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ORIGINAL RESEARCH PAPER

Bedrock and soil geochemistry influence the content of chemical elements in wild edible mushrooms (*Morchella* group) from South Italy (Sicily)

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Abstract
Chemical elements in the samples of wild edible mushrooms of the *Morchella* group collected from different unpolluted Sicilian sites was analyzed by the ICP-MS (method) to detect the content of their minerals and determine whether soil geology and geochemistry can influence the chemical composition in fungi. Results showed that the mushroom samples mainly contained a high concentration of K and P and a wide variety of minor and trace elements (V, Mo, Pb, Ce, Cs, Zr), including heavy metals. Statistical analysis showed that the mushrooms differed in their content of minor and trace elements based on the geological/geographic site of origin. Comparison with other studies showed differences in the content detected in the Sicilian morels with those collected from other geographical sites. Conversely, different fungal species collected from similar geological sites in Sicily showed different patterns of accumulation of the elements confirming that bioconcentration in fungi is species- and site-dependent.

Keywords
fungi; ICP-MS analysis; mineral content; accumulation factor; site geology

Introduction

It is well known that fungi accumulate chemical elements from their environment, particularly from soils and soil solutions [1–6]. The mineralogical composition of soil influences the availability of chemical elements and the mycelia of fungi absorb and accumulate all kinds of elements from their growth substrates. Although the capacity of accumulation and the presence of chemical elements in the fruiting bodies [7] of fungi depend on their nutritional requirements and can differ on the basis of genetic characteristics, several authors have confirmed that the content of elements in both micro- and macrofungi is mainly influenced by the chemical composition of the surrounding environment (water, air, and soil) [8–10].

The ability of fungi to take up elements makes them useful soil quality indicators as well as potential bioremediation agents for substrata contaminated with toxic elements such as heavy metals, metalloids, and radionuclides [11–16]. For instance, some authors [17] have observed that the macrofungal species *Agaricus macrosporus* Mont. may be effective in extracting heavy metals, such as mercury and cadmium, from contaminated soils.

In several studies, the presence of heavy metals in edible and nonedible fungal species from both, polluted and unpolluted sites, has been analyzed [1–3,5,9,17]. However, till date, very few studies have actually analyzed the influence of geology, soil-mineralogy,

and soil-chemistry on the chemical content of the fruiting bodies or investigated the correlation between chemical elements in fungi and their soil of growth [4,8,11,18]. For instance, Nikkarinen and Mertanen [8] analyzed the content of elements in two ectomycorrhizal species namely *Boletus edulis* Bull. and *Lactarius trivialis* (Fr.) Fr. from two different geological regions in Finland to determine whether any geochemical fingerprints can be observed in these fungi. They found that the macrofungal samples differed considerably in their content of trace elements based on the geological and geographic site of origin. Nonnis Marzano et al. [18] used chemical and radiochemical methodologies to analyze the concentrations of artificial radionuclides and trace elements in *Boletus* samples (known as "Porcini") collected from different geographical areas across the globe. Their results showed that the content of chemical and radiochemical elements in the fruiting bodies reflect the geological/chemical background of the environment and, therefore, can be used to determine the geographic site of origin.

Analyzing the mineral content and correlating it with the bedrock and soil geochemistry is particularly important for wild edible mushrooms due to their economic and social importance. Over 2,000 fungal species are known to produce edible fruiting bodies that are harvested, marketed and consumed in more than 85 countries around the world [19–23]. The global market value of edible mushrooms is estimated to be at least $2 billion, which is more than the value of timber [19,24,25]. When toxic elements (e.g., heavy metals) are present in the growth substrates, they can accumulate in the fungi and may pose a risk to human health. Particularly, the content of metals and metalloids is likely to be higher in mushrooms than those in agricultural crops, plants, vegetables, and fruits [6].

In this paper, the following approaches were undertaken to determine whether the bedrock and soil chemistry influences the content of elements in wild mushrooms: (***i***) we analyzed the chemical content in the wild edible fruiting bodies of morels (*Morchella* spp.) collected from different unpolluted sites in the south of Italy (Sicily). Our objective was to compare the content of major, minor, and trace elements in the fruiting bodies of mushrooms obtained from different geological sites in Sicily and investigate whether the observed differences could be correlated to those in the bedrock and soil geochemistry; (***ii***) moreover, using a set of literature data on the bedrock and (top) soil composition in Sicily, Italy, Europe and the rest of the world, we determined the accumulation factor (a.f.) of different elements in the fungal samples to assess which elements are accumulated in mushrooms at concentrations higher than those in the soil; (***iii***) finally, we compared the data thus obtained with those of mushrooms analyzed from other geographic/geological sites to determine whether soil geology and geochemistry can influence the chemical components present in the fungi; (***iv***) we also compared our data with those of other fungal species to understand whether different species of edible wild mushrooms originating from similar geological sites of Sicily differ in their preferences for the accumulation of specific elements.

