Journal of Radioanalytical and Nuclear Chemistry (2019) 320:475–483
https://doi.org/10.1007/s10967-019-06485-x

Elemental analysis of summer truffles *Tuber aestivum* from Germany

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Received: 4 February 2019 / Published online: 19 March 2019

Abstract
In an attempt to characterize inorganic constituents in *Tuber aestivum*, the summer truffle, samples from four different German states were collected by the "hypogea research group" for analytical investigations. Forty three elements in truffles, peridium and gleba were determined by three independent analytical techniques, PGAA, INAA and ICP-MS. PCA analysis of a set of data revealed a clear distinction of the different sampling sites. Results are discussed in view of the functional role of mycorrhizal fungi towards their host trees and the homeostatic control properties of the fungi.

Keywords *Tuber aestivum* · Major and trace elements · PCR and provenance analysis · Nuclear analytical techniques

Introduction

Summer truffle (*Tuber aestivum Vittad.*, synonym *T. uncinatum Chatin* [1], the Burgundy truffle) is considered the most common truffle in moderate climate of middle European countries. Although not as highly appreciated as the black Périgord truffle (*T. melanosporum Vittad.*) or the white Italian truffle (*T. magnatum Picco*), culinary value is still high and selling price for summer truffles varies between 160 and 300 €/kg [2]. Its fruiting bodies grow underground (hypogaeous) in calcareous soils and its mycelium forming mycorrhizal associations with host tree roots such as oak (*Quercus robur*), hazel (*Corylus avellana*), hornbeam (*Carpinus betulus*), and beech (*Fagus sylvatica*). The genus Tuber belongs to the family *Tuberacae* in the order of *Pezizales*. Together with e.g. morels they produce spores in a ´sac` or ´wineskin` forming the division of *Ascomycetes* [3]. Fresh fruit bodies of *T. aestivum* can weigh from 2 to 90 g, (in exceptional cases up to 755 g) with an average of 33 g [4]. The attempt to cultivate summer truffles using incubated tree seedlings can provide great revenues but is also prone to large risks as still not enough knowledge exists on the life cycle, optimal growing conditions, soil requirements and climatic obstacles. While growing underground, truffle fruit bodies producing an intense specific odour, hence, wild or cultivated species can only be detected by animals such as dogs with fine olfactory sense. This is probably the reason why scientific studies on truffle life cycle and constituents are rare and—exceptionally in Germany—truffles are listed in the red list of endangered species of the Bundesamt für Naturschutz, compiled by the DGfM (Deutsche Gesellschaft für Mykologie) [5]. Knowledge on the chemical composition of truffles hitherto is limited to the organic compounds forming taste and smell of the culinary highly esteemed mushrooms [6–8], inorganic constituents so far have been rarely documented. Comparative data with other ascomycetes can be found in e.g. [9].

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The samples

Mature fruit bodies of *T. aestivum* were collected in September 2016 and 2017 by members of the "hypogea research group" of Mobile Pilzschule (D.H. and S.S.) for scientific purposes at 4 different locations in Germany (see Fig. 1). Six samples from Bavaria (including one *T. mesentericum* and one *T. excavatum*), 8 samples from Thuringia, 8 samples from lower Saxony and 5 samples from North Rhine

