forests

MDPI

Article

Bioaccumulation and Health Risk Assessment of Nickel Uptake by Five Wild Edible Saprotrophic Mushroom Species Collected from Croatia

Ivan Širić [1], Katarina Rukavina [1], Boro Mioč [1], Valentino Držaić [1], Pankaj Kumar [2,*], Mostafa A. Taher [3] and Ebrahem M. Eid [4,5]

[1] University of Zagreb, Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia
[2] Agro-Ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to Be University), Haridwar 249404, India
[3] Biology Department, Faculty of Science and Arts, King Khalid University, Mohail Assir 61321, Saudi Arabia
[4] Botany Department, Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt
[5] Biology Department, College of Science, King Khalid University, Abha 61321, Saudi Arabia
* Correspondence: rs.pankajkumar@gkv.ac.in

Abstract: The current study investigates the bioaccumulation potential of the heavy metal nickel (Ni) in five edible saprotrophic mushroom species (*Agaricus campestris* L., *Armillaria mellea* (Vahl) P.Kumm., *Clitocybe inversa* (Sowerby) Vizzini, *Clitocybe nebularis* (Batsch), P.Kumm., and *Macrolepiota procera* (Scop.) Singer) collected from seven forest locations (Trakoscan, Medvednica, Petrova gora, Skrad, Krk, Labinstina, and Motovun) of Croatia. For this purpose, forest soil and mushroom samples (cap and stipe) were collected from January to December 2021 and analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES). The results showed that all soil samples showed an occurrence of Ni, ranging from 1.62 to 3.55 mg/Kg. On the other hand, the cap parts of all five mushroom species showed a higher Ni content as compared to those in the stipe parts. Overall, there is a substantial positive association between soil Ni availability and the mean Ni concentration (mg/Kg) in the cap and stipe parts of *A. campestris* (3.08 and 2.22), *A. mellea* (2.59 and 1.55), *C. inversa* (2.38 and 1.75), *C. nebularis* (2.56 and 1.91), and *M. procera* (2.94 and 1.94). Multivariate analyses using principal component analysis (PCA) and hierarchical cluster analysis (HCA) showed that the Skrad and Petrova gora locations had the highest Ni contents in the selected mushroom species. Moreover, the estimated daily intake of Ni from consuming these mushrooms was below the threshold limits as suggested by dietary intake modeling (DIM) and health risk index (HRI) values. Therefore, this study emphasizes the importance of examining the Ni bioaccumulation potential of wild edible mushrooms, as well as the health hazards associated with their consumption, which are useful for food safety rules and recommendations.

Keywords: bioaccumulation; forest fungi; health risk; heavy metals; metal elements; wild edible mushrooms

Citation: Širić, I.; Rukavina, K.; Mioč, B.; Držaić, V.; Kumar, P.; Taher, M.A.; Eid, E.M. Bioaccumulation and Health Risk Assessment of Nickel Uptake by Five Wild Edible Saprotrophic Mushroom Species Collected from Croatia. *Forests* **2023**, *14*, 879. https://doi.org/10.3390/f14050879

Academic Editor: Cate Macinnis-Ng

Received: 27 March 2023
Revised: 15 April 2023
Accepted: 23 April 2023
Published: 25 April 2023

1. Introduction

Mushrooms are spores forming fruiting bodies of fungi that are found in moist habitats such as forests and grasslands [1]. Saprotrophic mushrooms are those that grow on decaying or dead plant material. They are a sort of decomposer that aids in the breakdown of dead organic matter and the restoration of critical nutrients to the soil. They are necessary for a healthy and balanced forest ecosystem. Fungi produce several enzymes that help in the breakdown of complex organic substances such as lignin, cellulose, and chitin [2]. Thus, numerous soil activities, such as nutrient cycling, the breakdown of organic matter, and the development of soil structure, depend heavily on fungi [3]. Wild mushroom foraging is the practice of searching for, identifying, and collecting wild mushrooms in

their natural habitat for culinary or medicinal purposes [4]. It is a centuries-old activity that has been practiced in many different cultures around the world [5]. Foragers widely collect several mushroom species including, chanterelles, truffles, morels, shiitake, button mushrooms, oyster mushrooms, porcini, and hedgehog mushrooms [6]. Additionally, mushroom foraging can be a rewarding and educational experience, as it provides an opportunity to learn about the ecology and biology of fungi [7].

Nickel (Ni; atomic number 28, mass: 58.6934 u) is the sixth most abundant metal element on earth. Most often, Ni is found in combination with other mineral ores (pentlandite, millerite, nickeline, and galena) [8]. Ni is found in five natural isotopes, including Ni-58, Ni-60, Ni-62, and Ni-64 [9]. In addition, Ni occurs abundantly in soils in both soluble (chlorides and nitrates) and insoluble forms (oxides and sulfides) [10]. Being an essential micronutrient, Ni plays an important role in the proper functioning of cells in organisms, including microbes, fungi, plants, animals, and humans [11]. Ni acts as an important element for active sites of enzymes such as glyoxalase, urease, superoxide dismutase (SOD), hydrogenase, dehydrogenase, dioxygenase, etc. [12]. Therefore, Ni plays an important role in mediating different cellular mechanisms involved in oxygen transport, synthesis, fixation, and energy metabolism [13]. Low levels of Ni may result in inefficient cellular growth and deformities [14]. Despite the fact that Ni is an essential micronutrient for many life forms, it has several toxic effects at higher concentrations [15]. Some forms of Ni may be acutely toxic to living organisms if exposure duration and the dose are high [16].

Similar to other organisms, Ni is an essential trace element for the growth of mushrooms as it plays an important role in proper enzyme activity, cell wall formation, and metabolism [17]. Ni ions also aid in the scavenging of nitrogen from the environment and the formation of protective structures in fungal cells [18]. Ni uptake by mushrooms is a complex process in which multiple factors influence the amount of Ni absorbed from the soil or substrates on which they grow [19]. Ni toxicity in mushrooms occurs when soil or substrate contains excessive Ni content which is then absorbed and bioaccumulated by vegetative parts of the mushroom including cap, stipe, gills, volva, etc. through their mycelial network [20]. Generally, mushrooms absorb more Ni when grown in a medium deficient in other essential nutrients like phosphorus and potassium, or a medium with a low pH. Thus, Ni contents in forest mushrooms may vary significantly based on species, soil, substrate, climatic conditions, etc. [21]. Ni toxicity in forest mushrooms can be extremely detrimental to the health of consumers [22]. The sources of Ni pollution include industrial activities, mining, waste disposal, combustion of fossil fuels, etc. Global pollution with nickel is a concern due to its potential health and environmental impacts [15]. Consuming Ni-toxic foods can lead to symptoms such as nausea, vomiting, diarrhea, abdominal pain, headaches, and dizziness. In extreme cases, Ni toxicity can even cause liver and kidney failure [23]. Therefore, to avoid potential health risks, it is important to understand the bioaccumulation and risk associated with Ni uptake by edible saprotrophic mushroom species.

Nevertheless, there is a risk of Ni-contamination in wild saprotrophic mushrooms as it may affect consumer health. Considering the aforementioned, the present study focuses on the bioaccumulation and health risk assessment of Ni uptake by five wild edible saprotrophic mushroom species collected from Croatia. The risk associated with Ni intake was assessed by estimating the health risk index (HRI) and dietary intake modeling (DIM) indices.