Two species within the *Morchella* genus, *M. elata* Fr. and *M. esculenta* (L.) Pers., were chosen for this study because they are the ones that are majorly consumed globally [22] and give an estimated worldwide income of ~$1.67 billion [19,26,27].

Material and methods

Study sites

Ten sampling sites were selected from the province of Palermo in Sicily between the Natural Regional Park of Madonie and the Palermo Mountains (Fig. 1). Tab. 1 gives complete details on the study sites. Overall, we selected different Mediterranean vegetation types including both native (e.g., broadleaf and evergreen plants) and planted (e.g., conifers) wooded areas. The geology of the selected sites consisted of dolomitic limestone, carbonate rocks (S1–S3 and S6–S10), and flyschoid rocks (S4–S5).

The climate in the province of Palermo is the Mediterranean pluviseasonal-oceanic type [28]. The mean annual temperature is 18.4°C and varies from 26.2°C in August to 12.1°C in January. The mean annual rainfall is 605 mm and varies from 90 mm in December to 4 mm in July [29].

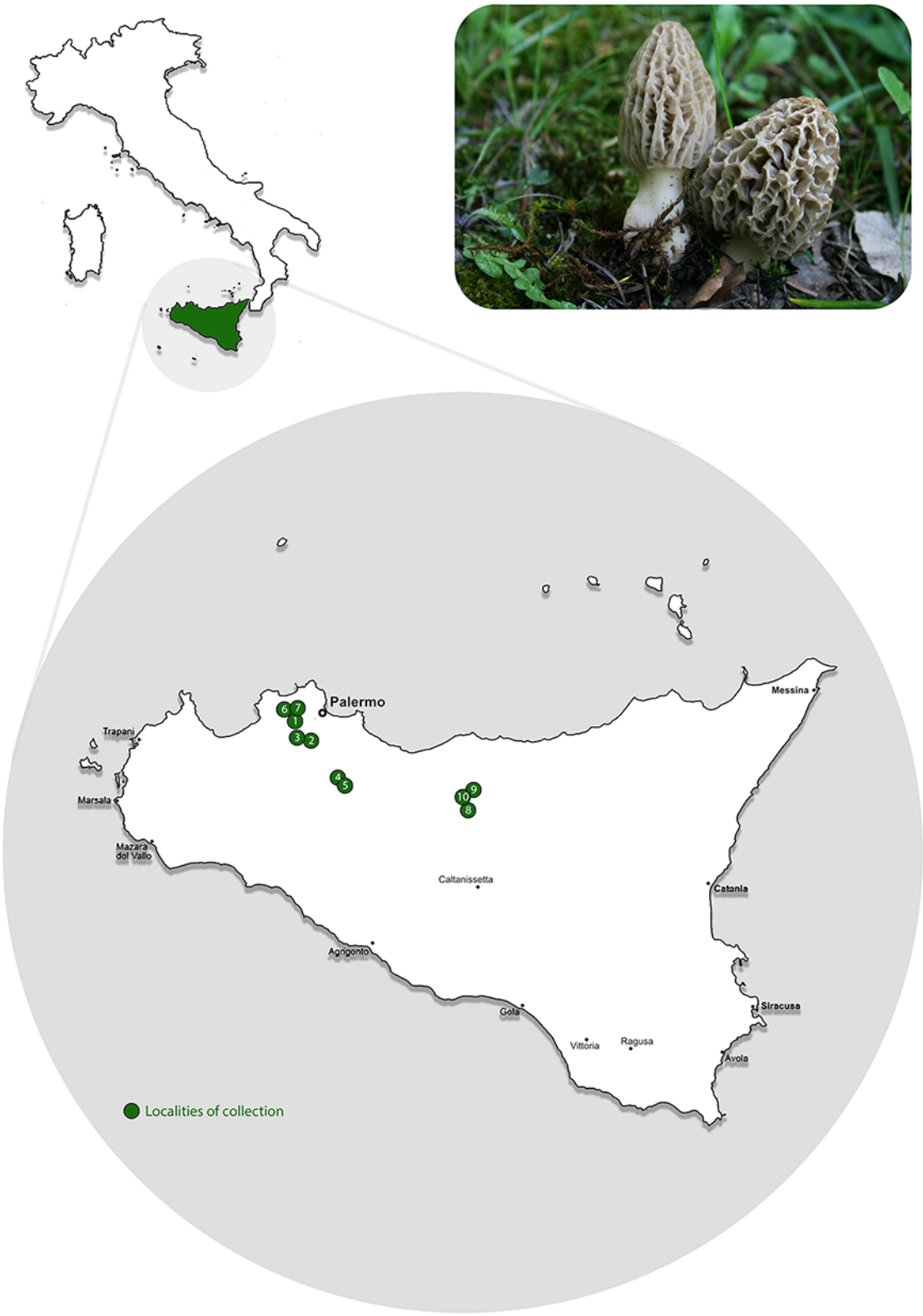

Fig. 1 Map of the study sites and the picture of *M. esculenta*.

