Content of major, minor and toxic elements of different edible mushrooms grown in Mekelle, Tigray, Northern Ethiopia

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Cogent Food & Agriculture (2019), 5: 1605013
Abstract: Major, minor and toxic elements were determined by flame atomic absorption spectrometry and flame photometry in edible canned, wild and cultivated oyster mushrooms grown in cotton waste, wheat straw and wood waste substrates in Mekelle, Tigray, Ethiopia. Dry ashing using mixtures of HNO₃:H₂SO₄:H₂O₂ (1:1:1 ratio) was used for digestion. The mean concentrations (mg/kg) of the studied elements in the mushroom samples were ranged from 964.66 to 4180.33, 2652.66 to 19918.66, 22.00 to 34.64, 16.00 to 30.38, 34.13 to 621.06, 40.25 to 120.91, 8.40 to 34.33, 4.22 to 30.63, 1.94 to 2.52 and 1.53 to 2.17 for Na, K, Ca, Mg, Fe, Zn, Cu, Mn, Cd and Pb, respectively. There were significant differences (p < 0.05) in the K, Fe, Zn and Mn levels of the mushroom samples which may be attributed to substrate variations. Moreover, significantly high correlations were observed between Na-K, Ca-Cd, Fe-Mn, Zn-Cu, Zn-Pb, Cu-Pb and Cd-Pb. Most of the results of this study were in good agreement with WHO recommended limits. However, the concentrations of Na and Cd in canned mushroom and all elements grown in wood substrate were recorded above the WHO recommended limits. The results of this study are significant for the food industry and can be used as a guideline for dietary recommendation.
study showed that, with the exception of canned and wood waste grown, the mushrooms can be used for human diets because of their acceptable content of many essential and minor elements and low content of toxic metals.

**Subjects:** Environmental Issues; Food Chemistry

**Keywords:** major elements; minor elements; toxic elements; mushroom; Ethiopia

1. Introduction

Nowadays, nutrition is becoming the most important subject for humankind. An adequate nutrition is particularly important from the point of taking in essential nutrients such as minerals, vitamins and high-quality proteins. Mushrooms as food can provide adequate diet for human nutrition as well as medicinal compounds (Çağlarîrmak, 2011). Mushrooms are peculiar in that they are neither plants nor animals (Feeney, Miller, & Roupas, 2014). Mushrooms like all other fungi lack chlorophyll and are non-green organisms. They cannot convert solar energy through the process of photosynthesis to organic matters as green plants do. For their survival, growth and metabolism, they rely on organic matter synthesized by the green plants around including organic products contained in agricultural crop residues (Chang, 2008).

The organic materials on which mushrooms derive their nutrition are referred to as substrates. These substrate materials are usually by-products from industry, households and agriculture and are usually considered as wastes. They are actual resources in the wrong place at a particular time, carelessly disposed of in the surrounding environment by dumping or burning. These disposal methods often lead to environmental pollution and consequently cause health hazards. However, mushroom cultivation can harness this waste or resource for its own beneficial advantage which plays an important role in managing and recycling of organic wastes (Semwal, Lemma, Dhyani, Equar, & Amhare, 2014).

Food security is a deep-rooted problem in Ethiopia in general and in Tigray in particular (van der Veen & Tagel, 2011). However, in Tigray, there is an abundance of agricultural waste products which are normally discarded. Thus, cultivation of mushroom can be used to transform this agricultural waste into a nutritious food and offer great opportunities for addressing the region's food security challenges. Mushroom can be cultivated on a large number of agro-wastes including sawdust (wood waste), straw (grass, cereals), cotton waste, leaves of maize, molasses, coffee waste, banana waste and cattle and horse dung. Thus, choosing the best substrate is the most important step in creating a successful mushroom cultivation program (Gargano et al., 2017).

Edible mushrooms once called the “food of the gods” and still treated as a garnish delicacy can be taken regularly as part of the human diet, being already considered healthy functional foods (Hunt, 2003). Mushrooms have been considered healthy food because they contain high-quality protein which contains all the essential amino acids, as well as vitamins B, B₂, C and D and minerals such as K, Ca, Na, Fe, Zn, Mg, P, and have low fat. These elements play important roles in chemical, biological, biochemical, metabolic and enzymatic reactions in the living cells of plants, animals and human beings (Hallberg, Brune, & Rossander, 1989).

Although mushrooms are used as supportive food sources, to the best of our knowledge, there is no study conducted on the determination of levels of major, minor and toxic elements in mushrooms grown in Mekelle and its surroundings, Tigray, Northern Ethiopia. Thus, the objective of this study was to determine the concentration of some major elements (K, Ca, Na and Mg), minor elements (Fe, Zn, Mn and Cu) and toxic elements (Cd and Pb) in wild, canned and cultivated edible mushrooms at different substrates collected from Mekelle and its surroundings.
2. Materials and methods

2.1. Substrate preparation and cultivation
The study was conducted in Mekelle city and its surroundings (Figure 1). Pure cultures of edible oyster mushroom species were obtained from YB Plants Micro-propagation (YBPM) PLC, Mekelle. The mushroom spawn was already prepared by the PLC. The wheat straw and wood waste substrates were collected from local market and wood workshops in Mekelle, respectively. Cotton waste was obtained from YBPM.