2. Materials and Methods

2.1. Description of the Study Area

The present study was carried out in two regions of Croatia, including Croatia proper and Istria. The study area lies between 45.00 and 46.50° N (latitude), and 13.50 and 16.00° E (longitude). The samples were collected from three regions (with localities): central and northwestern Croatia (Trakoscan, Medvednica, Petrova gora); mountain Croatia (Skrad); Istria and coastal Croatia (Krk, Labinstina, and Motovun) as shown in Figure 1. The areas were selected based on the expert knowledge of local mycologists. The sampling design aimed

to cover a wide range of forest habitats, including lowland deciduous forests, colline and submontane deciduous forests, montane deciduous mesophilic forests, and ultramontane mixed (coniferous/deciduous) forests. These areas are easily accessible and mushroom foragers frequently visit these forests for the collection of wild edible mushrooms as compared to other areas. The three areas were chosen to understand the variability in different forest land types. The average climate of the three regions is considered to be temperate, with warm summers and cold-snowy winters. The Adriatic Sea provides Croatia with a mild climate, moderating temperatures, and plenty of rain throughout the year. The annual temperature and humidity of the regions varies in the range of 1.60–15.49 °C and 62.50%–75.16% with an annual rainfall of 1195–8275 mm [24]. Such a climate results in a humid environment, thereby facilitating the growth of several forest mushroom species.

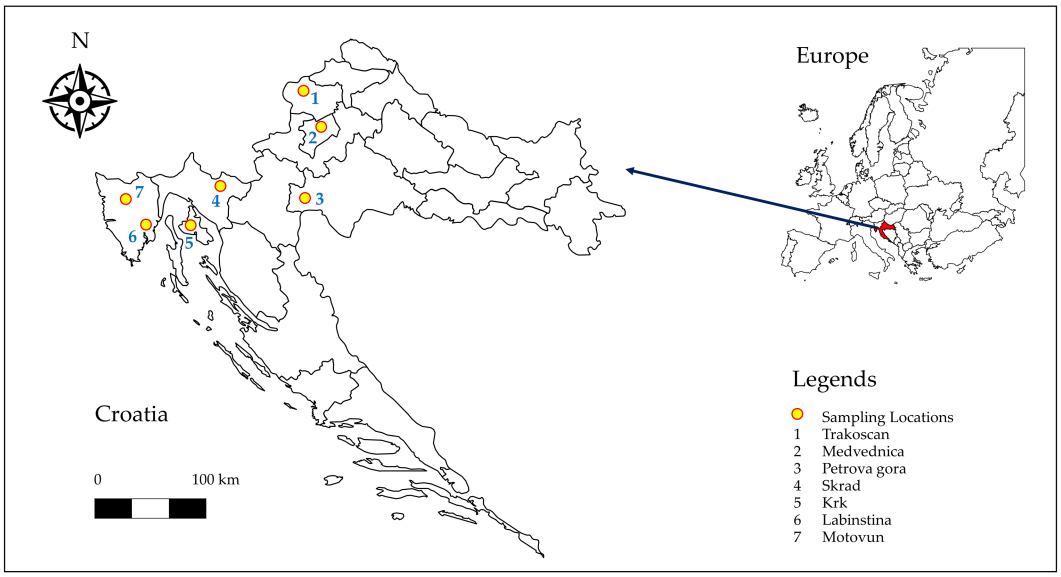

Figure 1. Map showing wild saprotrophic mushroom sampling locations.

2.2. Soil and Mushroom Sample Collection

For this study, the soil and mushroom samples were collected from several study locations across Croatia from January to December 2021. The five mushroom species collected in this study were meadow mushroom (*Agaricus campestris* L.), honey fungus (*Armillaria mellea* a (Vahl) P.Kumm.), funnel-shaped clitocybe (*Clitocybe inversa* (Sowerby) Vizzini), clouded agaric (*Clitocybe nebularis* (Batsch), P.Kumm.), and parasol mushroom (*Macrolepiota procera* (Scop.) Singer)). These foraged edible wild mushrooms are widely available in Croatia and are a favorite ingredient in the local cuisine. Even though they are generally regarded as safe to eat, it is crucial to correctly identify them before doing so because some species might be hazardous. It is also crucial to keep in mind that some mushrooms can accumulate dangerous heavy metals, including Ni, which can be harmful to your health if ingested in high amounts. Purposely, a total of ten (n = 10) separate soil and mushroom samples were collected for each species. For the mushroom sample collection, a sterile blade and tweezer were used to cut them near the volva part. Afterward, the stipe and cap parts were cut and placed in separate zip-locking clean plastic bags. For soil, the samples were obtained from the top 15 cm of the soil surface using a shovel and then placed in a clean plastic bag. All samples were collected between 10: AM and 4:00 PM (GMT+2), and morphologically identified based on shape, size, color, appearance, texture, stems, gills, and spores following the online identification guide (https://www.mushroom.world/mushrooms/list (accessed on 30 January 2021)). The mushroom and soil bags were labeled with the sample type, site and replicate number, date and time of sample collection, and sample depth [2]. The samples were immediately transferred to the laboratory and preserved at 4 °C until further analysis.

2.3. Nickel Analysis Using ICP-OES

Before the analysis, the soil samples were sifted to remove larger particles and the resulting soil was oven-dried at 105 °C for 1 h. For the Ni analysis, 1 g of the soil sample was mixed with 10 mL of a di-acid mixture in a 100 mL conical flask and digested by placing it on a hot plate for 2 h. After digestion, the volume of the contents was adjusted to 50 mL by the addition of 3% HNO_3 solution. Then, the sample was filtered (Whatman number 41) and finally used for Ni analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES, ICP-OES, Optima 8000, Perkin Elmer, SAD, Waltham, MA, USA). Similarly, the mushroom samples were also oven dried at 60 °C until constant biomass was achieved and then converted into powder form using a mechanical grinder (MSG-01; MPM Product, Milanówek, Poland, and Ultra FD1000 dehydrator, Ezidri, Australia). The mushroom samples were also digested using a di-acid mixture and analyzed for Ni content. A calibration verification check of the ICP-OES was performed with certified reference materials (CRM) to demonstrate the accuracy and precision of the analysis as previously outlined by Kalogiouri et al. [25] A total of two samples covering the range of analysis (99.50%–101.03% Ni recovery) were used for quality assurance of the obtained results. The results of the Ni content in soil and mushroom samples were expressed as mg/Kg (dwt.).