Tab. 1 Details of the study sites: code, locality with GPS coordinates, geology, and vegetation type. Fungal species and their corresponding codes are detailed in the last two columns.

Site	Locality – municipality	Geology	Vegetation type	Fungal species	Mushrooms code
S1	Monte Petroso – Monreale (38°06′03.1″ N, 13°15′43.2″ E)	Carbonate rocks	*Quercus ilex* – native wood	*Morchella elata*	F1
S2	Castellaccio – Monreale (38°04′49.5″ N, 13°15′48.9″ E)	Carbonate rocks	*Pinus pinea* – plantation	*M. elata*	F2
S3	Castellaccio – Monreale (38°05′05.4″ N, 13°15′58.2″ E)	Carbonate rocks	*Pinus pinea* – plantation	*M. elata*	F3
S4	Fontana Bosco – Palermo (37°53′34.8″ N, 13°23′28.4″ E)	Flyschoid rocks	*Quercus cerris* var. *gussonei* and *Fraxinus angustifolia* – native mixed wood	*M. esculenta*	F4
S5	Fontana Bosco – Palermo (37°53′21.7″ N, 13°23′33.4″ E)	Flyschoid rocks	*Quercus cerris* var. *gussonei* and *Fraxinus angustifolia* – native mixed wood	*M. esculenta*	F5
S6	Mandria Zarcati – Carini (38°09′12.5″ N, 13°16′04.3″ E)	Carbonate roks	*Pinus halepensis* – plantation	*M. elata*	F6
S7	Mandria Zarcati – Carini (38°09′08.8″ N, 13°15′52.9″ E)	Carbonate rocks	*Pinus halepensis* – plantation	*M. elata*	F7
S8	Pizzo colla – Polizzi Generosa (38°09′08.8″ N, 13°15′52.9″ E)	Carbonate rocks	*Fagus sylvatica* – native forest	*M. elata*	F8
S9	Bevaio del Faggio – Isnello (37°52′14.6″ N, 14°00′33.4″ E)	Carbonate rocks	*Pinus nigra* – plantation	*M. elata*	F9
S10	Bevaio del Faggio – Isnello (37°52′14.6″ N, 14°00′33.4″ E)	Carbonate rocks	*Fagus sylvatica* – native forest	*M. elata*	F10

Sampling

During the season favorable for fungal growth, in April and May 2014, several mycological surveys were carried out at the selected study sites. The fruiting bodies/mushrooms, namely *M. elata* Fr. and *M. esculenta* (L.) Pers. (F1–F10 in Tab. 1), were sampled from 10 unpolluted sites of Sicily (refer Fig. 1 and Tab. 1). Each sample consisted of 1–3 fungal specimens comprising a complete fruiting body (cap, stipe, and hymenium). Samples of the mushrooms were cleaned of forest debris (without washing) using a brush, transported to the laboratory and kept at −4°C for no more than 24 h prior to sample preparation.

Identification of the species and description of the fruiting bodies/ascomata were carried out for both fresh and dried specimens by macro- and microscopic observations. The macroscopic descriptions of the fresh ascomata were noted while the microscopic features were observed using an Olympus BH-2. The dried specimens were prepared for microscopic observations using a solution of 0.3% KOH and the Melzer reagent. Spore measurements were based on 50 observations conducted for each of the dried samples. For taxonomical identification, a series of monographs and keys were used by Breitenbach and Kränzlin [30], Courtecuisse R and Duhem [31], and Boccardo et al. [32].

We followed the methods of systematic classification described previously for the classification of mushrooms [7,33]. Nomenclature and author abbreviations were used in accordance with [34–36]. Studied specimens were deposited at the Herbarium Panormitanus (PAL), Italy.