After collecting the substrates, initially all the substrates were chopped to pieces and filled in bowls and then washed with water in order to remove unneeded materials like debris and dust particles. Then, it was soaked in water for about 12 h. Water was then drained off from the substrates. Thereafter, the substrates were soaked in a water bath for 45 min at a temperature of 120°C. The substrate materials were then taken out from the bath and drained of excess moisture applying squeezing by hands. When there was no dripping of solution, the chopped substrate material was considered as ready for spawning (Alam, Amin, & Sarker, 2007).

The wet substrate materials were then spread over a clean alcohol-washed polyethylene plastic sheet. The resulting spawn and substrates were thoroughly mixed with the additives of 40 g CaSO₄ and 200 g maize and then filled into a wooden box (about 3 kg per box) (Abrha, 2014). Following spawning, the wood boxes were kept in a dark place of cropping room. The temperature (25–30°C) and relative humidity (75–85%) of the cropping room were controlled by turning the electrical fluorescence on and off and spraying water to the walls and floors of the room. During cropping and harvesting periods, the mushroom was sprayed with water twice a day. Finally, after the cultivation period of 28 days, the fully matured mushroom grown on the substrates were harvested and collected for further treatment (Figure 2).

Experimental works were carried out at Ezana Analytical Laboratory for Fe, Zn, Cu, Mn, Cd and Pb analyses using flame atomic absorption spectrometry (FAAS) and Tigray Agricultural Research Institute for Na, K, Ca and Mg analyses using flame photometry and titrimetric methods.

Figure 1. Map of the study area: (a) Ethiopia, (b) Tigray and (c) Mekelle.
2.2. Instruments, equipment and chemicals

2.2.1. Instruments and equipment
FAAS (Model: Varian AA240 FS), flame photometer (Model: LT-67) using air acetylene, drying oven with forced air and timer (Model: WW16AN01), Austral analytical balance (FW 100, Yusung Industrial Ltd, China), bottles, volumetric flasks, conical flasks, measuring cylinders, beakers, safety devices, burettes with stands, crucibles, evaporating dishes, sample vials, mortar with pestle and mesh were used during the entire study.

2.2.2. Chemicals
The chemicals and reagents used were concentrated HNO$_3$ (98%, SD Fine Chem Limited, Mumbai, India), concentrated H$_2$SO$_4$ (98%, RFCL Limited, New Delhi, India), H$_2$O$_2$ (30%, SD Fine Chem Limited, Mumbai, India), EDTA (Ava Chemicals Private Limited, Mumbai, Maharashtra, India), Eriochrome Black T indicator (UNI SOURCE Chem Limited, Mumbai, India), and CaSO$_4$ (Uthaya Chemicals, Chennai, Tamil Nadu, India). The reagents used for the analysis were analytical grades, and double-distilled water was used throughout the study.

Stock standard solution of concentration 1,000 mg/L in 2% HNO$_3$ of the metals Na, K, Ca, Mg, Fe, Zn, Cu, Mn, Pb and Cd (Industrial Analytical (pty) Ltd, Johannesburg, South Africa) were used to prepare intermediate standard solutions. The working standard solutions of each metal were prepared by diluting the intermediate standard solution (i.e., 10 ppm). Then, the calibration curves were prepared by running series of working standards to determine the concentration of each element in sample solutions.

2.3. Mushroom cultivation and collection
The oyster mushroom cultivation was carried out in Daero Academy, Mekelle. The oyster mushrooms were grown in three different substrates—wood, cotton and wheat straw substrates—followed by collecting the matured mushroom for analysis. The wild and canned mushrooms were collected from Chelecot area, 17 km south of Mekelle and supermarkets found in Mekelle city, respectively. Identification of the wild edible and oyster mushrooms was made by making comparisons with authentic illustrations. Moreover, confirmations of the wild mushrooms were made by biotechnology experts at YB Plant Micro-propagation PLC.

2.4. Sample preparation and storage
The mushroom samples were cleaned out of forests debris with a plastic knife and sliced (cut) without separating the cap and the stipe of the mushroom. Then, they were dried in an oven at 60°C for 4-6 h. The dried samples were milled to a fine powder using a mill and homogenized using...
a gate homogenizer and were kept in precleaned plastic bottles until analysis (Chen, Zhou, & Qiu, 2009; Woldegiorgis, Abate, Haki, & Ziegler, 2015).

2.5. Analytical procedures

2.5.1. Digestion of mushroom samples

To minimize the risk of contamination, glasswares were washed with 10% HNO₃ and crucibles were soaked with 6 N HCl for 24 h after being washed with detergent and water. All materials were then rinsed with distilled water and dried in an oven before use.

Digestion of mushroom samples was carried out by the dry ashing method adopted from Chen et al. (2009) and Woldegiorgis et al. (2015). First, 2 g samples were placed in a porcelain crucible and ashed in an oven at 450°C for 24 h. Then, ashed mushrooms were dissolved in 2 mL of concentrated HNO₃ and evaporated to dryness and heated again at 450°C for 4 h until white residue was obtained. The residues were then dissolved in 2 mL portions of each concentrated H₂SO₄, concentrated HNO₃ and H₂O₂. Finally, the resulting solutions were transferred into 50 mL volumetric flask and made up to volume with distilled water. Similarly, a blank digestion was also carried out in the same way.

