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Determination of Muscimol Alpha Amanitin and Metal Ions in Some Poisonous Mushrooms

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Abstract: Mushrooms are an important nutritional source due to their richness in proteins, carbohydrates, fatty acids, vitamins, and minerals. They offer numerous health benefits, including blood pressure regulation, antitumor activity, immune system enhancement, cholesterol control, antioxidant properties, and the regulation of heart rhythm. However, cases resulting in severe bodily harm and even death have been reported due to the consumption of wild mushrooms by individuals lacking expertise in mushroom identification and relying on hearsay or inaccurate information. Even low doses of certain mushroom toxins can lead to poisoning. Given that the levels of compounds such as muscimol, alpha-amanitin, and various metals vary depending on the mushroom species and their growing environment, this study was conducted. Used for centuries as poisons, medicines, and food, mushrooms are now recognized as an indispensable source of protein. In addition to mushroom toxins, heavy metals present in mushrooms can damage various organs, lead to more severe health outcomes, and accelerate death. In this study, a quantitative analysis of different toxins and the metal content in various mushroom species was carried out. For this purpose, both edible and toxic mushroom species were analyzed, including Agaricus bisporus (J.E. Lange) Imbach, commonly consumed as a cultivated mushroom, and the toxic species Amanita phalloides (Vaill. Ex Fr.) Link, Amanita pantherina (DC.) Krombh., and Amanita muscaria (L.) Lam., collected from the provinces of Rize, Trabzon, and Düzce. The toxins alpha-amanitin in Amanita phalloides and muscimol in Amanita pantherina and Amanita muscaria were analyzed using High-Performance Liquid Chromatography (HPLC).The concentrations of elements Ag (ng/kg), Al (µg/kg), As (ng/kg), Ba (ng/kg), Ca (µg/kg), Cd (ng/kg), Co (ng/kg), Cr (ng/kg), Cu (ng/kg), Fe (µg/kg), K (µg/kg), Mg (µg/kg), Mn (ng/kg), Mo (ng/kg), Na (µg/kg), Ni (ng/kg), P (µg/kg), Pb (ng/kg), Sb (ng/kg), Sn (ng/kg), Sr (ng/kg), Ti (ng/kg), V (ng/kg), and Zn (ng/kg) in mushrooms were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), while Hg (µg/kg) was analyzed using graphite furnace Atomic Absorption Spectroscopy (AAS). Additionally, the elemental composition C, N, H, S, and O in terms of percentage was determined using an elemental analyzer. The binding forms of the metals were identified using X-ray Diffraction (XRD).

1. Introduction

Mushrooms, which have been used by humans as poisons, medicines, and nutritional substances, constitute one of the most important groups in the kingdom of living organisms. As they do not contain chloroplasts, they are not classified as plants; likewise, due to their lack of mobility, they are not considered animals. Instead, fungi are defined as a distinct kingdom separate from both plants and animals. Fungi are fundamentally divided into two groups:

microfungi and macrofungi. Macrofungi are further categorized into those that grow naturally in the wild and those that are cultivated. Wild-growing mushrooms are classified as either edible or poisonous. Poisonous mushrooms contain various toxic compounds such as ibotenic acid, muscimol, alpha-amanitin, beta-amanitin, gamma-amanitin, muscarine, and coprine, depending on the species. These toxins exert harmful effects on the nervous, respiratory, excretory, and gastrointestinal systems, varying by type. The latency period of these poisons also differs according to the species of the mushroom and the specific toxin involved. While the latency period can be as short as fifteen minutes in some species, it may extend up to one or two months in others. The amount of mushroom consumed and the individual's physiological resistance also play a critical role during the latency phase.

2. Materials and Methods

Among the mushroom species consumed as food, wild edible mushrooms are particularly rich in taste and aroma. Nevertheless, cultivated mushrooms are consumed more widely than wild mushrooms due to their scientific production processes, which ensure their non-toxic nature. Numerous studies have been conducted especially in East Asian countries on macrofungi used both as food and medicine, and the number of such studies continues to increase. Research on the medicinal properties of macrofungi encompasses a wide range of topics, including blood pressure regulation, antitumor activity, immune system enhancement, antimicrobial effects, cholesterol control, antibiotic properties, antioxidant activity, regulation of heart rhythm, antiulcer effects, influence on lipid metabolism, antidiabetic properties, anti-HIV activity, anti-obesity effects, protective functions on the liver and kidneys, antiviral effects, anti-aging properties, treatment of sexual hypofunction, and their impact on hematopoiesis (Erol, 2020).

