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EA conducted the fieldwork, did the species identification, analyzed the data, and wrote the manuscript; GMNB contributed to the statistical analysis; PM and MB performed the chemical analysis; GC and MZ contributed to the fieldwork; MGM coordinated the PhD project; EA, PM, and MZ contributed equally to the study plan

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ORIGINAL RESEARCH PAPER

Can the soil geology and chemistry analysis of a site predict the geographic origin of wild edible mushrooms (Porcini group)?

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Abstract

This study aimed to assess the element content of Porcini mushrooms collected from broadleaf Mediterranean forests (NW Italy) and underlying soil layers, and to elucidate the chemical connection between the mushrooms and their geographic site of origin. Comparing the elements in mushrooms with those in soil samples, we observed that the concentration of some microelements detected in mushrooms had similar distribution as that measured in both the soil layers assessed, especially with surface soil. Statistical analyses showed that the microelement pattern in mushrooms reflects the soil site of origin. Moreover, by comparing our results with other studies, we observed that the soil where Porcini grow is characterized by a high concentration of zinc. Some toxic elements were also detected in mushroom samples. Analysis of elements in mushrooms and soil layers can be used for quality assurance of natural products and help distinguish them from uncertified and unknown-origin products.

Keywords

wild edible mushrooms; *Boletus edulis* group; traceability; soil element content; mushroom safety

Introduction

It is well known that mushrooms are able to accumulate diverse chemical elements from the environment (e.g., air, water, and soil) or substrates (e.g., wood) where they grow [1–4]. Although accumulation capability and the presence of chemical elements in sporocarps [5] varies according to nutritional requirements and fungal genotypes, several authors have shown that the element content of mushrooms is mainly influenced by the chemical composition of the growing substratum, specifically the soil [6,7]. Despite these results, the majority of studies only analyze the presence of heavy metals inside a few fungal species from both polluted and unpolluted sites [4–8]. To the best of our knowledge, only few studies have analyzed the influence of geology, soil-mineralogy, and soil-chemistry on the element content in sporocarps, as well as the correlation between chemical elements in mushrooms and in the underlying soil layers [9–11].

Wild edible mushrooms are the most valuable nonwood forest products in the world [12,13]. Currently, more than 2,000 fungal species are known to produce edible sporocarps, which are harvested, consumed, and marketed in more than 85 countries [14,15]. Current estimations assign edible mushrooms a global market value that is at least $2 billion higher than that of timber products [16–20]. In particular, "Porcini" (*Boletus edulis* s. s., a group that also includes the species *B. aereus* Bull., *B. pinophilus* Pilát & Dermek, and *B. reticulatus* Schaeff) are among the most valuable and widely-consumed groups of wild mushrooms worldwide. From 20,000 to 100,000 metric tons of Porcini are estimated to be consumed annually, at fresh-mushroom prices that varied from $60 to $200/kg in 2009 [21,22].

Over the last few decades, consumer demand for specialty and high quality agri-food products has grown incredibly, and the market is greatly expanding [14–16]. For these reasons, the concept of traceability, in terms of detecting the origin and quality of products, has become an important criterion in the food sector [23–25]. Despite the existence of a legal system [26–28], there is a further need to distinguish between high- and low-quality produce and to develop new methods and technologies for food traceability. Identifying a product's geographic site of origin means that we can then identify possible risks to human health. Most fraudulent products do not satisfy food safety requirements, and they can profoundly damage confidence in typical food products.

Molecular analysis of proteins and fatty acid profiles as well as DNA barcoding have been widely used to identify the origin of food products [29–33]. Although molecular techniques are theoretically informative and precise, they involve high processing costs and do not allow us to clearly distinguish product authenticity, i.e., the geographical site of origin.

Different chemical and physical approaches, such as gas chromatography, mass spectrometry, NIR spectroscopy, and the analysis of trace elements and stable isotopes have been developed as alternatives to establish the geographical origin of agri-food products [34–38]. Several authors agree that soil elements can be favorably used as "signatures" of the geographic provenance of a sample [9,10,24]. For instance, Nikkarinen and Mertanen [9] analyzed the element content in a *Boletus edulis* group and *Lactarius trivialis* (Fr.) Fr., growing in two different geological regions in Finland, to determine whether geochemical fingerprints can be detected in mushrooms. They found that fungal samples differed considerably in trace content based on the geological and geographic site of origin. Because soil elements can be used as fingerprints of the geographic provenance of an organic sample, we analyzed the chemical content in mushrooms and soil layers to detect the origin, quality, and authenticity of the group of wild edible mushrooms known as Porcini.

The aims of this study was (***i***) to investigate the relationships between the element content of Porcini mushrooms and the underlying soil layers; (***ii***) to clarify the chemical connection between the mushrooms and their geographic site of origin; (***iii***) to assess the presence of toxic elements in the mushrooms; (***iv***) to identify possible soil-geochemical markers that can be used to trace Porcini mushrooms to their harvesting site; and (***v***) to chemically characterize the soil of selected Porcini sites.

Material and methods

Study area

This study was carried out in two sites, an oak wood and a beech forest, located in Liguria (province of Savona) in NW Italy (Fig. 1).

The first site (Site 1), near the province of La Maddalena (44°30′14″ N, 8°29′17″ E), covers an area of approximately 7,500 m^2 with an altitude ranging from 340 to 380 m a.s.l. The area is dominated by *Quercus cerris* L., and the site is classified as a high forest.

The second site (Site 2) is located near the province of Vereira (44°27′3″ N, 8°32′42″ E) and covers a total area of approximately 10,000 m^2. The altitude is 1,000 m a.s.l. The area is dominated by *Fagus sylvatica* L., and it is classified as a high forest.

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Fig. 1 Geographic location of the study sites.

Site 1 lies in a geologically complex area characterized by soils deposited on four different rocks: serpentine schists, calceschists, chlorite-actinolite schists, and conglomerates belonging to the Tertiary Piedmont Basin's stratigraphic succession. In contrast, Site 2 lies in a geologically homogeneous area characterized by soils deposited exclusively on calceschist rock.

The climate is temperate oceanic sub-Mediterranean for both sites [39]. The mean annual temperature is 12°C [from 0°C (min) in January to 25°C (max) in July)]. Mean annual rainfall is 912 mm [33 mm (min) in July; 122 mm (max) in October] [40].

Sample collection

In spring and fall 2014 and 2015, 20 samples of *Boletus reticulatus* Schaeff (Fig. 2), were collected from the two study sites. The mushroom samples are labeled in Tab. 1, Tab. 6, and Tab. 7 and Fig. 5–Fig. 7 with the identifiers "M1–M20". Species identification was carried out for both fresh and dried specimens by macro- and microscopic observations and a series of monographs and keys [41–43]. Nomenclature and author abbreviations were used in accordance with Hibbett et al. [44], Ima [45], and CBS [46]. Two soil samples (1 kg each) were collected beneath each mushroom: one soil sample from the surface layer (from 0 to 20 cm, labeled SL1–SL20), and the other from the deeper layer (from 20 cm to 40 cm, labeled DL1–DL20).

