



# Trace Metal Profiling In Mushrooms Using Inductively Coupled Plasma Mass Spectrometry

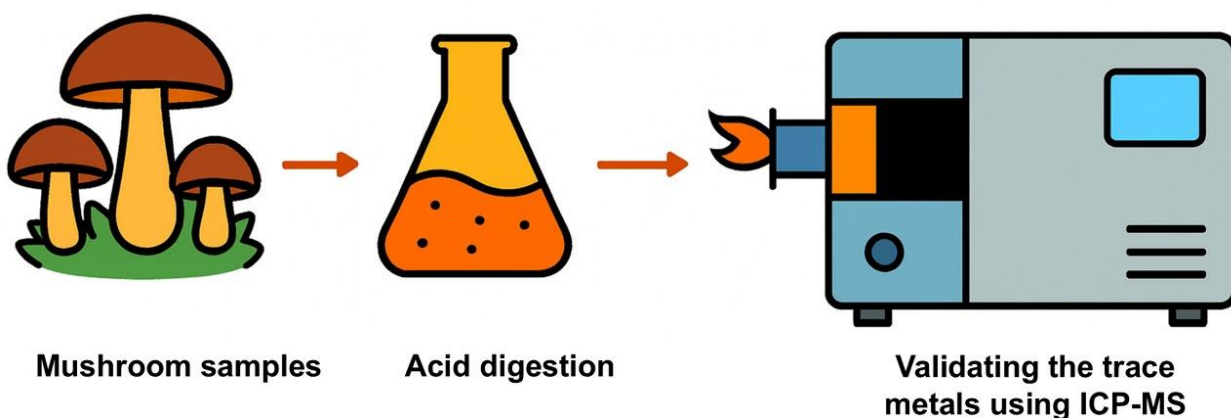
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## Abstract

This study focuses on the quantitative determination of trace metal concentrations in edible mushroom samples using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Mushrooms are known for their remarkable ability to accumulate essential and non-essential elements from their growth substrates, making them valuable bioindicators of environmental contamination. In this work, representative mushroom samples were collected from the market in Nellore, Andhra Pradesh, India. They are digested using a microwave-assisted acid digestion method before ICP-MS analysis. The concentrations of essential elements such as Fe, Zn, Cu, and Mn, along with potentially toxic metals including Pb and As, were accurately measured. Results show a considerable variation in metal accumulation among the samples, reflecting differences in soil composition and environmental exposure. Essential metals were found within acceptable nutritional ranges, while trace amounts of toxic elements were detected in certain samples, though generally below the permissible limits set by WHO and FAO. The precision and sensitivity of ICP-MS enabled reliable quantification even at sub-ppb levels, demonstrating its superiority for multi-elemental analysis. These findings highlight the importance of continuous monitoring of metal content in edible mushrooms to ensure food safety and to assess their potential role in environmental pollution studies.

**Key words:** Trace metals, toxicity, contamination, ICP-MS, spectrometry



**Scheme 1:** Schematic illustration of trace metals determination from mushrooms using ICP-MS.

## 1. Introduction

Edible mushrooms are important dietary components worldwide, valued not only for their nutritional composition but also for their well-documented capacity to accumulate trace elements from the growth substrate [1-3]. Mushrooms are a popular nutritional food due to their low calorie, carbohydrate, fat, and sodium content, and they contain no cholesterol. Additionally, they provide essential nutrients such as selenium, potassium, riboflavin, niacin, vitamin D, and fibre. Their increasing importance in our diets is attributed to their nutritional value, high protein content, and low fat/energy content [4-7]. Heavy metals are present in all parts of the Earth's crust. They are emitted in a continuous manner by various natural and anthropogenic sources. In recent decades, the contamination has increased dramatically because of continuous discharge of sewage and untreated industrial effluents. As they cannot be degraded or destroyed, they are continuously kept "in play", thus entering the physical and biological cycles. Common natural sources of heavy metals are volcanic activities, forest fires, erosion of rock and leaching processes [8]. The anthropogenic sources of heavy metals are mining and industrial wastes, vehicle emissions, municipal waste, fertilizers, paints, chemical conversion, and their modes of deposition to pollute the environment. The toxicity of heavy metals depends on various factors like the dose, route of exposure and chemical species, as well as the age, gender, genetics and nutritional status of exposed individuals [9]. The metal content of food, be of animal or plant origin, depends on many factors ranging from environmental conditions to methods of production and processing. Heavy metals can enter our food system via soil. These heavy metals are present in soil and are absorbed by the plants, and the heavy metals get accumulated in the plant products, thus entering our food. Mercury and Cadmium are the only metals which is capable of polluting our food supply after passage through water. These metals can easily penetrate cell membranes and internal organs and can cause health effects [10-12]. Mushrooms concentrate both essential micronutrients (Fe, Zn, Cu, Mn, Se) and potentially toxic elements (Fe, Zn, Cu, Mn, Se). Analytical advances, particularly the widespread adoption of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), have improved detection limits and multi-element throughput, enabling reliable quantification of trace metals at sub-ppb to low ppb levels in biological matrices [13-15]. ICP-MS, when combined with validated sample preparation such as Microwave-assisted or acid digestion and appropriate quality controls, provides the sensitivity and dynamic range required to assess both nutritional and toxic elements in mushroom tissue. This study addresses a rigorous ICP-MS analytical protocol to quantify a suite of essential and non-essential trace elements in representative mushroom samples. The objectives are (i) to determine concentrations of major nutritional elements and priority toxic metals in collected samples, (ii) to evaluate accumulation patterns, and (iii) to assess potential food-safety implications

