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RESEARCH ARTICLE

Metal concentration and health risk assessment of eight *Russula* mushrooms collected from Kizilcahamam-Ankara, Turkey

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Abstract

The aim of this study was to determine the essential element (Zn, Ca, K, Fe, Na, and Mg), essential trace element (Co, Mn, Cr, and Cu), and non-essential element (Pb, Ni, and Cd) contents of eight different *Russula* species (*R. risigallina* (Batsch) Sacc., *R. cyanoxantha* (Schaeff.) Fr., *R. delica* Fr., *R. vinosa* Lindblad, *R. olivacea* (Schaeff.) Fr., *R. velenovskyi* Melzer & Zvára, *R. turci* Bres., and *R. parazurea* Jul. Schäff.) collected from Soguksu National Park (Turkey), which is a region away from the city center (Kizilcahamam, Ankara). In addition to the metal contents of these species, daily intake and health risk index values of the metals in question were also calculated and discussed. As a result of elemental analysis, the major elements were K (28980–58,380 mg/kg), Mg (704–1404 mg/kg), and Ca (190–1662 mg/kg). Except for *R. risigallina*, *R. olivacea*, and *R. velenovskyi*, elemental concentrations were within the limits that can be safely consumed as nutrients in terms of their metal content. The daily intakes of metal (DIM) values of *R. risigallina* and *R. olivacea* for Cr exceed the reference dose limits (3.80 and 3.87 μg/kg body weight/serving, respectively). According to the health risk index (HRI) measurements, the HRI values of *R. risigallina* and *R. olivacea* for Cr and of *R. velenovskyi* for Cd were found to be above 1.0 and could pose a health risk. In order to analyze the mineral composition variability of the studied mushroom species, principal component analysis (PCA) and the hierarchical cluster analysis (HCA) techniques were also performed. Regarding the significant correlations between all descriptors ($r > 0.7$), there was a positive relationship between Mg–K, Ni–Co, Ni–Na, Cr–Ni, Cr–Co, Zn–Mg, Zn–K, Cd–Mg couples.

Keywords Wild edible mushrooms · *Russula* · Dietary intake · Metal concentration · Health risk index

Introduction

Scientists claim that by the end of the twenty-first century, the world population will approach 10 billion and consequently some problems will arise. It is predicted that the most important of these problems will be related to human health and nutrition. Therefore, alternative resources are needed to meet both nutritional and health requirements. According to the researchers, plants and mushrooms can be important alternatives in eliminating the food problems. Mushrooms are also thought to be important for meeting health needs. Although mushrooms have low fat and calories, they are rich in vitamins and proteins (Zengin et al. 2015). Diets rich in proteins and vitamins are essential for a balanced diet. Therefore, it is necessary to take these components regularly in order to have a healthy lifestyle (Georgescu et al. 2017). Many scientists believe that mushroom consumption protects people against many degenerative diseases, especially obesity and cardiovascular diseases (Kavishree et al. 2008). In studies so far, it has been that some mushrooms are edible and can be used as

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Highlights
- Metal contents and health risk assessments of eight *Russula* species were studied.
- Except *R. risigallina*, *R. olivacea* and *R. velenovskyi*, the samples were safe for the consumers.
- DIM and HRI values of *R. risigallina* and *R. olivacea* exceeded the legal limits.
- HRI value of *R. velenovskyi* was also found to be high than 1.0.

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functional foods (Akyuz and Kirbag 2009; Breene 1990; Sun et al. 2014).

Scientifically, metals with a specific density of more than 5 g/cm^3 are defined as heavy metals (Järup 2003). These metals are very harmful as they are not biodegradable and tend to accumulate within the living organisms (Georgescu et al. 2017; Radulescu et al. 2010a; Radulescu et al. 2010b; Stihi et al. 2011a; Stihi et al. 2011b). Therefore, authorities have focused on identifying toxic metals in many food sources, including mushrooms (Radulescu et al. 2013). As a result of the consumption of edible mushrooms rich in heavy metals, these metals enter the food chain and may cause serious problems on human health (Georgescu et al. 2017).

Due to the nutritional properties mentioned above, mushrooms have begun to gain worldwide popularity in recent years (Kosanić et al. 2016). Researchers in Turkey are in a huge competition to identify taxonomically the new mushroom species. As a result of the taxonomy studies carried out since the 1850s in Turkey, more than 2200 macrofungi have been identified (Akata and Sesli 2017; Hakan and Turkekul 2018; Keleş 2019; Uzun and Acar 2018; Uzun and Kaya 2018).

The *Russula* genus has about 250 species that are widely spread around the world (Isaka et al. 2017). Phytochemical analysis showed that *Russula* species contain some phytochemicals such as terpenoids and sterols (Clericuzio et al. 2012; Maarisit et al. 2017; Wang et al. 1994). Studies on these species are not limited to their chemical compositions. In addition to these studies, there are various reports that *Russula* species have excellent antioxidant (Zhao et al. 2019), antibacterial (Natori et al. 1994), anti-tumor (Zhao et al. 2019) activities. Generally, myceliums of the *Russula* species are smaller than the *Suillus* or *Cortinarius* species and contain fewer and shorter hyphae (Taylor and Alexander 2005). For this reason, it is thought that *Russula* species may contain lower amounts of minerals than other mushroom species (Kalac 2016) and better adapt to contaminated soils (Newbound et al. 2010).

The aim of this study was to determine the essential element, essential trace element, and non-essential element contents of eight different *Russula* species (*R. risigallina* (Batsch) Sacc., *R. cyanoxantha* (Schaeff.) Fr., *R. delica* Fr., *R. vinosa* Lindblad, *R. olivacea* (Schaeff.) Fr., *R. velenovskyi* Melzer & Zvára, *R. turci* Bres., and *R. parazurea* Jul. Schäff.) collected from Soguksu National Park (Turkey), which is a region away from industrial pollution. In addition to the metal contents of these species, daily intake and health risk index values of the metals in question were calculated and discussed. In order to analyze the mineral composition variability of the studied mushroom species, principal component analysis (PCA) and the hierarchical cluster analysis (HCA) techniques were also performed.