Fig. 2 Picture of *B. reticulatus* Schaeff.

To characterize each site from a geochemical and mineralogical perspective, we also randomly collected 10 soil samples (1 kg each) at each site, at a depth between 5 and 20 cm (labeled S1–S20). These soil samples were recorded in the center of 10 circular plots (4-m radius) selected along a transect line as proposed by Feest [47]. All soil samples were sieved in situ to remove particles >2 cm.

Chemical and physical analysis

The complete sporocarps (cap and stipe) and soil samples were brushed in the field, washed with distilled water, and dried at 60°C for 48 hours. They were then powdered, sieved and stored in hermetic plastic containers in the laboratory. Elements were detected by a field-portable energy dispersive X-ray fluorescence (FP-EDXRF) spectrometer (X-MET7500; Oxford Instruments). This is one of the simplest, most accurate, and most economic analytical tools to detect the chemical composition of many mineral and organic substrates [6,25,48–52]. A total of 5 g of each sample was exposed to X-rays and related element concentrations were expressed in ppm.

The pH was measured using the WTW Multiline P3 Set pH meter after equilibrating the soil fraction (<2 mm) in deionized water for 12–16 hours. The soil granulometry (% of clay, silt, and sand) was classified according to Folk [53].

Statistical analyses

For each mushroom and soil sample, descriptive statistics (min, max, mean, standard deviation) were calculated for element concentration, and differences in element content were displayed using boxplots.

Pearson's correlation (r) was used to test the hypothesis of linear independence between two variables. This coefficient indicates how well two data sets are interconnected (positively, negatively, or no connection) [54]. In this study, variables were represented by element concentration detected in the sporocarps and in both the soil portions (surface and deep layers).

A set of multivariate analyses was used to measure the degree of dissimilarity between mushrooms and soil samples. Specifically, the hierarchical cluster analysis (CA) (using the Bray–Curtis dissimilarity index and unweighted pair group method with arithmetic mean UPGMA) was performed to discern the geographic site of provenance of the samples using their degree of dissimilarity in chemical composition [54].

Principal component analysis (PCA) was used to reduce dimensionality and summarize all variables into a few principal components, which explain the greatest amount of variance in the data and can be visualized graphically. PCA was calculated in R using FactomineR and factoextra packages [54,55].

Finally, the indicator species analysis (ISA) technique was performed on the element concentration matrix in order to identify possible fungal and soil chemical markers [54,55].

Before performing statistical analyses, data were normalized using the formula $f(x)$ $x/sum(x)$.

Data analyses were performed using the R software environment for statistical computing and graphics version 3.5.1 [56].

Results

Element content in mushrooms and soil layers

Overall, we analyzed the elements in 80 samples: 20 sporocarps (10 recorded at Site 1 and 10 at Site 2) and underlying soil samples (20 from the surface layer and 20 from the deep soil). In addition, 20 soil samples were taken from a depth between 5 and 20 cm from the top of the soil, to perform a general geochemical characterization of the sites.

Chemical element concentrations measured in the *B. reticulatus* samples are summarized in Tab. 1. Full details on the elements detected in the mushroom samples (M1–M20) are provided in Appendix S1. Altogether, we detected the presence of 10 different elements: Ca, Ti, Mn, Fe, Cr, Ni, Cu, Zn, Sr, and Sb. In some samples, the concentration of certain elements (Ti, Fe, Sr) were below or very close to the detection limit [43 ppm, 10 ppm, and 1 ppm, respectively (Appendix S1)]. Conversely, high Zn concentration was found in most of the analyzed mushroom samples, specifically; 105 and 311 ppm were the maximum values detected at Site 1 and Site 2, respectively.

Tab. 1 Summary of the element content detected in 20 mushroom samples.

		Macroelements (wt %)				Microelements (ppm)					
		Ca	**Ti**	**Mn**	**Fe**	**Cr**	**Ni**	**Cu**	**Zn**	**Sr**	**Sb**
Site 1	Mean	0.128	0.004	0.007	0.022	40	55	48	81	1	60
	SD	0.061	0.000	0.008	0.032	9	60	17	18	1	20
	Min	0.061	0.004	0.002	0.001	30	5	24	40	1	13
	Max	0.269	0.004	0.025	0.095	58	209	73	105	3	84
Site 2	Mean	0.126	0.031	0.006	0.265	45	32	36	152	8	67
	SD	0.068	0.040	0.006	0.337	18	24	21	59	11	32
	Min	0.057	0.004	0.001	0.001	29	6	20	110	1	13
	Max	0.294	0.104	0.017	0.907	90	92	86	311	28	140

Mushroom samples are labeled M1–M20 in Appendix S1.

Element concentrations measured in soil samples (SL1–SL20 and DL1–DL20), collected beneath each mushroom sample, are summarized in Tab. 2 and Tab. 3, and detailed concentration values are listed in Appendix S2 and Appendix S3, respectively. Altogether, 26 elements were detected in both the soil layers. In some samples, levels of P, Mo, and Sb were below the detection limit, whereas the concentration of other elements such as Cr, Co, Ni, Cu, and Zn was very high in both surface and deep soil layers (Appendix S2 and Appendix S3).

Specifically, in the surface soil layer the concentration of Cr varied from a minimum value of 261 to a maximum of 683 ppm at Site 1, and from 595 to 1,129 ppm at Site 2. In contrast, Ni concentration varied from 308 to 554 ppm at Site 1, and from 385 to 932 ppm at Site 2. The presence of Zn was considerable and it also varied from 89 to 114 ppm at Site 1, and from 64 to 120 ppm at Site 2.

Similar to the surface layer, we detected a high concentration of Cr, Ni, and Zn in the deep soil layer as well. More precisely, in this soil portion, the content of Cr varied from 298 to 915 ppm at Site 1, and from 720 to 1,216 ppm at Site 2. The Ni concentration ranged from 516 to 915 ppm at Site 1, and from 413 to 959 ppm at Site 2. Finally, the content of Zn varied from a minimum value of 79 or 96 to a maximum of 115 ppm at Sites 1 and 2, respectively.

Soil mineralogy, lithology, and chemistry of the sites

In the soil samples collected at a depth between 5 and 20 cm, we identified the presence of 26 elements (Tab. 4), including a high concentration of some microelements (Cr, Ni, and Zr) (Appendix S4, S1–S20). More precisely, the highest range of Cr (75–1,871 ppm), Ni (257–1,371 ppm), and Zr (97–305 ppm) values were detected at Site 1. The distribution of macroelements was similar at both the sites.

The geology of Site 1 is different than that of Site 2 (Tab. 5). Site 1 is characterized by a high level of geodiversity: the parent rock consists of siliciclastic conglomerate, serpentine schist, and calceschist; whereas, the Site 2 is characterized by only calceschist.

The two sites showed variable pH values according to the parent rock (see caption in Appendix S4). The lowest pH value corresponds to siliciclastic conglomerate; whereas, the highest values were found on calceschist and serpentine schist. More precisely, Site 1 soil samples had pH values ranging from 4.27 to 5.83, whereas at Site 2, the pH varied from 4.20 to 4.90.