relative to international guideline values. The results will contribute to contemporary, high-sensitivity data to the literature and help clarify the suitability of ICP-MS for routine monitoring of trace metals in edible fungi.

## 2. Materials and methods:

The study was carried out for the detection of the concentration of toxic levels of trace elements in different types of edible mushrooms.

### 2.1. Chemicals

All chemicals of high-purity analytical grade reagents were employed. Heavy metal reference standards for digestion chemicals, including HCl, HNO<sub>3</sub>, and H<sub>2</sub>SO<sub>4</sub>, were of analytical grade and were purchased from Bross Scientifics (Tirupati, A.P.) and Milli-Q water was used throughout the experiment.

### 2.2. Sample collection

Samples were collected from supermarkets in Nellore city; these are different types of edible mushrooms, which are shown below in Table 1. The pre-cleaned plastic bottles were used to collect samples. It was thus necessary to clean them thoroughly so that they would not contaminate samples. Care must be taken that the sample containers do not contaminate the samples. Preservation and analysis of samples were based on the Standard methods proposed by the World Health Organization (WHO). The heavy metals of Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Copper (Cu), Arsenic (As), Cadmium (Cd), Lead (Pb), Selenium (Se), and Zinc (Zn) were analyzed for the selected and labelled Samples. The detection of heavy metals in the environment is accomplished by various methods, but here the ICP-MS technique was used, which is relatively simple, versatile, accurate, and free from interferences.

**Table 1:** Samples used for this study.

| SAMPLE | MUSHROOM TYPE                                  |
|--------|------------------------------------------------|
| M - 1  | Milky mushroom ( <i>Calocybe indica</i> )      |
| M - 2  | Oyster mushroom ( <i>Pleurotus ostreatus</i> ) |
| M - 3  | Button mushroom ( <i>Agaricus bisporus</i> )   |

### 2.3. Sample preparation

The glassware and polyethene containers used for analysis were washed with tap water and then with distilled water. 10g of each mushroom sample was taken for analysis. The samples were digested using the recommended method described by [Mustafa et al, 2005]. 10 mg of the mushroom samples were digested using 20 mL of HNO<sub>3</sub>. The mixture was heated on a Hot plate at 100°C for 20-30 minutes. The mixture was heated on a Hot plate until the solution turned yellow and gave out yellow fumes, and then the sample was filtered. The digested sample was transferred into a 100 mL standard flask, which was filled with distilled water to the mark. The concentration of Heavy metals was determined by using ICP-MS.

### 3. Results and Discussion:

**Table 2:** Concentrations of various trace and heavy metals in mushroom samples determined by ICP-MS.

\*ND = *Not detected*

| S.No | Elements    | Units | Milky (M-1) | Oyster (M-2) | Button (M-3) |
|------|-------------|-------|-------------|--------------|--------------|
| 1    | Potassium   | mg/L  | 22.30       | 125.1        | 33.21        |
| 2    | Magnesium   | mg/L  | 6.68        | 27.6         | 15.50        |
| 3    | Calcium     | mg/L  | 3.46        | 54.01        | 80.45        |
| 4    | Manganese   | mg/L  | 0.091       | 0.082        | 0.018        |
| 5    | Iron        | mg/L  | 5.571       | 0.420        | 0.333        |
| 6    | Copper      | mg/L  | 2.103       | 7.417        | 4.871        |
| 7    | Zinc        | mg/L  | 0.419       | 0.186        | 0.059        |
| 8    | Boron       | mg/L  | 0.575       | 0.282        | 0.252        |
| 9    | Phosphorous | mg/L  | 0.355       | 8.06         | 814.6        |
| 10   | Molybdenum  | mg/L  | 0.172       | 0.003        | 0.043        |
| 11   | Arsenic     | mg/L  | ND          | ND           | ND           |
| 12   | Cadmium     | mg/L  | 0.003       | 0.003        | 0.003        |
| 13   | Lead        | mg/L  | 0.019       | 0.005        | 0.007        |
| 14   | Selenium    | mg/L  | 0.101       | 0.1          | 0.109        |
| 15   | Thallium    | mg/L  | 0.022       | 0.057        | 0.041        |

The concentrations of macro- and microelements in M-1, M-2, and M-3 determined by ICP-MS are presented in Table 2. Marked variations were observed among species, indicating differences in metal uptake capacity, substrate composition, and physiological accumulation mechanisms.