2.5.2. Analysis of minerals

Analysis of minerals was conducted at Ezana Analytical Laboratory by using fully automated computer-controlled FAAS by setting the appropriate wavelength for each element. All the concentrations of minor and toxic element determinations were carried out in an air/acetylene flame mode of the spectrophotometer. The levels of Na and K elements in the mushroom samples were determined by microprocessor flame photometer using air/acetylene flame, and the levels of Ca and Mg were also determined using titration method (Isildak, Turkekul, Elmastas, & Aboul-Enein, 2007; Uzun, Genccelep, Kaya, & Akcay, 2011) at Tigray Agricultural Research Institute. All measurements were made in triplicates. The instrument working conditions for the FAAS is given in Table 1.

2.5.3. Validation of the analytical method

For the determination of limit of detection of the analytical method (LOD), 10 reagent blanks were prepared in parallel and analyzed for their metal contents. The standard deviation (SD) of the 10 blanks (Sᵇ) was calculated and multiplied by three (i.e. LOD = 3*Sᵇ) to determine the method detection limit. Calibration curve for each element was constructed using an appropriate standard at a series of concentrations. Regression equation for each metal was constructed, and the best fit of the equation was checked using correlation coefficient (R²) (Woldegiorgis et al., 2015).

The limit of quantification, LOQ, was also obtained from triplicate analyses of the blanks both for the mushroom and substrate samples, which were digested following the same procedures. The LOQ was then calculated using the relation; LOQ = 10*Sᵇ, n = 3.

| Table 1. Instrumental working conditions for FAAS |
|---------------------------------|
| Element | Lamp current (mA) | Flame type | Slit width (nm) | Wave length (nm) |
| Fe      | 5                 | Air/acetylene | 0.2           | 248.3 |
| Zn      | 5                 | Air/acetylene | 1             | 213.9 |
| Cu      | 4                 | Air/acetylene | 0.5           | 324.7 |
| Mn      | 5                 | Air/acetylene | 0.2           | 275.9 |
| Cd      | 4                 | Air/acetylene | 0.5           | 228.8 |
| Pb      | 5                 | Air/acetylene | 1             | 217.0 |
Recovery of the analytical method is one of the most commonly used techniques for validation of analytical results. It is the technique used for evaluating how far the analytical method is acceptable for its proposed purpose. Due to the absence of certified reference material (CRM) for the edible mushrooms in the laboratory, the validity of the digestion procedure was assured by spiking the sample with standard solutions of known concentrations of the analyte elements. Each recovery test for the sample was performed in triplicates. Standard metals solutions were used to fortify the sample to the specified metal, and the percentage recovery was calculated using the following equation:

\[
\text{% recovery} = \left( \frac{\text{Amount after spike} - \text{Amount before spike}}{\text{amount added}} \right) \times 100
\]

2.6. Statistical analysis
All experimental results were presented as mean ± SD of the three replicate measurements. The one-way analysis of variance (ANOVA) was calculated using the QI Macros Microsoft Excel Tool to evaluate the significance of the difference in mean concentration of each metal between different kinds of mushroom samples. A linear regression correlations test ($R^2$) was also performed to investigate correlations between metal concentration (Szenczi-Cseh, Horváth, & Ambrus, 2017).

3. Results and discussion

3.1. Validation of the analytical method
Values of correlation coefficient for each element studied are presented in Table 2. In all the cases, the regression coefficient ($R^2$) was found to be above the accepted linear range value of 0.96. Similarly, each recovery test for the samples performed in triplicates using the spiked mushroom samples was also obtained to be between 80% and 99.88% (Table 2). Thus, the mean percentage recoveries for all analytes were within an acceptable range (75–125%) (Flores-Magdaleno, Mancilla-Villa, Mejia-Saenz, Olmedo-Bolantilde, & Bautista-Olivas, 2011). The method detection limit (MDL) values for Fe, Zn, Cu, Mn, Cd, Pb, Na and K elements are given in Table 2. All the metals detected in this study from the mushroom samples were above detection limits. Besides, as it is shown in Tables 3, 4 and 5, the relative standard deviations (RSD%) were less than 10% for all elements and indicated that precise measurements were obtained using the intended intruments. Therefore, the laboratory performance for each analyte was under control.

3.2. Concentration of major elements in the mushroom samples
One of the groups of essential elements is the macroelements occurring in relatively large amounts such as Na, Ca, K and Mg. The concentration result of the major elements determined in five different mushroom samples is summarized in Table 3.