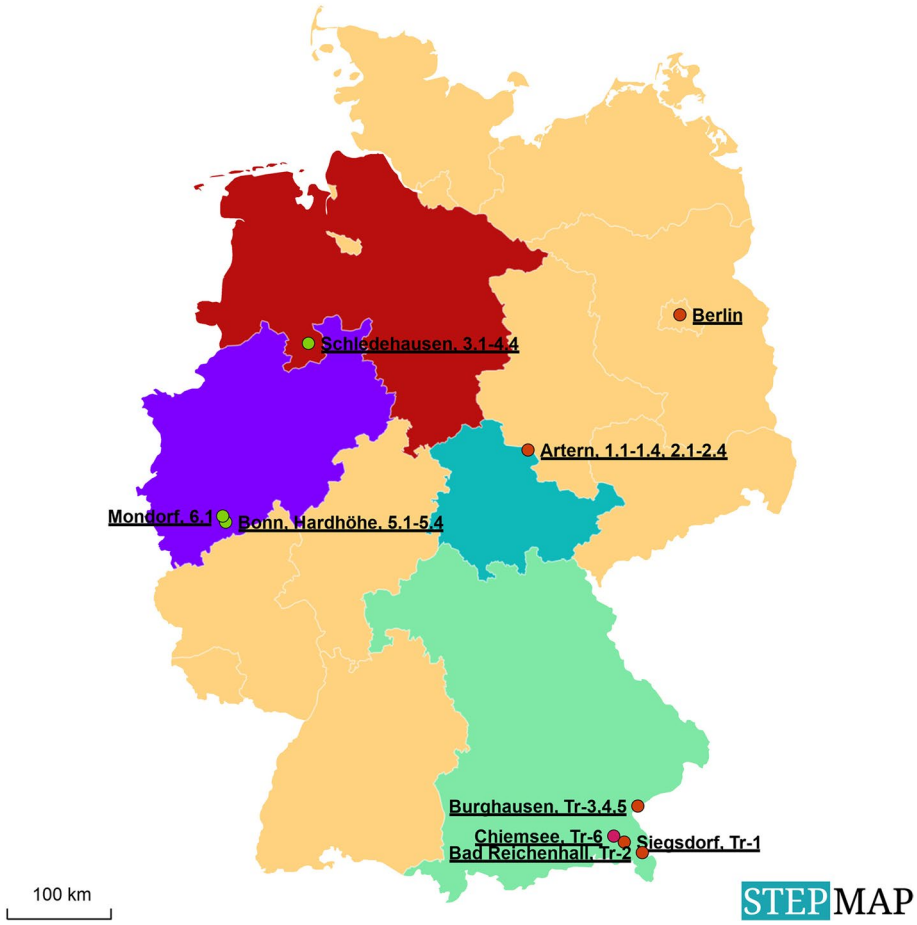

Fig. 1 Sampling locations of the *T. aestivum* samples used in this study

Fig. 2 Preparation of *T. aestivum* for analysis

Westphalia were collected and prepared. They were thoroughly cleaned in the field and sent freshly to M.R. for further treatment. A slice, 1–2 mm thick was cut from the central part of the mushroom using a ceramic knife and dried in the oven at 70 °C for 3–4 h (see Fig. 2).

From a few large fruit bodies additionally, the outer skin (peridium) and the inner soft part (gleba) was collected separately and dried as well. All dried samples were subsequently packed into ca. 2–3 cm wide Teflon©foil bags and heat sealed. These samples were then transferred to FRM II research reactor in Garching, Germany for PGAA analysis. When all samples were processed, they were forwarded to the Institute of Nuclear Chemistry and Technology in Warsaw, Poland for ICP-MS and INAA investigations. Additionally, a well described proficiency test material "Polish mushrooms" was used as a control material to check accuracy and comparability of the three analytical techniques employed in this study [10].

Experimental

PGAA analysis was performed at the FRM II in Garching, Germany. The basis of this method is the analysis of the prompt-gamma radiation which is induced by neutron capture during irradiation in a cold neutron beam. A comprehensive description of this method can be found in [11]. Each sealed Teflon bag was inserted in a sample holder

Table 1 Site specific mean values of truffle samples from individual techniques for elements Al, Fe, Zn, Cd in (mg/kg) dry weight

Analytical techniques	Element	Tr-1.1–Tr-1.4	Tr-2.1–Tr-2.4	Tr-3.1–Tr-3.4	Tr-4.1–Tr-4.4	Tr-5.1–Tr-5.4	Tr-1–Tr-6
ICP-MS	Al	1575 ± 627	811 ± 269	640 ± 468	847 ± 914	393 ± 89	490 ± 237
INAA							
PGAA		2175 ± 189	2225 ± 479	1725 ± 263	1450 ± 129	1600 ± 115	1250 ± 308
ICP-MS	Fe	398 ± 206	177 ± 92.8	127 ± 74	78.1 ± 57.2	115 ± 28.8	261 ± 209
INAA		574 ± 187	232 ± 125	159 ± 79.2	66.7 ± 45.4	118 ± 20.9	293 ± 26.5
PGAA		360 ± 110	223 ± 216	155 ± 144	243 ± 246	93.3 ± 51.3	425 ± 270
ICP-MS	Zn	108 ± 23.2	126 ± 27.2	155 ± 13.1	210 ± 25.1	167 ± 25.6	193 ± 41.5
INAA		123 ± 25.6	141 ± 28.6	177 ± 15.8	205 ± 35	172 ± 23.3	195 ± 57.3
PGAA							
ICP-MS	Cd	2.43 ± 0.35	2.98 ± 0.93	2.36 ± 0.86	2.7 ± 0.31	5.74 ± 1.11	2.71 ± 0.99
INAA							
PGAA		2.07 ± 0.3	2.44 ± 0.7	2.07 ± 0.72	2.13 ± 0.24	4.43 ± 0.84	2.13 ± 0.79