#### 3.1. Macro elements (K, Mg, Ca, and P)

Potassium (K) was the most abundant element across all mushroom species, ranging from 22.30 mg/L in milky to 125.1 mg/L in Oyster mushrooms. The high K level in Oyster mushrooms highlights their strong ability to accumulate this essential macronutrient, which plays a key role in osmoregulation and enzymatic activation. Similar dominance of potassium has been reported in numerous mushroom studies [14, 15]. Magnesium (Mg) concentrations followed a similar pattern, with the highest in Oyster 27.6 mg/L and the lowest in Milky 6.68 mg/L, reflecting both the nutrient availability in the substrate and the metabolic requirements of the species.

Calcium (Ca) concentration varied considerably, with Button mushrooms exhibiting the highest content 80.45 mg/L, followed by Oyster 54.01 mg/L and Milky 3.46 mg/L. This suggests that Button mushrooms have a higher affinity for Ca, possibly due to the calcareous nature of their compost substrate. Phosphorus (P) was extremely elevated in Button mushrooms 814.6 mg/L, compared with 8.06 mg/L in Oyster and 0.355 mg/L in milky mushrooms. The remarkably high P content in *A. bisporus* aligns with previous findings attributing this to phosphorous-rich compost substrates and efficient uptake mechanisms.

### 3.2. Micronutrients (Fe, Mn, Cu, Zn, B, and Mo)

Micronutrients, Iron (Fe) showed substantial interspecies variation: 5.571 mg/L in Milky, 0.420 mg/L in Oyster, and 0.333 mg/L in Button mushrooms. The higher Fe accumulation in Milky mushrooms indicates a stronger affinity for iron-rich substrates or enhanced metal transport activity. Manganese (Mn) content remained low across samples 0.018-0.091 mg/L, consistent with its role as a trace nutrient.

Copper (Cu) levels were markedly higher in Oyster mushrooms 7.417 mg/L compared with Button 4.871 mg/L and Milky 2.103 mg/L. The elevated Cu in Oyster mushrooms can be linked to the strong involvement of copper-containing oxidases such as laccase and tyrosinase in their metabolic system. Zinc (Zn) concentrations were modest, ranging from 0.059 mg/L in Button to 0.419 mg/L in Milky mushrooms, reflecting moderate bioaccumulation efficiency. Boron (B) and molybdenum (Mo) were detected at trace levels, with B ranging from 0.252 to 0.575 mg/L and Mo between 0.003 and 0.172 mg/L. The relatively higher Mo in milky mushrooms could indicate its role in nitrogen metabolism and enzyme regulation.

### 3.3. Toxic and Non-essential Elements (Pb, Cd, As, Se, Tl)

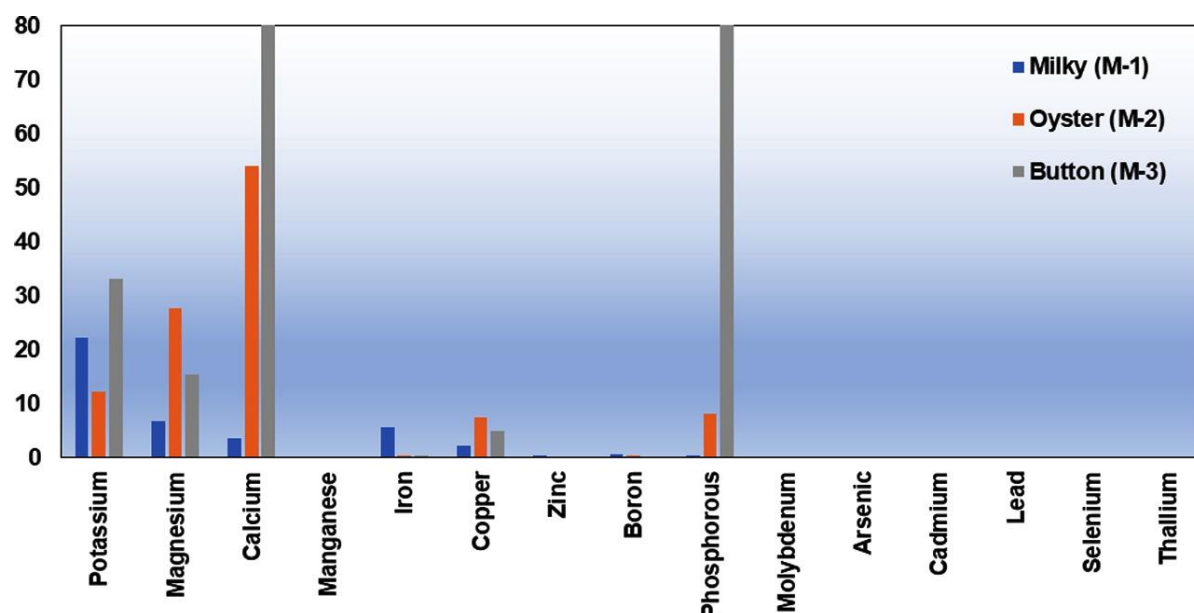
Arsenic (As) was not detected (ND) in any of the samples, signifying minimal contamination from industrial or agricultural sources. Cadmium (Cd) was present at 0.003 mg/L in all species, remaining far below the WHO/FAO permissible limit of 0.05 mg/L for edible mushrooms. Lead (Pb) concentrations ranged from 0.005 mg/L in Oyster to 0.019 mg/L in Milky mushrooms, also well within the acceptable range ( $\leq 0.3$  mg/L).

