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Proximate Composition and Micronutrient Mineral Profile of wild *Ganoderma lucidum* and Four Commercial Exotic Mushrooms by ICP-OES and LIBS

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Abstract Edible mushrooms are excellent food that can be incorporated into well balanced diets due to their low content of fat and energy, high content of dietary fibers and proteins. Proximate composition of mushrooms also varies within and among species due to agro-climate conditions and environmental factors. The current study was designed to analyze proximate composition and mineral profile of one local wild *Ganoderma lucidum*, two commercial local cultivated mushrooms species i.e. *Pleurotus ostreatus* and *Vovoriella volvacea*, and two commercial exotic mushrooms i.e. *Lentinus edodes* and *Hericium erinaceus* for their nutritive values. Minerals were analyzed by Inductivity Coupled Plasma Optical Emission Spectrometry (ICP-OES). Selected mushrooms were also analyzed by laser induced break down spectroscopy (LIBS) to identify any harmful element(s) present in these mushrooms. Proximate analysis showed that crude protein ranged from 15.04-24.8%, crude fat 0.53-2.02%, Fiber 6.11-54.12%, Ash 2.0-9.99% and total carbohydrates varied in a range of 65.34-82.47% on dry weight basis. Ca, Mg, Na, Zn, P and K were in elevated concentration. Al, B, Cu, Li and Mn were in the range of 2.5-8.1, 0.4-6.1, 0.9-1.4, 0.2-1.4, 0.4-1.3 mg/100 g on dry weight basis respectively. As, Ba and Se were in lower concentration whereas Pb, Cd, Mo, Be, Sn and Co were below detectable limits. LIBS also revealed some elements like, titanium, barium, calcium, iodine, carbon and hydrogen. The selected local mushrooms are safe for consumption, in accordance with the permissible tolerance limit of the toxic metals.

Keywords: *mineral analysis, proximate composition, Pleurotus ostreatus, Vovoriella volvacea*

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1. Introduction

Studies on the production of non-conventional protein as an alternative source of dietary supplement have increased with growing global demands for quality food [17]. Mushrooms are valued and appreciated for their sensory, nutritional and therapeutic properties as well as their mineral nutrients composition [12,24]. They are important owing to higher proteins, vitamins and mineral levels and lower levels of fat and calories [7]. Fruiting bodies of mushrooms are esteemed for taste and flavor and are also consumed as fresh and processed forms [26]. *Ganoderma* species don't have the fleshy texture, are not listed among the group of edible mushrooms because the fruiting bodies are thick, tough and corky. Although *Ganoderma species* could not be eating directly, they have been known all over the world as highly medicinal mushroom [9].

The tendencies to bioaccumulate the minerals from the growth medium and environment into fruiting bodies by mushrooms is well reported [13]. Principal factors influencing the accumulation of heavy metals in fruiting bodies are the development of mycelium, fungal structure, biochemical composition, nutritional needs, substrate decomposition activity, geological and environmental factors, whereas no evidence was found on an explicit role of soil pH or soil organic matter contents [3,5,12]. The essential macronutrient minerals are sodium, potassium, magnesium and calcium. The functions of macronutrient minerals are to maintain acid-base balance, the osmotic regulation of fluid and oxygen transport in the body [15]. The known essential micronutrient minerals are iron, zinc, selenium, manganese, cobalt and copper. These microminerals play an important role in the catalytic processes within the enzyme system that includes a wide range of enzyme activities associated with metabolic, endocrine and immune systems [10]. Living organisms require traces of some heavy metals, including iron, cobalt, copper, manganese, chromium and zinc. Excessive levels of these metals, however, can be detrimental to human health. Lead and cadmium are non-essential metals as they

are toxic, although their trace amounts are not known for any beneficial effects on organisms [11,19]. Furthermore, deficiency or imbalance of essential or non-essential minerals above threshold concentration levels can put the individual at risk of disease development [21]. Therefore, the adequate and accurate food composition data are inevitable for estimating the adequacy of intakes of essential nutrients and assessing the risk from toxic metals [20].

The mushrooms were collected to make a substantial contribution to food intake, in this context it would be worthwhile to evaluate the metal contents in mushrooms grown wild and on any substrate in Pakistan. Accordingly, the present study is focused on the analysis of five species of mushrooms collected from Pakistan for their mineral contents and to discuss the results generated on essential and trace elements in edible mushrooms, along with the limit of toxic metals. The obtained data would serve as useful basis to define the nutritional value of given mushrooms species cultivated in Pakistan in addition to any warnings of toxic minerals depending on the concentrations.