Materials and methods

Collection and identification of mushroom samples

Mature fruiting bodies of *Russula* species were collected in 2019 from Soguksu National Park, located in Ankara, Turkey. Information on the habitats from where mushroom species are collected is given in Table 1. In order to obtain a sufficient amount of research material, 80 samples were collected, ten random samples from each mushroom species. Dr. Ilgaz Akata (Department of Biology, Faculty of Science, Ankara University, Turkey) identified the mushroom species by using their microscopic and macroscopic characteristics (Sarikurkcu et al. 2011). Fruiting bodies were subjected to surface cleaning under gently running tap water in order to remove any dirt. The samples were then cut into small pieces and dried in a 105 °C oven for 24 h. The dried samples were homogenized using an agate homogenizer. They were then stored in pre-cleaned and dehumidified polyethylene bottles until experiments were performed.

Procedure of digestion and elemental analysis of mushrooms

In order to determine the essential element (Zn, Ca, K, Fe, Na, Mg), essential trace element (Co, Mn, Cr, Cu), and non-essential element (Pb, Ni, Cd) contents of *Russula* species, digestion procedure and elemental analysis methods defined in the literature were followed (Sarikurkcu et al. 2015; Sarikurkcu et al. 2012). Samples were digested using a closed system CEM Mars 5 microwave. Nitric acid (9 ml, 65%) and hydrogen peroxide (1 ml, 30%) were used as solvents for this process. Inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin-Elmer Optima 2000) device was used for elemental analysis. The metal contents of the samples were calculated based on dry weight (Table 2).

Both digestion and analytical method performed were validated using standard reference material (1573a Tomato Leaves) available from the National Institute of Standards and Technology (NIST). Accuracy in the range of 92–108% and precision in the range of 1–8% were considered sufficient. Data of the recovery and the analytical outputs of analytes in the reference material are given in Table S1 (supplementary file).

Health risk index

The Health Risk Index (HRI) provided here is a theoretical value. It refers to the daily amount of metal to be taken into the body as a result of the consumption of the mushroom species considered in the current study. The data given in Table 3 were calculated using the equation given below (Cui et al. 2004):

Table 1 Harvest dates and habitats of *Russula* mushroom species

Mushroom species	Habitat	Harvest date
Russula risigallina (Batsch) Sacc.	Under oak (*Quercus petraea*)	September 15, 2019
Russula cyanoxantha (Schaeff.) Fr.	Under oak (*Quercus petraea*)	September 20, 2019
Russula delica Fr.	Under oak (*Quercus petraea*)	August 30, 2019
Russula vinosa Lindblad	Under fir (*Abies nordmanniana* subsp. *equi-trojani*)	September 20, 2019
Russula olivacea (Schaeff.) Fr.	Under fir (*Abies nordmanniana* subsp. *equi-trojani*)	September 15, 2019
Russula velenovskyi Melzer and Zvára	Under fir (*Abies nordmanniana* subsp. *equi-trojani*)	August 30, 2019
Russula turci Bres.	Under fir (*Abies nordmanniana* subsp. *equi-trojani*)	August 30, 2019
Russula parazurea Jul. Schäff.	Under oak (*Quercus petraea*)	September 20, 2019

$$\mathrm{HRI}=\mathrm{DIM}/R_fD^{o}$$

In this equation, DIM refers to the daily amount of metal to be taken as a result of the consumption of mushroom species in question. R_fD^{o} means reliable level of metal that can be exposed orally throughout life. This value is usually taken into account in non-cancer health assessments (USEPA 2002).

The DIM value in the equation above was calculated by using the formula below (Cui et al. 2004; Liu et al. 2015):

$$\mathrm{DIM} = \mathrm{SM} \times \mathrm{MCM}/\mathrm{ABW}$$

SM, MCM, and ABW in the equation given above refer to the amount of mushrooms served (0.03 kg of dried mushrooms), metal concentration in mushrooms (mg/kg dry weight), and the average body weight of the individual (70 kg for a regular consumer), respectively.

In the current study, while calculating the HRI value, the daily consumption amount of dried mushrooms (1 serving) was considered as 30 g, while the average body weight of an ordinary consumer was accepted as 70 kg (Cui et al. 2004).

Statistical, PCA, and HCA analyzes

Variance (one-way ANOVA by Tukey's test at 5% significance level) analysis was performed by using SPSS Statistics 20.0 software. In order to evaluate the differences and/or similarities between the samples based on their mineral compositions, both PCA and HCA was performed by using FactoMiner R package. "Wards" and "Euclidean" were used as linkage rule and similarity measure respectively.

Results and discussion

Element content in mushrooms

The data obtained as a result of elemental analysis of *Russula* species were given in Table 2 and Fig. 1. Elemental analysis

Table 2 Element concentrations of *Russula* mushrooms (mg/kg dry weight)