| Table 2. Correlation coefficient ($R^2$) of calibration curves; IDL, MDL, LOQ and % recovery values |
|---------------------------------------------|
| Element | $R^2$ | IDL (mg/L) | MDL (mg/L) | LOQ (mg/L) | % Recovery |
|---------|-------|------------|------------|------------|------------|
| Fe      | 0.9974| 0.006      | 0.050      | 0.169      | 99.23      |
| Zn      | 0.9770| 0.001      | 0.014      | 0.047      | 95.90      |
| Cu      | 0.9989| 0.003      | 0.024      | 0.081      | 85.43      |
| Mn      | 0.9989| 0.002      | 0.014      | 0.047      | 98.34      |
| Cd      | 0.9918| 0.002      | 0.014      | 0.047      | 80.0       |
| Pb      | 0.9988| 0.010      | 0.024      | 0.081      | 83.0       |
| Na      | 0.9987| 0.50       | 3.00       | 10.00      | 99.88      |
| K       | 0.9984| 0.50       | 0.883      | 2.943      | 99.97      |
3.2.1. Sodium
The concentration of Na in the five mushroom samples ranged from 964.66 (wheat straw) to 4,180.33 mg/kg (canned). Sodium detected in canned mushroom samples was relatively high in comparison to the other mushroom samples (Table 3). The highest concentration of Na in canned mushroom may be attributed to the spices which are widely consumed for adding flavors and/or other additives such as sodium nitrite which is used as food preservative during canning. These additives generally provide sources of some important minerals to such canned foodstuffs (Szenczi-Cseh et al., 2017). This variation was also supported by the result of one-way ANOVA, indicating significant differences at p < 0.05. Similar results were also reported by Shah et al. (2009). In addition, the levels of sodium obtained in this study are also in agreement with other findings reported from Ethiopia by Woldegiorgis et al. (2015) which was reported in the ranges of 410–34,800 mg/kg and Mallikarjuna et al. (2013) in the ranges of 220.2–3,270.4 mg/kg. But Uzan et al. (2011) reported lower values of sodium in the ranges of 90.00–601.00 mg/kg.

The WHO permissible limit of Na in food is 300–1,340 mg/kg (FAO/WHO, 2001), which is in agreement for most mushroom samples studied. However, the concentration of sodium in canned mushroom was higher than the WHO recommended limit. As excess sodium intake is linked with high blood pressure and heart disease, great attention should be given to the consumption of canned mushrooms which could be considered as toxicological risk to human beings (Severoglu, Sumer, Yalcin, Leblebici, & Aksoy, 2013).

3.2.2. Potassium
The potassium content of the mushrooms studied in the present work ranged from 2,652.66 mg/kg (canned) to 19,918.66 mg/kg (wood waste). In the findings of this study, higher concentrations of potassium were recorded in comparison to the other nutrients studied which could be due to the high content of potassium in agro-waste substrate used and its high accumulation in mushroom (Wang, Zhang, Li, Wang, & Liu, 2015). The concentration trend of potassium in the studied mushrooms is recorded in the order of wood waste > wheat straw > cotton waste > wild > canned. One-way ANOVA also indicated that all mean concentrations of K obtained for the five mushroom samples were significantly different at p < 0.05. The observed variation in the K levels of the studied mushrooms may be due to the type of mushrooms and the substrate in which they were grown (Kalač, 2013).

The levels of potassium obtained in the present study were in agreement with reports from other studies such as Uzun et al. (2011) 5,950–29,230, Mallikarjuna et al. (2013) 590.30–36,340.00 and

| Mushroom type (substrate) | Na (mg/kg) RSD% | K (mg/kg) RSD% | Ca (mg/kg) RSD% | Mg (mg/kg) RSD% |
|---------------------------|-----------------|----------------|-----------------|-----------------|
| Wild                      | 994.66 ± 1.88; 0.19 | 13,486.00 ± 9.20; 0.07 | 22.00 ± 0.82; 3.735 | 16.00 ± 0.81; 5.06 |
| Canned                    | 4,180.33 ± 0.57; 0.01 | 2,652.66 ± 3.28; 0.12 | 24.00 ± 1.63; 6.79 | 29.76 ± 0.74; 2.45 |
| Cotton waste              | 1,066.66 ± 0.57; 0.05 | 16,161.00 ± 2.16; 0.01 | 30.50 ± 0.89; 2.92 | 24.26 ± 1.88; 7.75 |
| Wheat straw               | 964.66 ± 8.01; 0.83 | 17,502.00 ± 3.55; 0.02 | 26.12 ± 0.78; 2.97 | 19.06 ± 1.62; 8.50 |
| Wood waste                | 1,136.33 ± 2.86; 0.25 | 19,918.66 ± 104.09; 0.52 | 34.64 ± 1.35; 3.90 | 30.38 ± 1.50; 4.94 |
| FAO/WHO (2001)            | 300.0–1,340.0 | 190.0–5020.0 | 190.0–881.0 | 45.0–4520.0 |
Woldegiorgis et al. (2015) 3,660–42,400 mg/kg. Except for the canned mushroom samples, the overall data indicated elevated levels of K in the studied mushroom samples. This suggests that the mushrooms would be excellent in lowering blood pressure, reducing loss of appetite and maintaining bone health (Lott, Ockenden, Raboy, & Batten, 2000). However, control of the daily dietary intake of K requires due attention since the levels reported here exceeded the FAO/WHO specified values of 190–5,020 mg/kg.

3.2.3. Calcium
The maximum and minimum calcium concentrations determined in the mushroom samples were 34.64 and 22.00 mg/kg, respectively, which was generally lower than the permissible level set by FAO/WHO (2001), i.e., 8,810 mg/kg. The concentration of calcium in the studied mushrooms decreased in the trend of wood waste > cotton waste > wheat straw > canned > wild.