2. Material and Methods

2.1. Mushroom Collection

The exotic commercial mushrooms used in this study *Lentinus edodes* (Berk.) Sing., *Hericium erinaceu*s (Bull.) Pers. were purchased from local market (imported from China), local cultivated *Volvariella volvacea* (Bull, ex. Fr.) Sing. and *Pleurotus ostreatus* (Jacq. Ex. Fr.) Kumm. were collected from Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan. The wild *Ganoderma lucidum* (Fr.) P. Karst., was isolated from the stem of *Salmalia malabarica* plant from Jinnah Garden Faisalabad, Pakistan. Taxonomic identification was made by Prof. Dr. M. Asif Ali from medicinal mushroom laboratory, Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan. The collected mushrooms were shade dried, cleaned, sliced into small pieces and were ground into powder using a food processor (NR-56X). The ground mushrooms were stored at -20°C in sealed plastic bags for further use.

2.2. Proximate Analysis

Selected five mushroom species were analyzed for food composition according to the Association of Official Analytical Chemists [2]. These include the determination of crude protein, crude fat, ash, crude fiber, carbohydrate and minerals. The percentage of all the fractions (crude protein, crude fat and ash) were added together and subtracted from 100 to obtain the total carbohydrate percentage.

2.3. Preparation of Ash Solution

One gram of dry powdered mushroom was placed in a porcelain crucible and heated at 500°C overnight, then the ash was dissolved in 2 mL 70% HNO_3 (Merck, Germany), followed by centrifugation at 3000 x g for five minutes followed by the addition of distilled water to make the volume up to 10 mL. Control was also prepared using similar experimental procedure excluding ash. Three such replicates were performed for each of the mushrooms species [13].

2.4. Mineral Analysis

Analysis for Sodium (Na), Potassium (K), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Copper (Cu), Zinc (Zn), Selenium (Se), Molybdenum (Mo), Lead (Pb), Cadmium (Cd), Boron (B) and Barium (Ba) was carried out using inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perklin Elmer, optima 4300Dv, USA) with Argon plasma, at the Department of Nutrition Science, PURDUE University, Westlafayette, USA. Detection limit is defined as the concentration corresponding to three times the standard deviation of ten blanks. Calibration of ICP-OES was done using the working standard prepared from commercially available multimineral standard solution (100 mg/L, Spex CertiPrep, USA). The most appropriate wave length, argon gas flow, plasma stabilization, and other ICP-OES parameter for metals/minerals were selected, and measurements were made within linear range of working standards used for calibration [13].

2.4.1. Working conditions of ICP-OES were as follows:

Instrument: ICP-OES (Perklin Elmer, optima 4300Dv, USA),
Power: 40 MHZ (Free running solid state RF generator)
Plasma gas flow: 15 L/min
Auxillary gas flow: 0.8 L/min
Reading time: 3 sec

The concentration of all analyzed minerals was expressed as mg/100 g dry weight of sample.

2.5. Laser induced Breakdown Spectroscopic Analysis (LIBS)

The LIBS system used for experiment consisted of pulsed laser, a sample holding unit a spectrometer and a computer. A Nd: YAG laser was used as a light excitation source. The laser operated at the wave length of 1064 nm with a repetition rate of 10 Hz, a pulse width 10 ns and variable pulse energy of 4-40 mJ. The laser beam was focused on the sample surface by a quartz lens with a focal length of f=20 mm, to produce both ablation of the sample and formation of aluminum plasma. The plasma radiation light was imaged to the fiber input end through a quartz lens with a focal length of f=50 mm, which was coupled to spectrometer with a spectral range of 300-750 and a spectral resolution of 0.07 nm. The spectral data was processed directly with the associated analytical software [23].

2.6. Statistical Analysis

Minitab software was used for computation and analysis of different parameters. All the data was reported as the mean and standard deviation. Differences were analyzed by considering $p<0.01$ statistically significant. The collected data was analyzed by means and standard deviation.

3. Results and Discussion

3.1. Proximate Analysis

Proximate analysis was conceded out on selected mushroom species: *L. edodes*, *V. volvacea*, *H. erinaceus*, *P. ostreatus* and *G. lucidum*. The obtained data is assembled in Table 1.