Chemical analysis

The fruiting bodies were dried for 12 hours at 37°C in an electrically heated commercial dehydrator for mushrooms, fruits, and vegetables (Melchioni 118320000 Babele, 245 W). The dried fungal material was ground into powder using an agate mortar and stored in polyethylene bags under dry conditions. Powdered mushrooms (~0.700 g) were digested using 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). Ca, K, Na, and Mg were analyzed using the ion chromatograph Dionex 120, with a precision greater than ±5%. The presence of 24 elements (Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, Tl, U, V, and Zn) in the digested extract was determined using an inductively coupled plasma-mass spectrometer (ICP-MS) (Elan 6100 DRC-e, PerkinElmer). For the detection of As, Cr, Fe, Se, and V, the ICP-MS was operated in the DRC (Dinamic Reaction Cell) mode with methane as the reaction gas. All standard solutions were prepared with ultra-pure deionized water (18 MΩ) and reagent-grade chemicals (ICP multielement standard solution XXI Certi-PUR – Merck; Mo and Sb, CertiPUR standards – Merck). Calibration curves ranging from 0.05 µg/L to 500 µg/L were constructed. The standard addition technique was used for all analyses in order to minimize the matrix effects. Sample blanks were also analyzed and the operational detection limit for each element was calculated as three times the standard deviation of the analyte concentration in the blank samples. Values below the detection limit were set at one-third of the detection level and treated as real values. Analytical precision was in the range 1–11% for all the analyzed elements. For validation of the analytical procedure, the standard reference material NIST SRM 1515 Apple Leaves was analyzed for the corresponding elements. The rates of recoveries for metal were in good agreement with the certified concentrations, ranging between 94% and 111%.

All the analyzed elements are detailed as follows:

- Alkali metals: Li, Na, K, Rb, Cs.
- Alkaline earth metals: Mg, Ca, Sr, Ba.
- Transition metals or d-block elements: Y, Ti, Zr, Hf, V, Nb, Ta, Cr, Mo, W, Mn, Re, Fe, Co, Ni, Pd, Pt, Cu, Ag, Au, Zn, Cd, Hg.
- Semimetals: B, Ge, As, Te, Sb.
- Post-transition metals: Al, Ga, Tl, Sn, Pb, In, Bi.
- Lanthanides: La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu.
- Actinides: Th, U.
- Nonmetals: P, Se.

In total, the concentrations of 63 elements were determined (see Appendix S1 for a complete list) and converted from ppb to ppm for statistical analyses, as detailed in the following data analysis paragraph.

Data analysis

Quantitative data, referred to as the chemical concentrations of the elements in ppm, were analyzed statistically using the vegan package in R [37]. Descriptive statistical tools (histograms) were used to compare the concentrations of major, minor, and trace elements in each of the fungal samples.

We also used the whole dataset (see Appendix S1) to detect the a.f. in the fungal samples. The a.f. refers to the ratio of the concentration of a specific element in the mushroom samples to the concentration of that element in the soil (or native rock/bedrock). The a.f. was computed in accordance with Cocchi et al. [9] by comparing the concentration of each element detected in the fungal samples with the average concentrations of the same element in the soil. As a proxy for the average concentrations of elements in the soil, we used known concentrations of the elements in comparable soil types (Appendix S2). This information was obtained from a set of literature data on the bedrock and (top) soil composition in Sicily, Italy, Europe and the rest of the world [38–49]. Histograms were used to indicate the accumulation factor.

Finally, a hierarchical cluster analysis (CA) using the Bray–Curtis dissimilarity index and unweighted pair group method with arithmetic mean UPGMA [50] were carried out to discern the degree of similarity between the content of chemicals in the mushroom samples and the selected bedrocks or soil types.

Results

Appendix S1 lists all the 63 chemical elements detected in each of the mushroom samples (F1–F10).

The values of major elements (20 in total) measured in the mushroom samples ranged from 0.01 ppm (Sb) to 4,300 ppm (K) (Fig. 2) in the following order of abundance: K > P > Ca > Mg > Na > A > Fe > Zn > Cu > Mn > Rb > Ti > Cd > Au > Sr > B > Ba > Cr > Ni > Sb.