Generally, higher concentration of calcium was obtained in the cultivated oyster mushroom compared to the wild and canned mushrooms. This is likely to have resulted from the addition of CaSO\(_4\) to the substrate during the preparation stage. Nevertheless, the Ca concentrations in the mushroom samples were within the WHO safe limits for human intake (FAO/WHO, 2001) and pose no potential health risk (Zeleke, 2009). Levels of calcium determined in the present study were lower than reported values by different scholars such as Uzun et al. (2011) 40–5720, Mallikarjuna et al. (2013) 80.27–1740.9 and Woldegiorgis et al. (2015) 290–6450 mg/kg, respectively. Similarly, significant differences were observed in the level of Ca among the mushrooms cultivated in three different substrates (p < 0.05), which might be associated with the differences in chemical composition of the substrates.

3.2.4. Magnesium
The concentration of magnesium was highest in mushroom which was grown in wood substrate (30.38 mg/kg) and found lowest in wild mushroom (16.00 mg/kg). The concentration of Mg in the entire mushroom samples analyzed were below the WHO permissible limit value of 45–4,520 mg/kg (FAO/WHO, 2001). Thus, consuming mushroom as food source for humans provides the essential nutrient for bone and teeth (Freedman, Guenther, Dodd, Krebs-Smith, & Midthune, 2010). But, the levels of Mg obtained in the present study were less than the results reported by other scholars. For instance, magnesium concentration of mushroom samples have been reported in the ranges of 180–1,930 mg/kg in Turkey (Uzun et al., 2011), 210.10–400.70 mg/kg in India (Mallikarjuna et al., 2013) and 570–2120 mg/kg in Ethiopia (Woldegiorgis et al., 2015). The result of one-way ANOVA also showed that there were no significant differences for Mg among the mushroom samples studied, except the mushroom cultivated in the three different substrates. The variation could be attributed to species of mushroom, age of fruiting bodies, general climatic conditions, pharmaceuticals and the substrate influence on the nutrient composition of mushrooms (Kula, Solak, Uğurlu, İşiloğlu, & Arslan, 2011).

3.3. Concentration of minor elements in mushroom samples
Minerals occurring in small amounts and needed in a few milligrams or less per day are micro or minor elements which include Fe, Zn, Cu, Mn and Cr. The results of minor elements analyzed in this study are also summarized in Table 4.

3.3.1. Iron
The minimum and maximum values (mg/kg) of iron in the mushrooms analyzed were 34.13 for cotton waste substrate and 621.06 for wild. The high iron content of the wild mushroom could be due to the soil contribution to the iron content of the mushroom (Hunt, 2003). Next to the wild mushroom, the higher concentration of iron was detected in the canned mushroom (Table 4) which may be attributed to the use of iron and its alloys as food storage materials, the use of its organic and inorganic compounds as food additives and/or corrosion of the container upon long-term storage. The statistical analysis using one-way ANOVA also confirmed that all the mean
concentrations of Fe for the five different mushroom samples were significantly different at $p < 0.05$. The variations could be associated to the difference in the type of substrate used for cultivation.

Similarly, the levels of iron for most of the mushrooms studied in this present study were in agreement with the results reported by Uzun et al. (2011) $5.00–1,925.00$; Lalotra, Gupta, Yangdol, Sharma, and Gupta (2016) $118–1,411$ and Woldegiorgis et al. (2015) $32.50–6,835.90$ mg/kg, respectively. The levels of Fe recorded in cotton waste- and wheat waste-grown mushrooms were below the WHO permissible levels (Codex Alimentarius, 2015). Consumption of these mushrooms could, therefore, serve as a very good source of iron supplementation particularly in low-income countries where iron deficiency (e.g., anemia) is a serious health problem. However, the daily dietary intake should be monitored carefully with regard to consumption of the canned, wild and wood waste-grown mushrooms which contained levels of Fe much higher than the WHO permissible limits.

3.3.2. Zinc
In the present study, the levels of Zn in the different mushroom samples were from 40.25 to 120.91 mg/kg. The maximum concentration of Zn was recorded in wood substrate, while minimum concentration was obtained in the canned sample. The WHO permissible limit for Zn in foods is 60 mg/kg (Codex Alimentarius, 2015). Except for the mushrooms grown in wood and wheat substrates, all other mushrooms analyzed were in agreement with the WHO guideline values. Thus, consumption of the wood- and wheat substrate-grown mushrooms needs due attention and control of the daily dietary intake of Zn.

The statistical analysis using one-way ANOVA also showed that all the mean concentrations of Zn in the five different mushroom samples were significantly different at $p < 0.05$. This could possibly be due to differences in substrate composition among other factors (Kula et al., 2011). The results of Zn obtained in this study were also comparable with findings of other researchers such as Soylak et al. (2004) reported 15.00–450.00 mg/kg for mushroom obtained from Turkey, Lalotra et al. (2016) in India with zinc content of 87–299 mg/kg and Woldegiorgis et al. (2015) in Ethiopia with a value of 26.6–87.6 mg/kg.

