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Full Length Research Paper

Notes on selenium in mushrooms data determined by inductively coupled plasma atomic emission spectroscopy (ICP–AES) and hydride generation atomic absorption spectroscopy (HG–AAS) techniques

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This manuscript discusses the credibility of the selenium in mushroom data generated using inductively coupled plasma atomic emission spectroscopy (ICP–AES) compared to that from hydride generation atomic absorption spectroscopy (HG–AAS). Selenium (Se) was determined by two methods: one was the widely applied and well validated hydride generation atomic absorption spectroscopy (HG–AAS) that was a reference method, while the validity of using the ICP–AES was tested. We found that Se determination in fungal and plant materials by ICP–AES gives inaccurate and imprecise results. Hence, reports of rather high concentrations of Se determined by ICP–AES for mushrooms that do not accumulate Se or that have not emerged at selenoferous areas should be treated with caution, because such data could be highly biased.

Key words: Data validity, food, fungi, from hydride generation atomic absorption spectroscopy (HG-AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), nutrition, selenium.

INTRODUCTION

A quite common opinion among nutrition advisers is that mushrooms both cultivated and wild grown are excellent sources of selenium (Se). Are all mushrooms really rich in Se? This is an important question because Se in trace is a chemical element vital to animals and to human life. Due to essential nature of Se when taken in adequate amounts and chemical forms, the credibility of data produced and published on its occurrence in various media, including foods and human body fluids, is inestimable.

A growing number of data reporting on metals and metalloids contents of mushrooms worldwide have over time raised some articulated questions on accuracy and quality of some of these published data (Borovička and Řanda, 2007; Borovička et al., 2011; Jarzyńska and Falandysz, 2011).

Wild grown mushrooms are known to be usually rich in many metallic elements (Nnorom, 2011). Among the estimated worldwide 2000 species of edible wild mushrooms, some are rich in Se, for example, *Albatrellus* mushrooms (the goat's foot *Albatrellus pes–caprae* and some others) and *Boletus* mushrooms (king bolete, *Boletus edulis*; summer bolete, *Boletus aestivalis*; pinewood king bolete, *Boletus pinophilus*; oak bolete, *Boletus appendiculus*; and red–footed bolete, *Boletus erythropus*) (Falandysz, 2008; Stijve and Cardinale, 1974; Stijve et al., 1998). The reliability of certain data published on Se content in mushrooms has been questioned by some reviewers (Borovička and Řanda, 2007; Falandysz, 2008). This was because the concentrations reported by some scientists vary considerably from data obtained for the same mushroom species by well validated methods. These unrealistically high concentrations of Se were determined in

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Table 1. An example of accepted and questioned selenium data of mushrooms (µg/g dw).

Mushroom species	Se	
	Worldwide data[a]	Questioned data[b]
Morchella esculenta (Common Morel)	0.065–1.8[c] (0.67–2.0)	46
Gyromitra esculenta (False Morel)	0.035–0.39 (0.028–0.42)	6.1 (5.9–6.3)
Ramaria aurea (Golden Coral Fungus)	1.3–5.1 (0.73–11)	39
Cantharellus cibarius (Common Chanterelle)	0.04–1.3 (0.03–1.6)	6.3±0.5 (5.3–6.7)
Craterellus cornucopioides (Horn of Plenty)	0.12–0.4 (BDL[d]–1.0)	34
Suillus bovines (Cow Bolete)	0.62–2.5 (0.05–3.3)	35
Suillus grevillei (Larch Bolete)	0.37–1.1 (0.19–1.2)	42
Suillus luteus (Slippery Jack)	0.70–3.3 (0.45–4.3)	27
Suillus variegates (Variegated Bolete)	0.48–1.0 (0.36–1.1)	130
Boletus aestivalis (Summer Bolete)	11–21 (1.6–25)	50
Boletus appendiculatus (Butter Bolete)	7.3 (5.5–9.1)	53
Boletus erythropus (Dotted Stem Bolete)	2.7–7.3 (2.6–9.6)	35
Leccinum rufum (Red Aspen Bolete)	0.44–1.7 (0.34–1.7)	43
Agaricus campestris (Meadow Mushroom)	1.8–5.7 (0.99–22)	24 (20–28)
Agaricus silvicola (Wood Mushroom)	1.2–6.4 (0.27–7.7)	23
Coprinus comatus (Shaggy Mane)	0.60–0.79 (0.06–2.9)	36

