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Research Article

METAL AND RADIONUCLIDE ACCUMULATION OF SOME CULTIVATED MUSHROOMS

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ABSTRACT

Heavy metals and radionuclides of human origin and naturally occurring in nature are accumulated in plants, animals and fungi. In particular, some fungal species have a high capacity to absorb radionuclides. In this study, some metals (^{27}Al, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{111}Cd, ^{204}Hg, ^{206}Pb) and radionuclides (^{232}Th, ^{238}U, ^{40}K, ^{137}Cs) of culture mushrooms such as *Pleurotus eryingii*, *Pleurotus citrinopileatus* (cultivated on alder and walnut tree sawdust, separately) and *Pleurotus djamor* (cultivated on beech and walnut tree sawdust, separately) were investigated. Metal accumulations were determined using Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). Radioactivity measurements were performed by using High Purity Germanium (HPGe) detectors. Among the studied mushrooms, *Pleurotus citrinopileatus* has drawn attention with highest ^{52}Cr, ^{55}Mn, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{204}Hg, ^{206}Pb content. Among the radionuclides ^{232}Th, ^{238}U content were not determined in any mushroom species. ^{137}Cs was not detected in any mushrooms except *Pleurotus citrinopileatus* cultivated on alder tree sawdust (15 ± 3 Bq/kg.). The highest ^{40}K radionuclide content was determined in *Pleurotus eryingii* mushroom cultivated on alder tree sawdust with 947 ± 32 Bq/kg. It was concluded that the metal and radionuclide content of mushrooms were affected by mushroom type and cultivation conditions.

Keywords: Cultivated mushrooms, metal content, *Pleurotus*, radionuclide content.

1. INTRODUCTION

The medicinal properties of both wild mushrooms and cultivated mushrooms have been intensively studied in recent years [1-6]. With the increasing awareness of people about mushrooms, consumption of mushrooms has also increased [7]. Moreover, mushroom cultivation has been both a source of income and a nutritious food for people [8]. *Pleurotus* species have become more attractive as they can be grown more easily among the other cultivated mushrooms [9]. In addition, some types of *Pleurotus* have very beautiful colors (*Pleurotus citrinopileatus*-

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yellow, *Pleurotus djamor*-pink) and these mushrooms are also used for visual purposes, especially in restaurants.

Mushrooms cannot produce metal ions themselves but it is known that the edible mushroom species in nature or cultivated mushrooms can accumulate heavy metals and radionuclides [10-12]. Mushrooms directly or indirectly intake the nutrients and metal ions from the environment - soil or substrate- and thanks to their absorption properties, they store them in high concentrations [13, 14]. For this reason, it is important to determine whether metal ions, which have a detrimental effect on human health, are present in fungi taken directly as food. In this way, it can be determined whether there is heavy metal contamination in the growing area of mushroom.

Pleurotus species can be cultivated on many different lignocellulosic environments [15, 16] and the nutritional content of these mushrooms is affected by their subsrates [17-19]. In the literature it was reported that the chemical configuration of substrates could affect the mushrooms' nutritional elements, yield and the level of various toxic [20]. This study was made for highlight the required that substrates used for cultivation of mushroom species contain the lowest possible toxic levels to provide the safety of consumers. For that purpose, some metals (^{27}Al, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{111}Cd, ^{204}Hg, ^{206}Pb) and some radionuclides (^{232}Th, ^{238}U, ^{40}K, ^{137}Cs) of cultivation mushrooms such as *Pleurotus eryingii, Pleurotus citrinopileatus* (cultivated on alder and walnut tree sawdust, separately) and *Pleurotus djamor* (cultivated on beech and walnut tree sawdust, separately) were investigated.

2. MATERIALS AND METHODS

2.1. Mushroom

In this study, some culture mushrooms such as *Pleurotus eryingii, Pleurotus citrinopileatus* (cultivated on alder and walnut tree sawdust, separately) and *Pleurotus djamor* (cultivated on beech and walnut tree sawdust, separately) were investigated in terms of metal and radionuclide accumulations (Table 1, Table 2 and Table 3). *P. eryingii*, *P. citrinopileatus* and *P. djamor* myceliums were obtained from a commercial firm located in Istanbul. Alder, walnut and beech sawdust was supplied from workshop of Forest Industry Engineering, Karadeniz Technical University, Trabzon. All mushrooms were cultivated as detailed in our previous studies [21]. The mushroom names were coded from 1 to 6. The mushroom species and the substrate types on which they were cultivated were shown in Table 1 and Figure 1.

