Determination of heavy metals content in wild mushrooms and soil by EDXRF and FAAS techniques

Cristiana RADULESCU[a]*, Claudia STIHI[a], Ion. V. POPESCU[a,b,c], Gabriela BUSUIOC[d], Anca Irina GHEBOIANU[b], Valerica Gh. CIMPOCA[a,b], Ioana Daniela DULAMĂ[b] and Mihaela DIACONESCU[e]

[a] *Sciences Department, Faculty of Sciences and Arts, Valahia University of Targoviste, 130082, Targoviste, Romania*
[b]*Multidisciplinary Research Institute for Science and Technologies, Valahia University of Targoviste, 130082, Romania*
[c]*Academy of Romanian Scientists, 54 Splaiul Independentei, Bucharest 050094, Romania*
[d] *Environmental Engineering Department, Faculty of Environmental Engineering and Biotechnologies, Valahia University of Targoviste, 130082, Targoviste, Romania*
[e]*National College Nicolae Titulescu, Pucioasa, 135400, Romania*

Abstract The heavy metals (Cd, Cr, Ni, Pb, Zn, Cu, Fe, Mn) content of some edible wild mushrooms (*Amanita caesarea, Amanita rubescens, Amanita vaginata, Amanita spissa*) and soil samples, of ten sites from Dambovita county Romania, were analyzed. Elements concentrations were determined by Flame Atomic Absorption (FAA) spectrometry and Energy Dispersive X-ray spectrometry (EDXRF) in 40 samples of *Amanita* species and 40 underlying soil samples. In fruiting body of these mushrooms, the highest mean concentration of macroelements (dry mass basis) was found for Zn and Fe. Some metals (Cd, Pb, Cr and Zn) were bioconcentrated mainly in cap than the stipe of fruiting body. The mean concentration of heavy metals (Cd, Cr, Ni, Pb) was higher in mushrooms which was collected on sites near urban settlements Lead was determined at highest concentration in soil surrounding *Amanita vaginata*. The studied mushrooms were good bioaccumulators of zinc and cupper. The iso-concentration curves of heavy metals in samples of *Amanita sp*. and soil were realized with Surfer 9 Model.

Keywords wild mushroom, EDXRF, FAAS, heavy metal

1. Introduction

Numerous species of wild mushrooms have long been treated as a delicacy. *Amanita* is a large genus with 600 species, including some of the most toxic known mushrooms found worldwide, divided between two subgenera and several sections. This genus has been a subject of many studies over the past century, because is responsible for approximately 95% of the fatalities resulting from mushrooms poisoning (due the presence of amatoxins, phallotoxins and ibotenic acid which have been implicated in fatal human and animal poisonings). This genus contains some edible wild mushrooms of *Amanita species*, which are widely consumed in central and Eastern Europe, especially in Romania. It is true that the mycologists generally discourage amateur mushroom hunters from selecting these for human consumption [1].

Determination of the heavy metals level from fruiting body of several edible species of *Amanita* collected from Dambovita County was the subject of this study. Fruiting bodies really accumulated some heavy metals from soil and this is the consequence of many factors, for example, pH and the content of Zn from polluted sites. The ability to accumulate some elements from the substrate is species-dependent [2, 3]. Some originals papers were published on this topic [4 - 9].

The heavy metals (Cd, Cr, Ni, Pb) concentrations from fruiting body, separately in cap and stipe, for each species of *Amanita* were determined by Flame Atomic Spectrometry (FAAS) after microwave digestion. The essential elements

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(Zn, Cu, Fe, Mn) content of *Amanita* species was determined by EDXRF technique. The both analytical techniques were used to determinate the contents of heavy metals from forestry soil, as well.

2. Experimental

2.1. Materials

Four *Amanita sp.* young edible wild mushrooms (Table 1) were collected from ten forestry sites of Dambovita county, from well established locations (GPS coordinates). Collections of species were made at different times of the day: morning and mid-day (40 samples collected) by uprooting its substratum with the aid of a scalpel.

Table 1. Families, habitat and edibility of the mushrooms species under study.