Finally, according to grain size analysis (Tab. 5), the soil texture was classified as gravelly sand to muddy gravel for Site 1, and sandy gravel to gravelly mud for Site 2.

Tab. 2 Summary of the element content detected in 20 soil samples from the surface layer (0 to 20 cm).

	Macroelements (wt %)									Microelements (ppm)																
	Mg	Al	Si	P	K	Ca	Ti	Mn	Fe	S	Cr	Co	Ni	Cu	Zn	Rb	Sr	Zr	Nb	Mo	Sn	Sb	Ba	Pb	Cd	V
Site 1																										
Mean	5.04	12.64	47.06	0.11	1.29	3.80	0.89	0.50	7.51	6,597	438	57	433	41	100	70	92	154	122	40	27	21	98	74	18	97
SD	0.64	1.32	4.88	0.11	0.32	0.75	0.16	0.25	1.28	4,569	161	19	78	5	9	13	5	35	58	41	10	8	8	16	7	52
Min	4.06	10.29	38.55	0.05	1.00	2.77	0.74	0.26	5.62	1,763	261	18	308	34	89	47	82	94	45	2	10	13	83	40	9	23
Max	5.78	14.69	55.26	0.41	1.83	4.61	1.10	0.84	9.38	14,274	683	80	554	50	114	88	100	196	213	95	36	30	111	103	27	141
Site 2																										
Mean	11.91	12.09	45.12	0.09	1.29	2.51	1.09	0.18	9.08	2,645	845	69	624	33	94	83	97	174	54	43	37	21	124	46	25	170
SD	4.72	1.82	2.93	0.10	0.46	0.65	0.23	0.03	0.76	2,035	170	16	177	4	17	26	10	35	39	53	7	10	52	26	3	54
Min	5.07	9.28	39.29	0.05	0.59	1.52	0.78	0.13	8.00	935	595	39	385	25	64	42	83	117	2	2	27	2	72	13	21	23
Max	20.25	15.55	50.46	0.34	2.08	3.98	1.58	0.22	10.56	7,778	1,129	102	932	40	120	125	115	238	111	105	46	32	239	85	31	211

Surface soil layer samples are labeled SL1–SL20 in Appendix S2.

Tab. 3 Summary of the element content detected in 20 soil samples from the deep layer (20–40 cm from the top of the soil).

	Macroelements (wt %)									Microelements (ppm)																
	Mg	Al	Si	P	K	Ca	Ti	Mn	Fe	S	Cr	Co	Ni	Cu	Zn	Rb	Sr	Zr	Nb	Mo	Sn	Sb	Ba	Pb	Cd	V
Site 1																										
Mean	7.65	14.32	49.80	0.05	1.08	2.42	1.08	0.17	9.84	1,407	605	64	702	42	86	58	81	186	53	40	41	21	121	52	32	138
SD	1.04	0.52	2.20	0.00	0.16	0.30	0.09	0.03	0.65	386	278	8	176	6	5	12	11	16	7	21	5	7	28	14	3	19
Min	6.78	13.30	45.31	0.05	0.79	2.08	0.98	0.13	8.93	827	298	54	516	35	79	40	60	149	35	2	32	13	79	31	28	104
Max	9.85	15.08	52.56	0.05	1.35	3.01	1.28	0.21	11.11	2,023	915	80	915	55	96	67	93	202	60	60	50	29	170	82	37	170
Site 2																										
Mean	11.75	13.19	46.51	0.08	1.33	2.04	0.99	0.15	10.03	1,930	940	75	663	33	94	72	93	147	48	42	38	20	152	53	28	162
SD	5.18	1.45	1.73	0.03	0.56	0.55	0.14	0.04	0.57	1,002	158	28	168	6	12	30	10	29	22	23	7	9	39	27	5	58
Min	6.01	11.25	43.32	0.05	0.05	1.41	0.75	0.10	8.92	1,129	720	3	413	23	79	25	78	101	0	2	22	13	89	18	17	23
Max	20.44	15.82	49.95	0.11	2.26	3.29	1.20	0.22	11.08	4,210	1,216	109	959	44	115	136	107	212	85	70	51	30	239	111	35	243

Deep soil samples are labeled DL1–DL20 in Appendix S3.

Tab. 4 Summary of the element content detected in 20 soil samples collected at depths between 5 and 20 cm from the top of the soil.

	Macroelements (wt %)								Microelements (ppm)																
	Si	Ti	Al	Fe	Mn	Mg	Ca	K	S	V	Cr	Co	Ni	Cu	Zn	Rb	Sr	Zr	Nb	Mo	Cd	Sn	Sb	Ba	Pb
Site 1																									
Mean	48.57	1.75	15.61	10.91	0.17	7.73	2.53	1.01	1,200	160	819	94	678	53	78	48	103	190	35	16	31	44	18	167	40
SD	4.46	0.53	2.09	2.42	0.09	2.96	0.96	0.29	190	39	627	49	303	23	10	15	35	62	20	20	4	7	16	26	9
Min	39.83	1.02	12.69	8.49	0.10	3.09	1.90	0.70	1,009	99	75	45	257	28	65	17	64	97	0	0	26	31	0	115	17
Max	54.57	2.86	19.87	15.83	0.40	13.20	5.15	1.62	1,591	223	1,871	219	1,371	103	93	69	186	305	57	40	39	53	35	215	49
Site 2																									
Mean	49.11	1.37	16.62	10.40	0.17	5.82	1.47	2.00	87	2,061	180	214	59	152	32	91	120	117	206	84	45	29	41	154	63
SD	4.17	0.25	2.09	2.02	0.14	2.07	0.43	0.71	3	942	24	57	19	85	14	25	42	20	47	37	40	6	12	38	19
Min	42.68	1.09	11.93	6.94	0.06	4.45	1.03	0.82	83	1,012	146	146	28	30	13	41	62	90	137	51	0	17	27	103	38
Max	58.47	1.84	18.94	12.78	0.43	11.43	2.15	3.19	94	4,502	213	323	86	309	59	117	202	155	275	175	131	37	58	239	97

These samples are labeled S1–S20 in Appendix S4.

Tab. 5 Physical and geological soil features of each site.

			Granulometry			
		pH	% clay (<2 mm)	% silt (2 mm–0.63 μm)	% sand (<63 μm)	Rock type
Site 1	Mean	5.23	23.14	26.67	50.19	Serpentineschists Calceschists Chlorite-actinolite schists Conglomerates
	SD	0.52	6.90	5.86	10.46	
	Min	4.27	15.13	16.31	29.94	
	Max	5.83	35.64	35.07	68.56	
Site 2	Mean	4.62	50.08	32.28	17.65	Calceschists
	SD	0.22	15.99	10.19	14.03	
	Min	4.20	23.34	19.49	4.88	
	Max	4.90	75.62	45.32	55.34	

Chemical correlation among mushrooms and soil layers

In order to observe the differences between the element concentration detected in sporocarps and the underlying soil layers, we used a graph based on descriptive statistical measurements (see "Material and methods"). Fig. 3 and Fig. 4 display the results of the macro- and microelement content detected in mushrooms and soil samples from Sites 1 and 2, respectively.

