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Determination of trace elements on some wild mushroom samples encountered from Western ghats of Karnataka

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ABSTRACT

Eleven trace elements (Macro elements-Na, K, P, Ca, Mg and micro elements-Zn, Cu, Mn, Fe, Pb, Cd) in sixdifferent mushroom species were determined, using flame photometer, spectrophotometer and atomic absorption spectrometry after microwave digestion. The mushrooms samples were collected from Western Ghats of Shimoga forest regions, Karnataka. The ranges of element concentrations for macro elements Na, K, P, Ca and Mg were $1.3\pm0.1-3.9\pm0.3$, followed by $1.5\pm1.0-22.3\pm2.1$, $1.4\pm0.1-6.4\pm0.5$, $0.20\pm0.01-1.4\pm0.1$ and $0.10\pm0.02-1.5\pm0.1$ whereas micro elements such asZn, Cu, Mn, Fe, Pb and Cd were $42.1\pm3.3-65.4\pm5.3$, $9.4\pm0.5-43.9\pm3.9$, $13.9\pm1.2-94.3\pm8.1$, $179\pm10.8-381\pm25.4$, $7.9\pm0.8-12.2\pm1.1$ and $0.11\pm0.01-0.68\pm0.04$ mg/kg, respectively.

Keywords: Trace elements; Macro and micro elements; Wild Mushrooms; Atomic absorption spectrometry; Western Ghats

INTRODUCTION

Mushroom is a fleshy, spore bearing fruiting body, a fungus, typically produced above ground on soil or on its food source. Mushroom is most often applied to fungi (Basidiomycota, Agaricomycetes, order Boletales and family Boletaceae) that have stem (stipe), a cap (Pileus) and gills (Lamellae) on the other side of the cap. The seasons are normally wet and mild. The climate, especially in spring and autumn, is ideal for fungal growth [1]. Mushrooms such as the *Pleurotus* species are known to be among the largest fungi or saprophytic eukaryotes composed of hyphae filament that thrives very well in damp or moist condition, some mushrooms especially members of the genus *Amanita* sp are extremely poisonous [2].

Mushrooms are important in ecosystem because they are able to biodegrade the substrate and therefore use the wastes of agricultural production [3]. Heavy metal concentrations in mushroom are considerable higher than those in agricultural crop plants, vegetables and fruit [4]. Mushrooms are valuable health foods, low in calories, high in vegetable proteins, iron, zinc, chitin, fibre, vitamins and minerals. Mushrooms also have a longhistory of use in traditional Chinese medicine [5]. In general, their fruiting bodies, on a dryweight basis, contain about 39.9% carbohydrate, 17.5% protein and 2.9% fats with the rest constituting theminerals [6, 7]. Wild-growing macrofungi have been a favouritedelicacy in many countries. Some people collect macrofungito make a substantial contribution to food intake. Therefore, it is necessary to know the levels of toxic and essential elements in edible mushrooms [8].

Many wild growing species accumulate elements at high concentrations, especially cadmium, mercury, lead and copper, considerably exceeding those in other foods. Edible mushrooms may contain higher amounts of heavy

metals than plants. Mushrooms have been considered healthy food because they contain high quality protein which contains all the essential amino acids, vitamins B, B₂, C and D and minerals such as A, K, Zn, Na, Fe, Mg, P and low fat [9].

Mushrooms have been a food supplement in various cultures and they are cultivated and eaten fortheir edibility and delicacy. They fall between the best vegetables and animal protein source. Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals [10]. All essential amino acids are present as well as water-solublevitamins and all the essential minerals [11]. Mushroom are good sources of vitamins like riboflavin, biotin and thiamine [12,13], indicated that mushroom is about 16.5% dry matter out of which 7.4% is crude fiber, 14.6% is crude protein and 4.48% is fat and oil. Protein contents vary between 4 to 9% in *Auricularia* sp and between 24 to 44% in *Agaricus species*. The protein value of mushrooms is twice as that of asparagus and potatoes, four times as that of tomatoes and carrots, and six times as that of oranges [10].