Protein contents of *V. volvacea* were observed highest 24.12% on dry weight basis (DW), followed by the *L. edodes* (22.61%, DW), *P. ostreatus* (21.14%, DW), *G. lucidum* (15.04%, DW) and *H. erinaceus* (18.8%, DW). The difference in protein contents of mushroom could be due to a number of factors, namely the type of mushroom, the stage of development, the part samples, level of nitrogen available and the substrate/habitat [4]. Our results are consistent with the study of Hung and Nhi [8], that reported the protein contents of *P. ostreatus*, *V. volvacea*, *L. edodes*, *A. polytricha* and *G. Lucidum* were 28.6, 36.5, 26.3, 7.2, and 13.3% of DW, respectively. Mattila et al. *[14]* also studied several species of mushrooms grown in Finland and reported that protein content of *P. ostreatus* and *L. edodes* were 24.6 and 21.4% of dry matter, respectively. These results indicate that the *V. volvacea*, *P. ostreatus* and *L. edodes* were good sources of protein for humans [18].

Table 1. Proximate composition of selected wild and commercial mushrooms (% DW)

Mushrooms	Crude Protein	Crude Fat	Fiber	Carbohydrate	Ash	Energy Kcal/100g
L. edodes	22.61±.02^{b}	0.78±.01^{a}	9.38±.03^{a}	70.62±.03^{b}	5.99±.02^{b}	374.30±.02^{b}
H. erinaceus	18.80±.02^{a}	2.01±.01^{d}	7.10±.05^{a}	76.50±.02^{c}	7.51±.02^{c}	386.30±.02^{c}
P. ostreatus	21.14±.04^{b}	2.02±.02^{d}	6.11±.02^{a}	69.86±.02^{a}	7.02±.04^{c}	378.51±.02^{b}
V. volvacea	24.12±.03^{d}	0.67±.02^{a}	9.33±.03^{a}	65.34±.03^{a}	9.09±.04^{d}	363.80±.03^{a}
G. lucidum	15.04±.01^{a}	0.53±.03^{a}	54.12±.02^{d}	82.47±.02^{d}	2.01±.02^{a}	394.50±.02^{d}

Values are mean ± SD of carefully conducted triplicate experiments. Furthermore, mean carrying different superscripted alphabets vary (p<0.05) with 95% confidence.

Fat contents of the mushrooms varied from 0.53% to 2.02% on dry weight basis, in which *G. lucidum* had the lowest (0.53%, DW), while *P. ostreatus and H. erinaceus* had the highest fat contents 2.02-2.01%, DW. The fat content of edible mushrooms consists mostly of unsaturated fatty acids, which are less hazardous to the health than the saturated fatty acids of animal fats [26].

Major sources of fiber are cellulose and other indigestible cell wall polymers. Although fiber is indigestible, it plays significant nutritional role since, it helps clean and maintains the proper motility of the intestinal tract [17]. There is a great variation in the fiber contents of five mushrooms ranging from 6.11-54.12%. The maximum fiber contents were observed in *G. lucidum* and the lowest were in *P. ostreatus*.

The highest ash content was in *V. Volvacea* (9.99%, DW), followed by *H. erinaceus* (7.5%), *P. ostreatus* (7.0%, DW) and *L. edodes* (5.99%, DW), (2.5%, DW) and the lowest was in *G. lucidum* (2.0%, DW). The major reason for high ash content of the present species may be due to higher mineral contents. A recent study also reported the ash contents ranges similar to our findings [8].

Total carbohydrates varied from 65.34% to 82.47% on dry weight basis. The carbohydrates contents were 82.47% in *G. lucidum*, 76.5% in *H. erinaceus*, 70.62% in *L. edodes*, 69.86% in *P. ostreatus* and 65. 34% in *V. valvacea*, (Table 1). Mattila et al. *[14]* reported that carbohydrate contents in *P. ostreatus* were 62.5% and in *L. edodes* were 69% of the dry matter. Other studies reported that the carbohydrate contents of *L. edodes* varied from 67.5-78% on dry weight basis. Hung and Nhi [8] reported carbohydrate contents vary from 52.3% to 88.6% on dry weight basis, *V. Volvacea* contained 52.5%, *P. ostreatus* 61.3%, *L. edodes* 65.1%, *G. lucidum* 82.3% and *A. polytricha* 88.6% [16].