In both sites, the mushroom content differed from the soil samples for some macroelements (Fe, Ca) (Fig. 3). Regarding microelement content, the concentration of Zn, Cu, Sr, and Sb did not differ between the mushrooms and the soil layers (Fig. 4). Conversely, the content of Cr and Ni was lower in the mushrooms than in the soil portions, where we found a high concentration of these elements.

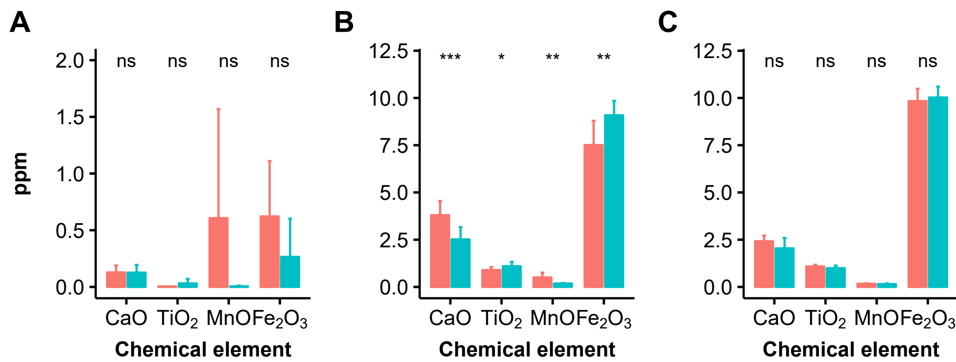

Fig. 3 Boxplot of macroelement content in mushrooms (**A**), surface soil layer (**B**), and deep soil layer (**C**) collected at Site 1 (red) and Site 2 (blue). The element concentration is expressed in ppm. Statistical significance: **** α = 0.001; *** α = 0.01; ** α = 0.05; * α = 0.1; ns – not significant values.

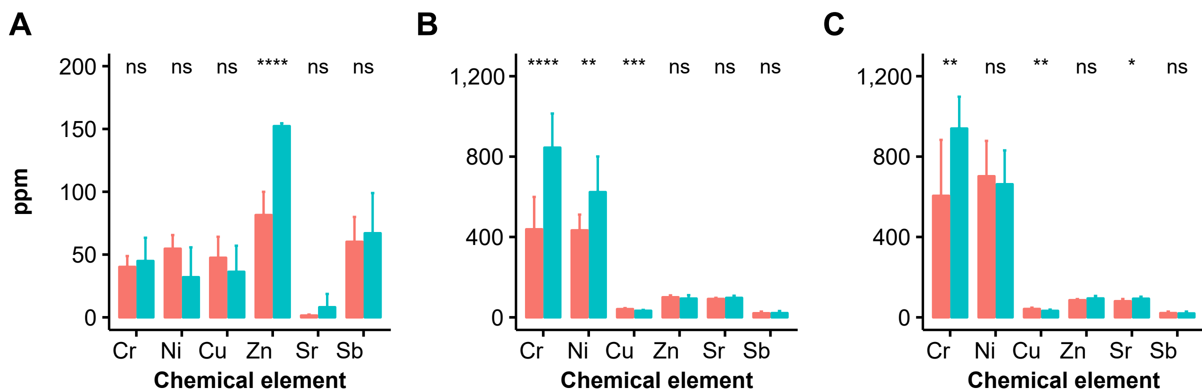

Fig. 4 Boxplot of microelement content in mushrooms (**A**), surface soil layer (**B**), and deep soil layer (**C**) collected at Site 1 (red) and Site 2 (blue). Element concentration is expressed in ppm. Statistical significance: **** α = 0.001; *** α = 0.01; ** α = 0.05; * α = 0.1; ns – not significant values.

The degree of correlation among the element contents detected in each sample level was also calculated by Pearson's coefficient. Overall, the highest correlation values were observed among the soil layers for the majority of the detected elements at both sites. For example, a strong positive correlation value ($r = 0.91$) was obtained for Cr and Zn detected in the soil layers in both Sites 1 and 2, respectively (Tab. 6).

The element content measured in the mushrooms, however, did not show strong positive correlation values compared to those detected in the underground soil samples. The highest positive correlation value in this group ($r = 0.57$) was observed between the Mn content quantified in the mushrooms and in the surface soil layer (Tab. 6).

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Tab. 6 Pearson correlation values (*r*) among the mushroom samples (M) versus the soil layers (surface soil – SL and deep soil – DL).

	Samples code	Elements									
		Ca	Ti	Mn	Fe	Cr	Ni	Cu	Zn	Sr	Sb
Total	M vs. SL	0.10	0.14	**0.40**	0.23	0.28	−0.34	−0.03	−0.47	−0.24	0.16
	M vs. DL	0.06	−0.55	−0.33	−0.30	0.04	−0.15	0.00	0.07	−0.09	0.36
	SL vs. DL	**0.48**	0.29	0.34	0.29	**0.87**	0.11	**0.73**	**0.65**	**0.45**	−0.19
Site 1	M vs. SL	0.35	0.00	**0.57**	−0.58	0.03	−0.30	−0.68	0.03	0.12	0.04
	M vs. DL	−0.21	0.00	−0.25	−0.47	−0.13	−0.10	−0.28	−0.28	−0.30	**0.56**
	SL vs. DL	−0.44	**0.79**	0.34	0.04	**0.91**	−0.71	**0.68**	**0.69**	−0.24	−0.04
Site 2	M vs. SL	−0.11	−0.11	−0.44	−0.08	0.37	−0.39	−0.08	−0.52	−0.51	0.22
	M vs. DL	0.18	−0.55	−0.52	−0.60	−0.08	−0.49	−0.24	−0.32	−0.64	0.29
	SL vs. DL	**0.79**	**0.50**	**0.78**	**0.64**	**0.68**	0.68	0.33	**0.91**	**0.71**	−0.28

Positive moderate ($0.40 < r < 0.59$), strong ($0.60 < r < 0.79$), and very strong ($0.80 < r < 1$) correlation values are indicated in bold.

Multivariate analyses

A set of multivariate analyses was used to measure the degree of dissimilarity among the mushroom and soil layer samples. In detail, the result of cluster analysis (Fig. 5, Fig. 6) showed that the mushrooms and soil samples do not form clearly distinctive clusters based on origin site with regard to macroelements (Fig. 5). Instead, microelement concentrations define separate groups based on geographic site of growth (Fig. 6).

The PCA analysis showed that two axes explained 76.1% of the total data variance (Fig. 7). Specifically, when considering all the samples together, the different concentration of some macro- (Ca, Fe, Ti) and microelements (Sb, Zn, Cu, Cr, Ni) separate the samples by spatial distribution (M vs. DL and SL).

The ISA results (Tab. 7) emphasize that some elements had a significant indicator value (IV). Specifically, we found that some macroelements, such as Fe, Sr, and Mn, were significant for mushroom samples, whereas Ca and Ti were significant elements for the deep soil layer. For both the surface and deep soil layers, Cr was a common significant element.