In recent times, mushrooms have assumed greater importance in the diets of both rural and urban dwellers. Mushrooms are now marketed along major highways and urban centers. They are also relatively much cheaper than beef, pork and chicken that contain similar nutrients [14]. Hence, the purpose of this study is to determination of trace elements (Na, K, P, Ca, Mg, Zn, Cu, Mn, Fe, Pb and Cd) in fruit bodies of some mushroom species from Western Ghats regions of Shivammogga, Karnataka.

EXPERIMENTAL SECTION

Collection of Mushrooms

Fresh and succulent mushroom samples were obtained from five different vegetation sites, tropical wet evergreen forest, tropical semi evergreen forest, moist deciduous forest, dry deciduous forest and tropical thorn forest [15] of Western Ghats forest regions of Shivanmogga (situated between 13°27' and 14°39' N latitude and 74°38' and 76°34' E longitude) district, Karnataka, India, in the rainy season, between the months of June-October, 2013.

Characterization, Identification and Preservation of Mushrooms

Each of the collected samples were characterized and identification was done by comparing their morphological, anatomical and physiological characteristics as described [16, 17] monographs with descriptions given in the manual [18] and also through the electronic data on identification keys of mushrooms [19]. All the specimens were preserved in the Department of Post Graduate Studies and Research in Applied Botany, Mycology Laboratory, Bio-Science Complex, Jnana Sahyadri, Kuvempu University, Shivammogga (Dist), Karnataka, India. The analysis was made at the same Department and the Central Coffee Research Institute (CCRI) Balehonnur, Chickamagalur district of Karnataka, India.

Mushroom handling procedures

Collected samples were sun-dried for two to four days as a means of preservation. The sun-dried mushrooms were homogenized and stored in acool, dry and air tight container to avoid growth of other fungi during the analysis. Sun-dried mushrooms were weighed in a crucible in quadruplicate, dried to constant weight in an oven at 35-40°C for 24hours and then cooled in desiccators and weighed again prior to grinding. The dried mushroom was ground to fine powder using clean pestle and mortar, this powder was stored in an air tight black polythene bag at room temperature, until required for use.

ANALYSIS OF TRACE ELEMENTS

The crude powder (2g), before subjecting it to various organic solvents, was ashes in an oven at 60°C for 3hours, 0.5g of the cooled ash was digested by heating for 2hours with a mixture of 10ml Hydrochloric acid (HCl), Nitricacid (HNO₃) and Hydrogen tetra oxochloric acid (HCLO₄). The digested mixture was evaporated down to 5ml using rotator evaporator; it was then made up to 10ml with 2M HNO₃ and to which was added 30ml of distilled water and kept in a 100ml beaker. Reagent blank samples were also prepared, these sample were analyzed for: Zn, Cu, Mn, Fe, Pb and Cd using Winlad 32 software flame atomic absorption spectro photometer (AAS), while Na, K, P, Ca and Mg, were determined by flame emission spectro photometry [20].

DETERMINATION OF MICRO ELEMENTS

Analysis of Zinc, Copper and Manganese

The 2ml of digested samples were taken and diluted to 50ml and the sample was aspirated of at the wavelength of 213.9, 324.75 and 279.5 of AAS to detect concentration of Zn, Cu and Mn respectively. Finally, the values of micronutrients are expressed in ppm by the help of following formula [21].

ppm of Zn / Cu / Mn = ppm / 1000 x Dilution factor x Volume of sample digestion made / Weight of the mushroom sample x 100

Analysis of Iron, Lead and Cadmium

The 2ml of digested samples were taken and diluted to 100ml. The presence of lead andcadmium were detected with the help of AAS by aspirating the sample at the wavelength of 217nm and 228nm with appropriate lamps. The ppm of lead and cadmium were calculated by the help of following formula [21].

ppm of Fe / Pb / Cd = ppm / 1000 x Dilution factor x Volume of sample digestion made / Weight of the mushroom sample x 100

DETERMINATION OF MACRO ELEMENTS

Determination of Sodium / Potassium

The concentration of potassium was determined with the help of flame photometer using separate standards of potassium. The yellow colored solution was aspirated at the wavelength of flame photometer to detect the concentration of potassium. Finally the percentage of potassium was calculated with the help of following formula.