Metal	*R. cyanoxantha*	*R. delica*	*R. risigallina*	*R. vinosa*	*R. olivacea*	*R. parazurea*	*R. turci*	*R. velenovskyi*
Co	0.06 ± 0.006^{c}	nd	0.34 ± 0.001^{b}	nd	0.45 ± 0.001^{a}	nd	nd	nd
Cr	0.49 ± 0.07^{f}	1.46 ± 0.01^{e}	8.86 ± 0.01^{a}	0.48 ± 0.05^{f}	9.02 ± 0.10^{a}	4.18 ± 0.01^{c}	3.18 ± 0.08^{d}	5.20 ± 0.14^{b}
Pb	1.51 ± 0.57^{a}	0.19 ± 0.03^{b}	0.85 ± 0.01^{ab}	1.59 ± 0.14^{a}	0.91 ± 0.01^{ab}	0.54 ± 0.10^{ab}	0.64 ± 0.18^{ab}	0.53 ± 0.19^{ab}
Cd	0.83 ± 0.01^{b}	0.69 ± 0.01^{c}	0.37 ± 0.03^{e}	0.50 ± 0.01^{d}	0.68 ± 0.01^{c}	0.88 ± 0.02^{b}	0.44 ± 0.02^{de}	1.50 ± 0.01^{a}
Ni	0.81 ± 0.14^{c}	0.78 ± 0.17^{c}	1.98 ± 0.17^{a}	0.77 ± 0.03^{c}	2.40 ± 0.07^{a}	0.82 ± 0.06^{c}	1.28 ± 0.01^{bc}	1.81 ± 0.14^{ab}
Zn	77.2 ± 0.5^{b}	47.2 ± 0.1^{c}	67.6 ± 0.4^{b}	165.4 ± 0.8^{a}	65.0 ± 0.2^{bc}	79.4 ± 1.3^{b}	81.0 ± 10.1^{b}	179.6 ± 0.8^{a}
Mn	40.2 ± 0.3^{a}	16.2 ± 0.1^{e}	35.8 ± 0.4^{b}	10.1 ± 0.1^{f}	19.9 ± 0.1^{d}	21.6 ± 0.1^{d}	29.6 ± 1.4^{c}	19.3 ± 0.2^{d}
Cu	53.8 ± 0.1^{b}	39.8 ± 0.3^{e}	44.0 ± 0.2^{d}	44.2 ± 0.4^{d}	46.4 ± 0.5^{c}	24.4 ± 0.1^{f}	44.4 ± 0.1^{d}	61.2 ± 0.1^{a}
Fe	224.0 ± 3.1^{b}	171.0 ± 0.1^{c}	248.0 ± 0.3^{a}	50.0 ± 0.2^{g}	151.0 ± 1.3^{d}	67.0 ± 0.6^{f}	73.0 ± 1.2^{f}	102.0 ± 1.3^{e}
Ca	614 ± 16^{c}	408 ± 1^{d}	626 ± 1^{c}	302 ± 2^{e}	190 ± 1^{f}	402 ± 7^{d}	1662 ± 10^{a}	820 ± 23^{b}
Mg	864 ± 8^{c}	778 ± 20^{cd}	874 ± 13^{b}	928 ± 8^{b}	704 ± 4^{d}	882 ± 1^{b}	934 ± 13^{b}	1404 ± 37^{a}
Na	318 ± 1^{d}	276 ± 4^{e}	432 ± 6^{a}	248 ± 1^{f}	364 ± 1^{bc}	224 ± 3^{g}	372 ± 5^{b}	354 ± 1^{c}
K	$29{,}980 \pm 35^{d}$	$28{,}980 \pm 438^{d}$	$33{,}230 \pm 212^{c}$	$41{,}860 \pm 919^{b}$	$29{,}380 \pm 1103^{d}$	$31{,}800 \pm 792^{cd}$	$34{,}900 \pm 233^{c}$	$58{,}380 \pm 156^{a}$

The values indicated by different superscripts within the same row shows significant difference at $p < 0.05$. *nd*, not detected

Table 3 Daily intakes of metal and health risk indexes in *Russula* mushrooms

Mushrooms	Daily intakes of metal (DIM, μg/kg body weight/serving)								Health risk indexes (HRI)							
	Pb	Cd	Zn	Fe	Mn	Cu	Cr	Ni	Pb	Cd	Zn	Fe	Mn	Cu	Cr	Ni
R. cyanoxantha	0.65	0.36	33.09	96.00	17.23	23.06	0.21	0.35	0.18	0.71	0.11	0.32	0.72	0.62	0.07	0.02
R. delica	0.08	0.30	20.23	73.29	6.95	17.06	0.63	0.33	0.02	0.59	0.07	0.24	0.29	0.46	0.21	0.02
R. risigallina	0.36	0.16	28.97	106.29	15.34	18.86	3.80	0.85	0.10	0.32	0.10	0.35	0.64	0.51	1.27	0.04
R. vinosa	0.68	0.21	70.89	21.43	4.35	18.94	0.21	0.33	0.19	0.43	0.24	0.07	0.18	0.51	0.07	0.02
R. olivacea	0.39	0.29	27.86	64.71	8.55	19.89	3.87	1.03	0.11	0.58	0.09	0.22	0.36	0.54	1.29	0.05
R. parazurea	0.23	0.38	34.03	28.71	9.26	10.46	1.79	0.35	0.06	0.75	0.11	0.10	0.39	0.28	0.60	0.02
R. turci	0.27	0.19	34.71	31.29	12.69	19.03	1.36	0.55	0.08	0.37	0.12	0.10	0.53	0.51	0.45	0.03
R. velenovskyi	0.23	0.64	76.97	43.71	8.27	26.23	2.23	0.78	0.06	1.29	0.26	0.15	0.34	0.71	0.74	0.04
R_fD^o [1] (μg/kg body weight/day)	3.6 [2]	1.0 [3]	300 [3]	300 [2]	140 [3]	40 [3]	3 [3]	20 [3]								

[1] R_fD^o, reference dose

[2] JECFA (1993)

[3] USEPA (2002)

has shown that Co is not present in *R. delica*, *R. vinosa*, *R. parazurea*, *R. turci*, and *R. velenovskyi*. It was understood that all the elements except Co were found to be in various amounts in all *Russula* species. According to the data obtained, the mushroom species richest in metals were *R. risigallina*, *R. olivacea*, *R. velenovskyi*, and *R. cyanoxantha*. In *R. risigallina*, significant amounts of elemental Fe, Mn, Cr, Ni, Co, and Na were measured (248.0, 35.8, 8.86, 1.98, 0.34, and 432.0 mg/kg dry weight, respectively). Cr, Ni, and Co were prominent in *R. olivacea* (9.02, 2.40, and 0.45 mg/kg dry weight, respectively). The amounts of Cu, Zn, Cd, K, and Mg were high in *R. velenovskyi* (61.2, 179.6, 1.50, 58,380.0, and 1404.0 mg/kg dry weight, respectively). As can be seen clearly from Fig. 1, in *R.cyanoxantha*, high amount of Pb (1.51 mg/kg) was determined in addition to Fe (224.0 mg/kg), Mn (40.2 mg/kg), and Cu (53.8 mg/kg). Another mushroom sample with high Pb content was *R. vinosa* (1.59 mg/kg). On the other hand, *R. delica* and *R. parazurea* were found to be poorer mushrooms in scanned metals than other mushroom samples.