Discussion

Our results supported the hypothesis that the soil lithology, mineralogy, and chemistry of a site can influence the element content in Porcini (here *Boletus reticulatus*). We showed that at both study sites, most elements detected in the sporocarps almost completely reflected the content detected in the underlying soil layers (Fig. 3, Fig. 4). In more detail, we showed that the concentration of some microelements (Cu, Zn, Sr, Sb) detected in the mushrooms is very similar to that measured in both the soil layers (Fig. 4). Conversely, some other elements (Cr and Ni) showed a different distribution.

The distribution of macroelements appeared to be variable among the mushrooms and the underlying soil samples, especially that of Ca and Fe. We surmised that the variation in the macroelement content is due to the wide distribution that these elements have in soil.

The correlation values obtained (Tab. 5) also emphasized the above-described element distribution. The highest correlation values were observed between the soil portions, SL and DL (Tab. 5), rather than the same elements compared between either soil layer and the mushroom samples. Moreover, the strongest Pearson's values ($0.61 < r < 0.91$) were obtained by performing the correlation between the element content in the surface layer and deep soil, confirming that some microelements have low mobility between soil layers.

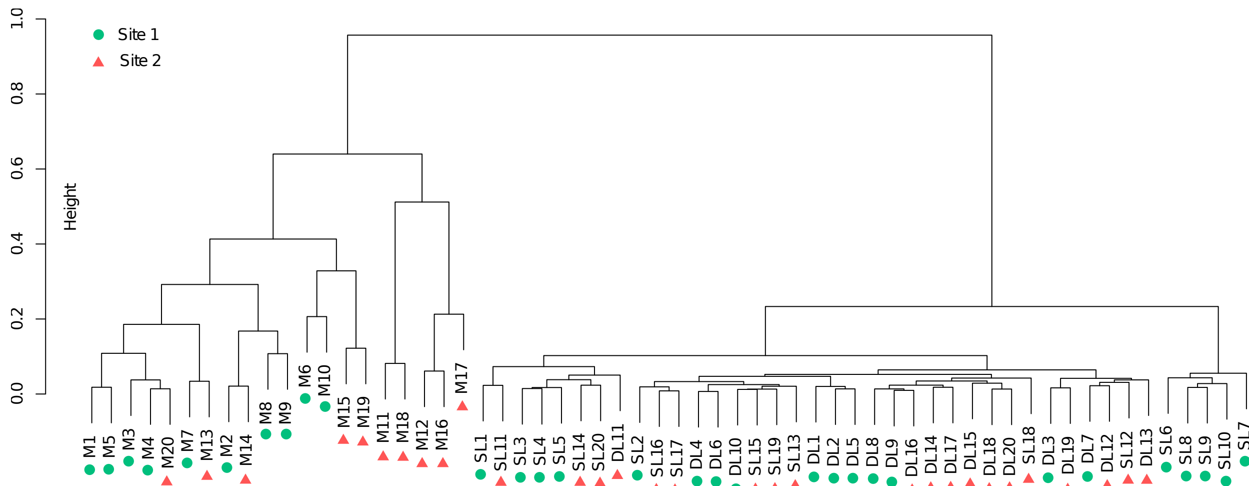

Fig. 5 Cluster dendrogram of the samples (mushrooms, surface soil layer, and deep soil) based on their macroelement content. Designations of M1–20, SL1–20, and DL1–20 indicate the mushrooms (M), surface soil layer (SL), and deep soil samples (DL), respectively.

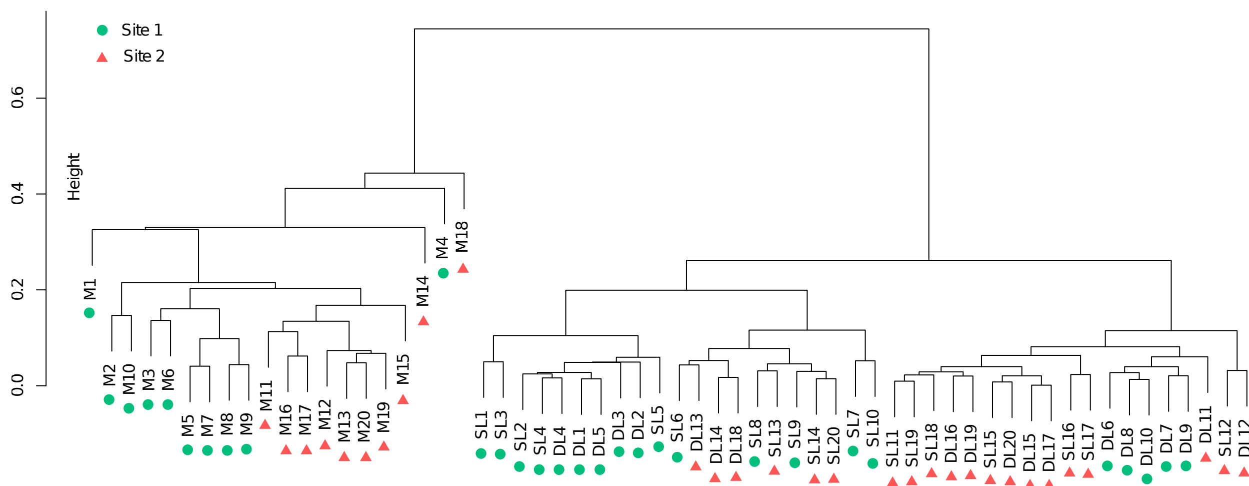

Fig. 6 Cluster dendrogram of the samples (mushrooms, surface soil layer, and deep soil) based on microelement content. Designations of M1–20, SL1–20, and DL1–20 indicate the mushrooms (M), surface soil layer (SL), and deep soil samples (DL), respectively.

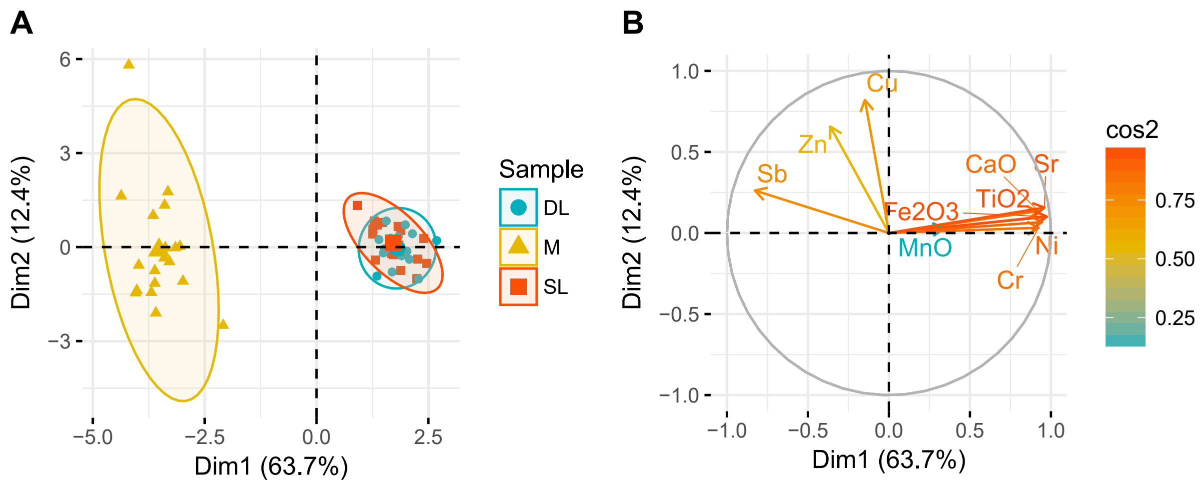

Fig. 7 Graphical representation of principal component analysis. (**A**) Ordination graph of the first two principal components. Ellipses are drawn around the 95% confidence interval for each sample group centroid. (**B**) Correlation circle between a variable and a principal component (PC). The cos2 value indicates the quality of representation of the variable on the principal component. M – mushroom samples; SL – surface soil samples; DL – deep soil samples.