2.4. Bioaccumulation and Health Risk Calculation

The relationship between Ni content in the forest soil and its uptake by wild saprotrophic mushrooms can be calculated by the bio-sediment accumulation factor (BSAF) index [26]. A value greater than one (>1) represents mushrooms that have accumulated Ni from the soil. In this study, BSAF was calculated to understand the Ni bioaccumulation efficiency of the selected wild saprotrophic mushroom species by using the formula given in Equation (1).

$$BSAF = Ni_{mushroom}/Ni_{soil} \quad (1)$$

On the other hand, dietary intake modeling (DIM) is a widely used method for calculating the risk of heavy metal exposure through dietary intake. This method utilizes food consumption data, such as heavy metal exposure to individuals of a particular age, weight, and duration [27]. Similarly, the health risk index (HRI) is an intensive tool that measures the potential risk factor associated with an individual's heavy metal exposure. HRI utilizes data from DIM and a value of >1 indicates a potential health hazard of consuming such foods [28]. In this study, DIM and HRI tools were used to evaluate the health risk of consuming Ni-contaminated wild saprotrophic mushrooms. For this, Equations (2) and (3) were used for the computation of DIM and HRI, respectively.

$$DIM = IR \times HMC \times CF/BW \quad (2)$$

$$HRI = DIM/RfD \quad (3)$$

where IR, HMC, CF, and BW refer to mushroom "Ingestion Rate" (30 g dried mushroom per person per day), "Heavy Metal Concentration" in the mushroom (mg/Kg dwt.), dry-to-fresh weight "Conversion Factor", and average "Body Weight" of consumer (70 kg), respectively. In HRI, RfD refers to safe levels of Ni oral "Reference Dose" exposure for a lifetime, as outlined by Sarikurkcu et al. [27].

2.5. Data Analysis

The data obtained in this study were analyzed using Microsoft Excel (Version 2019, Microsoft Corp., Redmond, WA, USA) and OriginPro (Version 2023, OriginLab, Wellesley Hills, MA, USA) software packages. Before the data analysis, the normality distribution was checked using the Shapro-Wilk normality test, which showed that the data was normally distributed, thus suggesting the use of parametric statistical tests. The data was processed using one-way Tukey's post-hoc analysis of variance (ANOVA, San Francisco, CA, USA), Pearson correction, principal component analysis (PCA), and hierarchically

clustered heatmap tools. The level of statistical significance was $p < 0.05$. An ANOVA test was performed to understand the significant differences between the mean concentrations in the selected mushroom species. The Pearson correlation was used to measure the linear correlation between the Ni concentrations in selected mushroom species with respect to their soil environments. PCA was used to reduce the dimensionality of the data to understand the most important factors contributing to the Ni concentrations in the selected wild edible saprotrophic mushrooms. Finally, cluster analysis was used to create a heatmap to visualize patterns of similarities between different mushroom species, their vegetative parts (caps and stipes), and sampling locations.

3. Results and Discussion

3.1. Results of Ni Content in Soil

The analysis of Ni in the soil collected from selected forest regions of Croatia showed that the Ni concentration varied across the sampling locations. The boxplots given in Figure 2 depict the range of Ni concentrations (mg/Kg) in the soil samples collected from the seven locations in Croatia. In this, the average Ni contents in the soils of selected regions i.e., Trakoscan, Medvednica, Petrova gora, Skrad, Krk, Labinstina, and Motovun were observed as 2.94 ± 0.42, 3.00 ± 0.35, 2.93 ± 0.46, 2.97 ± 0.19, 2.88 ± 0.20, 2.90 ± 0.13, and 2.65 ± 0.52 mg/Kg, respectively. However, the highest concentrations of Ni were observed in the soils of Medvednica (2.47–3.55 mg/Kg) while the lowest Ni contents were found in the soils of Motovun (1.62–3.33 mg/Kg). The highest concentration of Ni in the study area was detected in one site in northern Croatia. This could be due to increased urban and industrial activities in the region or to localized pollution sources.

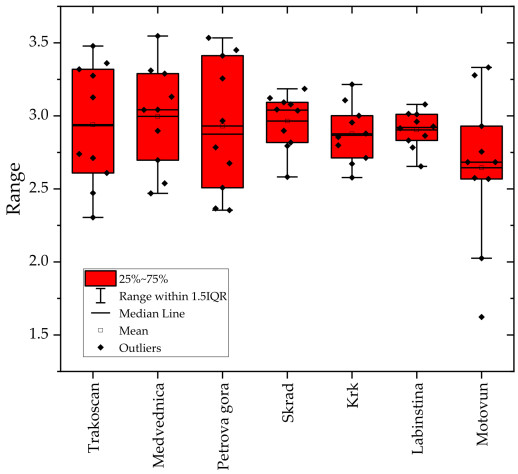

Figure 2. Boxplot showing the range of Ni concentration (mg/Kg) in soil samples (n = 10) collected from seven locations in Croatia.

As outlined by Michopoulos [29], the forest soil near the human settlements has higher Ni deposition as compared to that from non-affected areas. Barcan [30] confirmed that the levels of Ni and Cu in forest soils can be triggered by metallurgical dust deposition. Similarly, Pelkonen et al. [31] showed that Ni levels in the forest soils of Finland reached 6–40 mg/Kg which is much higher than those obtained in the current study. Overall, the results of the study demonstrate that Ni pollution in the forest soils of Croatia is generally low, with concentrations below the maximum allowable concentration of 40 mg/Kg [32]. Further research is needed to determine the specific sources and causes of Ni contamination in this area.

3.2. Results of Ni Contents in Selected Mushroom Species

The majority of the wild edible saprotrophic Croatian mushroom species belong to the class Basidiomycotina [2]. The mushroom species studied in this project are *A. campestris*,

A. mellea, *C. inversa*, *C. nebularis*, and *M. procera*. Results of Ni analysis in cap and stipe parts of these mushroom species are given in Table 1. The results showed that, in particular, the Ni contents in the cap and stipe parts of *A. campestris* ranged from 1.85 to 3.91 mg/Kg and 1.39 to 2.94 mg/Kg with a mean concentration of 3.08 and 2.22 mg/Kg, respectively. Similarly, the Ni contents in *A. mellea* were noted as 1.85–3.28 mg/Kg in the cap, 1.01–2.06 in the stipe with overall mean values of 2.59 and 1.55 for the cap and stipe regions, respectively. For *C. inversa*, the range of Ni contents in cap and stipe parts was noted as 1.22–3.28 and 0.91–1.74 mg/Kg with a mean concentration of 2.38 and 1.75 mg/Kg, respectively. Likewise, the Ni contents in *C. nebularis* ranged from 1.83–2.50 (with a mean value of 2.56) and 1.20–1.96 (with a mean value of 1.91) mg/Kg for cap and stipe parts. In *M. procera*, the Ni contents were determined as 2.39–4.39 (with a mean value of 2.94) and 1.15–1.90 (with a mean value of 1.94) mg/Kg for cap and stipe parts, correspondingly. Overall, the ANOVA test results showed that there were significant ($p < 0.05$) differences in the Ni concentrations in cap and stipe parts of the selected mushroom species. It was observed that the highest mean values for Ni were observed in both cap and stipe parts of *A. campestris* followed by *M. procera*, *A. mellea*, *C. nebularis*, and *C. inversa*. Based on the sampling locations, it was observed that the highest Ni contents were observed at Trakoscan, while minimum levels were detected in the case of Skrad. A low Ni content in these mushrooms is likely due to the fact that Ni is a trace essential element for mushroom growth and development. In addition, the Ni content in these five mushroom species may be influenced by the soil. As the Ni content of the soil or growing medium can vary, this could affect the amount of Ni taken up and accumulated by the mushrooms. A higher Ni uptake by cap parts indicates successful translocation of metal ions from fungal hyphae to the upper mushroom body as compared to those in the case of stipe which acts as a pileus supporting part, nutrient/water supply organ, and elevates the cap above the ground [22].