In addition to being a valuable nutrient, mushrooms provide important clues about the metal pollution of the environment in which they grow. For this reason, for researchers working on the metal content of mushrooms, the area where the samples are collected should be evaluated in terms of industrial pollution and the correlation between this data and the metal content should be interpreted together. The mushroom species examined in the current study were collected from Soguksu National Park, a region far from industrial pollution. Therefore, the majority of the metals were not above the level that can be considered as risky for health. To the best of our knowledge, apart from *R. cyanoxantha*, *R. delica*, and *R. vinosa*, the metal contents of other *Russula* species evaluated here have not been published anywhere before. Therefore, the data presented on the metal content of these mushroom species is an important contribution for the literature. However, the metal contents of *R. cyanoxantha*, *R. delica*, and *R. vinosa* have been previously reported by some researchers (Aloupi et al. 2012; Busuioc et al. 2011; Çayır et al. 2010; Demirbaş 2000; Demirbaş 2001a; Demirbaş 2001b; Isildak et al. 2007; Konuk et al. 2007; Kula et al. 2011; Mirończuk-Chodakowska et al. 2013; Murati et al. 2019; Ouzouni et al. 2009; Sarikurkcu et al. 2020; Sarikurkcu et al. 2012; Semreen and Aboul-Enein 2011; Singdevsachan et al. 2014; Tel-Çayan et al. 2017; Zsigmond et al. 2020) (Table 4). The regions, where the mushroom species given in the literature were collected, were mostly forested or mountainous area, where industrial pollution was minimized. However, the areas where samples were collected included areas where industrial activity is intensive, such as Kichevo valley (South-West Macedonia) (Murati et al. 2019), as well as areas close to high traffic road such as Targoviste city (Romania) (Busuioc et al. 2011) or Yumra (Trabzon-Turkey) (Demirbaş 2000).

Comparing the data obtained as a result of elemental analysis of *R. cyanoxantha* with the literature data, Co and Ni contents in the current study were lower (Demirbaş 2001a; Murati et al. 2019; Zsigmond et al. 2020). On the other hand, Cr, Mn, Cu, Fe, and Ca concentrations obtained from the current study were found to be higher than all elemental analysis data related to *R. cyanoxantha* given in the literature (Busuioc et al. 2011; Demirbaş 2001a; Demirbaş 2001b; Murati et al. 2019; Zsigmond et al. 2020). Some of the mushrooms that were found to have lower level of elements in question than the current study was collected from Kichevo valley (South-West Macedonia) (Murati et al. 2019) and

