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Major and trace elements in *Boletus aereus* and *Clitopilus prunulus* growing on volcanic and sedimentary soils of Sicily (Italy)

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ABSTRACT

The aim of this study was to determine and compare the content of 28 elements (Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Se, Sr, Tl, U, V and Zn) in fruiting bodies of *Boletus aereus* Bull. and *Clitopilus prunulus* P. Kumm collected from eleven unpolluted sites of Sicily (Italy) and, also to relate the abundance of chemical elements in soil with their concentration in mushrooms. Median concentrations of the most abundant elements in *Boletus aereus* ranged from 31,290 μg/g (K) to 107 μg/g (Zn) in caps and from 24,009 μg/g (K) to 57 μg/g (Zn) in stalks with the following abundance order: K > Na > Ca > Mg > Fe > Al > Rb > Zn. The same elements, in the whole fruiting body of *Clitopilus prunulus* samples, varied in the range 54,073–92 μg/g following the abundance order: K > Na > Mg > Ca > Fe > Al > Rb > Zn. Metal contents in *Boletus aereus* and in the whole fruiting body of *Clitopilus prunulus*, collected from the same sampling sites, showed statistically significant differences for most elements. In particular, *Clitopilus prunulus* contained around two to four times more Co, Cr, Fe, Mg, Mo, Pb, U and V than caps and stalks of *Boletus aereus* species which, in turn, was from two to four times more enriched in Cu, Se and Tl. Thus, the elemental content of *Boletus aereus* and *Clitopilus prunulus* appeared to be species-dependent. The distribution of chemical elements in *Boletus aereus* was not uniform throughout the whole fruiting body as most elements were significantly bioconcentrated in caps. Furthermore, the fruit bodies of *Boletus aereus* from the volcanic soil differed both in major and minor elements concentrations from those collected from sedimentary soils. Cadmium and lead concentrations were below the threshold limits for wild mushrooms proposed by EU Directives (2008 and 2015). The elemental content was not significantly influenced by soil pH.

1. Introduction

Fungi are eukaryotic unicellular and multicellular organisms, ubiquitous in natural environments. They are osmotrophic, symbiotic of plants and animals and possess the extraordinary ability to decompose organic material returning usable nutrients to soil or food chain. Mushrooms are fruiting bodies of some kinds of fungi, some of which are edible but others can be toxic and even fatal. Edible wild-grown mushrooms are popular food very appreciated as delicacy, especially in Europe and Asia, not only for their taste and low fat content, but also for their relatively high nutritional value, containing essential amino acids, proteins, vitamin D (Mattila et al., 2000), major and trace chemical elements (Alexopoulos et al., 1996; Bernas et al., 2006; Jonathan and Fasidi, 2003; Latiff et al., 1996; Siwulski et al., 2014). However, mushrooms fruiting bodies, as well known, are very effective in uptake of both essential and undesirable chemical elements from their natural habitat (Falandysz et al., 2001, 2003), even when growing on uncontaminated soils (Chudzyński and Falandysz, 2008; Chudzyński et al., 2009; Falandysz and Borovička, 2013; Giannaccini et al., 2012; Nikkarinen and Mertanen, 2004). Metal and metalloid contents in mushrooms may be considerably higher than those in agricultural crop plants, vegetables, and fruits (Zhu et al., 2011). All this raises important questions about the safety of edible mushrooms: if accumulation is species-specific, which elements are mainly accumulated by a single species, which part of the fruiting body contains higher concentrations of mineral elements, if soil geochemical background constitutes an important factor determining the content of potentially harmful metals and non metals.

The main factors influencing accumulation of trace elements in fungi may be summarized as follows:

1) geochemical background, presence of metalliferous areas and

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environmental pollution (Borovička et al., 2010a, 2010b; Drewnowska et al., 2012, 2013; Falandysz et al., 2008, 2014; Falandysz and Borovička, 2013; Falandysz, 2014; Soylak et al., 2005; Tüzen, 2003);
2) specificity of each particular fungal species in accumulation of metals and metalloids in fruiting bodies (Borovička et al., 2007; Falandysz et al., 2001, 2007);
3) ability to discriminate elements with similar properties and chemical behavior (Falandysz and Borovička, 2013).

This study was conducted to determine and compare the content of macro and trace elements in fruiting bodies (caps and stalks) of *Boletus aereus* Bull. and *Clitopilus prunulus* P. Kumm collected from several wooded areas of Sicily, and also to relate the abundance of chemical elements in soil with their concentration in mushroom. *Boletus aereus* Bull. *(*Family: *Boletaceae*; Genus: *Boletus)* is a thermophilous fungal species, typically Mediterranean, growing and widely consumed in Italy, France, Greece and Spain. Commonly it grows in mycorrhizal association with various broad-leaved trees, especially Quercus, Fagus and Castanea, and sclerophyllous shrubs. Its presence decreases gradually toward north Europe and it is harvested from late spring to early autumn. *Boletus aereus* is appreciated for its pleasant fragrant aroma, taste and nutritional value. These characteristics, along with its relatively easy availability, makes *Boletus aereus* one of the most sought mushrooms for human consumption.

Clitopilus prunulus P. Kumm (Family: *Entolomataceae*; Genus: *Clitopilus)* is a symbiont mushroom growing in meadows and under broad-leaved woods or conifers, from summer to autumn at all altitudes. The name derives from its odor of sweet flour and sometimes it is called by the nickname “spy mushroom”, as it is often found near the *Boletus edulis*, with which it shares habitats and climatic conditions. This mushroom is not marketed.

