



Determination of Mineral uptake and Heavy Metal Filtration of Machinga Forest *Cantherellus cibarius*

James Majamanda^{1,3*}, Rita Kumwenda², Placid Mpeketula¹, Lydia Umali², Chifundo Juta¹ and Joel B Njewa⁴

¹Department of Biological Sciences, Faculty of Science, Chancellor College, University of Malawi, Malawi

²Department of Human Ecology, Faculty of Science, Chancellor College, University of Malawi, Malawi

³Department of Biological Sciences, Faculty of Science, Domasi College of Education, Malawi

⁴Department of Chemistry, Faculty of Science, University of Malawi, Chancellor College, Malawi

*Corresponding author: James Majamanda, University of Malawi, Chancellor College, Faculty of Science, Department of Biological Sciences, Zomba, Malawi

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Abstract

The aim of the study was to measure the average mineral concentration of Ca, P, Se and as in a mushroom known as *C. cibarius* together with soil on which the mushroom grow so as to determine the bio-absorption of mineral elements as well as the filtration capacity of heavy metals. The samples were collected from Machinga forest and all the analyses were done in the laboratory at the University of Malawi. The mineral contents were determined by employing Atomic Absorption Spectrometer; other parameters that were also analysed are pH and also moisture content of the mushroom. Results show that there is more potassium in *C. cibarius* compared to calcium. There is less As in *C. cibarius* compared to the soil and also there was selenium in the soil but no selenium in the mushroom. *C. cibarius* is able to filter heavy metals (selenium and arsenic).

Introduction

Mushroom is a macro fungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eyes and to be picked by hand. Fungi lack the most important feature of plants that is the ability to use energy from the sun directly through chlorophyll. Thus, fungi depend on other organisms for food, absorbing nutrients from the organic material in which they live. The fungal organism which produces the mushrooms is called a mycelium [1]. It is composed of hyphae. The hypha starts its development from a spore. At certain temperature and humidity conditions, it increases its volume, the spore wall tears and from the inside emerges the hyphal tip. This is the beginning of the mycelium formation. Initially the hyphal growth is supported by the nutrients existing in the spore. For its further development however, the fungus needs external sources of nutrients [2]. Mushrooms are of high importance to both humans and the

environment. To humans they provide nutrients, proteins as well as vitamins. Mushrooms are important in the ecosystem because they are able to biodegrade the substrate and therefore use the wastes of agricultural production. Mushrooms are also involved in Myco-remediation which is a process of using fungi to return an environment (usually soil) contaminated by pollutants to a less contaminated state. Myco-remediation also help in removing heavy metals from the land by channeling them to the fruiting bodies for removal [3]. Several studies have been done on mushroom but not on *Cantherellus cibarius* in particular.

There are several species of mushroom, some are edible and some are not edible. The following are some of the edible mushroom species; *Pleurotus ostreatus*, *Cantharellus cibarius*, *Morchella esculenta*, *Lentinula edodes* and *Agaricus campestris*. The chemical composition of the edible mushrooms determine their nutritive value as well as their sensory characteristics [1]. Mushrooms may

be able to impact on cardiovascular disease risk through their ability to reduce blood cholesterol levels. The results of numerous studies indicate that mushrooms are a valuable source of lovastatin, which suppresses the activity of the main cholesterol synthesis enzyme, hydroxyl methyl glutaryl CoA reductase (HMG CoA reductase), and thus has a hypocholesterolaemic effect [4]. Another health benefit of mushroom is that they help in weight control. Mushrooms are low in energy and low in fat, for instance 80g serving provides only 10 kcals and 0.4 g of fat. In addition, mushroom is high in water content (over 90 percent) hence can contribute to a feeling of fullness, and low energy (calorie) density can help to promote weight maintenance [5]. Living organisms have evolved several mechanisms to respond to the toxic effects of heavy metals. One of the most common is the induction of metallothioneins after the uptake of such metals into the cell. The biological functions of copper-metallothioneins, which is a low molecular weight protein that contains a large amount of cysteine residue and binds heavy metal ions, have been investigated. These metallothioneins are assumed to play an important role in both metal storage and detoxification. When *Neurospora crassa* was cultivated in the presence of cadmium and mercury, metallothioneins were also found [6].

Mushroom is also associated with essential elements for human consumption like iron, potassium, sodium, phosphorus and many more. This mainly defers depending on the type of soil they mushroom grow on. This is so because different soils are associated with different elements depending on rock materials available and the level of weathering due to climatic factors. At the same time, the elements differ depending on the specie type [5]. On the other hand, mushrooms are also associated with heavy metals which might be harmful to the health of humans when consumed. Other mushrooms are poisonous, hence these negative and positive impact of mushroom on human makes mushroom a good area of study. This study was therefore aimed at determination of Mineral uptake and Heavy Metal Filtration by Machinga Forest *Cantherellus cibarius*.