The digestibility of mushroom protein ranges between 72% and 83%. In addition to essential amino acids required for human nutrition, mushrooms also contain all other amino acids. Although the fat content in mushrooms is very low, they include all types of fatty acids, including essential ones. Mushrooms are also an excellent source of folic acid, and diets containing mushrooms are used in the treatment of anemia caused by folic acid deficiency. Fresh mushrooms consist of approximately 92% water, while the remaining 8% is composed of protein, fat, carbohydrates, vitamins, calcium, phosphorus, potassium, iron, copper, fiber, and ash (Wang, 2023). Due to their significant mineral content, mushrooms may have beneficial effects for individuals with mineral deficiencies. Additionally, mushrooms are a source of thiamine (B1), riboflavin (B2), pantothenic acid (B5), nicotinic acid (B3, niacin), biotin (B7), ascorbic acid (C), and vitamin D (Assemie, 2022).

The macroelements found in the human body include calcium, phosphorus, potassium, sulfur, chlorine, sodium, and magnesium. Microelements consist of iron, manganese, cobalt, copper, zinc, molybdenum, vanadium, chromium, tin, fluorine, silicon, selenium, and iodine. Compared to microelements, macroelements are present in the body at higher concentrations. Among the trace elements found in living organisms, iron, manganese, cobalt, copper, zinc, molybdenum, vanadium, chromium, and tin are metals, whereas fluorine, silicon, selenium, and iodine are non-metals. Although these elements exist in relatively small amounts, they play crucial biological roles. Many of them are components of enzymes and are essential for vital physiological processes. Any imbalance either an increase or decrease in the levels of these elements in the human body can lead to serious health issues. Heavy metals exhibit increasing toxic effects on living organisms depending on their concentrations. In particular, cadmium (Cd), hexavalent chromium (Cr^{6+}), mercury (Hg), and lead (Pb) are not essential for biological systems and can exert toxic effects even at trace levels. The term "heavy metal" is used for metals with a density greater than 5 $g \cdot cm^{-3}$. This group includes over sixty metals such as

cadmium, chromium, lead, copper, iron, nickel, cobalt, mercury, molybdenum, tin, and zinc (Mood et al., 2021).

2.1. Researched fungi and the syndromes they cause amanita muscaria

The cap of *Amanita muscaria* ranges from 5 to 20 cm in diameter. Initially white in color, it develops red pigmentation as the partial veil disintegrates, leaving white remnants of the veil on the red surface. When young, the mushroom has a conical shape; as it matures, it becomes hemispherical, and in full maturity, it flattens. The cap surface is smooth and hairless, and its color can vary from red to orange. In immature specimens, the cap margin is inwardly curved, gradually flattening as the mushroom matures. The stipe (stem) ranges from 6 to 20 cm in length and is approximately 1 to 1.5 cm in diameter. *Amanita muscaria* is a gilled mushroom. The gills are initially white and transition to brownish hues with maturity. After the partial veil ruptures, a remnant known as the annulus remains on the upper part of the stipe. The spores are colorless, transparent, and measure approximately 9-11 µm in length and 6-9 µm in width. *Amanita muscaria* typically grows individually or in groups under both deciduous and evergreen trees, particularly pines and birches. It is especially fond of acidic soils. Fruiting bodies appear in late summer and autumn (Vendramin, 2014). In Türkiye, this species has been recorded in the Mediterranean region, the Eastern Black Sea part of the Black Sea region, and around Balıkesir, Bursa, Bolu, Düzce, and Istanbul. Red, Yellow Amanita muscaria, Amanita pantherina and Amanita phalloides are given in Figure 1.

Figure 1. Red, Yellow Amanita muscaria, Amanita pantherina and Amanita phalloides

2.2. Amanita pantherina

The cap diameter ranges between 5-10 cm. When young, the cap is hemispherical in shape; as it matures, it becomes convex and eventually flattens. In later stages of maturity, it may become depressed. The surface is shiny and sticky when moist, but turns matte when dry. It is light brown in color. Similar to *Amanita muscaria*, it bears white remnants of the partial veil on the cap. As the partial veil ruptures, an annulus, as seen in *Amanita pantherina*, forms on the upper part of the stipe. This species is lamellate, and the gills are white in color. The stipe is cylindrical, measuring 5-12 cm in height and 1-2 cm in width. When young, the stipe has a spongy texture, but it becomes grooved with age. The spores are colorless and elliptical, measuring 9-12 micrometers in length and 7-8 micrometers in width. Their walls are smooth. It is found singly or in groups under coniferous or deciduous trees during the summer and autumn months (Satora, 2006). This species has been observed in the Black Sea region, the Mediterranean region, and in the Bolu and Kaz Mountains.