% of Na / K = Graph ppm / 106 x Dilution factor x Volume of sample digestion made / Weight of the mushroom sample x 100

Determination of Phosphorous

Orthophosphate (phosphorous) present in the mushrooms was determined by vanadomolybdate yellow colour method. The 5ml of aliquot of mushroom digested was taken in 50ml volumetric flask and mixed with 10ml vanadomolybdate reagent. Having thoroughly mixed the final volume was adjusted to 50ml by distilled water. After 30min the developed yellow colour was measured on a spectrophotometer at 470nm. The concentration of phosphorous was calculated with the help of standard graph. The percentage of phosphorous is calculated with the help of following formula.

% of P = Graph ppm / 106 x Volume of digestion made / Aliquot x Volume of sampledigestion made / Weight of the mushroom sample x 100

Determination of Calcium and Magnesium

1ml of aliquot mushrooms digested material was taken in 50ml by volumetric flask, finalvolume was adjusted to 50ml by adding distils water. The presence of calcium and magnesium were determined at the wavelength 422.7 and 228.2nm of AAS respectively. The percentage of calcium and magnesium were calculated with the help of following formula.

% of Ca / Mg = Graph ppm / 106 x Dilution factor x Volume of sample digestion made / Weight of the mushroom sample x 100

STATISTICAL ANALYSIS

Experimental values are given as means \pm standard deviation (SD). Statistical significance was determined by oneway variance analysis (ANOVA). Differences at P < 0.05 were considered to be significant.

Sl. No	Vernacular Name	Species Name	Encountered forest regions	Habitat	Edibility
1	Kulebaddeanabe	Scleroderma bermudense	Sringeri	Terricolous	Edible
2	Haigenanabe	Hygrocybe parvula	Haniya	Terricolous	Not known
3	Nakshathranabe	Geastrum triplex	Kotegudda	Terricolous	Not known
4	Maradanabe	Ganoderma applannatum	Kodachadri	Lignicolous	Suspect
5	Maradanabe	Ganoderma sinense	Aagumbe	Lignicolous	Suspect
6	Kolikalanabe	Clavaria rosea	HulikalGhat	Terricolous	Not known

Table 1: Collected Wild Mushroom samples encountered in forest regions of Shivammogga

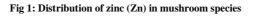
Table 2: Concentration of trace microelements (as mg/kg) in mushroom species

Mushroom species	Zn	Cu	Mn	Fe	Pb	Cd
Scleroderma bermudense	48.1±3.2	14.4±0.9	16.4±1.5	179±10.8	10.2±0.7	0.12 ± 0.01
Hygrocybe parvula	50.9±1.1	39.3±2.3	35.0±1.2	227±20.7	11.1±0.6	0.68 ± 0.04
Geastrum triplex	42.1±3.3	26.3±1.5	13.9±1.2	202±11.2	8.9±0.4	0.19±0.01
Ganoderma applanatum	52.8±4.6	12.9±1.0	21.9±1.3	331±22.6	9.9±0.3	0.11±0.01
Ganoderma sinense	65.4±5.3	9.4±0.5	24.8±1.3	376±21.0	7.9±0.8	0.13±0.01
Clavaria rosea	57.9±4.7	43.9±3.9	94.3±8.1	381±25.4	12.2±1.1	0.11±0.01

Table 3: Concentration of trace macro elements (as mg/kg) in mushroom species

Mushroom species	Na	K	Р	Ca	Mg
Scleroderma bermudense	1.3±0.1	10.2±0.7	2.4±0.1	0.22±0.07	0.10±0.02
Hygrocybe parvula	1.4±0.1	1.5±1.0	5.8±0.6	0.20±0.01	0.14 ± 0.04
Geastrum triplex	2.4±0.1	13.0±1.1	2.7±0.2	1.3±0.1	1.5±0.1
Ganoderma applanatum	3.2±0.2	9.2±0.6	1.4±0.1	0.27±0.08	0.19±0.01
Ganoderma sinense	1.6±0.1	9.5±0.8	1.6±0.7	0.36±0.02	0.21±0.4
Clavaria rosea	3.9±0.3	22.3±2.1	6.4±0.5	1.4±0.1	1.2±0.1