Table 1. Ni contents in cap and stipe parts of five mushroom species collected from seven locations in Croatia.

Location	Parts	Ni Concentration (mg/Kg)				
		A. campestris	***A. mellea***	***C. inversa***	***C. nebularis***	***M. procera***
Trakoscan	Cap	3.55 (3.48–3.62)	2.71 (2.65–2.79)	2.49 (2.43–2.58)	2.71 (2.63–2.78)	3.51 (3.47–3.57)
	Stipe	2.57 (2.44–2.66)	1.91 (1.75–2.06)	1.66 (1.60–1.75)	1.82 (1.72–1.96)	2.37 (2.27–2.42)
Medvednica	Cap	3.23 (2.67–3.89)	2.76 (2.26–3.28)	2.70 (2.13–3.28)	2.89 (2.60–3.20)	2.99 (2.39–3.49)
	Stipe	2.39 (1.99–2.94)	1.45 (1.16–2.03)	1.77 (1.23–1.75)	2.08 (1.54–3.22)	1.56 (1.15–2.04)
Petrova gora	Cap	2.91 (2.38–3.40)	2.79 (1.91–2.90)	2.49 (2.14–3.03)	2.68 (2.24–3.26)	2.85 (2.39–4.39)
	Stipe	2.28 (1.94–2.87)	1.47 (1.25–1.79)	1.72 (1.21–2.10)	2.00 (1.57–2.26)	1.56 (1.21–1.95)
Skrad	Cap	2.29 (1.85–2.61)	2.42 (1.91–2.90)	1.55 (1.22–1.94)	2.52 (2.04–2.95)	2.72 (2.40–3.16)
	Stipe	1.70 (1.39–2.03)	1.80 (1.46–2.16)	1.17 (0.91–1.74)	1.66 (1.20–2.02)	1.49 (1.18–1.90)
Krk	Cap	3.06 (2.74–3.40)	2.69 (2.29–3.03)	2.49 (2.05–2.82)	2.49 (2.03–2.90)	2.78 (2.39–3.24)
	Stipe	2.28 (2.01–2.46)	1.34 (1.01–1.57)	2.00 (1.61–2.21)	2.05 (1.62–2.51)	2.23 (1.91–2.61)
Labinstina	Cap	3.49 (3.14–3.91)	2.50 (2.23–2.73)	2.50 (2.15–2.76)	2.41 (2.02–2.50)	2.85 (2.60–3.24)
	Stipe	2.27 (2.01–2.67)	1.36 (1.00–1.69)	1.96 (1.67–2.36)	1.93 (1.62–2.21)	2.40 (2.01–2.70)
Motovun	Cap	3.03 (2.70–3.39)	2.25 (1.85–2.61)	2.43 (2.02–2.78)	2.22 (1.83–2.50)	2.89 (2.41–3.15)
	Stipe	2.06 (1.71–2.37)	1.56 (1.24–1.80)	1.93 (1.51–2.36)	1.82 (1.46–2.10)	2.00 (1.62–2.25)
Mean ± SD	Cap	3.08 ± 0.42 bc	2.59 ± 0.20 ab	2.38 ± 0.38 ab	2.56 ± 0.22 ab	2.94 ± 0.27 bc
	Stipe	2.22 ± 0.28 bc	1.55 ± 0.22 ab	1.75 ± 0.28 ab	1.91 ± 0.15 bc	1.94 ± 0.40 bc
Median	Cap	3.06	2.69	2.49	2.52	2.85
	Stipe	2.28	1.47	1.77	1.93	2.00

Values are the mean of ten samples ($n = 10$) followed by minimum and maximum values; a–c: the same letters indicate no significant difference between mushroom species at $p < 0.05$.

Previously, Muñoz et al. [33] studied Ni uptake by the *Agaricus bisporus* mushroom and found that different types of cells present in caps and stalks parts contribute to varied uptake of the Ni contents. They found that Ni accumulation in the cap part was higher than those in stalks. This might be because the cap serves as the main site for the transfer of nutrients, water, and other materials from the soil to the fruiting body. Heavy metals, which are frequently found in the soil and that the mushroom can absorb, frequently assemble in the cap as a result. Similarly, Širić et al. [2] also observed that Ni contents in ten ectomycorrhizal mushrooms were recorded between 2.34 and 3.62 mg/Kg, where *A. campestris* had the highest content. Moreover, Zhu et al. [34] studied the Ni contents in 14 wild edible mushrooms and found that all samples had significant contents of Ni ranging from 0.76 to 5.1 mg/Kg, which is in accordance with the limit observed in the present study. Another report by Mititelu et al. [35] revealed that the soil Ni levels have a significant impact on its uptake by two wild-growing mushroom species (0.32–0.71 mg/Kg for *Boletus* sp. and 0.22–0.68 mg/Kg for *Hymenochaete* sp.). Thus, the results of these reports are in line with those obtained in the present study, supporting the normal levels of Ni occurring in the wild edible mushroom species.

3.3. Results of Multivariate Analyses

Multivariate analysis is a powerful tool for understanding large data sets [36]. Results of the multivariate analyses using PCA are given in Figure 3a,b. Based on the PCA results, a total of two principal components, namely PC1 and PC2, with eigenvalues of 1.74 and 0.23, and variances of 80.96 and 10.86% were used to create a biplot matrix (Table 2). As given in Figure 3b, the first two PCs were most suitable for interpreting the study data. The PCA analysis of Ni in the five wild edible mushrooms revealed that Ni had the highest loading score for Medvednica, Petrova gora, and Skrad locations, indicating that they are the locations with the highest Ni contents in the overall composition of the samples. This is likely due to the fact that Ni in the soil of these locations is more abundant and thus more likely to be found in mushrooms. The loadings for other sampling locations were also significant but not as high as for these locations. In this, the highest loading values were noted for *A. mellea* (cap), *C. nebularis* (cap), and *M. procera* (cap). A large variation in the occurrence of Ni in the cap and stipe regions of selected mushroom species might be due to differences in their physiology, habitat, nutrient requirement, etc. On the other hand, the result of cluster analysis showed that sampling locations had significant similarities in terms of Ni contents in cap and stipe parts of the selected mushroom species (Figure 4). In this, it was observed that Krk-Labinstina and Medvednica-Petrova gora formed similar clusters which indicated that Ni contents in the mushrooms collected from these locations showed significant similarities. However, the Ni content similarities in the cap parts were higher than those in the stipe parts, as revealed by the clustered heatmap analysis. The similarity is less pronounced for the *A. campestris* and *M. procera* species, with both having similar concentrations of Ni in both parts. The hierarchical clustering results suggest that the Ni concentrations in the cap and stipe parts of the five mushroom species are highly correlated. This is an indication that the same environmental conditions or factors are likely to influence the Ni levels in both parts of the mushroom.