Pantherina Syndrome is caused by Amanita muscaria and Amanita pantherina, with a latency period ranging from 30 minutes to 3 hours. This syndrome manifests with symptoms such as speech impairment, gastrointestinal cramps, visual and auditory disturbances, hallucinations, abdominal pain, vomiting, diarrhea, and deep sleep. As it belongs to the first group of mushroom poisoning classifications, fatal outcomes are rare in pantherina syndrome. However, death may occur in sensitive individuals, particularly children (Yardan et al., 2010).

Additionally, pantherina syndrome primarily affects the central nervous system. The toxic compounds responsible for this syndrome are ibotenic acid and muscimol. Given in Figure 2. Ibotenic acid (α-amino-3-hydroxy-5-isoxazoleacetic acid) undergoes decarboxylation to form muscimol (3-hydroxy-5-amino methylisoxazole). In the treatment of Pantherina syndrome, the specific antidote physostigmine is used. Additionally, symptomatic and supportive therapies are administered as auxiliary treatments.

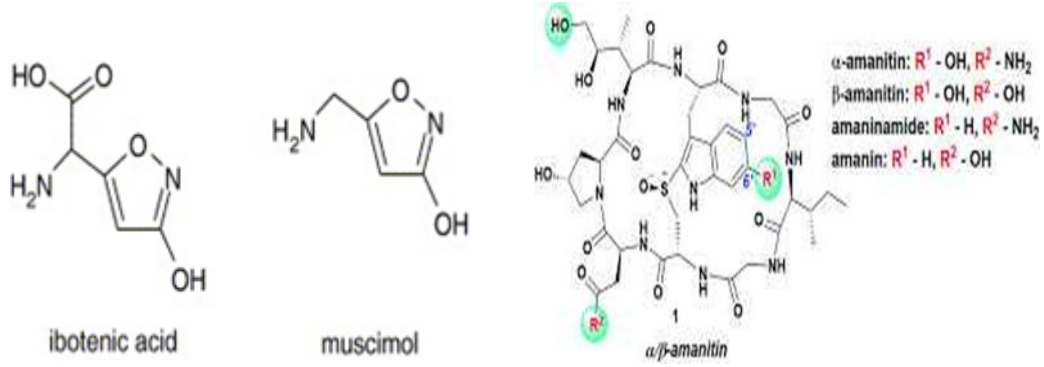

Figure 2. Molecular structure of Ibotenic acid, Muscimol and Chemical structure of α/β-amanitin and structure–activity relationships of natural and synthetic amatoxins with indole modifications

2.3. Amanita phalloides

The cap of *Amanita phalloides* (commonly known as the "death cap") measures 5-10 cm in diameter and has a yellowish to pale cream surface with a slightly rough texture. The stipe (stem) is cylindrical, 5-10 cm in length, 1-1.5 cm in width, and thicker at the base. As the mushroom matures, the central part of the stipe becomes hollow, resembling a tunnel. Like other *Amanita* species, it features an annulus. It is a gilled mushroom; the gills are white when young and gradually turn yellowish with age. The spores are colorless, elliptical, and have smooth walls, measuring 8-10 µm in length and 7-8.5 µm in width. *Amanita phalloides* typically grows under oak, beech, pine, and hornbeam trees, and is found during the summer and autumn months. In Türkiye, it has been recorded in regions such as Adana, Ordu, and Istanbul. *Amanita phalloides* is one of the most well-known toxic mushrooms and causes life-threatening illness due to liver and kidney failure.

Phalloides Syndrome The latency period in Phalloides syndrome is approximately one day. On the day the mushroom is consumed, no symptoms are observed. On the second day, poisoning symptoms emerge in the form of abdominal pain and diarrhea. These symptoms appear to subside by the third day, creating a deceptive sense of recovery. However, laboratory findings indicate severe liver damage. On the fourth day, gastrointestinal bleeding may occur; by the fifth day, neurological deterioration and the onset of coma are observed. Symptoms progress to kidney failure by the sixth day, and in most cases, result in death by the seventh day. Phalloides syndrome is classified into four degrees based on the severity of poisoning: First-degree poisoning involves the onset of gastrointestinal symptoms following the latent period, but no damage to the liver or kidneys is observed. Second-degree poisoning is characterized by moderate coagulopathy in the blood, with an increased white blood cell count, and gastrointestinal symptoms are also present. Third-degree poisoning results in severe liver damage, accompanied by elevated white blood cell count, while bilirubin levels remain unchanged. Fourth-degree poisoning shows increases in both white blood cell and bilirubin levels, accompanied by kidney failure and respiratory complications. The risk to life varies according to the degree of poisoning. First- and second-degree cases carry a low risk of fatality, whereas third-degree cases require more cautious management. In fourth-degree poisonings, due to kidney failure and respiratory distress, the chances of survival are significantly reduced.