MICRO ELEMENTS



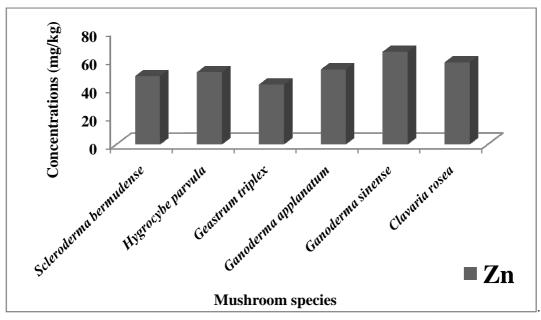


Fig 2: Distribution of copper (Cu) in mushroom species: 1. S. bermudense; 2. H. parvula; 3. G. triplex; 4. G. applanatum; 5. G. sinense and 6. C. rosea

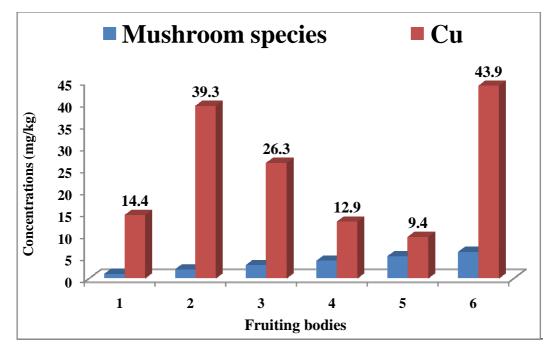
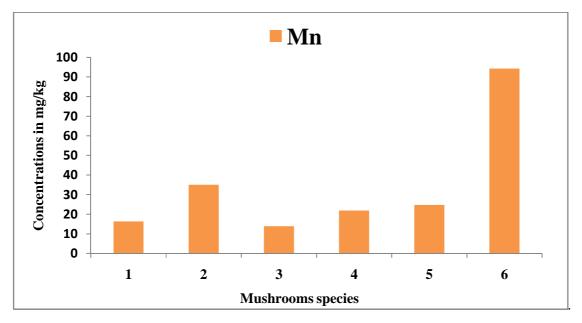


Fig 3: Distribution of manganese (Mn) in mushroom species: 1. S. bermudense; 2. H. parvula; 3. G. triplex; 4. G.applanatum; 5. G. sinense and 6. C. rosea



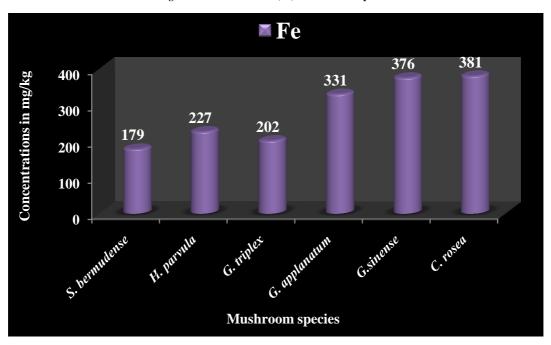
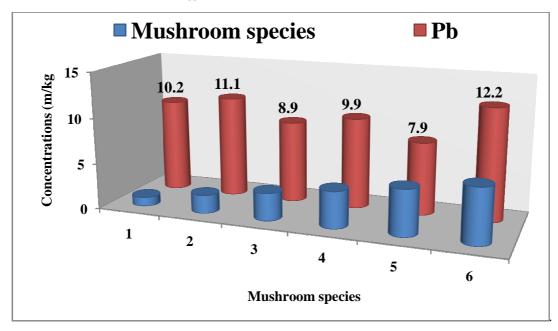


Fig 4: Distribution of iron (Fe) in mushroom species

Fig 5: Distribution of lead (Pb) in mushroom species: 1. Scleroderma bermudense; 2. Hygrocybe parvula; 3. Geastrum triplex; 4. Ganoderma applanatum; 5. Ganoderma sinense and 6. Clavaria rosea