3.3.3. Copper
The concentrations of copper recorded in the analyzed mushroom samples ranged from 8.40 in cotton waste substrate to 34.33 mg/kg in wood waste substrate (Table 4). The values for Cu obtained in this study were below the permissible limit of 73 mg/kg recommended by WHO.

| Mushroom type (substrate) | Fe (mg/kg) | Zn (mg/kg) | Cu (mg/kg) | Mn (mg/kg) |
|---------------------------|------------|------------|------------|------------|
| Wild                      | 621.06 ± 5.96; 0.96 | 47.9 ± 0.27; 0.56 | 18.67 ± 0.24; 1.29 | 30.63 ± 0.40; 1.31 |
| Canned                    | 138.56 ± 2.77; 1.44 | 40.25 ± 0.82; 2.04 | 18.39 ± 0.08; 0.44 | 4.22 ± 0.08; 1.90 |
| Cotton waste              | 34.13 ± 0.49; 1.44 | 45.99 ± 0.64; 1.39 | 8.40 ± 0.03; 0.36 | 7.92 ± 0.20; 2.53 |
| Wheat straw               | 48.96 ± 0.66; 1.35 | 94.25 ± 0.58; 0.62 | 27.91 ± 0.11; 0.39 | 10.86 ± 0.14; 1.29 |
| Wood waste                | 87.50 ± 1.91; 2.18 | 120.91 ± 0.92; 0.76 | 34.33 ± 0.12; 0.35 | 16.13 ± 0.03; 1.9 |
| Codex Alimentarius (2015) | 10.0–56.0 | 60.0 | 73.0 | 100.0 |

Table 4. Concentrations of some minor elements (mean±SD, mg/kg) in mushroom samples and RSD%; means followed by the same letter in the same column are not significantly different at $p < 0.05$.
(Codex Alimentarius, 2015). Copper concentration of edible mushroom samples in literature has been reported to be 5.00–83.00 mg/kg (Uzun et al., 2011), 83.60–290.00 mg/kg (Lalotra et al., 2016) and 5.69–45.90 mg/kg (Woldegiorgis et al., 2015), respectively, which are in good agreement with the results reported by Uzun et al. (2011) and Woldegiorgis et al. (2015) but below the values reported by Sharma et al. (2016). This variation could be due to differences in the substrate, the geographical location and types of mushroom species (Kula et al., 2011).

3.3.4. Manganese
The WHO acceptable limit for human consumption of manganese is 100 mg/kg (Codex Alimentarius, 2015). The present analysis revealed that the concentration of Mn varied from 4.22 mg/kg in canned to 30.63 mg/kg in wild mushroom, which lies below the safety limit of Mn set by WHO. Thus, eating mushroom as food can supplement the deficiency of manganese which may affect brain health, normal reproduction, skeletal and cartilage formation (Valko, Morris, & Cronin, 2005).

In some of the previous studies found in the literature, the concentration of Mn was reported to be 0.2–80 mg/kg (Uzun et al., 2011), 25.5–118 mg/kg (Lalotra et al., 2016) and 0.96–138.6 mg/kg (Woldegiorgis et al., 2015), indicating that the manganese content determined in this work is also comparable with that of Uzun et al. (2011) and Woldegiorgis et al. (2015). However, it was below the values reported by Lalotra et al. (2016). Similar to Cu, the variations between the results of this study and other literature values of Mn could be due to differences in sample size, soil type, genetic variation and environmental factors. The statistical analysis using one-way ANOVA also showed that there was a significant difference in the levels of Mn determined in the five different mushroom samples at p < 0.05.

3.4. Concentration of some toxic elements in mushroom samples
The levels of toxic elements analyzed in the samples of mushroom are presented in Table 5.

3.4.1. Cadmium
From this study, it was observed that the concentration of cadmium in mushroom samples ranged from 1.94 to 2.52 mg/kg. The highest concentration of Cd was recorded in mushroom cultivated at wood waste substrate and the lowest in mushroom grown at wheat straw substrate. The highest levels of Cd found in the mushrooms grown in wood substrate could be due to the geological nature of the site where the wood substrate used for mushroom cultivation was grown, weathering of Cd and phosphate-containing rocks, and the use of phosphates fertilizers such as monoammonium phosphate (MAP) and diammonium phosphate (DAP) in the field (Roberts, 2014). Some other literature also reported the values of Cd in the ranges of 0.03–19.00 (Uzun et al., 2011), 7.9.00–12.20 (Meghalatha, Ashok, Nataraja, & Krishnappa, 2014) and 0.0–4.08 mg/kg (Woldegiorgis et al., 2015), respectively. Thus, the Cd levels of this study are in agreement with those of literature values, but with lower concentration ranges. In addition, the values for Cd in the

| Mushroom type (Substrate) | Cd (mg/kg); RSD% | Pb (mg/kg); RSD% |
|---------------------------|-----------------|-----------------|
| Wild                      | 1.98 ± 0.04; 2.02 | 1.53 ± 0.12; 7.84 |
| Canned                    | 2.08 ± 0.02; 0.96 | 1.60 ± 0.60; 9.34 |
| Cotton waste              | 2.08 ± 0.03; 1.44 | 1.56 ± 0.05; 3.21 |
| Wheat straw               | 1.94 ± 0.00; 0.00 | 1.72 ± 0.15; 8.72 |
| Wood waste                | 2.52 ± 0.06; 2.38 | 2.17 ± 0.16; 7.37 |
| WHO (2011)                | 2.0             | 5.0             |
investigated mushrooms cultivated at wheat straw and wild mushroom samples were below the permissible limits recommended for food by WHO, i.e., 2 mg/kg (World Health Organization [WHO], 2011). From this, it can be concluded that the consumption of those mushrooms may not be considered as toxicological risk with regard to short-term effects. However, Cd concentration in the canned mushrooms and those cultivated in wood waste substrate were above the WHO recommended limit (WHO, 2011). Therefore, this study strongly recommends that use of canned mushroom and mushroom cultivated in wood waste substrate as a source of human diet may cause potential health risk to human beings (Kalač & Svoboda, 2000). Moreover, the result of one-way ANOVA showed that the mean concentrations of the canned mushroom and mushroom cultivated in cotton waste were not significantly different at p < 0.05.