Table 1. The mushrooms and substrate types

Mushroom codes	Mushroom types	Substrate types
1	*Pleurotus eryingii*	Alder tree sawdust
2	*Pleurotus eryingii*	Walnut tree sawdust
3	*Pleurotus citrinopileatus*	Alder tree sawdust
4	*Pleurotus citrinopileatus*	Walnut tree sawdust
5	*Pleurotus djamor*	Beech tree sawdust
6	*Pleurotus djamor*	Walnut tree sawdust

All the mushroom samples were sliced and dried on a drying machine at 40 °C until they were completely dehydrated. Then mushroom samples were crushed for passing a 40-mm mesh sieve. For radioactivity measurements, mushroom powders were put in a plastic cylindrical container of uniform size (50 mm in height, 60 mm in diameter) and sealed for a period of 4 weeks in order to allow for radon and its short-lived progenies to reach secular radioactive equilibrium prior to gamma spectroscopy [22].

2.2. Metal accumulation

Metal accumulations of mushrooms were determined using Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). One dissolution and three measurements were performed. Internal standard masses were continuously measured simultaneously with the samples during measurement: Li (6 no gas), Sc (45 Helium), Ge (72 Helium), Rh (105no gas), Rh (105 helium) Rh (103 no gas), In115 (no gas), Tb (159 no gas), Lu (175), Bi (209).

The microwave procedure was as follows: Weights not exceeding 0.5 g but close to that value were weighed (up to 0.5g) into the 50 ml heat and pressure resistant teflon containers. 8 ml of suprapur purity 65% HNO_3 and 2 ml of suprapur purity 30% H_2O_2 were added.

Microwave programming: Maximum 1000W energy and 45 bar pressure limit values are set. A temperature of 200 °C was reached from room temperature within 15 minutes. It remained at a constant temperature of 200 °C for 15 minutes. Then the heating process has ended. It was left to come to room temperature for half an hour.

Pleurotus eryingii cultivated on walnut tree sawdust

Pleurotus eryingii cultivated on alder tree sawdust

Pleurotus citrinopileatus cultivated on walnut tree sawdust

Pleurotus citrinopileatus cultivated on alder tree sawdust

Pleurotus djamor cultivated on beech tree sawdust

Pleurotus djamor cultivated on walnut tree sawdust

Figure 1. The mushroom species and the substrate types on which they were cultivated.

Samples dissolved in microwave were transferred from teflon cups to 50 ml polyethylene falcon tubes. Up to 50ml of ultra-pure water was added. Dilution factor for each sample was determined. For example, dilution factor for 0.4167g sample weighed: Final volume / weight of sample = 50ml / 0.4167g = 119.99. During the analysis, the calibration values and results of the elements were measured as ppb = μg/kg. Measurements were made in accordance with the standards of EPA 200.8, EPA 6020 for mushroom samples.

2.3. Radioactivity measurements

Radioactivity measurements were performed by using a HPGe computer-controlled detector having the resolution of 1.9 keV for the 1332 keV energy line of 60Co with conventional electronics and 15% relative efficiency (Canberra, GC1519 model) and Genie 2000 as the software. The detector was shielded with a 10 cm thick lead layer to reduce the background due to the cosmic rays and the radiation nearby the system [23].

Decay corrections were performed according to the sampling date. The energy calibration and absolute efficiency calibration of the spectrometer were carried out using calibration sources which contained ^{133}Ba, ^{57}Co, ^{22}Na, ^{137}Cs, ^{54}Mn, and ^{60}Co peaks for the energy range between 80 and 1400 keV (calibration sources supplied by Isotope Products Laboratories) [24]. The reference material of the International Atomic Energy Agency (IAEA-375) and the Gamma Acquisition & Analysis program were used to calibrate the efficiency of the gamma detector.