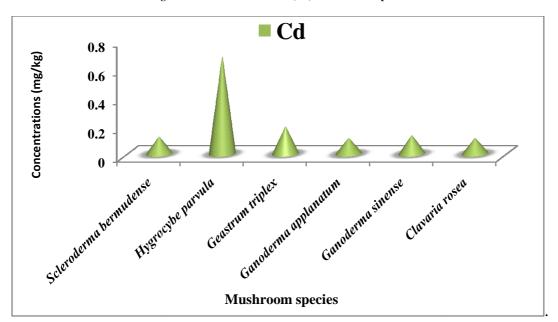
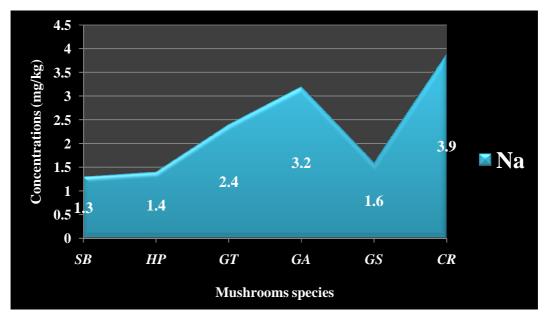


Fig 6: Distribution of cadmium (Cd) in mushroom species

MACRO ELEMENTS

Fig 7: Distribution of sodium (Na) in mushroom species: SB=S. bermudense; HP=H. parvula; GT=G. triplex; GA=G.applanatum; GS=G. sinense and CR=C. rosea



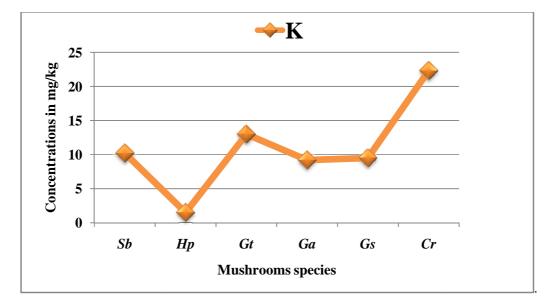
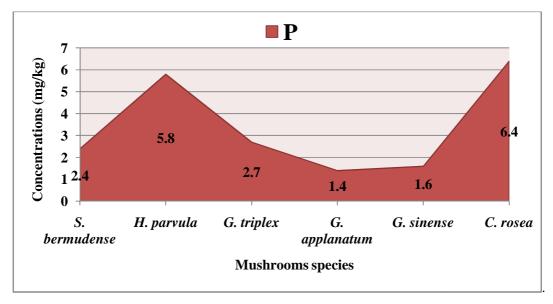


Fig 8: Distribution of potassium (K) in mushroom species: Sb=S. bermudense; Hp=H. parvula; Gt=G. triplex; Ga=G.applanatum; Gs=G. sinense and Cr=C. rosea

Fig 9: Distribution of phosphorous (P) in mushroom species: S. bermudense; H. parvula; G. triplex; G.applanatum; G. sinense and C. rosea



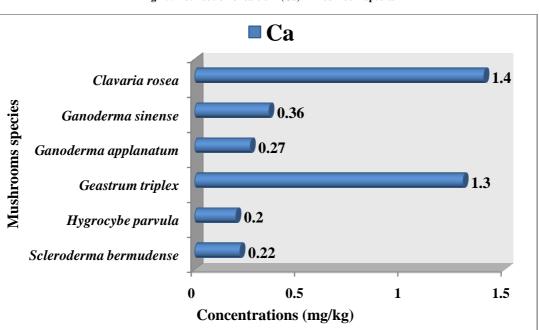
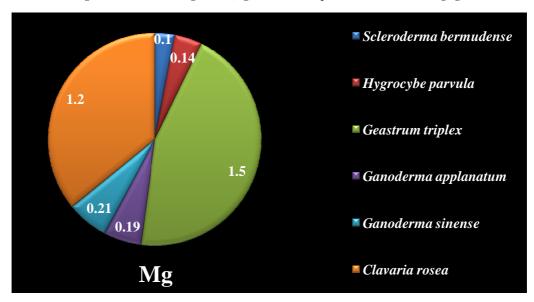


Fig 10: Distribution of calcium (Ca) in mushroom species

Fig 11: Distribution of magnesium (Mg) in mushroom species at concentrations (mg/kg)



RESULTS

Trace elements

Determination of trace elements onsix naturally growing wild mushrooms samples encountered from five different vegetation sites like, tropical wet evergreen forest, tropical semi evergreen forest, moist deciduous forest, dry deciduous forest and tropical thorn forests of Western Ghats regions of Shivammogga district, Karnataka, India. The vernacular names, species names, encountered forest places, habitats and edibility of mushroomspecies are given in Table-1.The concentrations of trace macro and microelements in wild mushroom species analyzed are shown in Table-2 and 3.