Table 2 Comparison of results for PT material Polish mushrooms

Element	RM polish mushrom reference	RM polish mushroom found		
		PGAA	ICP-MS	INAA
Na (%)	0.038 ± 0.0024	0.04 ± 0.0064		
Mg (%)	0.0819 ± 0.0065	0.1 ± 0.019		
K (%)	3.54 ± 0.2	3.4 ± 0.088		
Mn (ppm)	16.9 ± 1.35	18.5 ± 2.5	21.7 ± 0.6	
Co (ppm)	0.045 ± 0.003			0.042 ± 0.0077
Cu (ppm)	41.95 ± 3.05	70 ± 7.2	47.6 ± 0.13	
As (ppm)	0.417 ± 0.057		0.75 ± 0.036	
Rb (ppm)	381 ± 27			392 ± 23.3
Cd (ppm)	2.48 ± 0.27	1.83 ± 0.037	2.9 ± 0.049	
U (ppm)	0.34 ± 0.05		0.16 ± 0.0042	

consisting of an aluminum frame and FEP [12]. The samples were irradiated in a collimated neutron beam with a thermal equivalent flux of 1.35×10^9 cm^{-2} s^{-1} and simultaneously measured by a Compton-suppressed 60% HPGe detector for 4–8 h in the evacuated sample chamber (pressure below 0.3 mbar). More details about the PGAA setup at FRM II are documented in [13]. Spectrum evaluation was done with Hypermet-PC version 5.01 [14] and the final element concentrations were calculated with the Excel sheet ProSpeRo [15].

After completing PGAA analysis, the samples were prepared for analysis by ICP-MS and INAA method at the Institute of Nuclear Chemistry and Technology, Warsaw, Poland. Each sample was dried in a laboratory oven (BINDER) at 70 °C for 24 h. Then the samples were ground in a planetary mill (RETSCH) to grain size below 200 µm and homogenized by mixing. Part of the homogeneous sample was subjected to analysis by ICP-MS and the other part by INAA. RM Polish mushroom and CRM Oriental Basma Tobacco Leaves (INCT-OBTL-5) [16] were used for AQ/AC purposes.

INAA measurement

Samples of 70–100 mg mass were placed in PE capsules, firmly covered and irradiated together with CRMs and elemental standards in nuclear reactor MARIA (Świerk, Poland) at thermal neutron flux of 10^{14} cm^{-2} s^{-1} for 30 min. After appropriate cooling time, the gamma-ray spectroscopic measurements were performed with the aid of a 255 cm^3 HPGe well-type detector (Canberra) with associated electronics (resolution 2.15 keV for 1332 keV ^{60}Co line, efficiency approximately 40%), coupled to the multichannel analyser and Genie-2000 spectroscopy software (Canberra). Cooling time was from 3 to 7 weeks and measurement time between 10,000 and 50,000 s.