Mushroom species	Class/Subclass Family/Genus	Habitat and characteristics of fruiting body
Amanita caesarea	Homobasidiomycetae/ Hymenomycetes Amanitaceae / *Amanita*	Oak forest; Soil; Edible only young; orange-red cap 5 cm; stipe 8-12 cm long; 2-3 cm wide.
Amanita rubescens	Homobasidiomycetae/ Hymenomycetes Amanitaceae/ *Amanita*	Forest-soil; Edible if well cooked, white; cap 8-10 cm, convex; stipe: 8-11 cm long; 1.5-3 cm thick.
Amanita vaginata	Agaricomycetes/ Hymenomycetes Amanitaceae/ *Amanita*	Forest -soil; Edible young; cap 6-7 cm broad, convex; stipe 8-10 cm long, 1.5-2.0 cm thick
Amanita spissa	Basidiomycetes/ Agaricomycetidae Amanitaceae/ *Amanita*	Oak forest; Soil; Edible only young; Brown/grey cap 5 cm; stipe 7-9 cm.

The fruiting body samples were washed with deionised water then, with a plastic knife, they were chopped up in 1 mm portions; the samples were dried at 60^0C, 24 hours, then ground into fine powder and finally weighed. Soil samples were dried at 70^0C in 24 hours. After drying, the solid samples were ground into fine powder and weighed.

2.2. Chemicals and standard materials

The used chemicals included nitric acid (65% Aldrich), hydrochloric acid (37% Fluka), hydrogen peroxide (30% Fluka), and potassium chloride (Aldrich). Distilled deionised water had a rezistivity better than 17.5MΩcm. The solutions used for calibration of FAAS were prepared from standard solution (Merck) of the studied elements.

2.3. Analytical techniques

Energy Dispersive X-ray Fluorescence

Two grams of sample (n=6) for each collected species and soil collected from forest area, Dambovita County, Romania were pressed manually, without any chemical treatment, in a plastic vial with Mylar in the bottom and then were analyzed.

The Zn, Cu, Fe, Mn, Cd, Pb and Cr elements from samples were determined by Energy Dispersive X-Ray Fluorescence (EDXRF) technique, using the ElvaX spectrometer. Good agreements were achieved between certified values and got data, with recoveries ranging from 97 to 105%.

Flame Atomic Absorption Spectrometry

Dried samples were digested in an acid solution using a Berghof MWS-2 microwave digestion system. Dried fungus samples (300 mg) were introduced into the digestion vessels; then 3 mL nitric acid and 5 mL hydrogen peroxide were added. Certified Standard Reference Material SRM 1515 (Apple Leaves) from the National Institute of Standards and Technologies was used to verify the obtained values.

Dried solid substrates (500 mg) were introduced into the digestion vessels and then 3 mL nitric acid and 9 mL hydrochloric acid (aqua regia) were added. After digestion time (30 min) the vessels were cooled at room temperature and then each solution volume was made up to 50 mL for each sample using deionised water. Certified Standard Reference Material SRM 2710 and 2711 (Montana Soil) for soil was used, too.

The Cu, Cd, Cr, Ni and Pb elements from samples was determined using an AVANTA GBC

flame atomic absorption spectrometer with hollow cathode lamps.

2.4. pH and conductivity of soil

The pH of solid substrate was determined according to ISO 10390:2005 method. The solid samples (weight 10 g) were treated with 50 mL KCl 0.1N, under stirring for 30 min. After, 1 hour, the pH for the each sample was measured with a pH meter Consort P501 at room temperature. Electrical conductivity was measured in saturated extract of soil using HACH CO150 apparatus.

3. Results and Discussions

Eight metals including Pb, Cr, Cd, Ni, Cu, Mn, Zn and Fe were analysed in *Amanita caesarea, Amanita rubescens, Amanita vaginata, Amanita spissa* edible wild mushrooms and soils in selected zones of Dambovita county.

The characteristics of soil including pH and conductivity were measured. The pH of all examined soil samples was moderately acid. The result showed that the mean pH were between 5.8 and 6.5. For electrical conductivity, the measured conductivities of the water-extracted soils indicate the relative water-soluble salt content of the soil. The mean conductivity values of the water-extracted soil were between 652 and 915 μS/cm.

