	71.82257	241.9346	640.7574	281.14	706.00
	AB 9C	5	124.7600	86.63258	38.74327	17.1914	232.3286	44.40	244.13
	AV 4 C	5	105.7040	23.18140	10.36704	76.9205	134.4875	88.48	144.60
	AV 8 C	5	106.4240	1.98661	.88844	103.9573	108.8907	104.29	108.38
	AV 10C	5	831.2540	42.32201	18.92698	778.7043	883.8037	801.96	899.91
	ApH5C	5	892.7220	235.53358	105.33382	600.2684	1185.1756	705.67	1299.62
	PTR6C	5	541.9000	345.17660	154.36767	113.3066	970.4934	152.36	833.62
	Total	45	378.8784	340.18693	50.71207	276.6750	481.0819	24.00	1299.62

Mn	AB2 C	5	35.0980	15.33371	6.85744	16.0587	54.1373	22.28	60.50
	AB 3C	5	35.0980	15.33371	6.85744	16.0587	54.1373	22.28	60.50
	AB 7C	5	45.9000	34.42012	15.39314	3.1618	88.6382	21.91	101.74
	AB 9C	5	24.3020	4.35267	1.94657	18.8974	29.7066	20.33	30.24
	AV 4 C	5	53.3820	28.39392	12.69815	18.1263	88.6377	28.29	99.10
	AV 8 C	5	14.8240	.78682	.35187	13.8470	15.8010	14.22	16.12
	AV 10C	5	13.5180	.74573	.33350	12.5920	14.4440	12.36	14.36
	ApH5C	5	16.5360	1.39324	.62308	14.8061	18.2659	15.07	17.90
	PTR6C	5	15.7540	2.33861	1.04586	12.8502	18.6578	13.60	18.70
	Total	45	28.2680	20.59942	3.07078	22.0792	34.4568	12.36	101.74

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Ca	Between Groups	1767735.046	8	220966.881	3.198	.008
	Within Groups	2487142.549	36	69087.293		
	Total	4254877.596	44			
Co	Between Groups	436.894	8	54.612	20.430	.000
	Within Groups	96.233	36	2.673		
	Total	533.127	44			
Pb	Between Groups	150.853	8	18.857	4.398	.001
	Within Groups	154.343	36	4.287		
	Total	305.196	44			
Mg	Between Groups	7937852.329	8	992231.541	10.689	.000
	Within Groups	3341772.651	36	92827.018		
	Total	1.128E7	44			
Zn	Between Groups	806509.452	8	100813.681	1.977	.078
	Within Groups	1836188.294	36	51005.230		
	Total	2642697.746	44			
Cr	Between Groups	.000	8	.000	.687	.700
	Within Groups	.000	36	.000		
	Total	.000	44			
Fe	Between Groups	4054379.102	8	506797.388	5.182	.000
	Within Groups	3520724.841	36	97797.912		
	Total	7575103.943	44			
Cd	Between Groups	75.531	8	9.441	5.768	.000
	Within Groups	58.927	36	1.637		
	Total	134.457	44			
Cu	Between Groups	.000	8	.000	.	.
	Within Groups	.000	36	.000		
	Total	.000	44			
Ni	Between Groups	190.018	8	23.752	7.032	.000
	Within Groups	121.592	36	3.378		
	Total	311.610	44			
Hg	Between Groups	13740.490	8	1717.561	29.189	.000
	Within Groups	2118.370	36	58.844		
	Total	15858.860	44			

Na	Between Groups	3947028.102	8	493378.513	15.513	.000
	Within Groups	1144966.264	36	31804.618		
	Total	5091994.366	44			
Mn	Between Groups	8715.855	8	1089.482	3.940	.002
	Within Groups	9954.942	36	276.526		
	Total	18670.797	44			

Post Hoc Tests

Homogeneous Subsets

Ca

Duncan

Sample codes	N	Subset for alpha = 0.05			
		1	2	3	4
AV 10C	5	6.7680			
AB2 C	5	73.8300	73.8300		
AB 7C	5	135.2040	135.2040	135.2040	
PTR6C	5	299.9120	299.9120	299.9120	299.9120
AB 9C	5	377.1300	377.1300	377.1300	377.1300
AB 3C	5		437.7440	437.7440	437.7440
ApH5C	5			479.2140	479.2140
AV 4 C	5				539.8120
AV 8 C	5				584.2120
Sig.		.052	.056	.071	.142

Means for groups in homogeneous subsets are displayed.

Duncan **Co**

Sample codes	N	Subset for alpha = 0.05	
		1	2
AB 3C	5	.5580	
AB2 C	5	.6700	
AB 7C	5	1.1220	
AV 8 C	5	1.3160	
PTR6C	5	1.4180	
AV 10C	5	1.7220	
ApH5C	5	1.9340	
AB 9C	5	2.9340	
AV 4 C	5		11.1420
Sig.		.054	1.000

Means for groups in homogeneous subsets are displayed.

Pb

Duncan

Sample codes	N	Subset for alpha = 0.05	
		1	2
AB 9C	5	.5480	
AB2 C	5	.7360	
AV 8 C	5	.8760	
PTR6C	5	2.5360	
AV 4 C	5	2.6160	
AB 3C	5	2.6340	
ApH5C	5	2.7780	
AB 7C	5	3.4180	
AV 10C	5		6.9380
Sig.		.066	1.000

Means for groups in homogeneous subsets are displayed.