Table 3 Comparison of results for peridium/gleba

Element	Truffle peridium			Truffle gleba			Peridium/gleba ratio
	PGAA	ICP-MS	INAA	PGAA	ICP-MS	INAA	
H (%)	5.63 ± 0.17			6.29 ± 0.1			0.895
B (ppm)	11.5 ± 8.6			6.57 ± 4.2			1.75
C (%)	44 ± 4.6			42.7 ± 1.53			1.03
N (%)	4.93 ± 0.67			4.73 ± 0.75			1.04
Na (%)	0.073 ± 0.058			0.027 ± 0.058			2.7
Mg (%)	0.13 ± 0.03			0.12 ± 0.022			1.08
Al (%)	*0.47 ± 0.43*	3.37 ± 4.38		*0.16 ± 0.01*	0.548 ± 0.556		6.15
Si (%)	1.51 ± 2.0			0.0475 ± 0.039			31.8
P (%)	0.763 ± 0.09			0.977 ± 0.14			0.781
S (%)	0.293 ± 0.032			0.33 ± 0.036			0.888
Cl (ppm)	497 ± 275			133 ± 36.9			3.74
K (%)	2.77 ± 0.42			3.27 ± 0.65			0.85
Ca (%)	0.51 ± 0.23			0.143 ± 0.087			3.57
Sc (ppm)			0.388 ± 0.48			0.022 ± 0.016	17.6
Ti (ppm)	214 ± 251			15 ± 2.7			14.2
V(ppm)	24.5 ± 2.6			20 ± 2.8			1.23
Cr (ppm)		6.38 ± 3.63			3.14		2.03
Mn (ppm)	33 ± 3.25	98.9 ± 85.7			8.84 ± 4.88		11.2
Fe (ppm)	483 ± 165	1180 ± 1440	1450 ± 1884	60 ± 9.6	57.2 ± 32.7	78 ± 44.5	199
Co (ppm)			0.781 ± 0.85			0.113 ± 0.097	6.91
Ni (ppm)		3.23 ± 2.6			2.75 ± 2.5		1.17
Cu (ppm)	*70 ± 17.3*	53.7 ± 14.1		*70 ± 1.4*	41.5 ± 12.7		1.29
Zn (ppm)		150 ± 36.1	165 ± 42.8		140 ± 16.5	137 ± 15.1	1.14
As (ppm)		1.2 ± 0.9			0.364 ± 0.164		3.3
Se (ppm)		0.26 ± 0.17	*1.14*		0.28 ± 0.19		0.93
Rb (ppm)			8.43 ± 7.7			6.16 ± 5.5	1.37
Y (ppm)		1.49 ± 1.6			0.185 ± 0.061		8.05
Mo (ppm)		1.11 ± 0.47			1.07 ± 0.59		1.04
Ag (ppm)			1.1 ± 0.92			1.16 ± 1.38	0.95
Cd (ppm)	2.13 ± 0.79	2.38 ± 0.87		2.51 ± 1.04	3.03 ± 1.35		0.81
Sb (ppm)			0.098 ± 0.12			0.019	5.16
La (ppm)		0.522 ± 0.4			0.079		6.6
Ce (ppm)		4.0 ± 5.2	4.32 ± 5.74		0.141	0.35 ± 0.21	16.9
Pr (ppm)		0.655 ± 0.62					
Nd (ppm)		1.66 ± 2.1			0.042 ± 0.04		39.5
Sm (ppm)		0.385 ± 0.4			0.028 ± 0.05		13.8
Eu (ppm)			0.0064 ± 0.077			0.006	1.07
Gd (ppm)		0.35 ± 0.43					
Tb (ppm)		0.062 ± 0.054	0.068 ± 0.058				
Dy (ppm)		0.256 ± 031					
Ho (ppm)		0.068 ± 0.059					
Er (ppm)		0.15 ± 0.18					
Tm (ppm)		0.025 ± 0.02					
Yb (ppm)		0.213 ± 0.19					
Lu (ppm)		0.028 ± 0.021					
Hf (ppm)			0.56 ± 0.65			0.19 ± 0.19	2.95
Tl (ppm)		0.038 ± 0.049			0.004 ± 0.0014		
Pb (ppm)		1.81 ± 2.02			0.21 ± 0.2		8.62

Table 3 (continued)

Element	Truffle peridium			Truffle gleba			Peridium/gleba ratio
	PGAA	ICP-MS	INAA	PGAA	ICP-MS	INAA	
Th (ppm)			0.72 ± 0.99			0.064 ± 0.047	11.3
U (ppm)			0.27 ± 0.24				

Values in italics are not considered for ratio calculation

Table 4 Mean concentrations in *Tuber aestivum* from different German collection sites in (mg/kg) dry weight, n = 27, determined by PGAA, INAA, and ICP-MS