A report by Dimitrijevic et al. [37] explored the heavy metal concentrations in eleven mushroom species of the Boletaceae family. They used PCA and clustering methods in order to simplify the understanding of heavy metal availability. They concluded that the Ni contents were dependent on mushroom species. In their study, Cvetkovic et al. [38] found that the PCA and cluster tools were helpful in understanding the elemental composition of four wild edible mushrooms (*Marasmius oreades*, *Cantarellus cibarius*, *Boletus edulis*, and *Lactarius piperatus*) collected from Serbia. They covered a 95.51% cumulative variance in the first two components (PC1 and PC2), which is in line with results obtained in the current study.

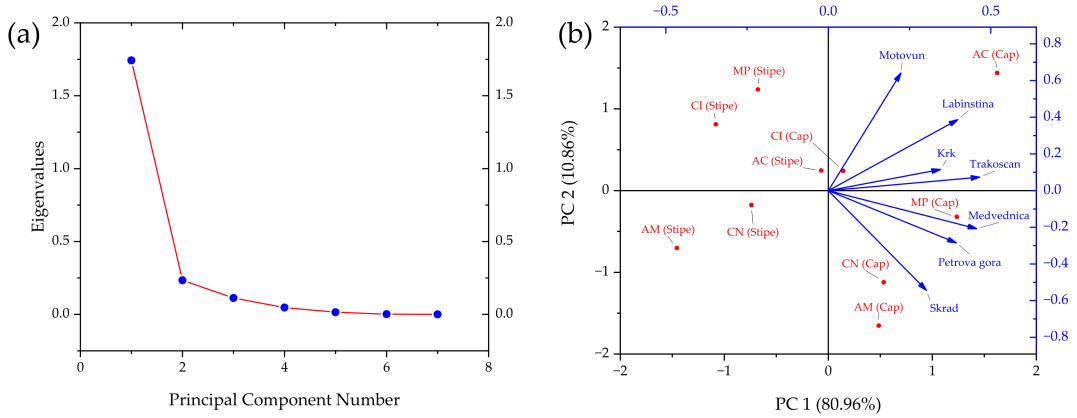

Figure 3. PCA scree (**a**) and biplot (**b**) for Ni concentration (mg/Kg) in cap and stipe parts of five mushroom species (AC: *A. campestris*; AM: *A. mellea*; CI: *C. inversa*; CN: *C. nebularis*; MP: *M. procera*) collected from seven locations of Croatia.

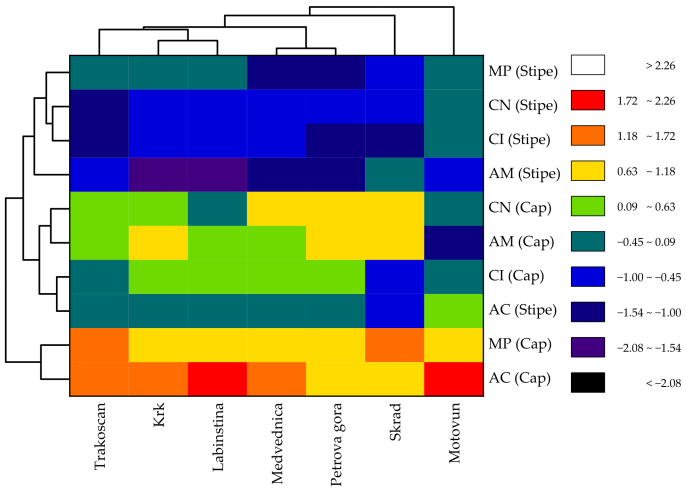

Figure 4. Hierarchically clustered heatmap for similarities in Ni concentration (mg/Kg) in cap and stipe parts of five mushroom species (AC: *A. campestris*; AM: *A. mellea*; CI: *C. inversa*; CN: *C. nebularis*; MP: *M. procera*) collected from seven locations of Croatia.

Table 2. PCA loading matrix for Ni concentration (mg/Kg) in cap and stipe parts of five mushroom species (AC: *A. campestris*; AM: *A. mellea*; CI: *C. inversa*; CN: *C. nebularis*; MP: *M. procera*) collected from seven locations of Croatia.

Variables	Principal Components	
	PC1	PC2
Eigenvalues	1.74	0.23
Variance (%)	80.96	10.86
AC (Cap)	1.62	1.43
AC (Stipe)	−0.06	0.24
AM (Cap)	0.48	−1.65
AM (Stipe)	−1.45	−0.70
CI (Cap)	0.14	0.24
CI (Stipe)	−1.08	0.81
CN (Cap)	0.53	−1.12
CN (Stipe)	−0.73	−0.17
MP (Cap)	1.23	−0.32

Table 2. *Cont.*

Variables	Principal Components	
	PC1	PC2
MP (Stipe)	−0.67	1.23
Trakoscan	0.46	0.07
Medvednica	0.45	−0.20
Petrova gora	0.39	−0.28
Skrad	0.30	−0.54
Krk	0.34	0.11
Labinstina	0.39	0.38
Motovun	0.22	0.64

3.4. Results of Ni Bioaccumulation and Correlation Analyses

The bioaccumulation of Ni by fungi is a natural process that helps in nutrient recycling [39]. In this study, it was observed that the selected wild saprotrophic mushroom species had a good potential for accumulating Ni contents in both cap and stipe parts. As given in Figure 5, the values of the BSAF were significantly higher in the cap than that in stipe parts. Specifically, the BSAF values exceed the level of unity (>1) in two mushrooms, i.e., *A. campestris* and *M. procera*. Overall, the BSAF values were a maximum at the Trakoscan location, whereas the lowest BSAF value was recorded for Skrad, which is in line with the results of the Ni contents in fruiting bodies of selected mushrooms. The results suggest that *A. campestris* and *M. procera* are most efficient in Ni accumulation from the forest soil, therefore, a higher contribution to soil remediation. Furthermore, the higher BSAF in the caps versus the stipes suggests that the fungal caps may be better suited for Ni accumulation. Moreover, the influence of soil Ni levels on their uptake by five wild saprotrophic mushrooms was studied using a Pearson correlation analysis. As given in Figure 6, a moderately positive (r = 0.20 to 0.80) or negative (r = −0.20 to −0.40) correlation between the Ni concentration in the soil and mushroom parts ($p < 0.05$). In addition, a non-significant correlation suggests that the Ni concentrations in both parts (caps and stipe) of the mushroom species are related, but are not necessarily interdependent. The correlation coefficient was positive for all mushrooms except for the stipe regions of *C. inversa* and *M. procera*. These results indicate that Ni concentrations in the caps and stipes of the five mushroom species growing in forest soil are moderately related. This could be because both parts of the mushroom are subjected to the same environmental conditions, such as soil composition and nutrient availability, which may influence Ni concentrations in both parts [40]. Overall, the moderate correlation between Ni concentrations in the mushroom parts suggests that they could be useful in monitoring Ni levels in the forest.

Bioaccumulation of Ni in wild edible mushrooms is widely explored by several researchers [41–43]. Out of them, Mazurkiewicz and Podlasińska [41] investigated Ni bioaccumulation in wild-growing edible mushrooms (*B. edulis* and *M. procera*) collected from Poland. They reported that BSAF values of Ni ranged from 0.8 to 8.3 in the cap while 1.0 to 8.0 in the stipe region. Similarly, Isildak et al. [42] also studied the BSAF of Ni in six wild edible mushroom species (*A. bisporus*, *Russula delica*, *Tricholoma terreum*, *B. badius*, *Lepista nuda*, and *Verpa conica*). They observed that the Ni contents in fruiting bodies of selected mushrooms were positively correlated (r = 0.06 to 0.40) with its concentration available in the soil. In the same vein, Giannaccini et al. [43] found that the contents of Ni in *B. edulis* and *M. procera* mushrooms showed a significant correlation with soil Ni content. Therefore, it was evidenced that the BSAF and correlation studies are useful tools for understand the metal element uptake behavior of wild edible mushrooms.