The toxic agents responsible for Phalloides syndrome are amatoxins (Butera, 2004). Amatoxins are classified into various types based on their radical side groups. The most common type is α-amanitin, which inhibits RNA polymerase II, thereby halting protein synthesis and leading to cell death. The molecular formula of α-amanitin is $C_{39}H_{54}O_{14}S$. The molecular structure of α-amanitin and the general structures of amanitin compounds are presented in Figure 2. The mushroom species used in this study are listed in Table 1.

Table 1. Mushrooms Used in the Experiment

Mushroom species	**Collection area**
Amanita phalloides	Düzce-Gümüşova Yeşilyayla
Amanita pantherina	Düzce-Gölyaka Çamlıbel plateau
Amanita muscaria/Trabzon	Trabzon-Akçaabat Isabel plateau
Amanita muscaria/Rize	Rize-Ayder Konifer plateau
Amanita muscaria/Düzce	Düzce
Agaricus bisporus **(**Culture)	Bought from the market

The ICP-MS instrument, the AAS system with graphite furnace, and the HPLC device were used in the experiment. Technical specifications of the instruments are given in Table 2, Table 3, and Table 4.

Table 2. Specifications and operating conditions of the ICP-MS device

Carrier gas	Argon
Flow rate	1.2 L/min.
Power	1350 W
Sampling depth	6,7 mm
Spraying chamber	Double chamber, quartz
Spraying temperature	2^0C
Internal standards	6Li, 45Sc, 72Ge, 89Y, 115In, 159Tb, 209Bi

Table 3. Features and operating conditions of the AAS device

System used	Graphite furnace
Lamp used	c-EDL Hg lamp
Matrix used	Pd, $Mg(NO_3)_2$
Carrier gas	Argon
Detector	5973 N
Temperature program	130^0C-150^0C for cleaning purposes 1150^0C-2450^0C output
Cleaning solution	0.2% nitric acid
Current	185 mA
Wavelength	253,7 λ

Table 4. HPLC device specifications and operating conditions

Column	Agilent Zorbax eclipse XDB-CB 4,6* 150mm 5µm column
Eluent	65% water, 10% methanol, 25% acetonitrile mixture
Eluent flow rate	0,5 mL/min
Intracolumn pressure	33 bar

3. Results and Discussions

The mushrooms, which were first left to dry at room temperature in open air for five days and then ground into powder using a glass mortar, were further dried in an oven at 50 °C for one hour and subsequently weighed. For metal determination, acid mixtures were added to each sample, followed by microwave-assisted digestion. To analyze the elemental composition of the mushroom samples using ICP-MS, six different digestion methods were tested by varying the types of acids and the ratios of the same acids. The acids were added to 0.4 grams of weighed mushroom powder.

1. 8 mL 65% HNO_3 ve 5 mL 35% H_2O_2

2. 12 mL 65% HNO_3

3. 12 mL 65% HNO_3 ve 5 mL 70% $HClO_4$

4. 8 mL 65% HNO_3, 4 mL 70% $HClO_4$, 1 mL 35% H_2O_2, 1 mL 37% HCl

5. 8 mL 65% HNO_3, 2 mL 95%-98% H_2SO_4, 1 mL 35% H_2O_2

6. Aqua regia was used. 9 mL 37% HCl ve 3 mL 65% HNO_3

In all six mixtures, the final volumes were brought up to 25 mL using deionized water. Complete dissolution of the prepared mushroom solutions was achieved using a microwave digestion system. The digestion process was carried out under a pressure of 1500 psi, with a stepwise temperature increase in the microwave oven: 100 °C for the first 10 minutes, 150 °C for the next 10 minutes, and 200 °C for the final 10 minutes, totaling 30 minutes. The samples were analyzed using ICP-MS, the specifications of which are given in Table 2, and the operating conditions of the AAS device in Table 3. Based on the sample results presented in Table 1, the elemental analyses obtained using the six solvent mixtures are shown in Table 5. Among these, Solvent Mixtures 1 and 3 were determined to be the most suitable. For *Amanita pantherina*, Mixture 1 was optimal, while Mixture 3 was the most appropriate for the other mushroom species. A graphical representation of the ICP-MS-determined concentrations of Ca (µg/kg), Al (µg/kg), P (µg/kg), and K (µg/kg) is provided in Figure 3, and the elemental concentrations for the six mushroom samples are presented in Table 7.