Materials and Methods

Sample Collection

The samples were collected during the rainy season in Machinga forest. This is so because thus when and where different kinds of mushrooms were found and *Cantherellus cibarius* is was likely found there. The mushroom samples were picked up by hands from different areas in the forest. In every 10cm depth, a sample of soil on which the mushroom grew was also collected ready for analysis. The GPS code of the area was also recorded.

Sample Drying

Mass of Aluminum Foil was measured and recorded, the mass of the aluminum foil and samples was also measured and recorded. Then they were put- in an oven drier at 60 degrees Celsius. The samples were weighed together with their foil up until a constant

mass was reached for all the samples. The samples were weighed using a well calibrated balance and the mass of the samples were recorded.

Determination of pH

The pH of the soil samples was measured using a pH meter. 10 grams of the woven dried soil samples was weighed and put in containers. 30 ml of deionized water was added to the containers and the mixture was shaken well. Then the samples were given 30 minutes of settling before getting the reading. After the 30 minutes, the samples were shaken whilst getting the reading using a pH meter and results were recorded.

Elemental Analysis

Ashing: Firstly, the crucibles were pre-heated in the furnace for 5 minutes at 550 degrees Celsius so as to remove any moisture in the crucibles. The crucibles were then weighed and their mass was recorded. One gram of each of the dried samples (both mushroom and the soil) were weighed together with their crucibles and the mass was recorded. After that, the samples were put in the furnace and were heated for 5 hours at a 550 degree Celsius. After the five hours, the samples were left to cool just for a short time before they start absorbing any moisture. The mass of both the crucible and ash were recorded and the weight of the ash was calculated. The samples were then taken for digestion. During digestion process, 10 ml of 55% Nitric Acid was put into the beakers where the ash was put then heated by using hot plates at 100 degrees Celsius until 3 to 4 ml of the Nitric acid evaporated. Then 10 ml of 32% Hydrochloric acid was put added into the beakers and heated until 3 to 4 ml of the HCL (mixture) evaporated. Then the digest was filtered by using filter papers then it was put into a 100ml conical flask. Then distilled water was added until it reached 100 ml. The samples were then ready for running process.

Elemental Detection: The mineral contents were determined by employing Atomic Absorption Spectrometer (AAS), with air-acetylene burner and Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) with Argon plasma. Aliquot of the ash solution was aspirated to the instrument (AAS/ICP-AES) for the determination of metals and minerals namely; Ca, K, Se and Ars. Calibration of AAS was also done using the working standards prepared from commercially available metal/mineral standard solutions with most appropriate wavelength, hallow cathode lamp current, gas mixture low rate, slit width, and other AAS instrument parameters for metals/minerals were selected as given in the instrument user's manual, and background correction was used during determination of metals/minerals. Measurements were made within the linear range of working standards used for calibration.

Statistical Analysis

In this research statistical approaches were used to organize and analyze the data. Data was analysed in Microsoft excel and

presented in tables, bar graphs. Means were used in the analysis of this data.

Results and Discussions

According to Figure 1, the results on pH showed that there was

coloration between the layer of the soil and the position. Top layer had almost a neutral pH (7) while acidic pH (< 7) was discovered in the bottom soils. There is a decrease in pH with depth. When the soil pH is homogenous, the concentrations of the selected mineral elements are supposed to be the same [7].