[a] Falandysz (2008), [b] Siobud–Dorocant et al. (1999), [c] the mean values or range of the means and total range – in parentheses; [d] BDL, below detection limit.

mushrooms, which are known as non–Se–accumulating species. Many of these imprecise data on Se were determined by using technique of inductively coupled plasma – atomic emission spectroscopy (ICP–AES) at *λ* 196.026 nm (Siobud–Dorocant et al., 1999). Some of these questioned data are illustrated in Table 1.

Having problems with the interpretation of these enormously high concentrations of Se reported in mushrooms (Table 1), we embarked on this study to check the credibility of the Se determinations in mushrooms by ICP–AES. A widely used, sensitive, accurate and precise method for the total Se determination in various materials is the hydride generation atomic absorption spectrometry (HG–AAS) (Dedina and Tsaley, 1995). We determined and examined the results of total Se analyses of several species of mushrooms as well as certified reference standard materials using the HG–AAS technique (Anan et al., 2001), and the doubtful ICP–AES technique (Siobud–Dorocant et al., 1999).

MATERIALS AND METHODS

The specimens of poison pax (*Paxillus involutus*), brown birch scaber stalk (*Leccinum scabrum*), fly agaric (*Amanita muscaria*) and king bolete (*B. edulis*) examined in this study were collected in Poland. Total Se content of the collected mushrooms was determined by HG–AAS as reported in earlier studies (Falandysz et al., 2007a, b, 2008a, b). In the present study, to determine Se, the sub–samples of dried caps of these mushrooms were examined by ICP-AES at *λ* 196.026 nm (Table 2).

The HG–AAS method used for Se determination was validated and the analyses were performed under the ultra clean laboratory conditions. In brief, the powdered mushrooms (caps) were dried to constant weight and sub–samples (3 replicates) were microwave digested with concentrated nitric acid (ultra clean; Wako Chemicals, Japan) in the polytetrafluoroethylene (PTFE) bakers placed into the PTFE pressurized vessels. The total Se content was determined using an external standard (Anan et al., 2001).

The following standard reference materials were used: dried fruiting bodies of cow bolete (*Suillus bovinus*) (CS-M-1), tea leaves (INTC-TL-1) and Oriental tobacco leaves (CTA–OTL-1). These certified reference materials were produced by the Institute of Nuclear Technology and Chemistry in Warsaw, Poland (Table 3) (Dybczyński et al., 1996, 2002; Polkowska-Motrenko et al., 2006).

Selenium determinations of mushrooms and certified reference materials in our study followed a digestion method with concentrated nitric acid solution and final Se measurement by ICP–AES at *λ* 196.026 nm (Siobud–Dorocant et al., 1999). In brief, in this quoted article, a dried fungal flesh was digested as follows: “Two different preparations [(a) 50 mg dry weight and (b) 150 mg dry weight approximately] of a given specimen were placed into all–propylene stopped vials free of mineral contamination and washed three times with dilute aqueous nitric acid (65% nitric acid, CARLO ERBA, RPE, density: 1.40) prior to analyses. The samples [(a) and (b)] were digested, respectively, with 5 and 10 ml of the acidic solution; the vials were warmed gradually from 35 up to 65°C in 90 min in a temperature–controlled heating block and kept at this temperature for 1 h with stirring. After cooling, volumes were adjusted to 5 ml (a) or 10 ml (b). Following centrifugation, the clear supernatants were transferred into all–propylene stopped vials and assayed ex tempore in the spectrometer. Standard solutions of each element were MERCK products (1±0.002 g/L in 0.5 N nitric acid)” (Siobud–Dorocant et al., 1999).