Mercury (Hg) analysis was performed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) on samples prepared from the following mushroom species: cultivated mushrooms, *Amanita phalloides*, *Amanita pantherina*, and *Amanita muscaria* collected from Rize, Trabzon, and Düzce. The results are presented in Table 6. According to the findings, the most effective digestion method for mercury analysis was the mixture of 9 mL of 37% pure HCl and 3 mL of %65 pure HNO_3. However, for *Amanita phalloides*, the most suitable method was found to be the mixture of 8 mL of 65% pure HNO_3 and 5 mL of 35% pure H_2O_2. As shown in Table 6, high levels of mercury were detected in all mushroom species, with *Amanita phalloides* exhibiting the highest mercury concentration. In a comparable study, the mercury level detected in cultivated mushrooms was lower than the levels observed in the present study. Mercury (Hg), which is a liquid at room temperature, exists in both inorganic and organometallic forms. All mercury compounds are toxic and pose significant risks to living organisms.

Table 5 Suitable Solvents and Analysed Elements in Elemental Analysis

Sample Solvent Mixture	**Element suitable for analysis**
1. 8mL 65% HNO_3 and 5mL 35% H_2O_2	Al,Ca,Co,Cd.Cu,Fe,K,Mg,Mn,Mo,Na,Ni,P,Zn
2. 12mL 65% HNO_3	Ba,K,Mn
3. 12mL 65% HNO_3 and 5mL 70% $HClO_4$	Al,Ca,Co,Cd,Cr, Cu,Fe,Mg,Mn,Pb,Sb,Sr,Ti,V
4. 8mL 65% HNO_3, 4mL 70% $HClO_4$, 1mL 35% H_2O_2, 1mL 37% HCl	Mn
5. 8mL 65% HNO_3, 2mL 95%-98% H_2SO_4, 1mL 35% H_2O_2	K,Mn,P
6. 9mL 37% HCl and 3 mL 65% HNO_3	Ag,As,Cr,K,Mn,Zn

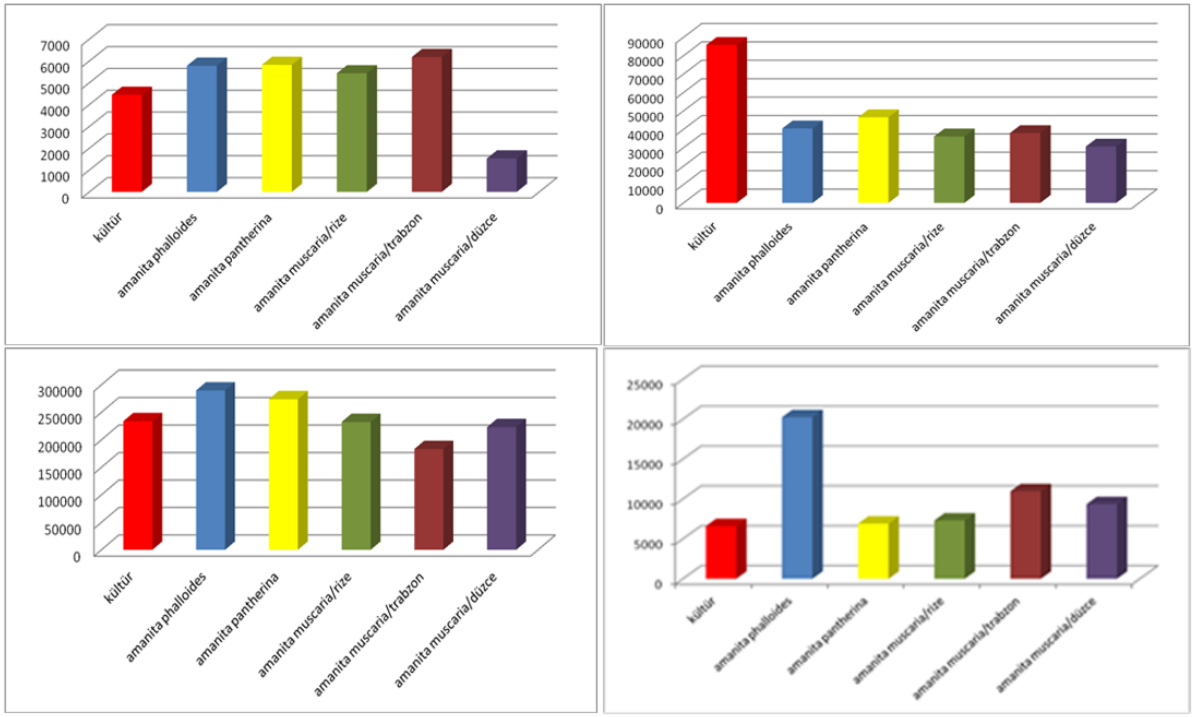

Figure 3 Graphical representation of the amount of Ca(µg/kg) P (µg/kg) K(µg/kg) element determined by ICP-MS and Hg(µg/kg) element analyzed by AAS