Our findings showed that calorie value of five mushrooms ranges from 363-394 Kcal/100 g. Calorie value was found to be highest 394.8 kcal/100 g and was lowest in *G. lucidum* 363 Kcal/100 g in *V. volvacea* (Table 1). Mushrooms are regarded as low fat calorie foods. This low calorie value is attributable to the content of high fiber, low fat, no cholesterol and no free fatty acids in mushrooms [26]. In this study, we established that the contents of crude proteins, fats, available carbohydrates, dietary fiber, total ash and energy contents are similar to the findings of Adejumo and Awosanya [1].

3.2. Mineral Analysis

As there is an emerging public interest in the essential, non-essential and toxic elements in foods consumed daily, therefore, this approach addresses the nutritional adequacy of essential nutrients present in food. There are also concerns regarding the toxic levels of some heavy metals in the food. Twenty four elements and heavy metals (K, Ca, P, Mg, Fe, Zn, Mn, Mo, As, Sn, Se, Sb, Na, Pb, Cd, As, Co, Al, B, Be, Ba, Mg, Cu and Li) were also determined in the selected species of mushrooms. The results are shown in Table 2. Our results of minerals analysis showed that the all five mushroom species were rich in K, P, Ca, Mg, Na, Fe, Zn. Li, Cu and Mn while in this study heavy metals Pb, Cd, Br, Cr, Co and Mo were below detection limit. K recorded higher contents followed by P, Mg, Na, Ca. Fe, Zn, Al, B and Li in all the mushrooms.

Potassium values ranged from 742-3547 mg/100 g along with phosphorus that was also detected at relatively higher contents (502-1221 mg/100 g) followed by magnesium (75.8-145.8 mg/100 g) and calcium 11.2-109.2 mg/100 g on dry weight basis (Table 2).

Among these five species of mushrooms *V. volvacea* proved to be comparatively richer in concentration of K, P, Mg, Fe and Zn, while Na was the highest in *P. ostreatus* and *G. lucidum* showed higher levels of Ca, Al and B (Table 2). Previously, Mallikarjuna et al. [13] studied four mushroom species (*L. cladopus*, and *P. djamor* growing wild, whereas *L. edodes* and *P. forida* cultivated), they reported potassium levels ranges from 59.3 to 3634 mg/100 g on DW. Phosphorous contents were relatively higher, followed by calcium. However, the level of Na was higher in *L. edodes*, and Mg contents ranged from 23.1- 40.7 mg/100 g DW.

Table 2. Major essential, non-essential and toxic element concentrations (mg/100g on dry weight basis) in five species of mushrooms

Minerals	*L. edodes*	*H. erinaceus*	*P. ostreatus*	*V. volvacea*	*G. lucidum*
Al	2.820±0.003^{a}	2.501±0.001^{a}	3.310±0.001^{b}	5.10±0.001^{c}	8.001±0.002^{d}
B	2.430±0.001^{c}	0.601±0.001^{a}	3.050±0.001^{c}	0.40±0.001^{a}	6.001±0.001^{d}
Ba	Nd	Nd	Nd	Nd	Nd
Be	Nd	Nd	Nd	Nd	Nd
Ca	12.106±0.01^{a}	11.002±0.02^{a}	61.33±0.001^{c}	32.80±0.03^{b}	109.200±0.02^{d}
Cu	1.101±0.001^{a}	0.900±0.00^{a}	1.420±0.001^{c}	1.90±0.001^{d}	1.200±0.008^{b}
Cd	Nd	Nd	Nd	Nd	Nd
Co	Nd	Nd	Nd	Nd	Nd
Fe	6.901±0.003^{a}	11.200±0.008^{c}	10.20±0.001^{c}	17.70±0.001^{d}	12.100±0.01^{c}
Li	0.202±0.01^{a}	0.900±0.000^{c}	0.80±0.001^{c}	1.40±0.001^{d}	0.200±0.00^{a}
Mg	102.01±0.10^{b}	75.810±0.001^{a}	125.40±0.001^{c}	145.60±0.020^{d}	89.100±0.01^{a}
Mn	1.30±0.007^{d}	0.80±0.02^{c}	0.4±0.00^{a}	0.6±0.001^{b}	1.10±0.001^{d}
Na	16.810±0.03^{a}	32.000±0.010^{a}	395.80±0.070^{d}	42.10±0.020^{a}	20.50±0.010^{a}
Zn	6.710±0.005^{c}	3.410±0.002^{b}	4.600±0.001^{b}	7.50±0.001^{d}	2.20±0.001^{a}
P	867.40±1.30^{b}	770.80±1.020^{a}	833.00±0.200^{b}	1221.01±0.20^{d}	502.50±0.030^{a}
Pb	Nd	Nd	Nd	Nd	Nd
K	2174.08±1.2^{c}	2912.3±0.010^{d}	2395.04±0.50^{c}	3547.01±1.20^{d}	742.10±0.100^{a}
Sb	Nd	Nd	Nd	Nd	Nd
As	Nd	Nd	Nd	Nd	Nd
Mo	Nd	Nd	Nd	Nd	Nd
Se	Nd	Nd	Nd	Nd	Nd
Sn	Nd	Nd	Nd	Nd	Nd