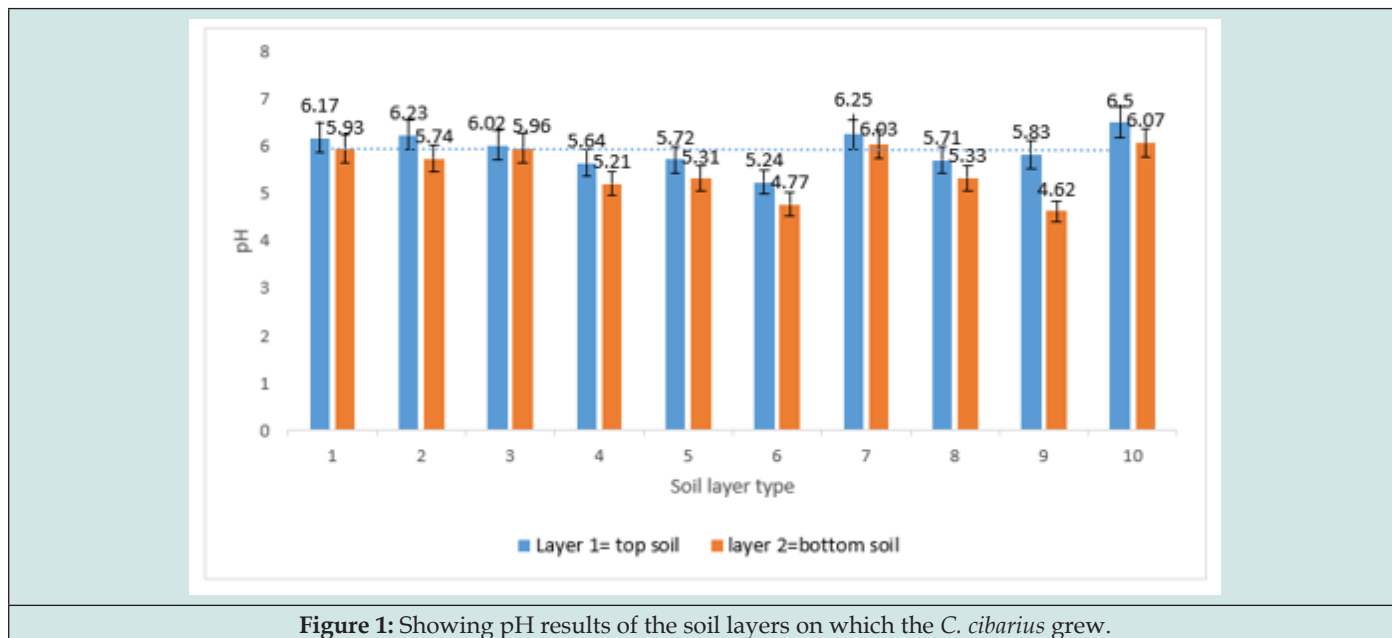


Figure 1: Showing pH results of the soil layers on which the *C. cibarius* grew.

Table 1 show that the average concentration of Arsenic in the mushroom (*C. cibarius*) is 0.24mg/Kg. A study that mainly focused on the total contents of Arsenic and associated health risks in edible mushrooms, mushroom supplements and growth substrates found that the wild mushroom had almost the same level of Arsenic as in our study [8]. It is of high importance to take note of concentration of Arsenic in the mushroom since it is a heavy metal and it is toxic if consumed in high amounts. Table 1 also shows that the average concentration of Calcium is 0.6 mg/Kg. According to a similar study that was conducted in India on the nutrient content of edible mushrooms, *Agaricus bisporus* and *Pleurotus ostreatus*, Calcium had least values in relation to the rest of the other trace elements hence having the same trend as in *C. Cibarius* [9]. Calcium plays a fundamental role in controlling functions in the body. Cereal grains, such as wheat (*Triticum aestivum*), finger millet (*Eleusine coracana*), and tea (*Eragrostis tef*), are relatively high in calcium. The best sources of calcium are dairy products and calcium-fortified beverages. Calcium can also be readily found in dark-green leafy vegetables [10].

One of the studies took place in south India, the aim was to measure the nutrient and trace minerals concentrations in two parts of the mushroom species, *Agaricus bisporus* and *Pleurotus ostreatus*. The pileus and stipes of the mushrooms were separated and analyzed individually. In the study, it was discovered that the

total lipid content of fresh and dry weight basis of *Agaricus bisporus* were highly significant when compared with *Pleurotus ostreatus*. The minerals like nitrogen, calcium, copper, iron, inorganic phosphorus, magnesium, manganese, nickel, phosphorus, silicon and titanium were found to be higher in pileus than stipes, while alumina, lead, potassium, sodium, selenium and zinc were found higher in stipes than pileus [9]. This indicate that mineral composition in parts of mushroom are differently concentrated. This would be very important to those that are nutritional conscious. It is informative on the diversity of nutrients and trace elements in two parts of mushroom [9]. The study also did not focus on the overall nutrient content of the two species of the mushroom.

Table 1 also shows the high average concentration of potassium in the mushroom (347.16 mg/Kg). According to a research of similar study that took place in Brazil on the analysis of essential elements in edible mushroom by Neutron Activation, the results showed highest value of potassium in [11]. Another study took place in Korea, this study aimed at determining mineral components in the cultivation substrates of edible mushrooms and their uptake into fruiting bodies, the results of potassium also showed high values of potassium in the mushroom similar to present study on *C. cibarius* [12]. The high bioavailability of potassium in the mushroom might be due to solubility of the mineral but also absorption capacity by mushroom variety.

Table 1: Showing Mineral bio-availability concentration in *C. cibarius* (mg/Kg).