In particular, methylmercury and other alkyl mercury compounds are highly toxic. Although poisoning from inhalation of metallic mercury or mercury vapor is rare, such cases can lead to severe health issues and even death. Mercury compounds tend to persist in the body, accumulating primarily in the liver and kidneys. Mercury also accumulates in the brain, where it particularly affects motor function systems, potentially leading to neurological syndromes. According to the World Health Organization (WHO), acceptable ambient mercury concentrations are 0.1-5 ng·m^{-3} in urban areas, 0.5-20 ng·m^{-3} in industrial zones, and 0.001-6 ng·m^{-3} in rural areas (Cocchi et al., 2006). Furthermore, mercury can cross the placenta, and even at very low doses, chronic exposure in pregnant women may adversely affect the developing fetus.

Table 6. Hg (µg/kg) concentrations determined by AAS

Mushroom Type	**Hg (µg/kg)**
Agaricus bisporus (Culture)	6600±23
Amanita phalloides	20236±101
Amanita pantherina	6908±22
Amanita muscaria/Rize	7292±18
Amanita muscaria/Trabzon	10960±23
Amanita muscaria/Düzce	9340±18

The toxins α-amanitin in *Amanita phalloides* and muscimol in *Amanita pantherina* and *Amanita muscaria* were analyzed using HPLC. The mushrooms, previously dried and ground into powder, were extracted in a methanol–water mixture at room temperature for approximately one hour. The extracts were then filtered through filter paper and prepared for analysis. For the extraction, 5 mL of methanol and 5 mL of deionized water were used. Standards for α-amanitin and muscimol analysis were similarly prepared using 2.5 mL of methanol and 2.5 mL of deionized water. Standard solutions were prepared for the analysis of the muscimol toxin. Peak areas were identified by HPLC. A calibration curve of concentration versus peak area was plotted for the standards. The calibration graph for muscimol is presented in Figure 4.a and for α-amanitin in Figure 4.b Using these calibration graphs, the concentrations

of muscimol and α-amanitin in the mushroom samples were calculated based on the obtained peak areas. The results are provided in Table 8. The graphical representation is given in Figure 5.

Table 7. Quantities of elements determined in mushroom samples by ICP-MS

	Agaricus bisporus (Culture)	**Amanita phalloides**	**Amanita pantherina**
Ag (ng/kg)	*	5,14±1,08	*
Al (µg/kg)	49,38±5,92	1111,81±28,99	3211,15±287,5
As(ng/kg)	18,42±1,69	20,26±1,1	23,95±1,71
Ba(ng/kg)	9,73±1,54	28,97±1,5	12,48±1,54
Ca(µg/kg)	4449±209,01	5778,7±1287,59	5819,7±118,78
Cd(ng/kg)	0,75±0,05	9,6±0,13	99,30±0,53
Co(ng/kg)	0,16±0,03	0,1±0,07	0,93±0,15
Cr(ng/kg)	9,44±3,06	10,28±1,85	8,68±2,04
Cu (ng/kg)	196,37±6,51	237,77±6,04	175,63±6,03
Fe(µg/kg)	*	1687,3±176,4	3054,7±455,5
K(µg/kg)	233733,3±27226,2	289633,3±26339	273600±43350,1
Mg(µg/kg)	10353,3±125,03	10396,7±179,26	9150,3±274,5
Mn(ng/kg)	34,06±0,9	234,1±10,07	154,13±5,02
Mo(ng/kg)	*	*	*
Na(µg/kg)	3727,8±97,61	287,3±62,62	2677,8±272,32
Ni(ng/kg)	*	*	0,036±0,06
P (µg/kg)	85830±1300	40430±384,32	46530±451,77
Pb (ng/kg)	158,2±17,45	242,77±4,25	254,6±7,89
Sb (ng/kg)	7,07±0,57	6,75±0,03	7,2±0,45
Sn(ng/kg)	87,07±28,73	90,14±102,68	33,63±25,17
Sr(ng/kg)	7,65±0,11	0,5±0,72	1,08±0,64
Ti (ng/kg)	121,58±1,18	77,36±0,9	133,08±1,14
V (ng/kg)	*	*	*
Zn (ng/kg)	389,48±27,37	431,62±23,82	1482,75±204,86
	Amanita muscaria/Rize	**Amanita muscaria/Trabzon**	**Amanita muscaria/Düzce**
Ag (ng/kg)	5,55±0,55	2,33±0,85	3,23±2,23
Al (µg/kg)	2401,48±264,86	659,28±65,76	1670,81±51,43
As(ng/kg)	26,01±0,78	23,55±1,6	22,96±2,33
Ba(ng/kg)	26,58±1,54	7,48±0,77	9,56±0,87
Ca(µg/kg)	5432,3±663,56	6177,7±434,36	1527±308,39
Cd(ng/kg)	88,45±1,9	51,52±1,74	51,45±1,86
Co(ng/kg)	1,45±0,61	0,29±0,06	*
Cr(ng/kg)	8,65±0,9	7,08±0,7	7,06±1,01
Cu (ng/kg)	223,23±0,95	144,37±8,05	127,53±5,49
Fe(µg/kg)	2727,3±289,86	527,23±279,46	349,6±71,96
K(µg/kg)	232333,3±40802,25	183100±6816,89	232033,3±8533,66
Mg(µg/kg)	7404,7±145,22	5816±324,73	6106±289,45
Mn(ng/kg)	137,37±3,11	72,36±17,26	53,7±2,00
Mo(ng/kg)	*	*	*
Na(µg/kg)	1677,1±487,01	628,8±92,29	779,8±316,75
Ni(ng/kg)	*	*	*
P (µg/kg)	36086,7±707,27	37943,3±1905,37	30633,3±1179,38
Pb (ng/kg)	190.07±6,11	207,9±5,96	226,13±3,79
Sb (ng/kg)	7,01±0,39	6,86±0,06	6,67±0,11
Sn(ng/kg)	137,68±17	52,18±7,9	109,67±8,9
Sr(ng/kg)	4,26±1,56	*	*
Ti (ng/kg)	131,14±12,9	92,918±4,31	75,582±1,66
V (ng/kg)	1374,7±20,1	537,8±29,6	896,4±36,00
Zn (ng/kg)	876,4±45,2	753,58±31,73	1084,08±68,65