Values are mean ± SD of carefully conducted triplicate experiments as mg/100 g, Furthermore, mean carrying different superscripted alphabets vary significantly (p<0.05) with 95% confidence, Nd- for not detected.

Iron, zinc, copper and manganese are classified as minor/trace elements. *V. volvacea* showed the highest concentration of zinc 7.5 mg/100 g, iron 17.7 mg/100 g, copper 1.9 mg/100 g whereas Mn was premier in *L. edodes* 1.3 mg/100 g on dry weight basis (Table 2). Concentration of Se, Ni and As was very low and those for Sb, Mo, Pb, Sn and Be were below detection level as have been reported earlier [22]. Some recent reports on trace elements like Cr, Ni, Li, Sr and Sb in mushrooms from China also support our findings that these trace elements are few and insufficient [25,27]. So the above findings showed that mushrooms are first-rate bioaccumulators and bioconvertors of such minerals from the growth substrate from inorganic forms to organic forms [6].

3.3. Elements Analysis of Mushrooms by LIBS

In the process of element identification, spectral information of the sample such as atomic emission wavelengths, intensities of the adjacent emission lines and line shapes were gathered to search for correspondence between elements and their specific spectral lines. In this experiment, we presented qualitative LIBS applied to mushroom samples as shown in Table 3.

LIBS spectrum of *L. edodes*, spectral lines are characteristic lines of scandium, cobalt, palladium, carbon and manganese. The spectrum of *P. ostreatus*, showed the spectral line for titanium, nitrogen, zinc, manganese and nitrogen. In spectrum of *H. erinaceus* the peak lines are for the elements of titanium, calcium, xenon and nitrogen while in the spectrum of *V. volvacea* titanium, barium, calcium, carbon and hydrogen were identified, titanium, copper, xenon, iodine lines were found from the spectrum of *G. lucidum* (Figure 1).

Table 3. Mineral contents of selected mushrooms by Laser Induced Breakdown Spectroscopy (LIBS)

Mushroom	Emission wave length (nm)	Element	Name
L. edodes	393.33	Sc	Scandium
	500.53	Co I	Cobalt
	567.88	Pd I	Palladium
	589.03	C II	Carbon
	656.37	Mn II	Manganese
P. ostreatus	399.56	Ti II	Titanium
	500.25	N II	Nitrogen
	567.91	Zn I	Zinc
	656.39	Mn II	Manganese
	746.86	N I	Nitrogen
H. erinaceus	399.49	Ti II	Titanium
	500.46	N II	Nitrogen
	567.83	Ca I	Calcium
	656.33	Xe II	Xenon
	746.918	N I	Nitrogen
V. volvacea	388.27	Ti I	Titanium
	493.40	Ba II	Barium
	563.57	Ca I	Calcium
	588.96	C II	Carbon
	656.27	H I	Hydrogen
G. lucidum	388.27	Ti I	Titanium
	500.32	Cu II	Copper
	567.80	I II	Iodine
	656.33	Xe II	Xenon
	786.86	N I	Nitrogen

I*= atomic, II*= ionic.