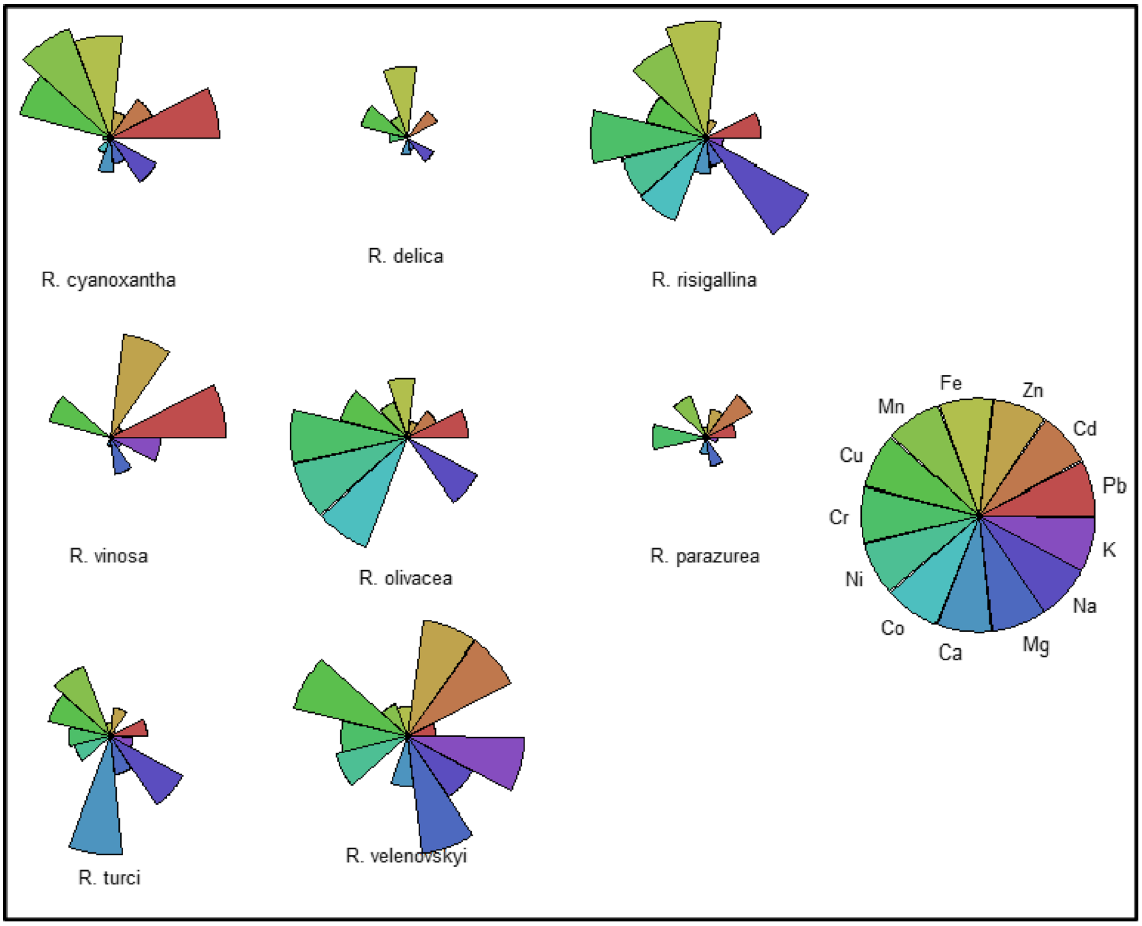


Fig. 1 Element concentrations of *Russula* mushrooms

Targoviste city (Romania) (Busuioc et al. 2011). As noted above, these zones are areas with high traffic flow or industrial pollution and the metal contents of the mushrooms collected from these areas is expected to be higher than that of samples collected from mountainous or forest regions. Due to the high metal content of the samples collected from the Soguksu National Park, which is a relatively remote region from human settlement, it was concluded that this area should be re-evaluated by the authorities in terms of industrial pollution. While the Na content of *R. cyanoxantha* was found very close to the upper limit of Na concentrations determined in the literature (Zsigmond et al. 2020), it was understood that the Pb, Cd, Zn, Mg, and K contents correspond to the mean of the literature data.

As far as our literature survey could ascertain, among the *Russula* species included in the present study, the most studied mushroom species in terms of metal contents is *R. delica*. As a result of the comparison with the literature data, Cr content determined in the current study was found to be higher than the samples collected from the forests of Cluj-Napoca (Romania) and Eastern Black Sea region (Turkey) (Demirbaş 2001a; Zsigmond et al. 2020). While there was only one report in the literature that *R. delica* contained Co (Demirbaş 2001a), this species did not contain Co in the current study. On the other hand, in the current study, it has been understood that the data obtained for all other metals were almost close to the average of the literature data.

It was seen in the literature that there was only one report regarding the metal contents of *R. vinosa* (Mirończuk-Chodakowska et al. 2013). In this study, only Pb and Cd contents of this mushroom were determined. The contents of these metals in the sample collected from a forested area of Podlaskie (Poland) were reported as 2.61 and 0.32 mg/kg, respectively. The Pb value obtained from the current study was found to be lower than the literature data, while the Cd value was found to be higher.

Health risk assessment

Daily intakes of metal (μg/kg body weight/serving) and health risk assessment index (HRI) values were calculated based on metal contents of *Russula* species in order to decide whether

Table 4 Literature data on metal contents of mushroom species examined in the current study

Metal	Concentration (mg/kg)	Sampling area and feature	Reference
R. cyanoxantha			
Co	0.35	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
Cr	0.25–0.40	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
	1.66	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
Pb	1.40	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	2.05	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001b)
	5.85	Kichevo valley, South-West Macedonia (industrial center)	(Murati et al. 2019)
Cd	0.39–14.1	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
	1.26	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	1.35	Kichevo valley, South-West Macedonia (industrial center)	(Murati et al. 2019)
	3.16	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001b)
Ni	1.07–2.49	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
	6.95	Kichevo valley, South-West Macedonia (industrial center)	(Murati et al. 2019)
	92.8	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
Zn	21.7	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	26.1	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001b)
	43.68	Kichevo valley, South-West Macedonia (industrial center)	(Murati et al. 2019)
	74.5–108.0	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
	94.54	Targoviste City, Romania (near high traffic road)	(Busuioc et al. 2011)
Mn	5.42	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	10.9–19.1	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
	12.6	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001b)
	22.86	Kichevo valley, South-West Macedonia (industrial center)	(Murati et al. 2019)
Cu	9.12	Targoviste City, Romania (near high traffic road)	(Busuioc et al. 2011)
	18.9	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	19.1	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001b)
	29.11	Kichevo valley, South-West Macedonia (industrial center)	(Murati et al. 2019)
	37.1–55.0	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
Fe	22.6–69.1	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
	63.2	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	110.17	Kichevo valley, South-West Macedonia (industrial center)	(Murati et al. 2019)
	212.82	Targoviste City, Romania (near high traffic road)	(Busuioc et al. 2011)
Ca	86.3	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	107–236	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
Mg	543–787	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
	1160	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
Na	110	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	115–337	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
K	15,800–21,400	Cluj-Napoca, Romania (forest)	(Zsigmond et al. 2020)
	46,000	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
R. delica			
Co	0.007	Similipal Biosphere Reserve, Odisha, India (tropical forest ecosystem)	(Singdevsachan et al. 2014)
	0.05	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)
	0.05	Western Black Sea Region (forest)	(Konuk et al. 2007)
	0.14	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	0.16	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)

Table 4 (continued)