The gamma-ray lines of 295.2 keV from ^{214}Pb, 352.0 keV from ^{214}Pb and 609.4 keV from ^{214}Bi were used to evaluate the ^{238}U activity concentration, while 583.1 keV gamma-ray from ^{208}TI, 238.6 keV from ^{212}Pb and 911. keV from ^{228}Ac were used to determine to the ^{232}Th activity concentration. The activity concentrations of ^{40}K and ^{137}Cs were determined by using their 1460 keV and 661 keV gamma-ray lines, respectively. After the samples and gamma spectroscopy system (with energy and yield calibrations) were prepared for measurement, the radioactivity analysis of each sample was performed for 80.000 seconds. At the end of this period, the spectra of the radioactive isotopes from the samples were calculated. The specific activity of each sample was then calculated utilizing the following Equation 1 [25].

$$A = \frac{C_{net}}{\varepsilon * I_{\gamma} * t * m} \tag{1}$$

where; C_{net} was the net area of the total absorption line, AA was the activity of the isotope in Bq/kg, I_γ was the absolute intensity of the transition, t was the sample measurement time, ε was the full energy peak efficiency and m was the mass of the sample.

The minimum detectable activity (MDA) of the present measurement system was calculated as follows Equation 2 [26].

$$MDA = \frac{\sigma\sqrt{B}}{\varepsilon * P * t * w} \tag{2}$$

where; MDA is in Bq/kg, σ was the statistical coverage factor equal to 1.645 (confidence level 95%), B was the background for the region of interest of a certain radionuclide, P was the absolute transition of gamma decay, ε was the full energy peak efficiency, t was the counting time in seconds and w was weight of the dried sample in kg.

2.3.1. Effective dose

A possible risk of radioactivity for human being that consume the mushrooms is expressed by the effective dose (E) given in μSv/y. The average annual effective dose equivalent that an individual receives due to the radionuclides ingestion from contaminated mushrooms was calculated using the following Equation 3 [27].

$$E = C * H * DF \tag{3}$$

where; E was annual effective dose from consumption of nuclide in foodstuff (μSv/y), C was the concentration of radionuclide in foodstuff (Bq/kg), H was the consumption rate for foodstuff p (kg/y) and DF was the dose coefficient for ingestion of radionuclide (μSv/Bq). The values of this conversion factor for adults were: 0.28, 0.23, 1.3×10^{-2} and 6.2×10^{-3} μSv/Bq for ^{238}U, ^{232}Th, ^{137}Cs and ^{40}K, respectively. In this study, the average annual consumption of mushrooms by adult Turkish people was taken as 0.360 kg.

3. RESULTS AND DISCUSSION

3.1. Metal accumulation

Metal accumulation of studied mushrooms was presented in Table 2.

Table 2. Metal accumulation of studied mushrooms

Metal contents (μg/kg)	Mushroom codes					
	1	2	3	4	5	6
Al	**47880,6**	32582,9	11480,3	12742,8	15511,6	17577,8
Cr	166,55	919,76	96,37	137,07	93,95	**1360,56**
Mn	8245,91	10571,9	8420,46	15067,4	13539,3	**59275,2**
Fe	49978,5	**232626**	41661,6	105689	122635	120888
Co	38,94	112,45	<0,00	6,29	6,65	**467,74**
Ni	225,79	853,76	522,88	**3027,39**	723,72	1359,45
Cu	5639,74	9410,69	9908,61	15359,2	6037,01	**31122,7**
Zn	34084,7	54583,7	81163,4	**115226**	105905	70250,6
As	37,83	152,39	2,43	27,06	15,59	**228,3**
Cd	175,96	43,5	269,78	284,07	421,49	**1786,33**
Hg	<0,00	10,46	6,03	8,5	9,09	**85,69**
Pb	<0,00	91,57	24,79	53,03	38,03	**760,34**

Aluminum (Al) is one of the most abundant metals in the earth's crust with a concentration of 80 g/kg [28]. In this study, Al content of studied mushrooms were ranged between 11480,3 and 47880,6 μg/kg and the highest Al content was determined in *P. eryingii* mushroom cultivated on alder tree sawdust. It was reported that Al contents of some wild mushrooms in Poland were 25,9-6,0 mg/kg [29]. Our Al results were found very lower than that of reported data for wild mushrooms.