In Table 2 are presented the mean concentrations and standard deviation of essential elements and heavy metals content in soil and edible wild mushrooms of *Amanita sp*.

The iso-concentration curves of heavy metals in samples of *Amanita sp*. and soil were realized with Surfer 9 Model (Fig. 1-6)

Table 2. Mean concentration of heavy metals in fruiting body (cap and stipe) of edible *Amanita* species and their substrate (mg/kg d.w)

Mushroom species and substrate (n=5)	Zn	Cu	Fe	Cd	Mn	Cr	Ni	Pb
Amanita caesarea (cap)	84.4±0.2	31.3±0.7	69.4±3.7	1.66±0.2	41.4±0.7	1.55±0.2	0.69±0.1	1.66±1.1
Amanita caesarea(Stipe)	50.8±0.3	17.3±0.8	51.9±3.3	0.33±0.1	37.9±0.4	1.12±0.1	0.79±0.4	0.89±1.3
Soil (S1)	1223±0.8	139.2±1.2	6.163*±5.7	2.17±0.4	239.5±1.3	3.17±0.3	11.57±1.5	12.9±1.8
Amanita rubescens(cap)	115.2±0.4	15.8±0.8	308.9±4.3	0.64±0.1	36.7±0.4	1.66±0.3	0.92±0.4	1.12±1.3
Amanita rubescens(Stipe)	98.3±0.2	12.9±0.7	289.4±3.5	0.39±0.2	33.4±0.5	0.33±0.2	0.43±0.8	0.68±2.2
Soil(S2)	363.7±0.8	53.56±1.7	3.589*±8.2	1.24±0.3	169.12±0.7	3.92±0.4	10.61±1.7	13.4±3.3
Amanita vaginata(cap)	132.1±0.7	27.11±0.4	227.4±5.6	0.72±0.1	31.4±0.4	0.66±0.1	0.56±0.2	1.93±1.4
Amanita vaginata (Stipe)	110.3±0.6	21.3±0.6	101.6±3.9	0.33±0.2	27.9±0.7	0.42±0.2	0.33±0.1	1.37±1.3
Soil(S3)	474.9±0.8	111.4±1.5	2.789*±6.8	2.57±0.7	112.6±0.9	1.19±0.3	7.9±1.6	8.03±3.5
Amanita spissa (cap)	193.5±0.5	37.8±0.4	192.3±3.3	1.46±0.4	38.2±0.8	1.72±0.1	0.98±0.4	2.69±2.2
Amanita spissa (Stipe)	89.4±0.2	24.9±0.7	108.3±3.4	0.83±0.2	31.2±0.6	1.34±0.3	0.66±0.1	2.31±2.3
Soil (S4)	915.6±0.9	410.5±1.6	6.007*±9.2	4.85±0.5	323.4±1.8	3.99±0.5	12.57±2.2	16.9±3.4

*Mass Fraction [%]

Fe, Zn and Mn concentrations were determined only by EDXRF technique; Cu, Cd, Cr and Pb concentration were determined by both spectrometric techniques; Ni concentrations was determined only by FAAS

The highest concentrations of Pb, Zn, Mn, Cu and Fe in soil were found in S1-Priseaca zone (43°30' lat N, 24°26' long E). This result is the fact that this region is close to urban settlements. Also, for Pb, Mn, Cu, Zn and Fe the highest concentration were obtained for samples collected from forestry sites (S4 – Vacaresti zone, 44°51' lat N, 25°29' long E), near roads and urban settlements. According with maximum levels as per the Romanian Law 756/1997, it can be seen that de mean concentration of soil S1 and S4 exceeds maximum levels for Zn and Cu. In the S2 Adanca (44°56' lat N, 25°33' long E) and S3 Ungureni (44°58' lat N, 25°17' long E) zones the levels of heavy metals are in the permissible limits of heavy metals according with Law 756/1997.

Essential elements and heavy metals in *Amanita* species were bioconcentrated mainly in caps (Table 2). For Pb, Cd, Cr and Zn highest concentration were obtained for *Amanita spissa* in cap, 2.69, 1.46, 1.72 and 193.5 mg/kg d.w. The highest concentration for Fe was obtained for *Amanita rubescens* in the both component of body fruiting, 308.9 mg/kg Fe in cap and 289.4 mg/kg in stipe. The Fe concentrations compared with the permissible limits of heavy metals in fruiting body of mushrooms from literature [1-3] are very high for *Amanita rubescens*.