In the present study, Se was determined by ICP–AES at *λ* 196.026 nm after digestion with concentrated nitric of dried and pulverized fungal material or certified reference materials. In details, the samples of about 500 mg were wetted with 7 ml of concentrated nitric acid solution (65%; Suprapur®, Merck) in the PTFE

Table 2. Total selenium content of mushrooms when determined by HG–AAS and ICP–AES, respectively (µg/g dw, mean±SD, range and median values).

Mushroom species, sampling site, year, morphological part and number of specimens examined	Se	
	HG–AAS	ICP–AES at λ 196.026 nm
Paxillus involutus (Poison Pax), Notecka Forest, Poland, 2000; whole fruiting bodies n=15	0.09±0.07 (0.03–0.24) 0.08	0.47±0.65 (0.005–1.4) 0.005[a]
Leccinum scabrum (Brown Birch Scaber Stalk), Augustowska Forest, Poland, 2000; caps n=15	0.52±0.33 (0.18–1.4) 0.44	0.64±0.83 (0.005–2.4) 0.005[a]
Amanita muscaria (Fly Agaric), Warmia Land, Poland, 2000; caps n=15	4.7±1.2 (2.2–6.4) 5.0	4.0±1.3 (1.7–6.3) 4.0[b]
Boletus edulis (King Bolete), Sudety Mountains, Poland, 2000; caps n=10	32±20 (18–70) 26	33±19 (20–73) 24[b]

Mann–Whitney *U* test: (a) $p < 0.001$ and (b) $p > 0.05$.

Table 3. Total selenium content of selected certified reference standard materials determined by HG–AAS and ICP–AES, respectively (µg/g dw, mean±SD).

Material	Se	
	Certified value	ICP–AES at λ 196.026 nm
CS–M–1 Dried fruiting bodies of cow bolete (*Suillus bovinus*)	1.37±0.11	1.7±0.3
CTA–OTL–1 Oriental tobacco leaves	0.153±0.018	< 0.001
INTC–TL–1 Tea leaves	0.076[a]	0.73±0.02

[a] Informative value.

pressurized vessels and left to stand for 24 h. Further, the PTFE vessels were closed and the mixture was heated in a microwave digestion system type MARS Xpress (Microwave Accelerated Reaction System, CEM Corp., Matthews, NC, USA). After cooling, the digested volume was adjusted to 25 ml using double–deionized water. With every set of 15 samples of fungal or plant material digested were run three blank samples. The spectrometer used was model Optima 2000™ DV (Perkin Elmer, Waltham, Massachusetts, USA). Yttrium was used as internal standard.

The statistical analyses were performed using the student's *t*–test and Mann–Whitney *U* test. The statistically significant level is reported for p-value less than 0.05 and highly significant level is p–value less than 0.001. Statistical analyses were performed using the Statistica 8.0 software.

RESULTS AND DISCUSSION

Data on Se content of mushrooms determined by HG–AAS and ICP–AES are given in Table 2. Table 3 gives information on certified and informative contents of Se as well as own results of this element determinations in four certified reference materials by ICP–AES.

Mushrooms examined (Table 2) can be described as species very poor (poison pax; *P. involutus*), poor (brown birch scaber stalk; *L. scabrum*), moderate (fly agaric; *A. muscaria*) and relatively rich (king bolete; *B. edulis*) in Se (Falandysz, 2008). Analysis by HG-AAS confirmed known opinion on Se content in fruiting bodies of these four mushroom species, as summarized in Table 2. Also, measurements by ICP–AES in this study showed moderate abundance of Se in fly agaric and its abundance in king bolete. Nevertheless, the ICP–AES data obtained for Se in poison pax or brown birch scaber stalk disagree with data obtained by HG–AAS (Table 2).

Data on Se in poison pax obtained by ICP–AES are highly scattered when compared to those obtained by HG–AAS. Median Se concentration in poison pax after ICP–AES measurement is significantly smaller ($p < 0.001$; Mann–Whitney *U* test), while the arithmetic mean value is greater, an order of magnitude, when compared to the HG–AAS results.