Tab. 7 Summary of the ISA technique.

Element	Sample	IV	*p*
Ca	DL	0.746	0.03*
Ti	DL	0.730	0.03*
Fe	M	0.997	0.010**
Sr	M	0.926	0.020*
Mn	M	0.890	0.040*
Cr	DL	0.853	0.005**
Cr	SL	0.848	0.005**
Ni	DL	0.759	0.015*

M, SL, and DL indicate mushrooms, surface soil layer, and deep soil samples, respectively. IV – indicator value. Significance: *** $p = 0$; ** $p = 0.001$; * $p = 0.01$.

The degree of chemical dissimilarity among the samples is also shown by the cluster analysis (CA) result (Fig. 5, Fig. 6). Both dendrograms indicate that mushroom samples form a distinctive group apart from the soil layers. This confirms that our collected mushrooms had a different chemical content for some elements (Ca, Fe, Ni, and Cr), than that of the soil layers. The analyzed mushrooms have accumulated, in fact, a minority of elements (10 of the 26 analyzed) from the soil. Moreover, the CA results also displayed a high degree of similarity between the surface and deep soil samples, establishing a unique group in the cluster dendrograms.

The result of CA also showed that considering the microelement concentration, mushroom samples (M1–M10 and M11–M20) as well as soil samples established distinctive groups on the basis of their geographical sites of origin (Fig. 6). This result supported our hypothesis that soil geology of origin influences the chemical composition of wild edible mushrooms, and suggested that some microelements can be used as fingerprints to indicate the geographic provenance of a sample.

Additionally, the PCA result confirmed that when considering the whole chemical element pattern, the mushrooms samples formed a distinctive group (Fig. 7A) because of their higher content of Sb, Zn, and Cu than the soil layers (Fig. 7B).

The chemical analysis performed on our mushrooms and soil samples also revealed the presence of some toxic elements in the two sites: Zn, Ni, Cr, Co, and Cu. However, it is important to highlight that the high concentrations of some heavy metals (e.g., Cr) in the two Ligurian sites is due to natural geological background factors (in this case, the presence of serpentine schist parent rocks) of this geographic area, rather than an anthropogenic source of pollution. The concentration of these elements is in fact very common, and high in soil developed on ophiolitic and ultrabasic rocks [57–59]. The presence of toxic elements in mushrooms confirms the mushrooms' ability to take up heavy metals from the growing substratum [1–4,7]. However, it should also be emphasized that the content of Cr detected in the sporocarps samples did not exceeded the limits of the law, in which tolerable intake values for heavy metals are set by regulatory agencies [14,15,60].

Comparing our results with those from different studies is quite difficult, since very few studies fully describe the chemical content of wild edible mushrooms, especially Porcini, as well as the geology of their sites of growth/origin. Based on the available literature, interesting aspects emerge from the comparison of our results with those obtained by Nonnis Marzano et al. [10]. These authors analyzed trace element concentration in some Boletus species recorded in Central Italy. Despite a different geology in their studied area, concentrations of Zn in both the mushrooms and the soil samples were very similar to our results. Also, Giannaccini et al. [61] analyzed the content of microelements in the (top)soil and in some edible mushrooms (viz. *B. edulis* and *Macrolepiota procera*), growing on sedimentary-clastic rocks (prevalently limestone), in Central Italy (Tuscany). Specifically, this group found a level of Zn similar to our results in both *B. edulis* and soil samples. Based on these similarities, it may be supposed that some specific Italian sites favorable for the growth of Porcini are characterized by similar levels of Zn ([Zn] > 100 ppm ca. in the mushrooms and [Zn] < 100 ppm ca. in the soil), despite differing in soil geology (for parent rock). In contrast, as variables influenced by natural geological background factors, the Cr, Ni, and Sb content detected in our sites (and mushroom samples) was higher compared to the other Italian areas [7,10,61]. Moreover, in another recent study on the element content of *Boletus aereus* from volcanic areas in South Italy (Sicily), the authors found a high concentration of Z, Zn, Cu, Se, and Ti in the analyzed fungal samples [62].

Despite some evidence that emerged from comparing our results with those of Nonnis Marzano et al. [10], characterization of the soil chemistry of naturally-growing Porcini habitats is quite difficult to perform. Based on these gaps in knowledge, we performed statistical analysis (Tab. 7) to detect possible soil and mushroom chemical

indicators. Based on our results, some elements (Ca, Ti, Mn, Sr, and Cr), had a significant value, which indicated that they may be considered potential soil and mushroom geomarkers.

In conclusion, the results obtained by our study highlight that mushrooms and soil samples, with different substrata of origin, differ considerably in their chemical components, especially with regard to microelements. This finding leads us to recommend that geological and chemical soil information should be included in food traceability.

Based on our results, the application of this method could be the basis for performing quality assurance on natural products. The study of chemical content in the mushrooms and in the soil layers can protect genuine products and distinguish them from uncertified and unknown-origin products.

Since the application of geochemical approaches in the mycological field has not been widely adopted, future efforts should include more extensive sampling to implement this method in protecting human health and food safety.

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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.1130/0:

Appendix S1 Element content detected in the mushroom samples.

Appendix S2 Element content in surface soil layer (0–20 cm) samples (SL1–SL20).

Appendix S3 Element content in deep soil layer (20–40 cm) samples (DL1–DL20).

Appendix S4 Element content in soil layer (5–20 cm) samples (S1–S20).