Mg

Duncan

Sample codes	N	Subset for alpha = 0.05				
		1	2	3	4	5
AB 9C	5	86.4840				
AV 10C	5	291.6740	291.6740			
AB 7C	5		648.9460	648.9460		
AB2 C	5		670.2400	670.2400		
AB 3C	5			848.7800	848.7800	
AV 4 C	5				1132.6520	1132.6520
ApH5C	5				1151.1040	1151.1040
AV 8 C	5					1300.5780
PTR6C	5					1349.7740
Sig.		.294	.070	.336	.147	.313

Means for groups in homogeneous subsets are displayed.

Zn

Duncan

Sample codes	N	Subset for alpha = 0.05	
		1	2
AV 4 C	5	16.3260	
AV 8 C	5	18.0160	
PTR6C	5	37.6840	
ApH5C	5	41.3280	
AB 9C	5	45.3960	
AV 10C	5	50.5220	
AB2 C	5	68.0000	
AB 7C	5	317.6320	317.6320
AB 3C	5		395.5420
Sig.		.077	.589

Means for groups in homogeneous subsets are displayed.

Cr

Duncan

Sample codes	N	Subset for alpha = 0.05
		1
AV 8 C	5	.0008
AB 3C	5	.0010
AB 7C	5	.0010
AB 9C	5	.0010
AV 4 C	5	.0010
ApH5C	5	.0010
PTR6C	5	.0010
AB2 C	5	.0024
AV 10C	5	.0028
Sig.		.179

Means for groups in homogeneous subsets are displayed.

Fe

Duncan

Sample codes	N	Subset for alpha = 0.05		
		1	2	3
AB 3C	5	61.1960		
AB2 C	5	128.2520		
AV 8 C	5	163.9280	163.9280	
AB 7C	5	199.1420	199.1420	
AB 9C	5	317.0000	317.0000	
AV 4 C	5	317.0000	317.0000	
PTR6C	5	424.4960	424.4960	
ApH5C	5		598.4180	
AV 10C	5			1094.3820
Sig.		.119	.060	1.000

Means for groups in homogeneous subsets are displayed.

Cd

Duncan

Sample codes	N	Subset for alpha = 0.05		
		1	2	3
AB2 C	5	1.2760		
AB 3C	5	2.1380	2.1380	
AB 7C	5		3.5400	3.5400
AB 9C	5		3.9160	3.9160
AV 4 C	5		3.9160	3.9160
ApH5C	5			4.1740
PTR6C	5			4.9960
AV 8 C	5			5.2360
AV 10C	5			5.3020
Sig.		.294	.050	.065

Means for groups in homogeneous subsets are displayed.

Ni

Duncan

Sample codes	N	Subset for alpha = 0.05		
		1	2	3
AV 8 C	5	.0000		
AB 9C	5	.0100		
AV 4 C	5	.0100		
PTR6C	5	2.0820	2.0820	
AB 7C	5	2.1080	2.1080	
AB2 C	5		3.7820	3.7820
AB 3C	5		3.7820	3.7820
AV 10C	5			4.9380
ApH5C	5			5.6300
Sig.		.113	.191	.155

Means for groups in homogeneous subsets are displayed.

Hg

Duncan

Sample codes	N	Subset for alpha = 0.05	
		1	2
AV 8 C	5	.0080	
AB2 C	5	.0100	
AB 3C	5	.0100	
AB 7C	5	.0100	
AB 9C	5	.0100	
AV 10C	5	.0100	
ApH5C	5	.0100	
PTR6C	5	.0100	
AV 4 C	5		55.6120
Sig.		1.000	1.000

Means for groups in homogeneous subsets are displayed.

Na

Duncan

Sample codes	N	Subset for alpha = 0.05		
		1	2	3
AV 4 C	5	1.0570E2		
AV 8 C	5	1.0642E2		
AB 9C	5	1.2476E2		
AB2 C	5	1.8290E2		
AB 3C	5	1.8290E2		
AB 7C	5		4.4135E2	
PTR6C	5		5.4190E2	
AV 10C	5			8.3125E2
ApH5C	5			8.9272E2
Sig.		.549	.379	.589

Means for groups in homogeneous subsets are displayed.

Mn

Duncan

Sample	N	Subset for alpha = 0.05		
		1	2	3
AV 10C	5	13.5180		
AV 8 C	5	14.8240		
PTR6C	5	15.7540		
ApH5C	5	16.5360		
AB 9C	5	24.3020	24.3020	
AB2 C	5	35.0980	35.0980	35.0980
AB 3C	5	35.0980	35.0980	35.0980
AB 7C	5		45.9000	45.9000
AV 4 C	5			53.3820
Sig.		.082	.067	.120

Means for groups in homogeneous subsets are displayed.

APPENDIX X

LABORATORIES USED

1. Quality Control Laboratory, Technology Incubation Centre, Nnewi, PMB 5081 Nnewi, Anambra State.
2. NAFDAC Zonal Complex Laboratory, Agulu, Anambra State
3. Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos
4. Life Breweries PLC, Onitsha, Anambra State.
5. National Research Institute for Chemical Technology (NARICT) Zaria.
6. Anal Concept Ltd, Flat 6, Royal Estate, Plot 18 Oroigwe Road, By Pipeline, PH, River State.
7. Springboard Research Laboratory, I-7 Udoka Housing Estate, Awka, Anambra State.