Metal	Concentration (mg/kg)	Sampling area and feature	Reference
	0.25	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	1.44	Ajloun Valley, Jordan (mountainous region)	(Semreen and Aboul-Enein 2011)
	3.14	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
Cr	0.10	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	0.12	Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	0.12	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)
	0.15	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	0.16	Western Black Sea Region (forest)	(Konuk et al. 2007)
	0.27	Camyayla village, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	0.32	Ezine, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	0.38	Lapseki, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	0.57	Lesvos island, North-East Aegean, Greece (forest)	(Aloupi et al. 2012)
	0.88	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	7.23	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
Pb	0.03	Western Black Sea Region (forest)	(Konuk et al. 2007)
	0.078	Lesvos island, North-East Aegean, Greece (forest)	(Aloupi et al. 2012)
	0.10	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	0.59	Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	0.77	Ezine, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	0.82	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	0.865	Between Trabzon-Yomra, Turkey (highway)	(Demirbaş 2000)
	1.42	Mugla, Turkey (not specified)	(Kula et al. 2011)
	1.56	Camyayla village, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	2.06	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
	2.22	Ajloun Valley, Jordan (mountainous region)	(Semreen and Aboul-Enein 2011)
	2.5	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	3.05	Lapseki, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	3.15	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
Cd	0.034	Western Black Sea Region (forest)	(Konuk et al. 2007)
	0.22	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)
	0.40	Ezine, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	0.42	Camyayla village, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	0.64	Lesvos island, North-East Aegean, Greece (forest)	(Aloupi et al. 2012)
	0.654	Between Trabzon-Yomra, Turkey (highway)	(Demirbaş 2000)
	0.83	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
	0.90	Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	1.04	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	1.14	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	1.54	Ajloun Valley, Jordan (mountainous region)	(Semreen and Aboul-Enein 2011)
	1.77	Mugla, Turkey (not specified)	(Kula et al. 2011)
	3.88	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	4.23	Lapseki, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
Ni	0.079	Similipal Biosphere Reserve, Odisha, India (tropical forest ecosystem)	(Singdevsachan et al. 2014)
	0.08	Western Black Sea Region (forest)	(Konuk et al. 2007)
	0.25	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	1.90	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)

Table 4 (continued)

Metal	Concentration (mg/kg)	Sampling area and feature	Reference
	1.95	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	2.43	Lesvos island, North-East Aegean, Greece (forest)	(Aloupi et al. 2012)
	2.56	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
	5.4	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	116.0	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	12.65	Ajloun Valley, Jordan (mountainous region)	(Semreen and Aboul-Enein 2011)
Zn	0.34	Western Black Sea Region (forest)	(Konuk et al. 2007)
	32.6	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	33.40	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	33.45	Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	36.8	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	38.93	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
	39.9	Lesvos island, North-East Aegean, Greece (forest)	(Aloupi et al. 2012)
	41.44	Ajloun Valley, Jordan (mountainous region)	(Semreen and Aboul-Enein 2011)
	52.0	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	56.58	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)
	58.08	Ezine, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	72.28	Camyayla village, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	83.0	Mugla, Turkey (not specified)	(Kula et al. 2011)
	100.17	Lapseki, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
Mn	0.180	Similipal Biosphere Reserve, Odisha, India (tropical forest ecosystem)	(Singdevsachan et al. 2014)
	0.55	Western Black Sea Region (forest)	(Konuk et al. 2007)
	5.4	Lesvos island, North-East Aegean, Greece (forest)	(Aloupi et al. 2012)
	6.62	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	7.86	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	9.19	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	16.61	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)
	35.08	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
	36.55	Ajloun Valley, Jordan (mountainous region)	(Semreen and Aboul-Enein 2011)
	66.0	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
Cu	0.20	Western Black Sea Region (forest)	(Konuk et al. 2007)
	0.51	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	10.8	Between Trabzon-Yomra, Turkey (highway)	(Demirbaş 2000)
	13.6	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	19.55	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
	25.0	Lesvos island, North-East Aegean, Greece (forest)	(Aloupi et al. 2012)
	27.0	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	37.0	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	37.07	Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	51.71	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)
	52.01	Camyayla village, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	57.46	Ezine, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
	58.41	Ajloun Valley, Jordan (mountainous region)	(Semreen and Aboul-Enein 2011)
	164.2	Lapseki, Canakkale, Turkey (not specified)	(Çayır et al. 2010)
Fe	2.54	Western Black Sea Region (forest)	(Konuk et al. 2007)
	2.78	Similipal Biosphere Reserve, Odisha, India (tropical forest ecosystem)	(Singdevsachan et al. 2014)

Table 4 (continued)

Metal	Concentration (mg/kg)	Sampling area and feature	Reference
	24.6	Lesvos island, North-East Aegean, Greece (forest)	(Aloupi et al. 2012)
	59.0	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	74.8	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	81.80	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)
	203.32	Tokat (Middle Black Sea region), Turkey (forest)	(Isildak et al. 2007)
	235.4	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	288.54	Ajloun Valley, Jordan (mountainous region)	(Semreen and Aboul-Enein 2011)
	470.0	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	704.0	Mugla, Turkey (not specified)	(Kula et al. 2011)
Ca	3.91	Western Black Sea Region (forest)	(Konuk et al. 2007)
	24.7	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	72.8	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	1188.0	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	1416.0	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
Mg	7.3	Western Black Sea Region (forest)	(Konuk et al. 2007)
	395.8	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
	688.7	West Macedonia and Epirus, Greece (forest)	(Ouzouni et al. 2009)
	1060.0	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	1124.0	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	1438.0	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
Na	2.1	Western Black Sea Region (forest)	(Konuk et al. 2007)
	82.9	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
	436.3	Mugla, Turkey (not specified)	(Tel-Çayan et al. 2017)
K	1.60	Similipal Biosphere Reserve, Odisha, India (tropical forest ecosystem)	(Singdevsachan et al. 2014)
	99.0	Western Black Sea Region, Turkey (forest)	(Konuk et al. 2007)
	100.0	Mediterranean region, Turkey (forest)	(Sarikurkcu et al. 2020)
	402.0	Egirdir, Isparta, Turkey (forest)	(Sarikurkcu et al. 2012)
	34,000.0	Eastern Black Sea region, Turkey (forest)	(Demirbaş 2001a)
R. vinosa			
Pb	2.61	Podlaskie, Poland (forest)	(Mirończuk-Chodakowska et al. 2013)
Cd	0.32	Podlaskie, Poland (forest)	(Mirończuk-Chodakowska et al. 2013)

The concentrations of the elements are given in ascending order. In none of the literature data given above, health risk assessment value was calculated by the researchers

their consumption was risky for human health. DIM values of fungi for Pb and Fe remained below the reference doses prescribed by JECFA (1993). On the other hand, Cd, Zn, Mn, Cu, and Ni contents of all mushroom species were found to be lower than the maximum legal DIM limits published by USEPA (2002). Cr content of *R. risigallina* and *R. olivacea* exceed the DIM values prescribed by USEPA (2002), but the DIM values calculated based on the Cr content of other mushroom species were within the safe dose range.