References

1. Demirbas A. Accumulation of heavy metals in some edible mushrooms from Turkey. Food Chem. 2000;68:415–419. https://doi.org/10.1016/S0308-8146(99)00210-1
2. Svoboda L, Havlíčková B, Kalač P. Contents of cadmium, mercury and lead in edible mushrooms growing in a historical silver-mining area. Food Chem. 2006;96:580–585. https://doi.org/10.1016/j.foodchem.2005.03.012
3. Yamac M, Yildiz D, Sarikurkcu C, Celikkollu M, Solak MH. Heavy metals in some edible mushrooms from the Central Anatolia, Turkey. Food Chem. 2007;103(2):263–267. https://doi.org/10.1016/j.foodchem.2006.07.041
4. Zhang D, Gao T, Ma P, Luo Y, Su P. Bioaccumulation of heavy metal in wild growing mushrooms from Liangshan Yi Nationality Autonomous Prefecture, China. Wuhan University Journal of Natural Sciences. 2008;13(3):267–272. https://doi.org/10.1007/s11859-008-0302-2
5. Kirk PM, Cannon PF, Minter DW, Stalpers JA. Dictionary of the Fungi. 10th ed. Wallingford: CAB International; 2008
6. Campos J, Tajera NA, Sanches CJ. Substrate role in the accumulation of heavy metals in sporocarps of wild fungi. Biometals. 2006;22:835–841. https://doi.org/10.1007/s10534-009-9230-7
7. Cocchi L, Vescovi L, Petrini LE, Petrini O. Heavy metals in edible mushrooms in Italy. Food Chem. 2006;98:277–284. https://doi.org/10.1016/j.foodchem.2005.05.068
8. Garcia M, Alonso J, Melgar MJ. *Agaricus macrosporum* as potential bioremediation agents for substrates contaminated with heavy metals. Journal of Chemical Technology and Biotechnology. 2005;80:325–330. https://doi.org/10.1002/jctb.1203
9. Nikkarinen M, Mertanen E. Impact of geological origin on traces element composition of edible mushrooms. J Food Compost Anal. 2004;17:301–310.

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https://doi.org/10.1016/j.jfca.2004.03.013

10. Nonnis Marzano F, Bracchi PG, Pizzetti P. Radioactive and conventional pollutants accumulated by edible mushrooms (*Boletus* sp.) are useful indicators of species origin. Environ Res. 2001;85:260–264. https://doi.org/10.1006/enrs.2001.4233
11. Alaimo MG, Saitta A, Ambrosio E. Bedrock and soil geochemistry influence the content of chemical elements in wild edible mushrooms (*Morchella* group) from South Italy (Sicily). Acta Mycol. 2019;54(1):1122. https://doi.org/10.5586/am.1122
12. Pettenella D, Kloehn S. Mediterranean mushrooms: how to market them. In: Berrahmouni N, Escuté X, Regato P, Stein C, editors. Beyond cork – a wealth of resource for people and nature. Madrit: WWF Mediterranean; 2007. p. 52–65.
13. Sardeshpande M, Shackleton C. Wild edible fruits: a systematic review of an under-researched multifunctional NTFP (non-timber forest product). Forests. 2019;10:467. https://doi.org/10.3390/f10060467
14. Ambrosio E. Analysis and characterization of *Boletus edulis* group: an ecological and economic resource [PhD thesis]. Genoa: University of Genoa; 2015.
15. Sitta N, Davoli P. Edible ectomycorrhizal mushrooms: international markets and regulations. In: Zambonelli A, Bonito G, editors. Edible ectomycorrhizal mushrooms. Current knowledge and future prospective. Berlin: Springer; 2012. p. 355–380. (Soil Biology; vol 34). https://doi.org/10.1007/978-3-642-33823-6_20
16. Boa E. Wild edible fungi: a global overview of their use and importance to people. Rome: Food and Agricultural Organization of the United Nations (FAO); 2004.
17. Cai M, Pettenella D, Vidale E. Income generation from wild mushrooms in marginal rural areas. For Policy Econ. 2011;13:221–226. https://doi.org/10.1016/j.forpol.2010.10.001
18. Ortega-Martinez P, Martinez-Pena F. A sampling method for estimating sporocarp production of wild edible mushroom of social and economic interest. Investigacion Agraria: Sistemas y Recursos Forestales. 2008;17(3):228–237. https://doi.org/10.5424/srf/2008173-01037
19. de La Verga H, Agueda B, Agreda T, Martinez-Pena F, Parlade J, Pera J. Seasonal dynamics of *Boletus edulis* and *Lactarius deliciosus* extraradical mycelium in pine forests of central Spain. Mycorrhiza. 2013;23:391–402. https://doi.org/10.1007/s00572-013-0481-3
20. Agreda T, Cisneros O, Agueda B, Fernandez-Toiran LM. Age class influence on the yield of edible fungi in a managed Mediterranean forest. Mycorrhiza. 2014;24:143–152. https://doi.org/10.1007/s00572-013-0522-y
21. Ambrosio E, Zotti M. Mycobiota of three *Boletus edulis* (and allied species) productive sites. Sydowia. 2015;67:197–216.
22. Dentinger BTM, Ammirati JF, Both EE, Desjardin DE, Halling RE, Henkel TW, et al. Molecular phylogenetic of Porcini mushroom (*Boletus* section *Boletus*). Mol Phylogenet Evol. 2010;57(3):1276–1292. https://doi.org/10.1016/j.ympev.2010.10.004
23. Opara L. Traceability in agriculture and food supply chain: a review ofbasic concepts, technological implications, and future prospects. J Food Agric Environ. 2003;1(1):101–106.
24. Schwägele F. Traceability from a European perspective. Meat Sci. 2005;71:164–173. https://doi.org/10.1016/j.meatsci.2005.03.002
25. Ambrosio E, Marescotti P, Mariotti MG, Cecchi G, Brancucci M, Zotti M. Food traceability: a novel approach for wild edible mushrooms. In: II International Plant Science Conference (IPSC) – 110° Congresso della Società Botanica Italiana (SBI); 2015 Sep 15–18; Pavia, Italy. [publication place unknown]: [publisher unknown]. p. xxvi.
26. Gazzetta ufficiale dell'Unione europea UE. Reg.931/2011 [Internet]. 2011 [cited 2019 Dec 23]. Available from: http://www.izs.it/bollettino_segn_legislative/bollettini_2011/settembre_11/9.alimenti.pdf
27. Gazzetta ufficiale dell'Unione europea UE. UE Reg. 209/2013 [Internet]. 2013 [cited 2019 Dec 23]. Available from: http://www.izsto.it/images/stories/Osservatorio/alimenti/reg_209_2013.pdf
28. Gazzetta ufficiale delle Comunità europee. UE Reg. 178/2002 [Internet]. 2012 [cited 2019 Dec 23]. Available from: https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:031:0001:0024:IT:PDF
29. Leonardi M, Paolocci F, Rubini A, Simonini G, Pacioni G. Assessment of inter- and intra-

specific variability in the main species of *Boletus edulis* complex by ITS analysis. FEMS Microbiol Lett. 2005;243:411–416. https://doi.org/10.1016/j.femsle.2005.01.003