*below the determination limit

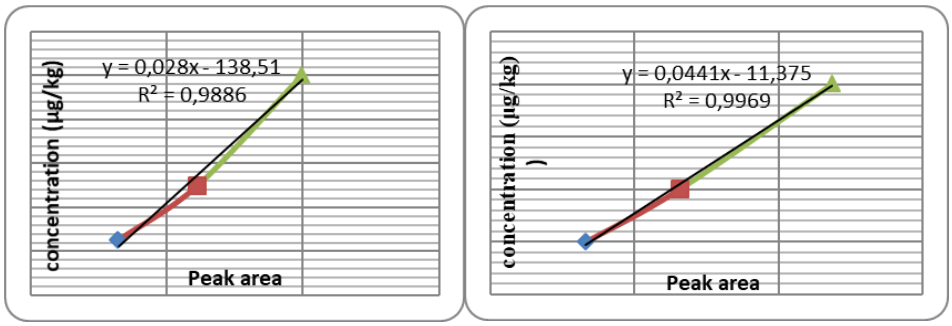

Figure 4. a-Muscimol calibration graph **b** -Alpha amanitin calibration graph

Table 8. Muscimol and Alpha Amanitin determined amounts by HPLC

Mushroom Type	**Muscimol (µg/kg)**
Amanita muscaria/Rize	1493,74
Amanita muscaria/Trabzon	2048,02
Amanita muscaria/Düzce	1387,28
Amanita pantherina	-
	Alfa amanitin (µg/kg)
Amanita phalloides	1223,21

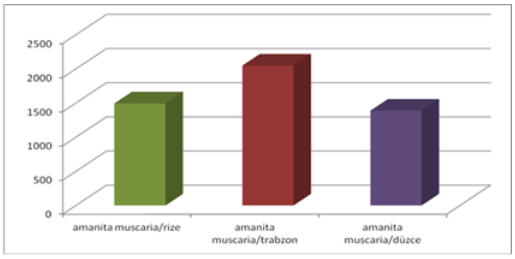

Figure 5. Graphical representation of muscimol amounts in Amanita muscaria / Rize, Amanita muscaria / Trabzon, Amanita muscaria/Düzce

An elemental analyzer was used to determine the percentage composition of carbon, hydrogen, and sulfur in our mushroom samples using an infrared absorption detector, while nitrogen was quantified using a thermal conductivity detector. The results are presented in Table 9. The elemental analyzer is capable of simultaneously analyzing the percentage composition of carbon, hydrogen, nitrogen, and sulfur in pharmaceuticals, chemicals, plastics, resins, rubbers, and all homogeneous organic compounds using approximately 2 mg of sample within an average of 3 minutes. In the case of toxic mushrooms, the results were relatively similar, with only slight differences observed in *Agaricus bisporus* (cultivated mushroom).