Chromium (Cr) essential for human metabolism in low concentrations because it is enzyme activators, but it can be toxic as its concentration increase [30]. In the literature, Cr content of 25 higher mushroom species were reported in the range of 0,05 and 4,51 mg/kg [31]. In this study, the highest Cr content was determined in *P. djamor* cultivated on walnut tree sawdust with 1360,56 μg/kg.

Manganese (Mn) is an essential element for the activity of a group of enzymes called phosphotranferases [32]. Researchers have been reported that Mn contents of wild edible and cultivated mushrooms were 4.8-65.4 mg/kg [33]. In this study, Mn content of studied mushrooms were ranged between 8420,46 and 59275,2 μg/kg and the highest Mn content was determined in *P. djamor* mushroom cultivated on walnut tree sawdust with 1360,56 μg/kg.

Iron (Fe) is an integral part of many proteins and enzymes that maintains various physiological functions [34]. It has been reported that Fe content of 15 mushrooms were 467–3,280 mg/kg and also the tolerable daily intake (PTDI) for iron is 48 mg for an average adult (60 kg body weight) [35]. In this study, the highest Fe content was determined in *P. eryingii mushroom* cultivated on walnut tree sawdust with 232626 µg/kg.

Cobalt (Co) can be either toxic or essential for living organisms depending on its concentration level. [36] have reported that Co content of macro-fungi is relatively low (mostly below 0.6 ppm), rarely in units of ppm. In this study, the highest and lowest Co content was determined in *P. djamor* cultivated on walnut tree sawdust (467,74 µg/kg) and *P. citrinopileatus* cultivated on alder tree sawdust, respectively (<0,00 µg/kg).

Nickel (Ni) is a nutritionally essential trace metal for at least several animal species, micro-organisms and plants, and therefore either deficiency or toxicity symptoms can occur when, respectively, too little or too much Ni is taken up [37]. In a previous study, Ni content of 25 higher mushroom species were reported in the range of 0,81 and 9,9 mg/kg and and the Ni content was noted under the toxicological limits (20mg/kg) [31]. In this study, the highest Ni content was determined in *P. citrinopileatus* mushroom cultivated on walnut tree sawdust with 3027,39 µg/kg.

Copper (Cu) is an essential element and adverse effects can potentially be associated with both very low and very high intakes (toxic levels) [29, 38]. It was determined the Cu content of 6 wild mushrooms in Poland in the range of 8,20- 26.33 mg/kg. In this study, Cu content of the studied mushrooms were ranged between 5639,74 and 31122,7 µg/kg.

Zinc (Zn) is a masculine element that balances copper in the body, and is essential for male reproductive activity [39]. PTDI for Zn is 60 mg for an average adult (60 kg body weight) [35]. The Zn content of 6 wild mushrooms have been reported as 31.92-88.71 mg/kg [29]. In this study, Zn content of cultivated mushrooms were determined in the range of 34084,7-115226 µg/kg.

Arsenic (As) is a toxic element and one of the most notorious compounds [40]. It has been reported that As content of 37 common edible mushroom taxa was 0,0-146,9 mg/kg [41]. In this study, the highest As content (228,3 µg/kg) was 100 times higher than the lowest As content (2,43 µg/kg).

Cadmium (Cd) is toxic at extremely low levels and in this study Cd content of mushrooms were determined in the range of 43,5-1786,33 µg/kg. In a previous study, ten times higher cadmium concentrations were determined in cultivated *P. ostreatus* than in *A. bisporus* [42].

Mercury (Hg) is highly toxic for microorganisms, animals, and humans [43]. It has been reported that Hg content of 6 wild mushrooms were 0.9-1.71 mg/kg [29]. In this study, Hg content of studied mushrooms were ranged between 0,00 and 85,69 µg/kg and the highest Hg content was determined in *P. djamor* cultivated on walnut tree sawdust.

Lead (Pb) is the most significant toxin of the heavy metals. Its inorganic forms are absorbed through ingestion by food and water, and inhalation [44]. In a previous study, the Pb content of 15 higher mushrooms have been described as 0.69-9.15 mg/kg [35]. The highest Pb content was determined in *P. djamor* cultivated on walnut tree sawdust with 760,34 µg/kg.