Mineral type	Bio-concentration in <i>C. cibarius</i>
As	0.24
Se	0.00
Ca	2.46
K	347.16

According to Table 2 the results of Arsenic in the soil show that the average concentration was 3.54mg/Kg, indicating that *C. cibarius* was able to filter this high amount to 0.24mg/Kg (Table 2). This is also similar to the trend of in selenium. Mushroom absorbed more of non-heavy metal minerals from soil compared to the heavy metals. According to results of a similar study that took place in Korea on the determination of mineral components of the cultivation of substrates of edible mushrooms and their uptake into Fruiting bodies, the results showed high concentration of calcium in the substrates compared to the mushrooms that were analyzed

[12]. In a study that was done in Japan, *Pleurotus* species strains showed higher resistance to the heavy metals, copper, cadmium, zinc, nickel, cobalt, mercury than the other species. *Pleurotus ostreatus* exhibited the highest resistance to all these heavy metals. *Pholiota species*, *Flammulina vertices*, *Lyophyllum ulmarium*, *Agaricus bisporus* and *Polyporus arcularius* were rather sensitive to all the metals tested. The uptake of heavy metals into the mycelia of *P. ostreatus* increased proportionally to an increasing concentration of these metals in the medium [13]. It can therefore be concluded that not all the mushrooms have the capacity to filter heavy metals.

Table 2: Showing concentration of Mineral elements of the soil on which *C. cibarius* grew (mg/Kg).

Mineral type	Mean Concentration in soils where <i>C. cibarius</i> grew
Ars	3.54
Se	5.60
Ca	2.56
K	348.76

Another study on mushroom took place in Brazil though they are not consumed in large quantities. The study intended to contribute to a better understanding of the essential element content in edible mushrooms, which are currently commercialized in São Paulo state. Br, Fe, K, Na and Zn concentrations were determined by Instrumental Neutron Activation Analysis in the following mushroom species: Shiitake (*Lentinula edodes*), Shimeji (*Pleurotus* spp), Paris Champignon (*Agaricus bisporus*), *Hiratake* (*Pleurotus* spp) and *Eringue* (*Pleurotus Eryngii*). It was discovered that large variability can be observed among mushroom species in relation to their Br, K, Na, Fe and Zn content [14]. The edible mushrooms analyzed presented high K and Zn content, confirming that mushrooms can be considered a good source for these essential elements. The study was significant since it analyzed the essential trace elements. The main gap of the study is that it did not make analysis of the heavy metals like selenium, lead, copper and many more associated with the mushroom, another thing is that the researcher only analyzed five elements but there are several of them. The elements were detected using a Gamma Spectrometry but the same study would have been done by using an Atomic Absorption Spectrometer. The upcoming study will focus much on both the essential metals as well as the heavy metals. This is so because the mushrooms are not only associated with the essential elements but also heavy metals.

A comparative study on selected nutrient and mineral contents of two cultivated and two indigenously grown edible mushrooms was done at Bunda in Malawi. The results showed that, nutrient and mineral content of the two edible indigenous mushrooms namely *Cantharellus cibarius* and *Termitomyces letestui* and two cultivated mushrooms namely *Agaricus bisporus* and *Pleurotus florida*, protein, fat, fiber, calcium, iron and magnesium were significantly different but contained considerable amount of proteins and minerals which are vital in supplementing nutrition to mankind [15]. Another study was on the levels of arsenic (As) in the main commercial species of mushrooms present in Galicia, in their growth substrates, and mushroom supplements have been analyzed by ICP-MS, with the intention of assessing potential health risks involved with their consumption. Samples were analyzed by an ICP-MS spectrometer, Varian 820. The sensitivity of this method was determined according to the detection limits established for this spectrometer, which for arsenic was 20 mg/Kg [8]. The main discoveries were that the data collected for the studied element indicated that the detected levels of arsenic were common in samples that were analyzed in unpolluted areas [16, 17]. Significantly higher concentrations were found in soil samples from the province of La Coruña due to geochemical causes. However, no significant correlations between levels of Arsenic in fungi and their growth substrates were observed which indicated that there was no toxicological risk associated with

regular consumption of the analyzed mushroom species collected in Galicia or with the indicated dosages of mushroom supplements [8].

Conclusion

The mineral uptake of *C. cibarius* depends on the type of element, others are high and others are low as evident in potassium which have a higher mineral uptake compared to Calcium. There was less Arsenic in *C. cibarius* compared to that was in the soil and also the there was almost no Selenium in *C. cibarius* but the soil had these metals providing us with evidence that mushroom *C. cibarius* helps in filtering heavy metals.

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