Table 9. Elemental analysis results

Mushroom Type	C%	H%	N%	S%	O%
Agaricus bisporus (Culture)	37,78	6,109	3,761	0,212	52,138
Amanita phalloides	42,06	6,285	6,456	0,473	44,726
Amanita pantherina	44,02	6,583	4,812	0,260	44,325
Amanita muscaria/Düzce	45,41	6,813	4,230	0,222	43,325
Amanita muscaria/Rize	44,89	6,740	4,338	0,249	43,783
Amanita muscaria/Trabzon	44,54	6,582	4,851	0,236	43,791

X-ray diffraction (XRD) was used to determine the structural composition of the mushroom samples. Electromagnetic radiation of a specific wavelength was directed onto the surface of the material, and the reflected rays were interpreted according to Bragg's Law to obtain information about the structure. In addition to distinguishing whether the material is amorphous or crystalline, XRD can also determine the crystal parameters and unit cell types of

the formed crystals. However, in the analysis performed using the XRD device to observe the structural forms of elements in crystalline structures, no structural observations could be made due to the amorphous bonding of the elements. Only in *Amanita phalloides* was potassium detected in a crystalline form.

4. Conclusion

ICP-MS results showed the analysis of Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mn, Na, Ni, P, Pb, Sb, Sn, Sr, Ti, V, and Zn in samples prepared from the mushroom species *Agaricus bisporus* (cultivated), *Amanita phalloides*, *Amanita pantherina*, and *Amanita muscaria* collected from Rize, Trabzon, and Düzce. The concentrations of the elements were found at the µg/kg and ng/kg levels, as shown in Table 9. Additionally, suitable acid mixtures were determined for each element, as presented in Table 6. These six mixtures varied depending on the mushroom species and the element to be analyzed (Jasinska et al., 2024). Overall, the analysis results indicated that the mixture of 12 mL of 65% pure HNO_3 and 5 mL of 70% pure $HClO_4$ was the most suitable for metal analysis in mushroom species. In the cultivated *Agaricus bisporus*, which is consumed as food, no toxic elements were detected or were only found at trace levels, while essential and beneficial elements such as calcium, potassium, magnesium, sodium, and phosphorus were present in significant amounts. Although *Amanita muscaria* mushrooms collected from Rize, Trabzon, and Düzce belong to the same species, variations were observed in both muscimol content and elemental composition. Among these, the *Amanita muscaria* sample with the highest muscimol content was the one collected in Trabzon. Furthermore, the sample collected from Rize was observed to contain higher levels of all elements except calcium, mercury, lead, and zinc. The *Amanita muscaria* from Trabzon was found to contain higher amounts of calcium and mercury, while the one from Düzce had higher levels of lead and zinc. It was observed that all toxic mushroom species contained significant amounts of essential and beneficial elements such as calcium, potassium, magnesium, sodium, and phosphorus, indicating that these mushrooms possess high nutritional value from this perspective. However, due to both the elevated levels of metals such as aluminum, iron, mercury, and zinc, and the presence of lethal doses of toxins, these mushroom species are deemed entirely unsuitable for consumption as food. High levels of mercury were detected in all mushroom species, with *Amanita phalloides* showing the highest mercury content. In a similar study, the levels observed in cultivated mushrooms were lower than those found in our study (WHO, 1996). Muscimol analysis was conducted on samples prepared from Amanita pantherina, Amanita muscaria (collected from Rize, Trabzon, and Düzce), and α-amanitin analysis was performed on Amanita phalloides. The results showed that the highest muscimol concentration was found in Amanita muscaria collected from Trabzon (2048.02 µg/kg). Rize (1493.74µg/kg) and Düzce (1387.28 µg/kg) mushrooms contained higher amounts of Amanita muscaria, although the levels were lower than in Trabzon. Muscimol could not be detected in Amanita pantherina. In contrast, Amanita phalloides was found to contain a high concentration of α-amanitin. Among these highly toxic compounds, muscimol begins to exhibit toxic effects when 6 mg is ingested—an amount typically found in 2 to 20 mushroom caps. Amatoxins, including α-amanitin, are fatal when orally ingested at doses as low as 0.1 mg/kg. Given that a single Amanita phalloides cap contains approximately 10-15 mg of amatoxins, even one mushroom can be lethal (Cocchi, 2006; Tsujikawa, 2007). The muscimol levels determined in our study were found to be higher than those reported in similar studies. In recent years, studies have been carried out to isolate the poisons of poisonous mushrooms (Lee et al., 2024).

Declaration of Author Contributions

All authors declare that they have contributed equally to this manuscript, have reviewed the final version, and have approved it for publication.

Declaration of Conflicts of Interest

The authors declare that there is no conflict of interest regarding this study.

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