Element	Method	Mean	± SD	= %
H (%)	P	6.16	0.277	4.5
B	P	8.44	0.73	8.7
C (%)	P	43.2	1.6	3.7
N (%)	P	4.79	0.778	16.2
Na	P	500	320	65
Mg	P	1300	396	30.5
Al	M	771	559	72.5
Si	P	1900	1660	87
P	P	8860	1800	20
S	P	4200	1250	30
Cl	P	254	83.6	34
K (%)	P	2.9	0.45	15.6
Ca	P	2300	740	32
Sc	I	0.0635	0.0598	94
Ti	P	42	26,4	63
V	P	28.1	21.2	75
Cr	M	3.56	1.83	51.4
Mn	P, M	22.5	12.1	53.7
Fe	P, I, M	213	187	87.8
Co	I	0.182	0.138	75.6
Ni	M	1.87	1.57	84
Cu	P, M	61.6	15	24.4
Zn	I, M	166	43	25.9
As	M	0.829	0.946	114
Se	M	0.488	0.241	49.4
Rb	I	4.66	3.6	77
Y	M	0.415	0.439	106
Mo	M	1.13	0.676	60
Ag	I	1.1	1.5	138
Cd	P, M	2.84	1.2	42
Sb	I	0.0282	0.0213	75.7
La	M	0.31	0.276	89.2
Ce	I, M	0.6	0.527	87.9
Pr	M	0.062	0.06	96.4
Nd	M	0.31	0.257	88.6
Sm	P, M	0.058	0.0345	42
Eu	I	0.0134	0.0075	56
Gd	P, M	0.066	0.039	61.2
Hf	I	0.138	0.179	130
Tl	I	0.0106	0.0018	17
Pb	M	0.706	0.855	121
Th	I	0.0928	0.0928	100
U	M	0.107	0.049	45.8

ICP-MS measurements

In the preparation of all solutions 18 MΩ cm grade water from Milli-ORG Millipore Co. purification system was used.

Samples of 250 mg mass were mineralized in a high-pressure microwave digestion system (Anton Paar Multiwave 3000, USA) with two-step procedure: first—by mixture of 6 mL HNO_3 + 2 mL HF (60 bar, 240 °C, 1400 W, 0.75 h) and then by 12 mL 4% H_3BO_3 (60 bar, 220 °C, 600 W, 0.75 h) to remove fluoride ions. The reagent blanks were prepared in the same manner. The details of the mineralization procedure have been published elsewhere [17]. The resulting solutions were analysed by ICP-MS method using a PerkinElmer Elan DRC II instrument. The solutions were diluted with 0.7% HNO_3 by weighing and In-115 was added as an internal standard prior to analysis. The following nuclides ^{27}Al, ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{63}Cu,^{66}Zn, ^{75}As, ^{82}Se, ^{89}Y, ^{98}Mo, ^{111}Cd,^{139}La, ^{140}Ce, ^{141}Pr, ^{142}Nd, ^{152}Sm, ^{153}Eu, ^{158}Gd, ^{159}Tb, ^{164}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{174}Yb, ^{175}Lu, ^{205}Tl,^{208}Pb, ^{238}U, were selected since they are free from interference and are sufficiently abundant for quantitative measurement by ICP-MS. The applied instrument operation conditions are as follows: RF power 1050 W, plasma gas flow—13.0 L min^{-1}, auxiliary gas flow—1.2 L min^{-1}, nebulizer gas flow—0.92 L min^{-1}, lens voltage—6.25 V, detector mode—dual, working mode—standard, Ni cones.

Results and discussion

A suit of 20 elements were determined by PGAA, 29 by ICP-MS and 13 by INAA, some of them by more than one technique. For comparison of results from different techniques mean values for each sampling site were compiled in Table 1. Elements with results from more than one technique generally are in good agreement except for Al which showed systematically higher values in all samples by PGAA compared to ICP-MS.