Data obtained by ICP–AES for brown birch scaber stalk are highly scattered and not precise, when compared to those obtained by HG–AAS (Table 2). The median values of Se concentration obtained in this study by ICP–AES and HG–AAS vary significantly ($p < 0.001$; Mann Whitney *U* test). On the other side, the arithmetic mean values obtained by ICP–AES and HG–AAS are of the same magnitude ($p > 0.05$; *t*–test; Table 2). Nevertheless, the majority of Se results for Brown Birch Scaber Stalk by ICP–AES are at the lowest range, and the median concentration (all at 0.005 µg/g dry weight) is smaller, when compared to that obtained by the HG–AAS

measurement (Table 2). These results by ICP–AES are also much lower than the smallest value of 0.12 µg/g dw reported for cap of this fungus in a worldwide search (Falandysz, 2008).

In the case of fly agaric, data obtained by ICP–AES are comparable to data by HG–AAS, that is, means ($p > 0.05$; *t*–test) and medians ($p > 0.05$; Mann–Whitney *U* test). For king bolete, the HG–AAS and ICP–AES data are also comparable, both the means ($p > 0.05$; *t*–test) and medians ($p > 0.05$; Mann–Whitney *U* test).

King bolete contained Se at mean concentration of 32±20 µg/g dw by HG–AAS and 33±19 µg/g dw by ICP–AES, while the median values were 26 and 24 µg/g dw, respectively (Table 2). These results imply that if Se content of fungal material is high (> 20 µg/g dw), the interferences from a well oxidized sample during the ICP–AES measurement at λ 196.026 nm can be negligible.

Three certified reference materials examined can be characterized as fungal and plant products of moderate to low Se content. The certified and the informative mean values of Se concentrations in these materials are in the range from 0.076 to 1.37 µg/g dw, respectively (Table 3).

Selenium content of dried fruiting bodies of cow bolete (*S. bovinus*) (CS–M–1) standard reference material obtained by ICP–AES was 1.7±0.3 µg/g dw, which is a value close to the certified value of 1.37±0.11 µg/g dw. For oriental tobacco leaves (CTA–OTL–1) standard reference material, the certified Se concentration is 0.153±0.018 µg/g dw. The ICP–AES result is below the limit of detection (0.001 µg/g dw) – a result/value far below the certified Se content. For tea leaves (INTC–TL–1) standard reference material, Se concentration when determined by ICP–AES was 0.73±0.02 µg/g dw – an order of magnitude greater than the informative value declared by the producer which is 0.076 µg/g dw. Selenium determination in fungal and plant materials by ICP–AES at λ 196.026 nm after wet–digestion of organic matrix with nitric acid gives inaccurate and imprecise results. This, most probably, is due to some matrix effect even for well oxidized samples. The matrix effects of biological samples is due to the presence of carbon but also sulfur, phosphorus and bromine that cause non–spectral interferences, and these have been observed in the determination of Se by ICP–AES by earlier studies (Grindla et al., 2007; Machat et al., 2002). These effects result in the rise of emission signal for Se especially when an increased amount of carbon is reaching the plasma (Grindla et al., 2007; Machat et al., 2002). In our experiment, and probably due to a perfect fungal or herbal samples mineralization, these possible matrix effects from carbon seem small but still, the ICP–AES results of Se determination are erroneous (Tables 2 and 3). An exception is data for king bolete, for which Se content was the same after the HG–AAS and ICP–AES determination, that is, 32±20 and 33±19 µg/g dw, respectively ($p > 0.05$; Table 2). The findings in this study imply also that if the fungal matrix is well oxidized, the matrix effects in Se rich materials such as flesh of king bolete can be negligible and Se data for this mushroom by ICP–AES can be acceptable. Hence, reports of very high concentrations of Se in mushroom species that do not accumulate Se or that have not emerged at selenoferous areas, when determined by ICP–AES, should be treated with caution, because the data could be highly biased.

Conclusion

Selenium determination in fungal and plant materials by ICP–AES at λ 196.026 nm after wet–digestion with nitric acid gives inaccurate and imprecise results. Hence, reports of very high concentrations of Se in mushroom species that do not accumulate Se or that have not emerged at selenoferous areas, determined by ICP–AES, should be treated with caution, because the data could be highly biased.

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