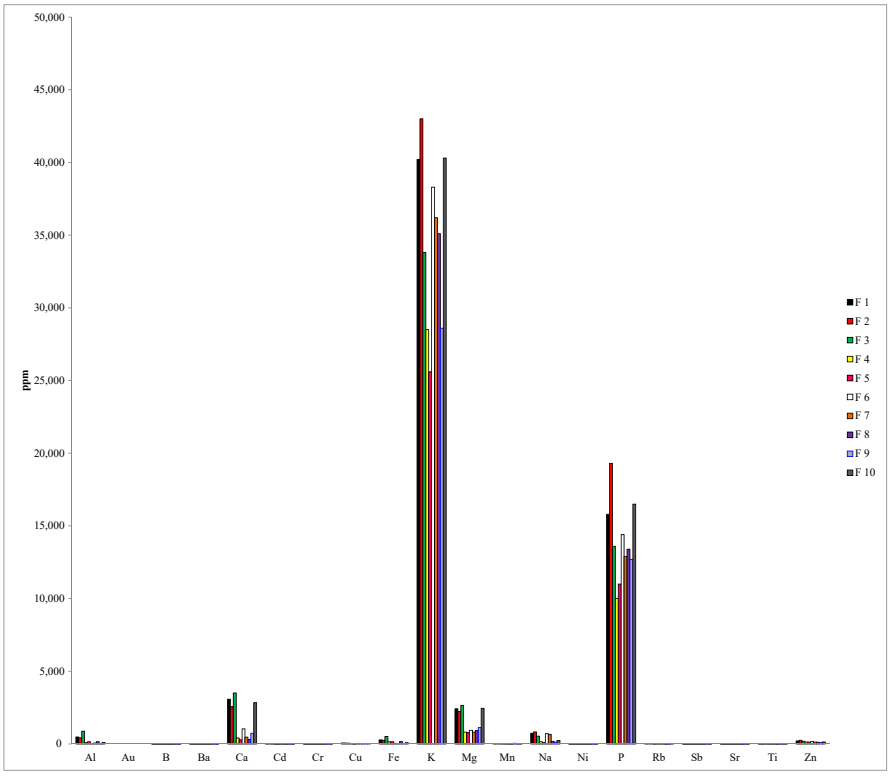

Fig. 2 Major elements content in mushroom samples (F1–F10).

Minor and trace elements (26 in total) ranged from 0.001 ppm (U) to 1.26 ppm (V) (Fig. 3) in the following order of abundance: V > Mo > Pb > Ce > Cs > Zr > Ag > Li > Se > Y > Co > La > As > Nd > Ga > Tl > Hg > Nb > Th > Pr > Sm > Gd > Ge > Yb > Te > U.

Seventeen other elements were detected under the limits of this detection method (see Appendix S1).

Overall, the concentrations of the major elements showed a very similar distribution across the mushroom samples (Fig. 2); however, in the cases of minor and traces elements (Fig. 3), different patterns were observed in the collected mushrooms. For instance, in F3, we found a higher concentration of Pb, V, Zr, and Ce than that in the other samples.

When comparing the concentrations of elements in the mushroom samples (F1–F10, see Appendix S1) with those in the carbonate rocks, according to the selected literature datasets, flyschoid rocks + clays, Earth's crust and topsoil of Sicily, Italy, Europe, and the rest of the world (Appendix S2), we could not conclude whether some elements