Selenium (Se), an essential trace element with antioxidant significance, appeared in moderate concentrations 0.1-0.109 mg/L, consistent with reported natural levels in wild and cultivated mushrooms, a rarely measured element in fungal studies, was detected in all samples at low levels 0.022-0.057 mg/L) Fig. 1.

### 3.4. Comparative Assessment and Environmental Implications

**Table 3:** WHO permissible limit for metal content in foods.

| Element    | Unit | Permissible Limit |
|------------|------|-------------------|
| Potassium  | mg/L | N/A               |
| Magnesium  | mg/L | N/A               |
| Calcium    | mg/L | N/A               |
| Manganese  | mg/L | 2 [16]            |
| Iron       | mg/L | 15[16]            |
| Copper     | mg/L | 10 [16]           |
| Zinc       | mg/L | 50 [16]           |
| Boron      | mg/L | N/A               |
| Phosphorus | mg/L | N/A               |
| Molybdenum | mg/L | 0.1 [16]          |
| Arsenic    | mg/L | 0.1 - 0.2 [16]    |
| Cadmium    | mg/L | 0.05 - 2.0 [16]   |
| Lead       | mg/L | 0.01 - 3.0 [16]   |
| Selenium   | mg/L | 0.01 [16]         |
| Thallium   | mg/L | 0.002 [16]        |



**Fig. 1.** Image showing the various concentrations of metals in three mushroom samples

Overall, Oyster mushrooms exhibited higher concentrations of key macronutrients (K, Mg, Cu), indicating robust uptake and efficient mineral assimilation from their lignocellulosic substrates. Button mushrooms showed exceptional accumulation of calcium and phosphorus, suggesting species-specific nutrient preference and substrate dependency. In contrast, Milky mushrooms, while lower in major nutrients, displayed elevated iron and molybdenum levels, highlighting selective bioaccumulation pathways. Importantly, all measured toxic elements (Cd, Pb, Tl) (Table 3) remained well below international food-safety thresholds, confirming that the studied mushroom samples are safe for human consumption. The low detection of hazardous elements suggests limited industrial contamination in the cultivation environment.

#### 4. Conclusion

This study successfully quantified the elemental composition of *Calocybe indica* (Milky), *Pleurotus ostreatus* (Oyster), and *Agaricus bisporus* (Button) mushrooms using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The analytical results revealed significant variations in elemental accumulation among the three species, reflecting their distinct physiological uptake mechanisms and substrate affinities. Among the macronutrients, potassium, calcium, and phosphorus were dominant, with Oyster mushrooms exhibiting the highest potassium and magnesium levels, while Button mushrooms accumulated exceptionally high amounts of calcium and phosphorus. Milky mushrooms showed relatively lower macronutrient content but displayed elevated iron and molybdenum concentrations, suggesting selective bioaccumulation behavior. The concentrations of potentially toxic elements such as cadmium, lead, and thallium were minimal and remained well below the permissible limits set by WHO/FAO, confirming the safety of the analyzed mushrooms for human consumption. The absence of detectable arsenic further indicates that the cultivation soils in the studied regions are not significantly contaminated by industrial pollutants.

These findings highlight the capability of edible mushrooms to act as both nutritious food sources and reliable bioindicators for environmental trace metal assessment. The precision and sensitivity of ICP-MS proved valuable for simultaneous multi-element detection at trace levels, ensuring accurate environmental and food-safety evaluation. Continued monitoring of metal accumulation in cultivated and wild mushrooms is

recommended, particularly areas exposed to industrial or agricultural activity, to ensure safe consumption and to track potential ecological changes.

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