The data on the DIM values of the mushroom species mentioned above were also compatible with the HRI data. HRI values of *R. risigallina* and *R. olivacea* related to Cr contents were found to be 1.27 and 1.29, respectively. In addition, Cd content of *R. velenovskyi* was found to be risky for the human health (HRI, 1.29). Therefore, it was considered that the consumption of *R. velenovskyi* was not safe due to their high Cd and Cr contents.

Multivariate analysis

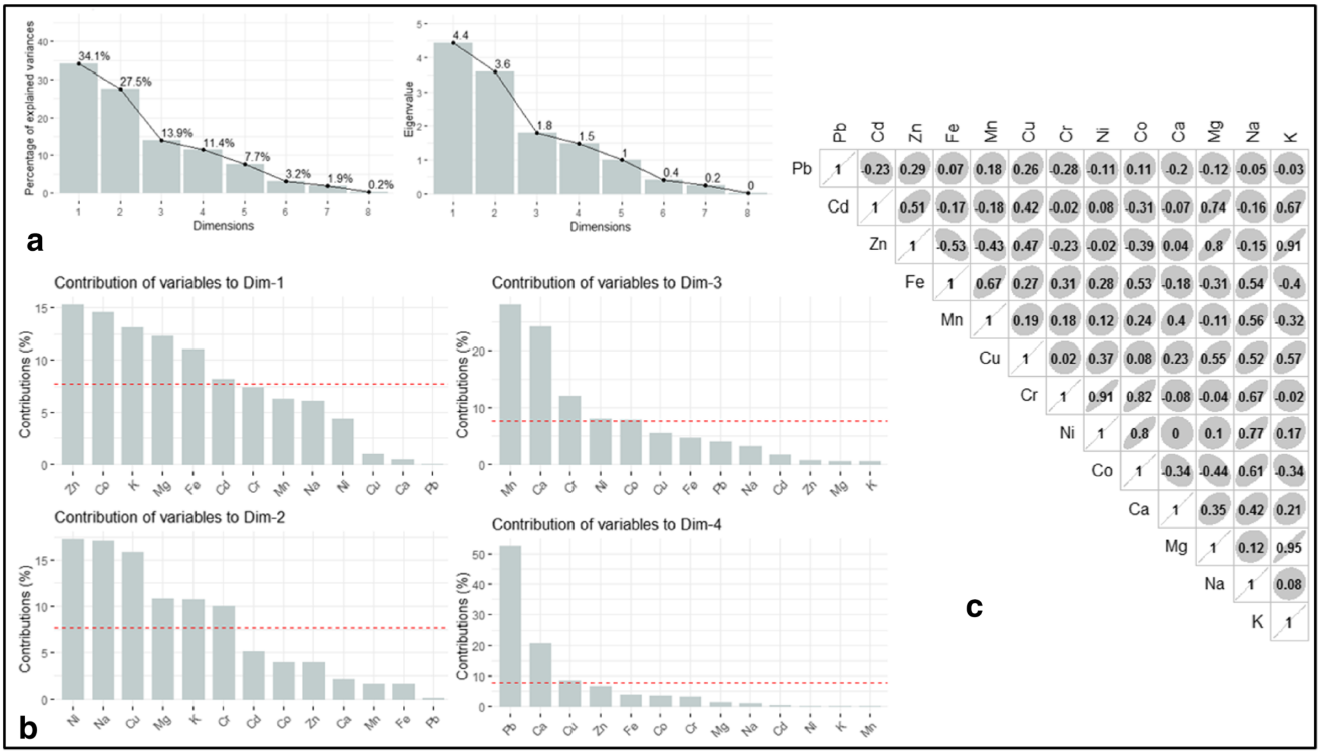
The best way to analyze the mineral composition variability of the studied mushroom species is to use exploratory statistical tools to reveal patterns between these species based on similarities between the input datasets. PCA and the HCA are the most frequently used multivariate methods to perform such analyzes. PCA is a multivariate analysis that reduces dimensionality. It is a method that decomposes a large number of variables into a fewer number of principal components (PCs) and preserves the maximum amount of information while doing this. Results of statistical analysis were reported in Figs. 2 and 3. To determine the number of PCs that keep the most information in the dataset, the percentage of variance and eigenvalues explained in the PCs were analyzed. Accordingly, only four PCs having eigenvalue greater than or equal to 1 and showing a cumulative percentage of variances of 86.9% were retained. The contribution of each descriptor to the first four PCs is shown in Fig. 2. As seen in the figure, PC1 is related to Zn, Co, K, Mg, and Fe. PC2 was predominantly associated with Ni, Na, and Cu. Mn and Ca were dominant in PC3, while PC4 was associated with Pb. The average of the correlation coefficients between the descriptors is shown in Fig. 2. Regarding the significant correlations between all descriptors ($r > 0.7$), there was a positive relationship between Mg–K (0.95), Ni–Co (0.80), Ni–Na (0.77), Cr–Ni (0.91), Cr–Co (0.82), Zn–Mg (0.80), Zn–K (0.91), Cd–Mg (0.74) couples. This high positive coefficients of correlation imply that the abovementioned metals are directly related, i.e., as the concentration of one metal increases, the concentration of the metal associated therewith also trend to increase. Additionally, this reflects the possibility to predict what happens to the concentration of one metal based on the knowledge of the concentration of the other metal.