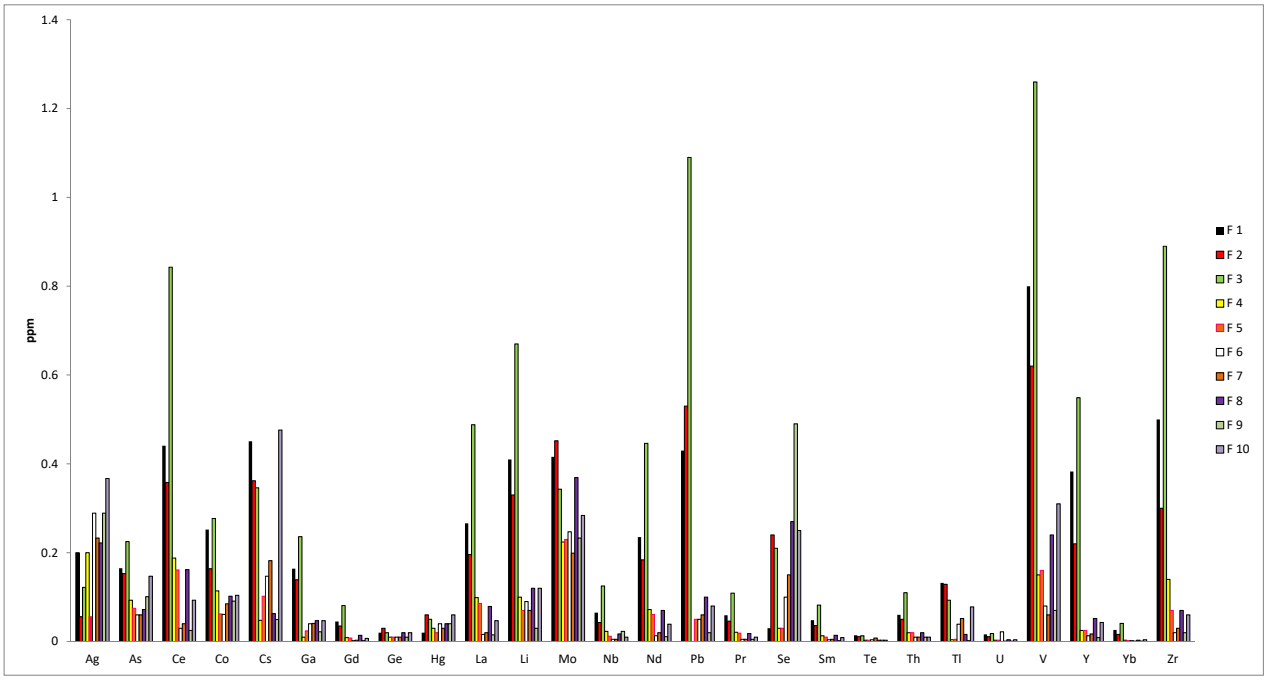

Fig. 3 Minor and trace elements in the mushroom samples (F1–F10). Elements very close or under the detection limit were not included in this graph.

are accumulated better than the others, because the concentrations of such elements in the analyzed mushroom samples were below the detection limits. Some other elements show an a.f. that can be clearly correlated to the average value of those elements detected in their corresponding bedrocks and topsoil (Fig. 4). Particularly, an accumulation of the following six elements was detected in the sampled mushrooms: Ag, Cd, Cu, K, P, and Zn (see Fig. 4). Appendix S3 represents the a.f. for each mushroom sample and the reference soil type.

Results of the CA in Fig. 5 show that the samples of morels (F1–F10) and the rock/soil types used for comparison (carbonate rocks, Earth's crust and topsoil from Sicily, Italy, Europe, and the rest of the world) form two distinctive clusters and the mushroom samples are placed into clusters on the basis of the geographic and geologic site of collection.

Discussion

Based on the results on samples of *Morchella* spp. obtained in this study, we can confirm that fungi can accumulate different elements from their substrata (especially soil) of the areas where they grow. The content of elements in the morels used for this study consisted of high concentrations of K and P and a wide pattern of minor and trace elements (e.g., V, Mo, Pb, Ce, Cs, Zr), including heavy metals.

Moreover, when the content of elements in the studied specimens was compared with the average concentration values in the bedrock and soil types (see Appendix S2), it was observed that the morels had accumulated six elements in particular which included the ones found abundantly in the Earth's crust (K, P, and Zn; Fig. 4) and heavy metals such as Ag, Cd, and Cu. These results confirm that fungi are characterized by the presence of high concentrations of K in their structures, which are comparable with those found in some agricultural crops, plants, and vegetables such as spinach and potatoes [6], as well as the presence of (heavy) metals like Cd and Pb. It is worth noting that the content of Cd in the soil is mainly influenced by the native rock (e.g., high in carbonate rocks) and is particularly abundant in anthropized soil. Moreover, the concentrations of Cd and Pb in the analyzed mushrooms were below the threshold limits for wild mushrooms specified by the European directives [51,52].