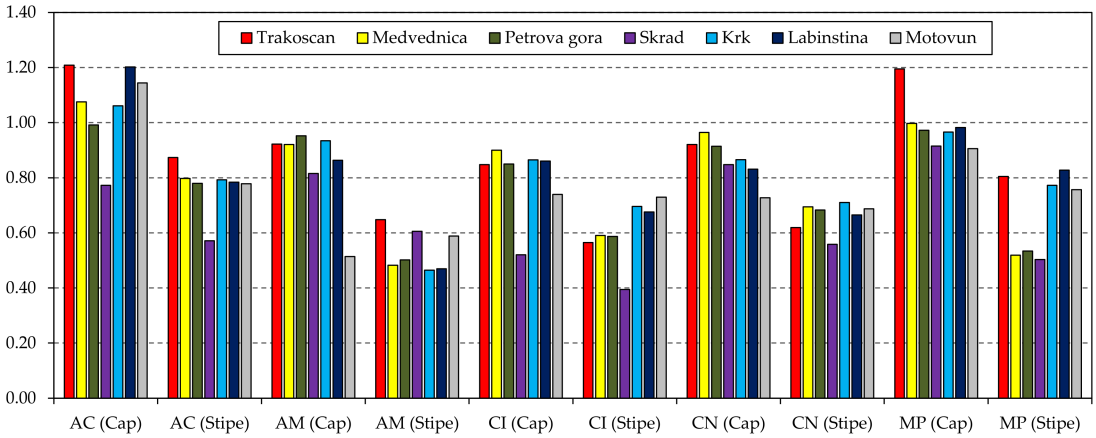

Figure 5. Bio-sediment accumulation factor (BSAF) of Ni in five mushroom species (AC: *A. campestris*; AM: *A. mellea*; CI: *C. inversa*; CN: *C. nebularis*; MP: *M. procera*) collected from seven locations in Croatia.

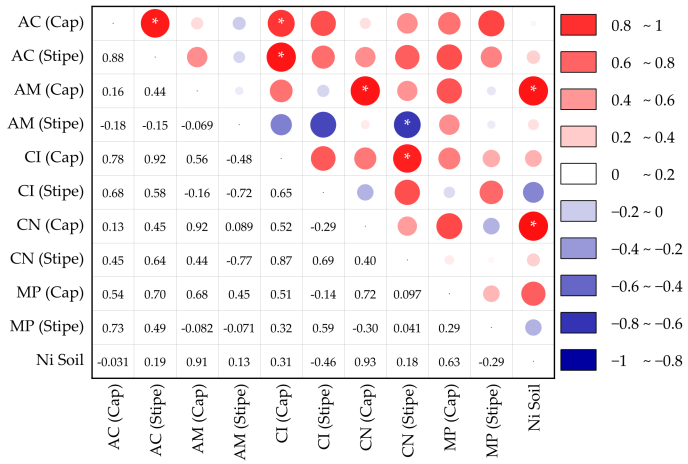

Figure 6. Pearson correlation matrix for Ni concentration (mg/Kg) in soil, cap, and stipe parts of five mushroom species (AC: *A. campestris*; AM: *A. mellea*; CI: *C. inversa*; CN: *C. nebularis*; MP: *M. procera*) collected from seven locations of Croatia (* significant at $p < 0.05$).

3.5. Results of Health Risk Studies

Although Ni is an essential trace element required for maintaining several biochemical and physiological cellular processes, its exposure at higher levels may cause severe health issues [22]. Health risk studies are very important in order to understand potential health hazards associated with the consumption of heavy metal-contaminated wild edible mushrooms. HRI and DIM are two crucial tools used to evaluate the risks to consumers' health posed by consuming particular foods, such as wild mushrooms. While HRI is used to evaluate the potential health risks connected with a substance, DIM is specifically used to estimate the quantity of a specific substance that an individual is likely to consume over a specific amount of time [27]. In this study, HRI and DIM indices were used to understand the health impact of Ni exposure through the consumption of sampled wild edible mushrooms. Based on the daily intake amount of mushrooms (30 g dry weight), the results were compared to the standard levels of the European Union (EU) [44], U.S. Environmental Protection Agency (USEPA, Washington, DC, USA) [45], and the World Health Organization (WHO, Geneva, Switzerland) [46]. In this regard, Table 3 shows the HRI and DIM values of Ni (mg/Kg) in cap and stipe parts of the five mushroom species (collected from the seven locations in Croatia). It was depicted from the values of indices that Ni contents in selected mushrooms were safer as they did not exceed the maximum permissible limits of the EPA. In this, the maximum DIM was noted as 0.1295 in the cap

part of *A. campestris* at the Trakoscan location, whereas the minimum DIM value was 0.0427 in the stipe part of *C. inversa* at the Skrad location. The greater values of DIM and HRI at these locations might be due to the higher availability of Ni in the soil as its further uptake by fruiting bodies of selected wild edible mushrooms. However, the HRI values did not exceed the threshold limit of one, indicating that the consumption of collected wild edible mushrooms was safe. An HRI value equal to or less than one is considered safe as previously explained by Sarikurkcu et al. [47]. If the HRI is greater than one, it means that the estimated intake of Ni is higher than the safe level of exposure and that eating wild mushrooms with high levels of Ni may be harmful to one's health. It might be advised to limit or avoid consuming these mushrooms in such circumstances.

Table 3. Health Risk Index (HRI) and Dietary Intake Modeling (DIM) values for Ni (mg/Kg) in cap and stipe parts of five mushroom species (collected from seven locations in Croatia).

Mushroom spp.	Part	Index	Locations						
			Trakoscan	Medvednica	Petrova Gora	Skrad	Krk	Labinstina	Motovun
A. campestris	Cap	HRI	0.0026	0.0024	0.0021	0.0017	0.0022	0.0025	0.0022
		DIM	0.1295	0.1176	0.1058	0.0836	0.1113	0.1270	0.1105
	Stipe	HRI	0.0019	0.0017	0.0017	0.0012	0.0017	0.0017	0.0015
		DIM	0.0935	0.0872	0.0832	0.0618	0.0831	0.0828	0.0751
A. mellea	Cap	HRI	0.0020	0.0020	0.0020	0.0018	0.0020	0.0018	0.0016
		DIM	0.0988	0.1006	0.1016	0.0882	0.0981	0.0912	0.0821
	Stipe	HRI	0.0014	0.0011	0.0011	0.0013	0.0010	0.0010	0.0011
		DIM	0.0694	0.0527	0.0535	0.0655	0.0487	0.0496	0.0568
C. inversa	Cap	HRI	0.0018	0.0020	0.0018	0.0011	0.0018	0.0018	0.0018
		DIM	0.0908	0.0984	0.0907	0.0563	0.0908	0.0909	0.0886
	Stipe	HRI	0.0012	0.0013	0.0013	0.0009	0.0015	0.0014	0.0014
		DIM	0.0605	0.0645	0.0627	0.0427	0.0730	0.0714	0.0704
C. nebularis	Cap	HRI	0.0020	0.0021	0.0020	0.0018	0.0018	0.0018	0.0016
		DIM	0.0986	0.1054	0.0976	0.0917	0.0908	0.0878	0.0807
	Stipe	HRI	0.0013	0.0015	0.0015	0.0012	0.0015	0.0014	0.0013
		DIM	0.0663	0.0759	0.0729	0.0604	0.0745	0.0702	0.0663
M. procera	Cap	HRI	0.0026	0.0022	0.0021	0.0020	0.0020	0.0021	0.0021
		DIM	0.1280	0.1090	0.1038	0.0990	0.1014	0.1038	0.1051
	Stipe	HRI	0.0017	0.0011	0.0011	0.0011	0.0016	0.0017	0.0015
		DIM	0.0862	0.0567	0.0570	0.0544	0.0811	0.0874	0.0730