Comparison of results in the Polish mushroom PT material with recommended values and results from peridium and gleba samples are documented in Tables 2 and 3. From Table 2 one can see that Cu is too high and Cd seems slightly low in PGAA, whereas As is too high and U too low in

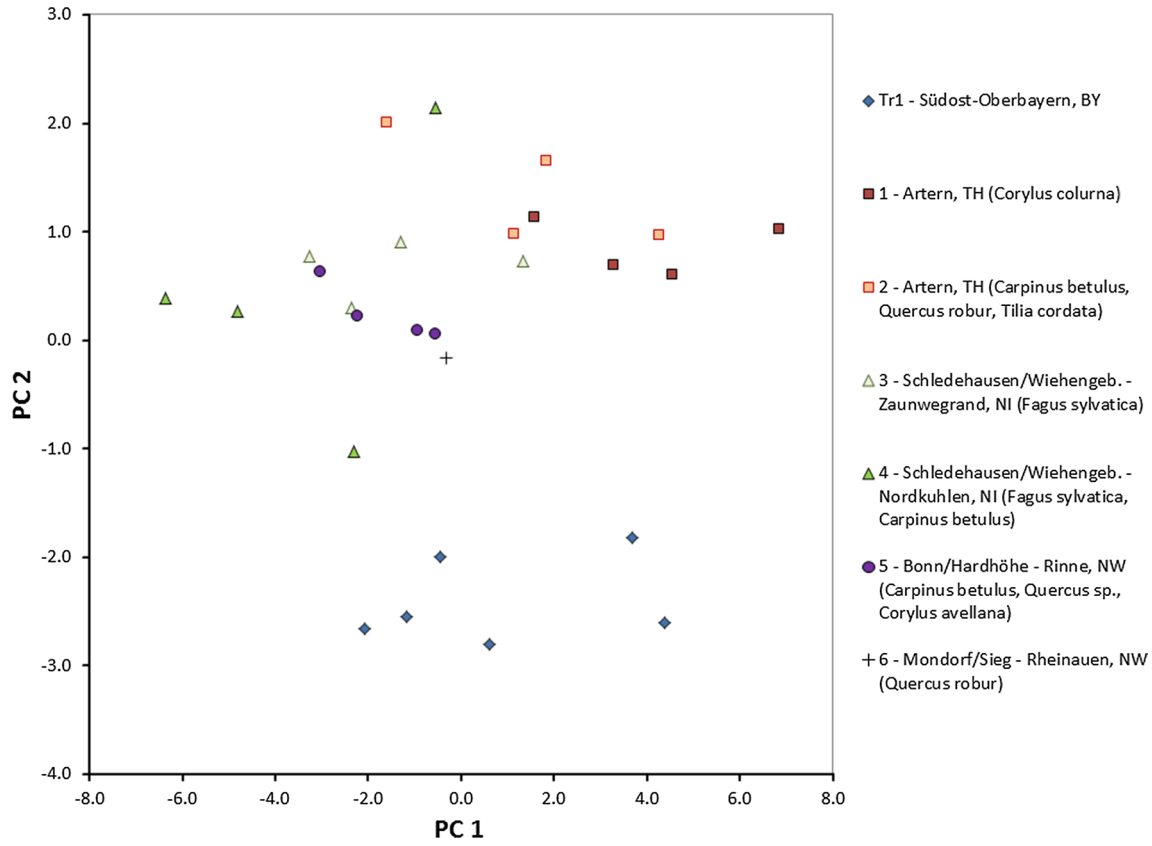

Fig. 3 Principal component analysis of all truffle samples

ICP-MS. Only Co and Rb are comparable for INAA and seem to match the given values well. Cu, as well as Al elevated results seem to be due to structure materials in the sample chamber of the PGAA facility. Our hydrogen rich samples give rise to elevated background induced by scattered neutrons which could not be reduced mathematically.

Comparison of peridium and gleba samples show that most elements are enriched in peridium, however, a number of elements having ratios between 0.8 and 1.5. Among these are C, N, Mg, P, S, K, V, Ni, Cu, Zn, but also Se, Mo, Ag and Cd. It seems, that essential elements show a comparably low variability (SD close to 10%), whereas other elements, such as Sc, Ti, As, Sb or the REE show standard deviations of close to 100%. Na, Cl. Al, Sc, Fe and Co show considerable enrichment in peridium (possibly soil contamination?) and large variability from sampling site to sampling site.