In Italy picking mushrooms is regulated by regional and locals laws according to which the professional mushroom/fungi expert can collect a limited amount of mushrooms (Sicily Region, Law 02/, 2006, no. 4, art. 2).

Focus of the research was also to investigate possible variations in elemental concentrations due to different bedrock soil geochemistry.

2. Materials and methods

2.1. Mushrooms

The fruiting bodies of 41 composite samples (made up of 1–3 specimens) of *Boletus aereus* Bull. and 15 of *Clitopilus prunulus* P. Kumm were collected from eleven unpolluted sites of Sicily (Italy) during the period September–November 2016 (Fig. 1).

Samples from the volcanic area (VA: 11 and 4 samples of *Boletus aereus* and *Clitopilus prunulus,* respectively) were separated from those of other areas characterized by flyschoid or calcareous substrates (FCS: 30 and 11 samples of *Boletus aereus* and *Clitopilus prunulus,* respectively). The sampling locations, mushroom species and vegetation type are reported in Table 1.

Fruiting bodies were thoroughly cleaned directly after picking using clean plastic knives. *Boletus aereus* were separated into caps and stalks whereas the samples of *Clitopilus prunulus* were not subdivided in caps and stalks because of their small size which did not allow separate analysis. They were dried (37 °C, overnight) in an electrically heated commercial dehydrator for mushrooms, fruits and vegetables (Melchioni 118320000 Babele, 245 W). After that, the dried fungal material was ground into powder using an agate mortar and then kept in polyethylene bags under dry conditions. Powdered mushrooms (~ 0.700 g) were digested with a mixture of 5 mL of 65% HNO_3 (Suprapure Merck,) and 2.5 mL of 33% H_2O_2 (Suprapure Merck). The digest was diluted to 50 mL using deionized water (18 MΩ). Analyses were carried out at Dept. Scienze della Terra e del Mare, University of Palermo. Ca, K, Na, Mg were analysed by ion chromatograph Dionex 120, with precision better than ± 5%. Twentyfour elements (Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, Tl, U, V, Zn) were determined using an Inductively Coupled Plasma Mass Spectrometer (Elan 6100 DRC-e, PerkinElmer). Isotopes of ^{45}Sc, ^{89}Y and ^{186}Re prepared from individual solutions with contents of 1000 mg/L were applied as internal standards in order to effectively correct random variations in signal intensity (ICP Standard CertiPUR, Merck). For As, Cr, Fe, Se and V, the ICP-MS was operated in DRC (Dinamic Reaction Cell) mode with CH_4 as reaction gas. All standard solutions were prepared with ultra-pure deionised water (18 MΩ) and reagent-grade chemicals (ICP Multi Element Standard Solution XXI CertiPUR – Merck and Mo and Sb CertiPUR Standards – Merck). Calibration curves ranging from 0.05 µg/L to 500 µg/L were constructed. To minimize matrix effects, the standard addition technique was used for all determinations. Sample blanks were also analysed, and the operational detection limit for each element was calculated as three times the standard deviation of the analyte concentration in blank samples. Values below the detection limit were set at one-third the detection level and treated as real values. Analytical precision, estimated from triplicate analyses every tenth sample, was in the range 1–11% for all the analysed elements. In order to validate the analytical procedure the standard reference material NIST SRM 1515 Apple Leaves was analysed for corresponding elements. The metal recovery rates resulted in good agreement with the certified concentrations, ranging between 94% and 111%. The calculated median concentrations of macro and trace elements are reported on a dry weight basis in Table 2.

2.2. Soil samples

A total of 11 surface soils (depth of 0–20 cm) were collected from the same areas where mushroom were taken or in close vicinity by using a plastic scoop. The substrate soil samples were placed in plastic bags and transported to the laboratory. They were dried at 110 °C in a stove (AG System, mod. G-therm) overnight; stones and plant materials were then removed and each sample was sieved using 500 µm nylon mesh and then milled in agate mortar. Soils analysis was carried out at Activation Laboratories Ltd. (Ontario, Canada). Portions of each sample were analysed for major and trace elements by FUS-ICP mod. Varian Vista ICP, (0.2g of sample were mixed with a mixture of lithium metaborate/lithium tetraborate and fused in a graphite crucible. The molten mixture was then poured into a 5% nitric acid solution and shaken until dissolved), by ICP-OES mod. Varian Vista ICP (0.25 g of sample were digested with three acids beginning with hydrofluoric, followed by a mixture of nitric and perchloric acids) and INAA (1 g of sample was encapsulated in a polyethylene vial and irradiated with flux wires). The whole methodology is described in Hoffmann (1992). Employed standard reference materials were GBW 07113 (FluXana), GXR-4 (USGS) and BIR-1a (USGS). The analytical data are reported in Table 3.

The measurement of pH values were performed potentiometrically on the soil suspensions obtained by adding 25 mL of potassium chloride 1 M to 10 g of soil (dry weight). The solution was kept agitated for at least two hours and then allowed to sit for 30 min.

2.3. Data analysis

Data were analysed statistically by the STATISTICA program, StatSoft version 6.0 (2001). All the tests, in this study, were considered significant at $p < 0.05$. The Shapiro-Wilk test was used to verify the normality of data distributions. The Mann–Whitney test, which makes no assumptions about the distributions and do not rely on distribution parameters, was used to verify the statistical significance of observed differences between group medians. The Spearman's correlation coefficients (r_s-value) were used to modelling linear relationships between couple of variables.