3.4.2. Lead
Lead concentration levels in mushroom for this study was also ranged from 1.53 to 2.17 mg/kg. The highest concentration was found in mushroom cultivated in wood substrate and the lowest in wild mushroom. The concentration of Pb in all the mushroom samples analyzed in this study were below the WHO recommended limit (i.e., 5.0 mg/kg) (FAO/WHO, 2001). Therefore, from this, it can be concluded that the mushrooms may not pose health risk to consumers due to the toxic effects of Pb. Similarly, other literature also reported the value of Pb in the range of 0.01–2.30 mg/kg (Uzun et al., 2011), 0.20–0.68 mg/kg (Meghalatha et al., 2014) and 1.52–18.00 mg/kg (Woldegiorgis et al., 2015), respectively. Thus, the results of these findings are in good agreement with those results reported for similar studies. The statistical analysis using one-way ANOVA also showed that there was no significant difference in the levels of Pb among the studied mushroom samples at p < 0.05. This could be due to the similarity in environmental conditions of cultivation and substrate compositions.

The overall ranges of results analyzed for major, minor and trace elements in mushroom samples with different substrates are compiled in Table 6 with other literature values.

3.5. Correlation analysis
The correlation coefficient of experimental analysis indicated how strongly two variables are related to each other. A correlation coefficient of +1.0 indicated a perfect positive correlation, while a correlation coefficient of −1.0 indicated a perfect negative correlation. The correlation values are categorized as no correlation ($R^2 = 0.00–0.19$), low correlation ($R^2 = 0.20–0.39$), medium correlation ($R^2 = 0.40–0.59$), higher correlation ($R^2 = 0.60–0.79$) and highest correlation ($R^2 = 0.80–1.00$) (Melina, Craig, & Levin, 2016). Thus, a linear regression correlations test was performed to investigate the correlations between metal concentrations in the mushroom samples and are summarized in Table 7.

According to the present study higher correlations were observed between K-Ca ($r = 0.62$), K-Zn ($r = 0.70$), Ca-Fe ($r = −0.63$), Ca-Zn ($r = 0.66$), Ca-Pb ($r = 0.77$), Mg-Mn ($r = −0.61$), Zn-Cd ($r = 0.63$) and highest correlation also seen in Na-K ($r = −0.92$), Ca-Cd ($r = 0.82$), Fe-Mn ($r = 0.87$), Zn-Cu ($r = 0.89$), Zn-Pb ($r = 0.91$), Cu-Pb ($r = 0.83$) and Cd-Pb ($r = 0.88$) (Melina et al., 2016). The reason could be due to chemical similarities and competition for binding stage of each element (Soylak, Tuzen, Narin, & Sari, 2004).

4. Conclusions
Mushrooms play an important role in human life due to various benefits. Generally, the studied mushrooms contained minerals required in the human diet such as K, Ca, Mg, Na, Fe, Zn, Mn and Cu. Thus, the edible mushrooms analyzed showed high K, Fe and Zn content, confirming that the mushrooms can be considered as a good source for these essential elements and are very good at balancing the nutrient supply scarcities which is common in developing countries like Ethiopia. The level of toxic elements was lower than the other major and minor elements in the entire five different mushrooms that have been studied.

The element (major, minor and toxic) concentration varied significantly not only among the mushroom species but also within the oyster mushroom cultivated in different substrates (p <
## Table 6. Comparison of the results of the present study with literature values