The DIM and HRI were previously used by Sarikurkcu et al. [27] for the estimation of 13 essential and non-essential elements present in the 19 wild mushrooms collected from the Mediterranean region of Turkey. They found that the HRI and DIM values for Ni in all mushrooms did not exceed the threshold limits except for *Auricularia auricula-judae*, which had DIM and HRI values of 16.89 and 3.38, respectively, which indicate a high health risk. In another study by Sarikurkcu et al. [47], wild mushroom samples were collected from human-affected and non-affected areas and assessed for HRI and DIM of eight heavy metals, including Ni. They found that the DIM and HRI values of Ni did not exceed the safe limits of USEPA [48]; however, the values in human-affected areas were higher than those reported in non-affected areas. Similarly, Badshah et al. [49] also evaluated the health risk of Ni in three wild edible mushroom species, i.e., *Morchella crassipes*, *M. pulchella*, and *M. eohespera*. Their results concluded that the DIM and HRI values for Ni in all three mushroom species were < one, indicating their consumption was safe. Overall, it was observed that DIM and HRI are useful tools in assessing the risk associated with Ni-contaminated wild edible mushrooms. These results may be further used for designing and preparing appropriate recommendations to limit or avoid consumption.

4. Conclusions

The results of this study concluded that Ni contents in collected soil and mushroom species (*A. campestris*, *A. mellea*, *C. inversa*, *C. nebularis*, and *M. procera*) sampled from seven locations in Croatia showed the occurrence of Ni at moderate levels. However, the cap parts of all mushrooms showed relatively higher Ni contents as compared to those in the stipe parts. The bio-sediment accumulation factor (BSAF) values approaching a level of one showed that wild edible mushrooms had a good potential for Ni uptake from the forest soils. Pearson correlation analysis showed that the Ni contents of mushrooms were positively correlated with the available Ni concentration in forest soil. Moreover, the multivariate studies were helpful in understanding the patterns of Ni distributions in wild mushroom species across Croatia. The health risk studies also showed that Ni contents in the cap and stipe parts of mushrooms did not exceed the safe limits. The results of this study can be utilized to help shape food safety standards and recommendations to reduce the danger of ingesting hazardous heavy metals. To build a more thorough framework for risk assessment, additional investigation is required to broaden the study's focus and evaluate the accumulation of other heavy metals in edible mushrooms from various geographic locations.

Author Contributions: Conceptualization, I.Š.; data curation, P.K.; formal analysis, I.Š.; funding acquisition, I.Š. and M.A.T.; investigation, I.Š.; methodology, I.Š.; resources, I.Š.; software, P.K.; supervision, I.Š.; validation, K.R., B.M., V.D., P.K., M.A.T. and E.M.E.; visualization, P.K.; writing—original draft, P.K.; writing—review and editing, I.Š., K.R., B.M., V.D., M.A.T. and E.M.E. All authors have read and agreed to the published version of the manuscript.

Funding: The University of Zagreb dedicated multi-year institutional funding of scientific and artistic activities. This research was funded by King Khalid University, grant number RGP.2/220/44.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The University of Zagreb dedicated multi-year institutional funding of scientific and artistic activities. The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University, Abha, Saudi Arabia, for funding this work through the Research Group Project under grant number RGP.2/220/44.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Li, H.; Tian, Y.; Menolli, N.; Ye, L.; Karunarathna, S.C.; Perez-Moreno, J.; Rahman, M.M.; Rashid, M.H.; Phengsintham, P.; Rizal, L.; et al. Reviewing the World's Edible Mushroom Species: A New Evidence-Based Classification System. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*, 1982–2014. [CrossRef] [PubMed]
2. Širić, I.; Humar, M.; Kasap, A.; Kos, I.; Mioč, B.; Pohleven, F. Heavy Metal Bioaccumulation by Wild Edible Saprophytic and Ectomycorrhizal Mushrooms. *Environ. Sci. Pollut. Res.* **2016**, *23*, 18239–18252. [CrossRef] [PubMed]
3. Wendiro, D.; Wacoo, A.P.; Wise, G. Identifying Indigenous Practices for Cultivation of Wild Saprophytic Mushrooms: Responding to the Need for Sustainable Utilization of Natural Resources. *J. Ethnobiol. Ethnomed.* **2019**, *15*, 1–15. [CrossRef]
4. Tsing, A. Arts of Inclusion, or, How to Love a Mushroom. *Aust. Humanit. Rev.* **2011**, *22*, 191–203. [CrossRef]
5. Bertelsen, C.D. *Mushroom: A Global History*; Reaktion Books: Clerkenwell, UK, 2013; ISBN 9781780231754.
6. Jones, B.; Jones, W.A. *The Deerholme Mushroom Book: From Foraging to Feasting*; TouchWood Editions: Victoria, BC, Canada, 2013; ISBN 9781771510042.
7. Tibuhwa, D.D. Wild Mushroom- an Underutilized Healthy Food Resource and Income Generator: Experience from Tanzania Rural Areas. *J. Ethnobiol. Ethnomed.* **2013**, *9*, 49. [CrossRef] [PubMed]
8. Morgan, J.W.; Anders, E. Chemical Composition of Earth, Venus, and Mercury. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 6973–6977. [CrossRef]
9. Cheltsov, A.N.; Sosnin, L.Y.; Khamylov, V.K. Centrifugal Enrichment of Nickel Isotopes and Their Application to the Development of New Technologies. *J. Radioanal. Nucl. Chem.* **2014**, *299*, 981–987. [CrossRef]
10. Begum, W.; Rai, S.; Banerjee, S.; Bhattacharjee, S.; Mondal, M.H.; Bhattarai, A.; Saha, B. A Comprehensive Review on the Sources, Essentiality and Toxicological Profile of Nickel. *RSC Adv.* **2022**, *12*, 9139–9153. [CrossRef] [PubMed]