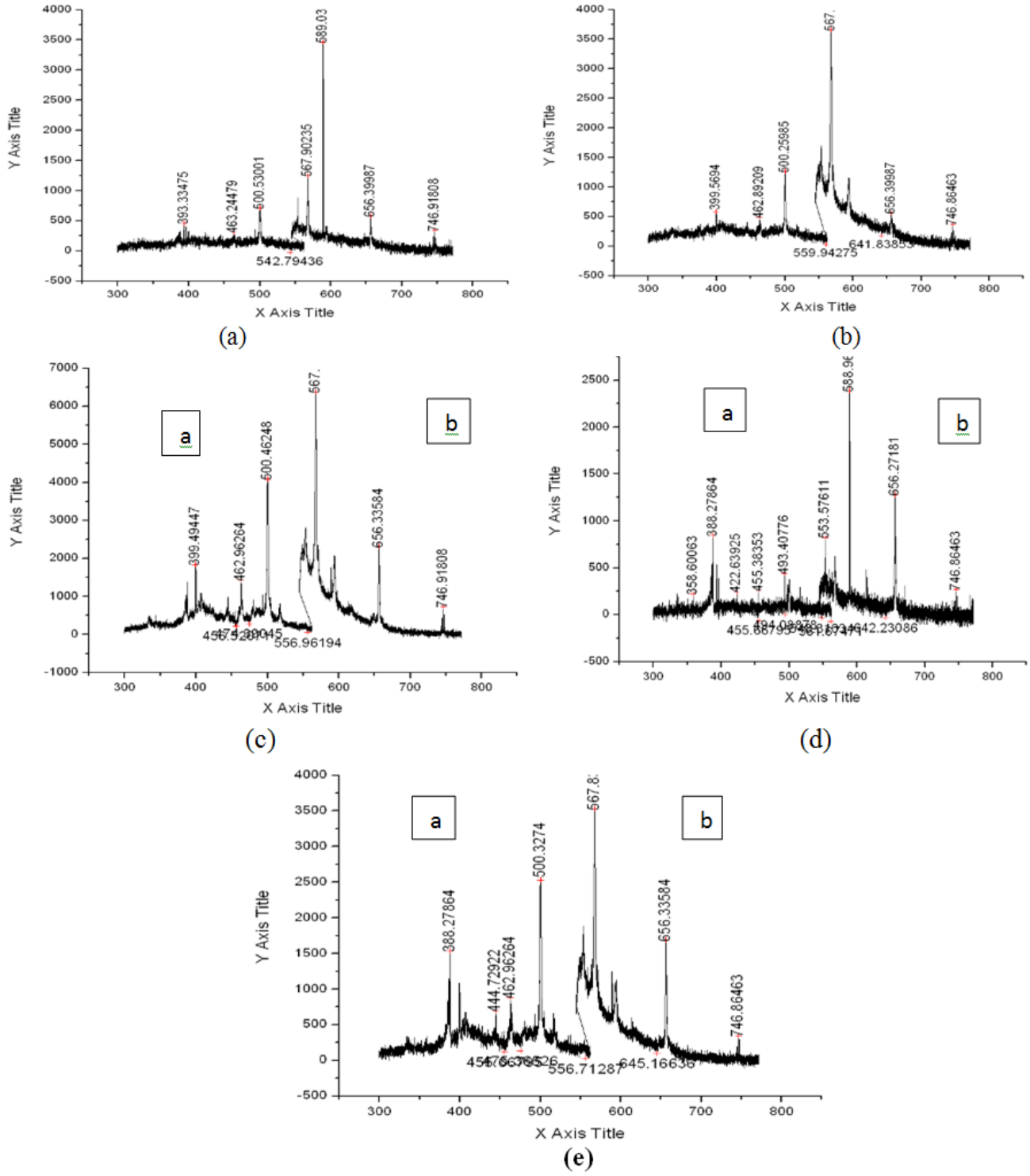

Figure 1. LIBS Spectra of mineral contents by laser induced breakdown spectroscopy (a) *L. edodes*; (b) *P. ostreatus*; (c) *H. erinaceus*; (d) *V. volvacea*; (e) *G. lucidum*

From the LIBS analysis it was also revealed that all these mushrooms are safe for consumption.

4. Conclusion

We observed that these five edible mushrooms especially the local cultivated mushrooms (*P. ostreatus*, *V. volvacea*) harbor tremendous promise in complementing the protein and mineral supply deficits prevalent in developing countries like Pakistan. The amount of proteins, carbohydrate, essential minerals and low energy contents make many wild-grown mushrooms as excellent functional food source for the consumers. So it can be concluded that mushrooms could be a good and cheaper replacement of present day nutritional sources (meat, eggs and milk) especially in developing countries. LIBS is also a useful technique that provide chemical analysis without sample preparation. Thus, local people eating these mushrooms might be beneficial to provide themselves these nutrients and also have prospective therapeutic value in controlling blood glucose and lipids due to high fiber contents.

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