Trace element concentrations were determined on dry weight as mg/kg and the relative standard deviations were less than 10% for all elements. The heavy metal concentrations in mushrooms are mainly affected by acidic and organic matter contents of the ecosystem and soil. The uptake of metal ions in mushrooms is in many respects different from plants; thus, the concentrations of metals depend on mushroom species and their ecosystems and soil [22].

Micro elements

Results for macro elemental composition of wild mushrooms is presented in Table-2, these results showed high concentrations of microelements such aszinc 48.1mg/kg, followed by 50.9, 42.1, 52.8, 65.4 and 57.9mg/kg respectively (Fig-1), copper 14.4mg/kg, respectively 39.3, 26.3, 12.9, 9.4 and 43.9 (Fig-2), manganese 16.4mg/kg, 35.0, 13.9, 21.9, 24.8 and 94.3mg/kg respectively (Fig-3), iron (179mg/kg, 227, 202, 331, 376 and 381mg/kg (Fig-4). However, other elements such as lead 10.2mg/kg, 11.1, 8.9, 9.9, 7.9 and 12.2mg/kg (Fig-5) and cadmium (0.12mg/kg, followed by 0.68, 0.19, 0.11, 0.13 and 0.11mg/kg respectively) Fig-6, were found inmoderately slight concentrations in all six mushrooms species such as, *Scleroderma bermudense, Hygrocybe parvula, Geastrum triplex, Ganoderma applanatum, Ganoderma sinense* and *Clavaria rosea*.

Macro elements

Results for macro elemental composition of wild mushrooms is presented in Table-3, these results showed moderate concentration of calcium 0.22mg/kg, followed by 0.20, 1.3, 0.27, 0.36 and 1.4 (Fig-10) and magnesium 0.10mg/kg, followed by 0.14, 1.5, 0.19, 0.21 and 1.2 respectively (Fig-11). Highly presence of potassium 10.2mg/kg, respectively 1.5, 13.0, 9.2, 9.5 and 22.3 (Fig-8), phosphorus 2.4, 5.8, 2.7, 1.4, 1.6 and 6.4mg/kg (Fig-9) and sodium 1.3mg/kg, followed by 1.4, 2.4, 3.2, 1.6 and 3.9 respectively (Fig-7) were observed in all six encountered mushrooms samples such as *S.bermudense, H.parvula, G. triplex, G.applanatum, G.sinense* and *C. rosea*.

DISCUSSION

The contents of macro elements like, Na, K, P, Ca and Mgin mushroom species were found to be $1.3\pm0.1-3.9\pm0.3$, followed by $1.5\pm1.0-22.3\pm2.1$, $1.4\pm0.1-6.4\pm0.5$, $0.20\pm0.01-1.4\pm0.1$ and $0.10\pm0.02-1.5\pm0.1$ where as micro elements such as Zn, Cu, Mn, Fe, Pb and Cd were $42.1\pm3.3-65.4\pm5.3$, $9.4\pm0.5-43.9\pm3.9$, $13.9\pm1.2-94.3\pm8.1$, $179\pm10.8-381\pm25.4$, $7.9\pm0.8-12.2\pm1.1$ and $0.11\pm0.01-0.68\pm0.04$ mg/kg, respectively.

Minimum and maximum values of iron were 179 and 381mg/kg. The highest and lowest levels of iron were found in *Claveria rosea* and *Scleroderma bermudense* (Fig-4). The highest content of manganese was 94.3mg/kg in *Claveria rosea*, where as the lowest manganese content was 13.9mg/kg in *Geastrum triplex* (Fig-3). Zinc levels were determined to be 65.4mg/kg in *Ganoderma sinense* and 42.1mg/kg in *Geastrum triplex* (Fig-1). Zinc is wide spread among living organisms due to its biological significance. Mushrooms are known as zinc accumulators and the sporophore: substrate ratio for Zn ranges from 1 to 10mg/kg [23, 8]. In this study, the highest copper content was 43.9mg/kg in *C. rosea*; the lowest was 9.4mg/kg in *G. sinense* (Fig-2). The lead concentrations were high in *C. rosea* (Fig-5) and ranged from 7.9 to 12.2mg/kg. Cadmium contents were between 0.11 and 0.68mg/kg in the samples. The highest cadmium content was found in *H. parvula* (Fig-6). The average sodium concentration was 1.3-3.29mg/kg. The lowest and highest sodium values were observed in *S. bermudense*, *H. parvula* and *G. sinense* (Fig-7). Maximum potash level was 22.3mg/kg in *C. rosea* and minimum potash level was 1.5 mg/kg in *H. parvula* (Fig-8). The range of phosphorous concentrations was 1.4-6.4 mg/kg in *G. applanatum* and *C. rosea* (Fig-9).