Also, all *Amanita* species accumulate high levels of Zn and Fe. Mainly Cd and Pb contents accumulate in cap of these *Amanita* species, especially *Amanita caesarea* and *Amanita spissa*, may pose a risk. Consumption of those species, as well mushrooms gathered from polluted forest sites (S1 and S4), represents a risk for human health.

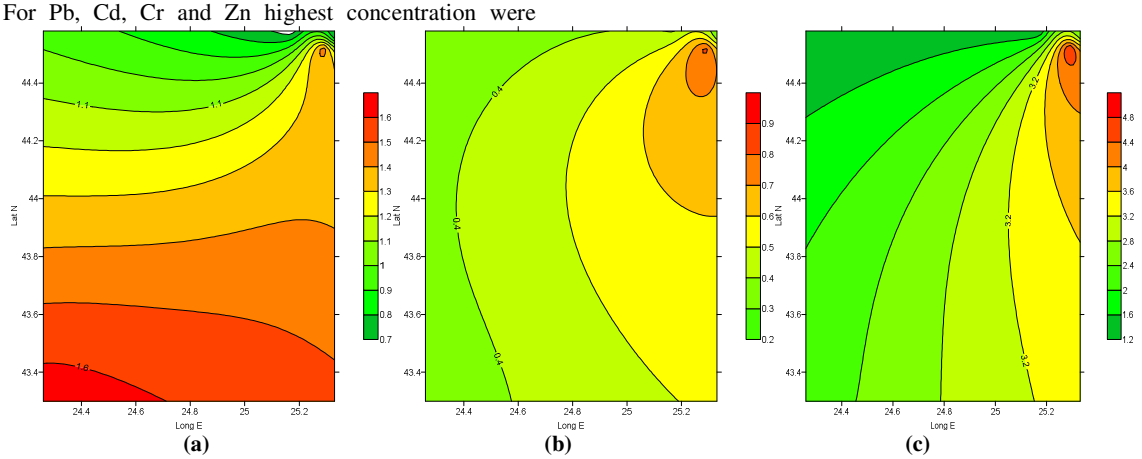


Fig.1. Iso-concentrations curves of Cd in *Amanita sp.*, cap (a), stipe (b) and soil (c).