A distinction was made between the species by observing the graph of samples. Six clusters were then determined by calculating the Euclidean distance between the samples (cluster 1: *R. velenovskyi*; cluster 2: *R. vinosa*; cluster 3: *R. parazurea* and *R. delica*; cluster 4: *R. cyanoxantha*; cluster 5: *R. turci*; cluster 6: *R. risigallina* and *R. olivacea*) (Fig. 3).

The result of cluster multivariate analysis based on PCA and HCA techniques has proven to be a very useful method for investigating the variability of the metal composition of the studied mushroom species. As clearly shown, it offers the ability to identify clusters of certain types of mushrooms of similar composition.

Conclusions

According to the results obtained from this study, it was concluded that the consumption of *R. risigallina* and *R. olivacea* may have harmful effects due to the Cr content exceeding the reference DIM level and high HRI value. It was also thought that consumption of *R. velenovskyi*, which has a high HRI value in terms of Cd element, may pose a risk to human health.



	Pb	Cd	Zn	Fe	Mn	Cu	Cr	Ni	Co	Ca	Mg	Na	K
Pb	1	-0.23	0.29	0.07	0.18	0.26	-0.28	-0.11	0.11	-0.2	-0.12	-0.05	-0.03
Cd		1	0.51	-0.17	-0.18	0.42	-0.02	0.08	-0.31	-0.07	0.74	-0.16	0.67
Zn			1	-0.53	-0.43	0.47	-0.23	-0.02	-0.39	0.04	0.8	-0.15	0.91
Fe				1	0.67	0.27	0.31	0.28	0.53	-0.18	-0.31	0.54	-0.4
Mn					1	0.19	0.18	0.12	0.24	0.4	-0.11	0.56	-0.32
Cu						1	0.02	0.37	0.08	0.23	0.55	0.52	0.57
Cr							1	0.91	0.82	-0.08	-0.04	0.67	-0.02
Ni								1	0.8	0	0.1	0.77	0.17
Co									1	-0.34	-0.44	0.61	-0.34
Ca										1	0.35	0.42	0.21
Mg											1	0.12	0.95
Na												1	0.08
K													1

Fig. 2 Principal component analysis outcomes. (**a**) Scree plot of percentage of explained variances and eigenvalue of each principal component. (**b**) Contribution of descriptors to the first four retained principal component. (**c**) relationship between the descriptors

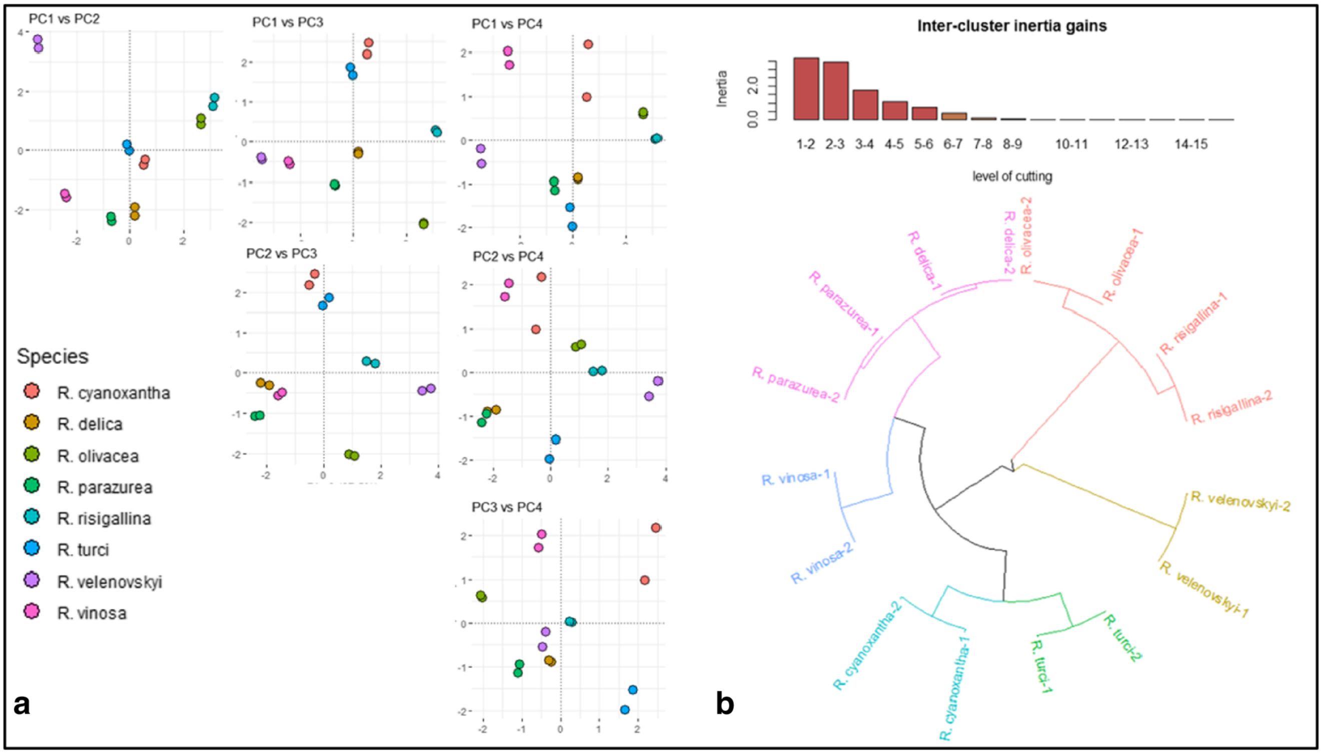


Fig. 3 A pairs Samples plot of PCA (comparison of PC1–PC4 on a pairwise basis) and circular dendogram derived from hierarchical clustering analysis

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information file.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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