A list of mean values with standard deviation from all 27 *Tuber* samples is given in Table 4. For some elements the biological variation in composition can reach up to 100% or slightly more. Main constituents, e.g. H, C, N, P, S, but also B and K (essential elements) seem to be rather evenly contained. As, Ag, Hf and Pb however, show the largest spread between our samples. Uptake of these elements seem to be strongly dependent on site specific conditions. It is also interesting to compare individual samples with the mean of all. Sample TR-2 was collected within the spa park of Bad Reichenhall, southern Bavaria. Almost all elements seem to be enriched compared to mean values, particularly Pb, with 4.2 ppm is 5.7 times higher than the overall mean concentration. This may be explained by previous Zn and Pb mining in the area and possibly forgotten waste deposits in the region [18]. Rare earth elements are all a factor of 2 higher, Cr 6.1 ppm, Mn 39 ppm, Fe is 565 ppm and Zn 219 ppm.

Statistical analysis

Principle component analysis (PCA) is a widely known and frequently used multivariate statistical method for exploratory data analysis. An optimum projection of a high dimensional data set into 2–3 dimensions allows data reduction while maintaining relevant information of the data structure. Mathematically, the method is a coordinate transformation taking the covariances into account. It was first described in [19]. In our case, the new principle components (PC) are linear combinations of the element concentrations. Before starting the PCA calculations, the data have to be conditioned using missing-value treatment,

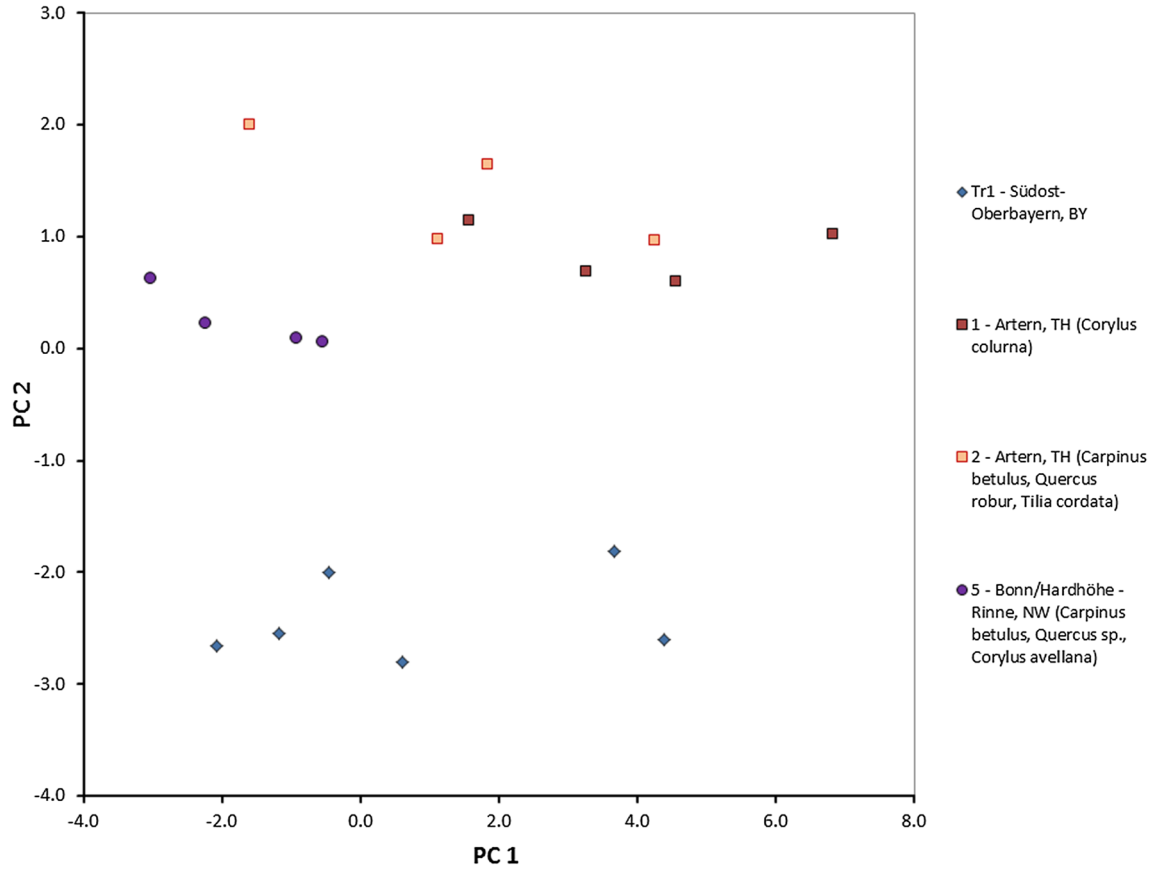

Fig. 4 PCA analysis for samples from Bavaria, Arthern, Thuringia and Bonn, Hardhöhe