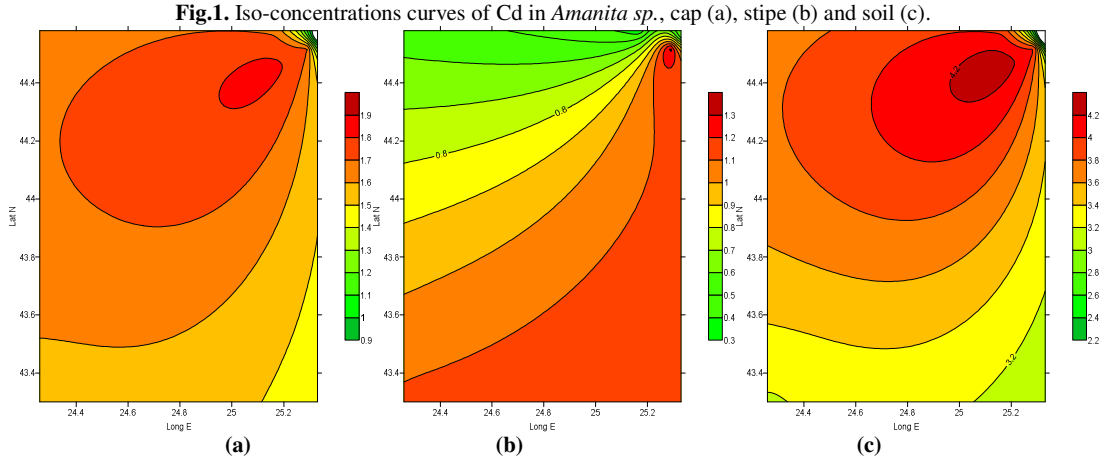
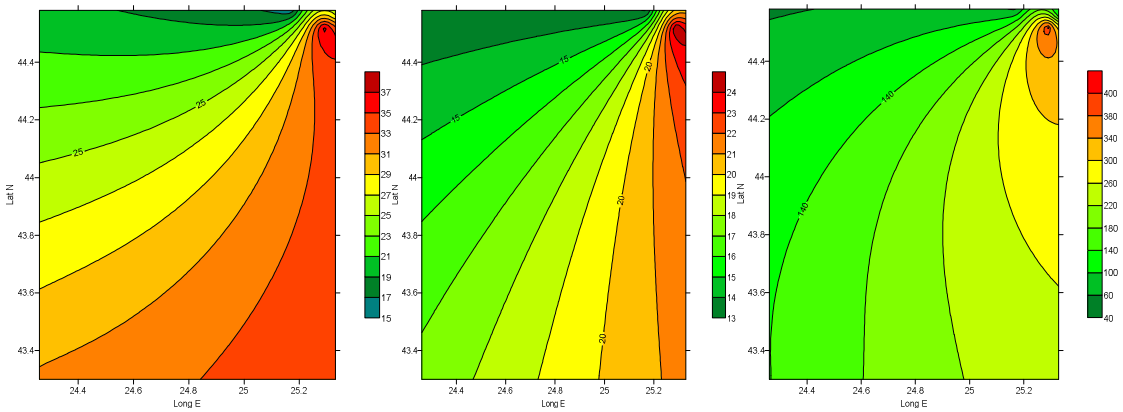
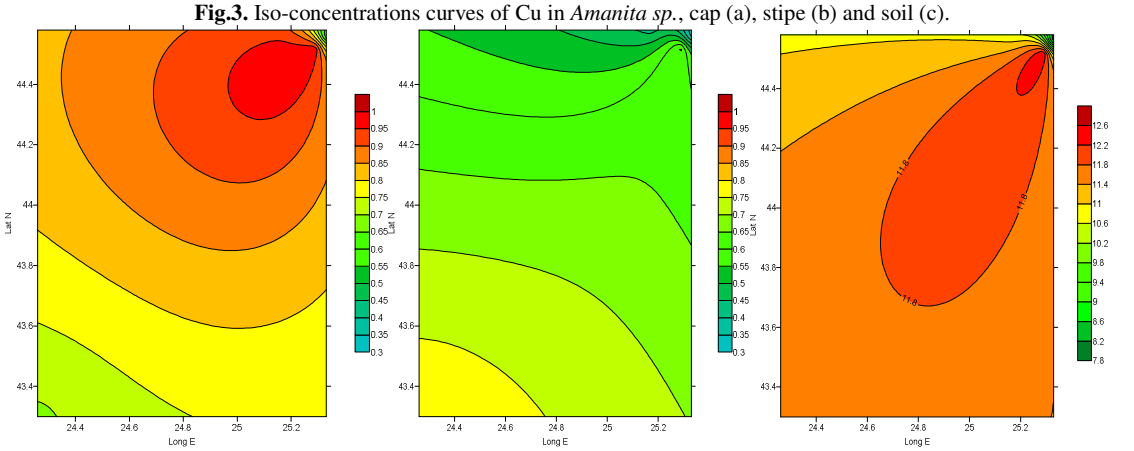


Fig.2. Iso-concentrations curves of Cr in *Amanita sp.*, cap (a), stipe (b) and soil (c).