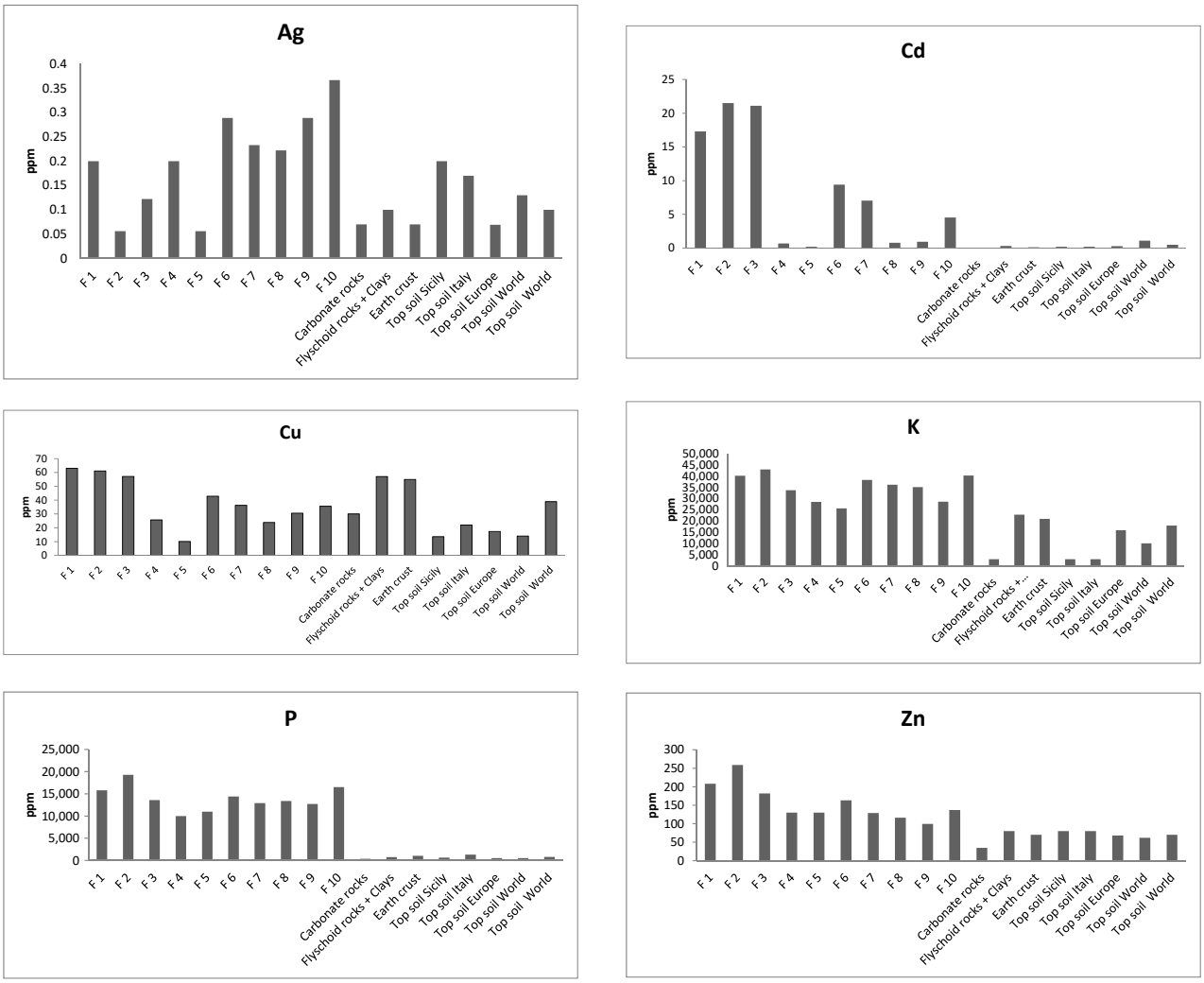

Fig. 4 Content of Ag, Cd, Cu, K, P, and Zn in mushroom samples (F1–10) and the rocks and soil types.

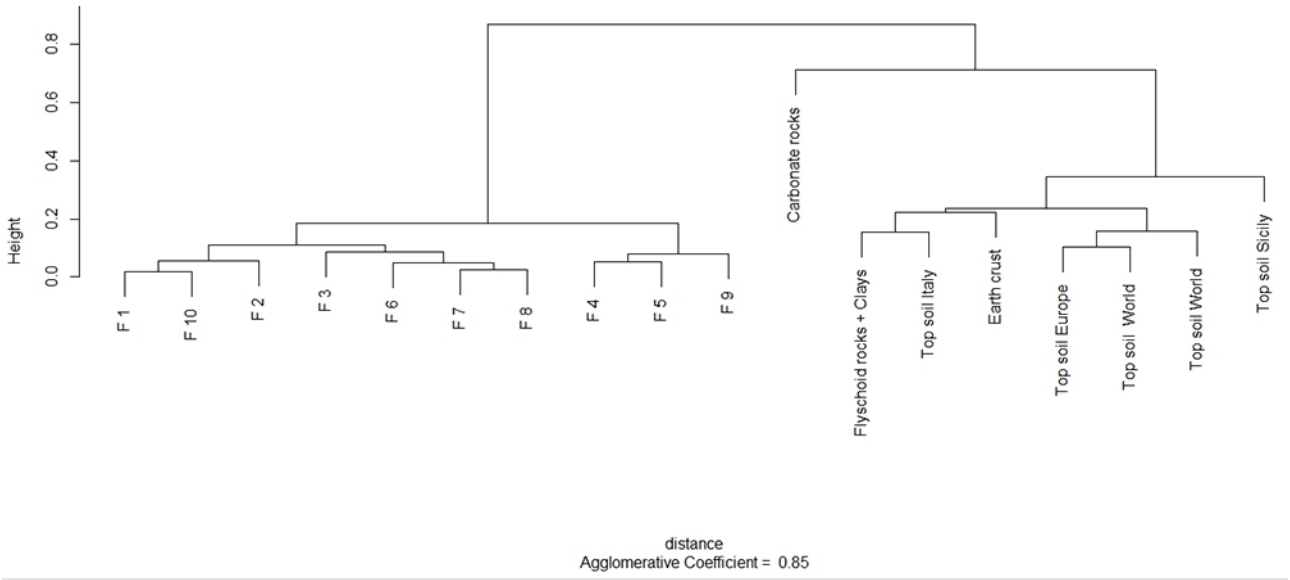

Fig. 5 Cluster dendrogram of matrix distance of the mushroom samples and soil types (carbonate rocks, Earth's crust and topsoil from Sicily, Italy, Europe, and the rest of the world) based on the content of their elements.