Iron contents of mushrooms were lower than ours results in other studies [24]. The reported manganese values for mushrooms were 6.78-63.6mg/kg, 7.6-56.2mg/kg and 15-19mg/kg, respectively [7, 8, 24]. Our zinc results are lower than those reported earlier [8, 25]. Copper contents were similar in other studies [5, 25]. Copper values were reported as 34.5-83.0mg/kg, 10.0-14.0mg/kg and 21.1-42.6mg/kg, respectively [1, 8, 24]. Lead concentrations are very low compared to our results [26, 27]. Reported cadmium levels are very highcompared to our results [1, 25, 28]. Calcium contents were lower than literature values and magnesium contents were higher than literature values. Phosphorous values have been reported as 10.5-12.5mg/kg for different mushroom species [24].

The above observed elements have physiological importance and maintenance of cellular enzymatic functions; these elements are required for normal growth, muscular activity and skeletal muscle development, especially calcium [29], blood viscosity; Calcium, manganese and cobalt, oxygen transport and cellular activity are enhanced by elements such as copper and iron. Manganese is found with lecithin and needed as a co-factor for some enzymes, especially in the synthesis of fatty acids and cholesterol, it is also involved in chemical reaction the body and assists

in intestinal nutrients absorption; it is also an important cofactor in energy production through the ATPase channel and by supporting immune system [30]. Manganese complex with Vitamin K to enhance blood clotting factor and with Vitamin-B complex to reduce effect of stress [30], thus providing astrong anti-oxidant effect as claimed [31]. Sodium and potassium are required for the maintenance of fluid balance, while potassium and calcium are important in stimulating action potential across nerve endings, and also to enhance heart contractile rate. Iron is highly required physiologically formation and to enhance oxygen carrying capacity of red blood cells. Zinc is an important requirement in protein synthesis, normal body development and recovery from illnesses. it is a cofactor in the function of the enzyme carbonic anhydrase required for carbon dioxide transport and as part of peptidases needed for protein digestion [30], it is also a necessary part of DNA, for cell division and synthesis hence its importance in wound healing [32].

This result is not a surprise because the vegetation of these areas is typical of wild fungi. Specific mushroom tropical rainforest, which support the luxuriant growth of species were collected from different forest study areas. The results only provide indication of the areas where the sporocarps could be collected in large quantities [33].

CONCLUSION

The results of the determination of trace micro and macro elements of the six wild mushroom species showed that *G. sinense* had the highest levels of zinc, followed by *H. parvula* (cadmium), *C. rosea* recorded the highest copper, manganese iron, lead, sodium potassium, phosphorous and calcium contents. It can be reasonably concluded following the results of this study that these wild mushrooms hold tremendous promise in complementing the trace elements supply deficiencies prevalent in developing countries.

The results of the study showed appreciable levels of iron, manganese and copper, which is known as antitumorigenic and hypochlestrolaemic agent. This implies that mushrooms hold special attraction and may be recommended for people with Cholesterol related ailment [34]. Due to high level of most mineral elements in the species, the three mushrooms (*G. sinense, H. parula* and *C. rosea*) can be essential for normal metabolic reaction, transmission of nerve impulse, regulation of water and salt balance and rigid bone formation.

The results suggest that the mushrooms hold great promise of alleviating the problem of human beings, since mushrooms are highly nutritional and can compare favourably with egg, meat and milk. However, for the nutritional potential of mushrooms to be realized, sustained efforts must be geared towards the husbandry (cultivation) and popularization of the more nutritious species.

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