logarithmization and standardization. Missing values were treated by imputation using the geometric mean in the case of $\leq 25\%$ missing values in each sub dataset. If there are more missing values than 25%, in at least one sub-set, the chemical element was completely excluded from the statistical analysis. The reason for choosing the geometric mean and also the logarithmization is based on the assumption that element concentrations are mostly log-normal distributed in the environment due to multifactorial influences (see also [20]). All calculations concerning the data preparation were done using Excel. The PCA was performed with R version 3.5.1 [21] using the prcomp function.

Sufficiently complete data sets are available for the elements:

- B, Na, Si, P, S, Cl, K, Ca (all analyzed by PGAA).
- Sc, Co, Rb, Hf, Th (NAA).
- Al, Cr, Cu, Mn, Ni, As, Y, Mo, La, Nd (ICP-MS).

Data for Fe (weighted arithmetic means of PGAA, NAA and ICP-MS), Zn (NAA and ICP-MS), Cd (PGAA and ICP-MS), and Ce (NAA and ICP-MS), were used. Major constituents, such as H, C, N, and calculated values for O were not part of the multivariate analysis. Values for Al determined by PGAA were possibly biased by higher Al background due to neutron scattering (sample: high H conc. + Al frame) and were not used. Certain elements show a higher variability of concentration data within a geographical group than between different groups. In these cases, the typical log-normal characteristics with a high skew are probably interfered. Therefore, we filtered out elements with a deviation less than 15% of the geometric mean relative to the arithmetic mean (as a rough estimate of the skewness).

As can be seen from Figs. 3 and 4 PCA analysis can clearly separate samples from different sampling sites on the basis of elemental concentrations. However, component PC 2 seems to have more influence on separation compared to component PC1 (see Fig. 4 from only 4 sampling sites).

Samples from Schledehausen/Wiehengebirge, Nordkuhlen in Lower Saxony scatter most, all other samples are grouped relatively close.

Comparably to plants, ectomycorrhizal fungi have a range of homeostatical mechanisms that ensure an optimal cellular

micronutrient concentration [22]. The dual role of mycorrhizal fungi to supply limited essential nutrients and, at the same time to filter toxic elements in excess to their host plants in exchange for carbohydrates requires sofisticated transport and detoxification mechanisms. Biotransformation of solubilized metals into insoluble organic forms is a phenomenon observed in mycorrhizal fungi [23]. Trace elements can be revesibly bound in the cell wall on caboxyl- and hydroxyl groups. Cd and Pb have been detected associated with glomalin in the fungal cell wall. Metallothioneins were also detected in ectomycorrhizal fungi after Cd treatment. The high concentrations of non-essential elements in sporocarps of some fungi suggest an additional detoxification process in mycorrhizal fungi to secure detrimental effects in their host tree plants [22]. Except a few essential elements all trace and micronutrients in our *T. aestivum* samples were concentrated in peridium compared to gleba supporting the idea of detoxification and immobilization of minerals. These capabilities of mycorrhizal fungi may be important to normalize and/or adjust nutrient concentrations for the host plant in spite of natural external fluctuations.

Conclusions

A suit of 43 elements, including main, major, and trace elements were determined in up to 27 individual *T. aestivum* samples from four different German states using three independent analytical methods. General enrichment of elements in peridium compared to gleba was found supporting the idea of actively controlling transport to the host plant. PCA analysis of our data suggest a possible distinction of provenance according to elemental analysis. In view of the augmented market value of truffles this could render as a suitable technique for fake identification.

Acknowledgements This work was carried out without any institutional support, scientific cooperation was based on good will and friendly assistance. However, we thank all supporters, known and unknown that have made this work possible. Many thanks to Robert Neagu, who helped in preparing the data for PCA analysis. Without the help of Sammy, Jette, Jule, Milli, and Loki, the dogs that located the truffles, this work could not be materialized at all.

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