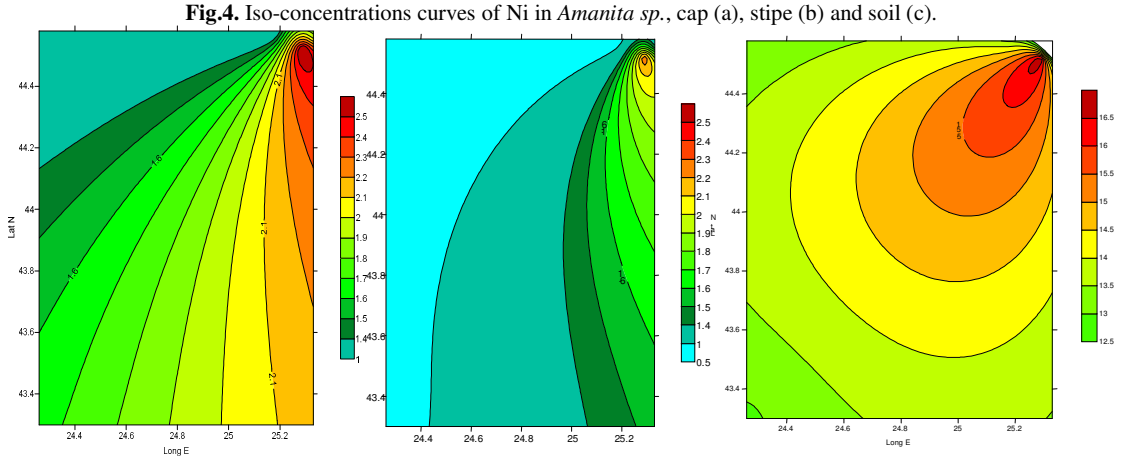
(a) **(b)** **(c)**

Fig.3. Iso-concentrations curves of Cu in *Amanita sp.*, cap (a), stipe (b) and soil (c).



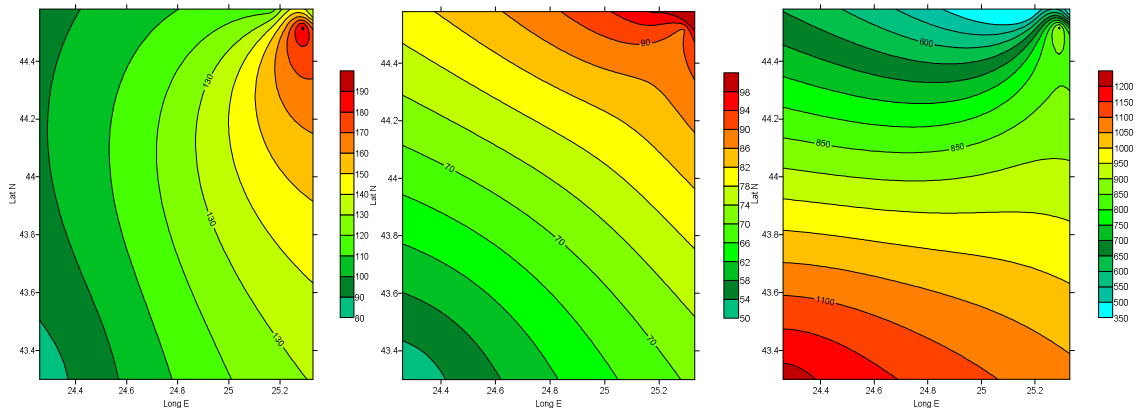
(a) **(b)** **(c)**

Fig.4. Iso-concentrations curves of Ni in *Amanita sp.*, cap (a), stipe (b) and soil (c).



(a) **(b)** **(c)**

Fig.6. Iso-concentrations curves of Pb in *Amanita sp.*, cap (a), stipe (b) and soil (c).



(a) **(b)** **(c)**

Fig.6. Iso-concentrations curves of Zn in *Amanita sp.*, cap (a), stipe (b) and soil (c).

4. Conclusions

The variation of heavy metals content between *Amanita sp*. is dependent upon the ability of the species to extract elements from the soil and on the selective uptake and deposition of metals in tissue. The moderately acid pH value of soil influenced the accumulation of Zn and Cd inside of the *Amanita sp.* Edible wild mushrooms *Amanita caesarea, Amanita rubescens, Amanita vaginata, Amanita spissa* have been widely consumed as a delicacy by part of Romanian population. All heavy metals were bioconcentrated mainly in cap than the stipe of fruiting body High Pb and Cd levels in all *Amanita sp*., in cap, can induce abdominal pains, convulsion, drowsiness, vomiting in the case of the peoples. The maximum level for certain contaminants in foodstuffs established by the commission of the European Committees (EC No 466/2001) [10] is set at 0.2 and 0.3 mg/kg wet weight for Cd and Pb respectively in cultivated fungi.

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*E-mail address: radulescucristiana@yahoo.com

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