Statistical analysis showed that the mushroom samples (F1–F10) were clustered on the basis of the soil type. Samples F1–F3 and F6–10 (except F9) were collected from the carbonate rock and formed a separate group (viz. sister group) from samples F4–F5, which were collected from sites with a substratum developed on flyschoid rocks. Similarly, the second cluster (see right side in Fig. 5) was established by bedrock and soil types (e.g., topsoil, Earth's crust, carbonate and flyschoid rocks) and samples therein formed sister groups on the basis of their geological differences. Based on these results, we can observe that both site geology and geochemistry can influence the chemical composition in wild mushrooms.

It is challenging to compare our results with those of others because, till date, very few studies are available that have analyzed all the elements in morels and evaluated the influence of bedrock or soil geochemistry in this fungal group. For instance, Cenci et al. [53] studied heavy metals in samples of some morels collected from Central Italy (in the Emilia-Romagna region) and found a higher content of Hg (0.28–2.7 mg/kg) than that observed in the Sicilian morels of our study (Hg = 0.05–0.33 mg/kg). Conversely, the content of Cd detected in our samples (0.18–21.5 mg/kg) was higher than that in the morels of Central Italy (Cd = 0.19–4.12 mg/kg [53]), or in some *Morchella* species found in Turkey (Cd = 0.036–1.43 mg/kg [54]).

Despite a differences in the geological sites, the Cu content in our samples (10.6–63 mg/kg) was very similar to that detected from the morels of Central Italy (43.65–63.39 mg/kg), whereas the content of Ag in the Sicilian samples (0.05–0.22 mg/kg) was lower than that found by Cocchi et al. [9] (Ag = 0.28–2.7 mg/kg).

We were also interested in understanding whether different species of edible wild mushrooms collected from similar geological sites would exhibit differences in the accumulation of elements. We compared our dataset with that of Venturella et al. [4] and Alaimo et al. [6], including information on the mineral contents of some bolets [e.g., *Boletus aereus* Bull., *B. reticulates* Schaff., *B. impolitus* Fr., *B. lupinus* Fr., *B. queletii*, *B. rhodoxanthus* (Krombh.) Kallenb., *B. satanas* Lenz, and *Leccinum lepidum* (H. Bouchet ex Essette) Bresinsky & Manfr. Binder] and *Clitopilus prunulus* P. Kumm collected in the sedimentary sites (flyschoid or calcareous substrates) of Sicily. Although all species had a high concentration of K and Na [4,6], the bioconcentration of the other elements appeared to be element- and species-depended. For example, Co, Cr, Fe, Mg, Mo, Pb, U, and V was four folds higher in *C. prunulus* than that detected in the bolets analyzed from similar geological sites.

To summarize, the results obtained in this study confirmed that fungi (here, the *Morchella* group) accumulate all kinds of elements (including heavy metals) and that the bedrock or soil geochemistry can influence patterns of their minerals. The chemical content of elements in our morel samples was characterized by high concentrations of K and P. Additionally, a wide number of minor and trace elements, including heavy metals, were accumulated in the fruiting bodies.

A comparison of our results with those of other studies demonstrated that the same fungal species (in this case, *Morchella* spp.), collected from different geological and geographic sites was characterized by a differing content of minor and trace elements in their bodies. Conversely, different fungal species collected in similar geological sites (in Sicily) exhibited different patterns of accumulation of the elements. This confirms that, in fungi, the bioconcentrations are species-dependent and site-dependent. In fact, different fungal species have shown different responses to metal pollution. On the other hand, the content of elements in the soil depends on both physical characteristics (e.g., the grain of soil) and the impact human activities [55,56].

Future studies should include samples from different geographical/geological sites and other species of edible mushrooms. With growth in the consumption and demand of edible wild mushrooms, the analysis of mineral content in both mushrooms and soils can be an important tool in identifying the possible risks to human health.

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Supplementary material

The following supplementary material for this article is available at http://pbsociety.org.pl/journals/index.php/am/rt/suppFiles/am.1122/0:

Appendix S1 List of elements detected in each mushroom sample (F1–F10).

Appendix S2 Element content in carbonate rocks, Earth's crust and topsoil from Sicily, Italy, Europe, and the rest of the world.

Appendix S3 List of the mushroom samples (F1–F10) with the accumulation factor based on the types of bedrocks and soils (soil and rock from Sicily, Italy, Europe, and the rest of the world) were considered.

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