| Element | Present study | Literature values (mg/kg dry weight of mushroom) |
|---------|---------------|--------------------------------------------------|
| Na      | 964.66–4,180.33 | 220.20–3,720.40 (Meghalatha et al., 2014) | 410.0–3,800.0 (Woldegiorgis et al., 2015) |
|         |                | 90.00–601.00 (Uzun et al., 2011)               | 5,950.00–29,230.0 (Soylak et al., 2004) |
| K       | 2,652.66–19,918.66 | 590.3–36,340 (Meghalatha et al., 2014) | 3,660.0–4,240.0 (Woldegiorgis et al., 2015) |
|         |                | 5,950.00–29,230.0 (Soylak et al., 2004)       | 180.0–1,930.00 (Soylak et al., 2004) |
| Ca      | 22.00–34.64    | 80.27.00–1,740.90 (Meghalatha et al., 2014) | 290–6,650 (Woldegiorgis et al., 2015) |
|         |                | 40.00–572.00 (Soylak et al., 2004)            | 5,00–192.500 (Soylak et al., 2004) |
| Mg      | 16.00–30.38    | 210.10–400.70 (Meghalatha et al., 2014) | 570–2,120 (Woldegiorgis et al., 2015) |
|         |                | 180.0–1,930.00 (Soylak et al., 2004)          | 5,00–192.500 (Soylak et al., 2004) |
| Fe      | 34.13–621.06   | 118.00–1,411.00 (Lalotra et al., 2016) | 32.5–6,835.9 (Woldegiorgis et al., 2015) |
|         |                | 5,00–192.500 (Soylak et al., 2004)           | 5,00–192.500 (Soylak et al., 2004) |
| Zn      | 40.25–120.91   | 87–299 (Lalotra et al., 2016) | 26.6–87.6 (Woldegiorgis et al., 2015) |
|         |                | 15.00–45.00 (Soylak et al., 2004)            | 15.00–45.00 (Soylak et al., 2004) |
| Cu      | 8.40–34.33     | 83.60–290.00 (Lalotra et al., 2016) | 5.69–45.9 (Woldegiorgis et al., 2015) |
|         |                | 5,00–83.00 (Soylak et al., 2004)              | 5,00–83.00 (Soylak et al., 2004) |
| Mn      | 4.22–30.63     | 25.50–118.00 (Lalotra et al., 2016) | 0.96–138.5 (Woldegiorgis et al., 2015) |
|         |                | 0.20–80.00 (Soylak et al., 2004)              | 0.20–80.00 (Soylak et al., 2004) |
| Cd      | 1.94–2.52      | 7.90–12.20 (Meghalatha et al., 2014) | 0.04–4.08 (Woldegiorgis et al., 2015) |
|         |                | 0.03–19.00 (Soylak et al., 2004)              | 0.03–19.00 (Soylak et al., 2004) |
| Pb      | 1.53–2.17      | 0.2–0.68 (Meghalatha et al., 2014) | 1.52–18.0 (Woldegiorgis et al., 2015) |
|         |                | 0.0–2.30 (Soylak et al., 2004)                | 0.0–2.30 (Soylak et al., 2004) |
Among the studied elements, only the mean concentrations of Pb did not show any significant differences in all the studied mushroom samples at p < 0.05. Moreover, most of the results in this study were in agreement with values from the literature. In addition, the wild mushroom, mushroom cultivated from wheat straw and cotton waste substrate were within the WHO safe limits for human intake for all the studied elements. Hence, these mushroom types do not pose any health risk to consumers. However, the elements such as Cd and Na in canned mushroom and Cd and Zn analyzed from mushroom cultivated in wood waste substrate were above the WHO recommended limits for food. Therefore, this study concludes that the use of canned mushroom and mushroom cultivated in wood waste substrate as a source of human diet may cause potential health risks.

Acknowledgements
This study was conducted in the Department of Chemistry, Mekelle University, Mekelle, Ethiopia as MSc thesis work. The authors thank Mekelle University for granting this thesis work; Daero Academy of Mekelle, Ethiopia, for providing equipment and place for mushroom cultivation; and Ezana Analytical Laboratory of the Ezana Mining Development PLC, Mekelle, Ethiopia, and Tigray Agricultural Research Institute for their constant help of metal analysis.

Funding
The authors received no direct funding for this research.

Competing Interests
The authors declare no competing interests.

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Table 7. Metal-to-metal correlation. Very strong correlations (positive/negative) are shown in bold.

|          | Na    | K     | Ca    | Mg    | Fe    | Zn    | Cu    | Mn    | Cd    | Pb    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Na       | 1.000 |       |       |       |       |       |       |       |       |       |
| K        | −0.927| 1.000 |       |       |       |       |       |       |       |       |
| Ca       | −0.338| 0.629 | 1.000 |       |       |       |       |       |       |       |
| Mg       | 0.552 | −0.235| 0.591 | 1.000 |       |       |       |       |       |       |
| Fe       | −0.124| −0.162| −0.635| −0.596| 1.000 |       |       |       |       |       |
| Zn       | −0.445| 0.709 | 0.661 | 0.223 | −0.364| 1.000 |       |       |       |       |
| Cu       | −0.168| 0.400 | 0.344 | 0.204 | −0.126| 0.898 | 1.000 |       |       |       |
| Mn       | −0.536| 0.328 | −0.266| −0.617| 0.872 | 0.070 | 0.172 | 1.000 |       |       |
| Cd       | −0.052| 0.349 | 0.822 | 0.731 | −0.296| 0.639 | 0.545 | −0.029| 1.000 |       |
| Pb       | −0.213| 0.532 | 0.778 | 0.544 | −0.373| 0.913 | 0.837 | −0.005| 0.890 | 1.000 |

0.05), with some exceptions. Among the studied elements, only the mean concentrations of Pb did not show any significant differences in all the studied mushroom samples at p < 0.05. Moreover, most of the results in this study were in agreement with values from the literature.

In addition, the wild mushroom, mushroom cultivated from wheat straw and cotton waste substrate were within the WHO safe limits for human intake for all the studied elements. Hence, these mushroom types do not pose any health risk to consumers. However, the elements such as Cd and Na in canned mushroom and Cd and Zn analyzed from mushroom cultivated in wood waste substrate were above the WHO recommended limits for food. Therefore, this study concludes that the use of canned mushroom and mushroom cultivated in wood waste substrate as a source of human diet may cause potential health risks.

Citation information
Cite this article as: Content of major, minor and toxic elements of different edible mushrooms grown in Mekelle, Tigray, Northern Ethiopia, Mulu Berhe Tsegay, Abraha Gebrekidan Asgedom & Masho Hilawie Belay, Cogent Food & Agriculture (2019), 5: 1605013.

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