30. Mello A, Ghignone S, Vizzini A, Secchi C, Ruiu P, Bonfante P. ITS primers for the identification of marketable boletes. J Biotechnol. 2006;121(3):318–329. https://doi.org/10.1016/j.jbiotec.2005.08.022
31. Pafundo S, Agrimonti C, Maestri E, Marmiroli N. Applicability of SCAR markers for food genomics: olive oil traceability. J Agric Food Chem. 2007;55(15):6052–6059. https://doi.org/10.1021/jf0701638
32. Rizzello R, Zampieri E, Vizzini A, Autino A, Cresti M, Bonfante P, et al. Authentication of prized white and black truffle in processed products using quantitative real-time PCR. Food Res Int. 2012;48(2):792–797. https://doi.org/10.1016/j.foodres.2012.06.019
33. Vita F, Lucarotti V, Alpi E, Balestrini R, Mello A, Bachi A, et al. Proteins from *Tuber magnatum* Pico fruiting bodies naturally growth in different areas of Italy. Proteome Sci. 2013;11:7. https://doi.org/10.1186/1477-5956-11-7
34. Anderson KA, Magnuso BA, Tschirgi ML, Smith B. Determining the geographic origin of potatoes with traces element analysis using statistical and neural network classifiers. J Agr Food Chem. 1999;47(4):1568–1574. https://doi.org/10.1021/jf980677u
35. Sharma A, Weindorf DC, Man T, Aldabaa A, Chakraborty S. Characterizing soils via portable X-ray fluorescence spectrometer: 3. Soil reaction (pH). Geoderma. 2014;232–234:141–147. https://doi.org/10.1016/j.geoderma.2014.05.005
36. Casale M, Bagnasco L, Zotti M, Di Piazza S, Sitta N, Olivieri P. A NIR spectroscopy-based approach to detect fraudulent additions within mixture of dried porcini mushrooms. Talanta. 2016;160(1):729–734. https://doi.org/10.1016/j.talanta.2016.08.004
37. Gioacchini AM, Menotta M, Guescini M, Saltarelli R, Ceccaroli P, Amicucci A, et al. Geographical traceability of Italian white truffle (*Tuber magnatum* Pico) by the analysis of volatile organic compounds. Rapid Communications in Mass Spectrometry. 2008;22(20):3147–3153. https://doi.org/10.1002/rcm.3714
38. Li Y, Zhang J, Li T, Liu H, Li H, Wang Y. Geographical traceability of wild *Boletus edulis* based data fusion of FT-MIR and ICP-AES coupled with data mining methods (SVM). Spectrochim Acta A. 2017;177:20–27. https://doi.org/10.1016/j.saa.2017.01.029
39. Rivas-Martinez S. Bioclimatic and biogeographic maps of Europe [Internet]. 2008 [cited 2019 May 3]. Available from: http://www.globalbioclimatics.org/form/maps.htm
40. climate-data.org [Internet] 2019 [cited 2019 May 3]. Available from: https://it.climate-data.org/
41. Courtecuisse R, Duhem B. Guide des Champignons de France et d'Europe. Lausanne: Delachaux et Niestlé; 1994.
42. Boccardo F, Traverso M, Vizzini A, Zotti M. Funghi d'Italia. Bologna: Zanichelli; 2008.
43. Muñoz AJ. *Boletus* s. l. Alassio: Edizione Candusso; 2005. (Fungi Europei; vol 2).
44. Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, et al. A higher-level phylogenetic classification of the Fungi. Mycol Res. 2007;111:509–547. https://doi.org/10.1016/j.mycres.2007.03.004
45. MycoBank [Internet]. 2019 [cited 2019 May 3]. Available from: http://www.mycobank.org/
46. CBS-KNAW Collections [Internet]. 2019 [cited 2019 May 3]. Available from: http://www.westerdijkinstitute.nl/Collections/DefaultInfo.aspx?Page=Home
47. Feest A. Establishing baseline indices for the quality of the biodiversity of restored habitats using a standardized sampling process. Restor Ecol. 2006;14(1):112–122. https://doi.org/10.1111/j.1526-100X.2006.00112.x
48. Brancucci G, Brancucci M, Marescotti P, Solimano M, Vagge I, Vegnuti R. The geodiversity of the Ligurian DOC vineyards and its relationships with the terroir. International Journal of Environmental Science and Development. 2017;8(9):686–690. https://doi.org/10.18178/ijesd.2017.8.9.1039
49. Bosco GL. Development and application of portable. hand-held X-ray fluorescence spectrometers. TrAC Trends in Analytical Chemistry. 2013;45:121–134. https://doi.org/10.1016/j.trac.2013.01.006
50. Weindorf DC, Zhu T, McDaniel P, Mitchell V, Lynn L, Michaelson G, et al. Characterizing soils via portable X-ray fluorescence spectrometer: 2 Spodic and Albic horizons. Geoderma. 2012;189–190:268–277.

https://doi.org/10.1016/j.geoderma.2012.06.034

51. Weindorf DC, Paulette L, Man T. In-situ assessment of metal contamination via portable X-ray fluorescence spectroscopy: Zlatna, Romania. Environ Pollut. 2013;182:92–100. https://doi.org/10.1016/j.envpol.2013.07.008
52. Weindorf DC, Bakr N, Zhu Y, Mcwhirt A, Ping CL, Michaelson G, et al. Influence of ice on soil elemental characterization via portable X-ray fluorescence Spectrometry. Pedosphere. 2014;24(1):1–12 https://doi.org/10.1016/S1002-0160(13)60076-4
53. Folk RL, Andrews PB, Lewis DW. Detrital sedimentary rock classification and nomenclature for use in New Zealand. New Zealand Journal of Geology and Geophysics. 1970;13(4):937–968. https://doi.org/10.1080/00288306.1970.10418211
54. Legendre P, Legendre L. Numerical ecology. 3rd ed. Amsterdam: Elsevier Science; 2012.
55. Dufrêne M, Legendre P. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr. 1997;67(3):345–366. https://doi.org/10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2
56. R Core Team. R: a language and environment for statistical computing. Version 3.5.1 [Software]. Vienna: R Foundation for Statistical Computing; 2018.
57. Marescotti P, Solimano M, Beccaris G, Scotti E, Crispini L, Poggi E, et al. La presenza naturale di metalli nei suoli: criticità operative e possibili soluzioni. ECO. 2014;29:60–63.
58. Venturelli G, Contini S, Bonazzi A. Weathering of ultramafic rocks and element mobility at Mt Prinzera Northern Apennines Italy. Mineral Mag. 1997;61(6):765–778. https://doi.org/10.1180/minmag.1997.061.409.02
59. Oze C, Fendorf S, Bird DK, Coleman RG. Chromium geochemistry of serpentine soils. Int Geol Rev. 2004;46(2):97–126. https://doi.org/10.2747/0020-6814.46.2.97
60. Decreto legislativo 3 aprile 2006, n. 152. Norme in materia ambientale [Internet]. 2013 [cited 2019 May 3]. Available from: http://www.isprambiente.gov.it/it/garante_aia_ilva/normativa/normativa-ambientale/Dlgs_152_06_TestoUnicoAmbientale.pdf
61. Giannaccini G, Betti L, Palego L, Mascia G, Schmid L, Lanza M, et al. The trace element content of top-soil and wild edible mushrooms samples collected in Tuscany Italy. Environ Monit Assess. 2015;184(2):7579–7595. https://doi.org/10.1007/s10661-012-2520-5
62. Alaimo MG, Dongarrà G, La Rosa A, Tamburo E, Vasquez G, Varrica D. Major and traces elements in *Boletus aereus* and *Clitopilus prunulus* growing on volcanic and sedimentary soils of Sicily (Italy). Ecotoxicol Environ Saf. 2018;157(15):182–190. https://doi.org/10.1016/j.ecoenv.2018.03.080