3.2. Radionuclide accumulation

Radionuclide accumulation of studied mushrooms was presented in Table 3.

Uranium (^{238}U), thorium (^{232}Th) and potassium (^{40}K) are primordial radionuclides, while cesium (^{137}Cs) is anthropogenic radionuclide which are available in diverse environments such oceans, rivers, streams, soils, rocks, vegetable, animals and human body [45]. In this study, uranium and thorium was not detected in any studied mushrooms. In our previous study, uranium and thorium content of cultivated mushrooms were determined 14,6-26,6 and 2,1-9,2 Bq/kg, respectively [46]. Cesium radionuclide was detected in only *P. citrinopileatus* mushroom cultivated on alder tree sawdust. In a previous study, cesium (^{137}Cs) content of 27 wild mushrooms were described as 2,5- 2763 Bq/kg [47]. Potassium content of studied mushrooms were ranged between 306-947 Bq/kg and the highest potassium content was determined in *P. eryingii* cultivated on alder tree sawdust. It has been reported that the average concentrations of ^{40}K of 6 wild mushrooms varied from 254.17 to 416.07 Bq/kg [48].

Table 3. Radionuclide accumulation of studied mushrooms

Mushroom codes	**Radionuclides (Bq/kg)**			
	^{238}U	^{232}Th	^{137}Cs	^{40}K
1	ND*	ND	ND	**947 ± 32**
2	ND	ND	ND	350 ± 13
3	ND	ND	**15 ± 3**	367 ± 14
4	ND	ND	ND	495 ± 18
5	ND	ND	ND	306 ± 12
6	ND	ND	ND	455 ± 17

*ND: Not detected

3.3. Effective dose

Effective dose values of studied mushrooms were presented in Figure 2.

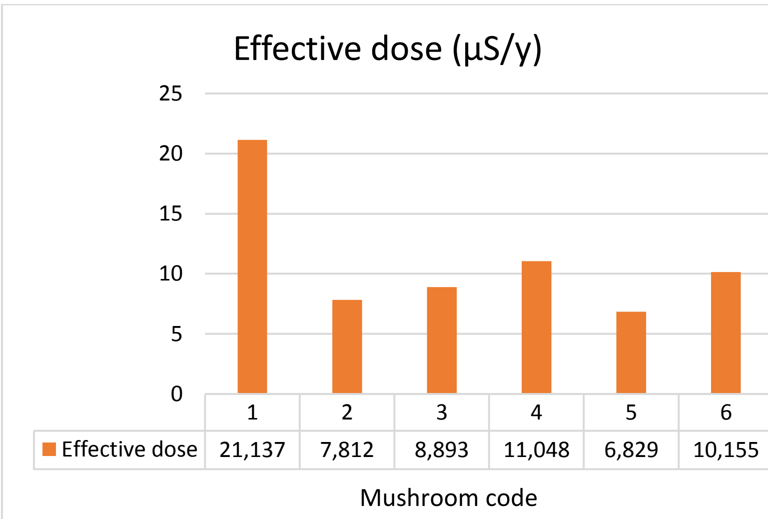

Figure 2. Effective dose values of studied mushrooms

A possible risk of radioactivity for human being that consume the mushrooms is expressed by the effective dose [27]. In the study, effective dose values were ranged between 6,829 and 21,137 µS/y. *P. eryingii* cultivated on alder tree sawdust and *P. djamor* cultivated on beech tree sawdust reached the highest and the lowest effective dose values, respectively. All effective doses were found below the world average value (290 µSv/y) [27]. The low doses levels in analyzed mushrooms reveals that the consumption of these mushrooms will not cause any health problems for human body.

4. CONCLUSIONS

Important findings of this study can be sorted as below;

- In this study, among the studied mushrooms, *P. djamor* cultivated on walnut tree sawdust had drawn attention with highest Cr, Mn, Co, Cu, As, Cd, Hg and Pb contents. But there is no risk for human life because very low accumulation of metals.
- *P. citrinopileatus* cultivated on alder tree sawdust had drawn attention with Cs (Cesium) content.
- All effective doses were found below the world average value (290 µS/y).
- It was concluded that the metal and radionuclide content of mushrooms were affected by mushroom type and cultivation conditions.

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