11. Uchida, R.; Silva, J.A. Essential Nutrients for Plant Growth: Nutrient Functions and Deficiency Symptoms. *Plant Nutr. Manag. Hawaii's Soils* **2000**, *4*, 31–55.
12. Soetan, K.O.; Olaiya, C.O.; Oyewole, O.E. The Importance of Mineral Elements for Humans, Domestic Animals and Plants: A Review. *Afr. J. Food Sci.* **2010**, *4*, 200–222.
13. Saravanan, V.S.; Kumar, M.R.; Sa, T.M. Microbial Zinc Solubilization and Their Role on Plants. In *Bacteria in Agrobiology: Plant Nutrient Management*; Maheshwari, D., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 47–63. [CrossRef]
14. Welch, R.M. The Biological Significance of Nickel. *J. Plant Nutr.* **1981**, *3*, 345–356. [CrossRef]
15. Sidhu, P.; Garg, M.L.; Dhawan, D.K. Protective Role of Zinc in Nickel Induced Hepatotoxicity in Rats. *Chem. Biol. Interact.* **2004**, *150*, 199–209. [CrossRef]
16. Chakrabarti, S.K.; Bai, C. Role of Oxidative Stress in Nickel Chloride-Induced Cell Injury in Rat Renal Cortical Slices. *Biochem. Pharmacol.* **1999**, *58*, 1501–1510. [CrossRef] [PubMed]
17. Ważny, R.; Rozpądek, P.; Domka, A.; Jędrzejczyk, R.J.; Nosek, M.; Hubalewska-Mazgaj, M.; Lichtscheidl, I.; Kidd, P.; Turnau, K. The Effect of Endophytic Fungi on Growth and Nickel Accumulation in Noccaea Hyperaccumulators. *Sci. Total Environ.* **2021**, *768*, 144666. [CrossRef] [PubMed]
18. Gerwien, F.; Skrahina, V.; Kasper, L.; Hube, B.; Brunke, S. Metals in Fungal Virulence. *FEMS Microbiol. Rev.* **2018**, *42*, 1–21. [CrossRef] [PubMed]
19. Congeevaram, S.; Dhanarani, S.; Park, J.; Dexilin, M.; Thamaraiselvi, K. Biosorption of Chromium and Nickel by Heavy Metal Resistant Fungal and Bacterial Isolates. *J. Hazard. Mater.* **2007**, *146*, 270–277. [CrossRef]
20. Tel, G.; Çavdar, H.; Deveci, E.; Öztürk, M.; Duru, M.E.; Turkoğlu, A. Minerals and Metals in Mushroom Species in Anatolia. *Food Addit. Contam. Part B Surveill.* **2014**, *7*, 226–231. [CrossRef] [PubMed]
21. Nnorom, I.C.; Eze, S.O.; Ukaogo, P.O. Mineral Contents of Three Wild-Grown Edible Mushrooms Collected from Forests of South Eastern Nigeria: An Evaluation of Bioaccumulation Potentials and Dietary Intake Risks. *Sci. Afr.* **2020**, *8*, e00163. [CrossRef]
22. Doğan, H.H.; Şanda, M.A.; Uyanöz, R.; Öztürk, C.; Çetin, Ü. Contents of Metals in Some Wild Mushrooms: Its Impact in Human Health. *Biol. Trace Elem. Res.* **2006**, *110*, 79–94. [CrossRef]
23. Pereira, M.C.; Pereira, M.L.; Sousa, J.P. Evaluation of Nickel Toxicity on Liver, Spleen, and Kidney of Mice after Administration of High-Dose Metal Ion. *J. Biomed. Mater. Res.* **1998**, *40*, 40–47. [CrossRef]
24. Državni Hidrometeorološki Zavod Dhmz DHMZ—Državni Hidrometeorološki Zavod (The State Hydrometeorological Institute). Available online: https://meteo.hr/index.php (accessed on 24 March 2023).
25. Kalogiouri, N.P.; Manousi, N.; Mourtzinos, I.; Zachariadis, G.A. Multielemental Inductively Coupled Plasma–Optical Emission Spectrometric (ICP-OES) Method for the Determination of Nutrient and Toxic Elements in Wild Mushrooms Coupled to Unsupervised and Supervised Chemometric Tools for Their Classification by Species. *Anal. Lett.* **2022**, *55*, 2108–2123. [CrossRef]
26. Alonso, J.; Garcia, M.A.; Parez-Lopez, M.; Melgar, M.J. The Concentrations and Bioconcentration Factors of Copper and Zinc in Edible Mushrooms. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 180–188. [CrossRef]
27. Sarikurkcu, C.; Popović-Djordjević, J.; Solak, M.H. Wild Edible Mushrooms from Mediterranean Region: Metal Concentrations and Health Risk Assessment. *Ecotoxicol. Environ. Saf.* **2020**, *190*, 110058. [CrossRef] [PubMed]
28. Liu, B.; Huang, Q.; Cai, H.; Guo, X.; Wang, T.; Gui, M. Study of Heavy Metal Concentrations in Wild Edible Mushrooms in Yunnan Province, China. *Food Chem.* **2015**, *188*, 294–300. [CrossRef] [PubMed]
29. Michopoulos, P. Nickel in Forests—A Short Review on Its Distribution and Fluxes. *Folia Oecologica* **2021**, *48*, 205. [CrossRef]
30. Barcan, V. Leaching of Nickel and Copper from Soil Contaminated by Metallurgical Dust. *Environ. Int.* **2002**, *28*, 63–68. [CrossRef]
31. Pelkonen, R.; Alfthan, G.; Järvinen, O. *Cadmium, Lead, Arsenic and Nickel in Wild Edible Mushrooms*; Finnish Environment Institute: Helsinki, Finland, 2006; ISBN 9521122749.
32. Holmgren, G.G.S.; Meyer, M.W.; Chaney, R.L.; Daniels, R.B. Cadmium, Lead, Zinc, Copper, and Nickel in Agricultural Soils of the United States of America. *J. Environ. Qual.* **1993**, *22*, 335–348. [CrossRef]
33. Serafín Muñoz, A.H.; Corona, F.G.; Wrobel, K.; Soto, G.M.; Wrobel, K. Subcellular Distribution of Aluminum, Bismuth, Cadmium, Chromium, Copper, Iron, Manganese, Nickel, and Lead in Cultivated Mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*). *Biol. Trace Elem. Res.* **2005**, *106*, 265–277. [CrossRef] [PubMed]
34. Zhu, F.; Qu, L.; Fan, W.; Qiao, M.; Hao, H.; Wang, X. Assessment of Heavy Metals in Some Wild Edible Mushrooms Collected from Yunnan Province, China. *Environ. Monit. Assess* **2011**, *179*, 191–199. [CrossRef]
35. Mititelu, M.; Ghica, M.; Ionită, A.C.; Morosan, E. The Influence of Heavy Metals Contamination in Soil on the Composition of Some Wild Edible Mushrooms. *Farmacia* **2019**, *67*, 398–404. [CrossRef]
36. Širić, I.; Kumar, P.; Adelodun, B.; Abou Fayssal, S.; Bachheti, R.K.; Bachheti, A.; Ajibade, F.O.; Kumar, V.; Taher, M.A.; Eid, E.M. Risk Assessment of Heavy Metals Occurrence in Two Wild Edible Oyster Mushrooms (*Pleurotus* Spp.) Collected from Rajaji National Park. *J. Fungi* **2022**, *8*, 1007. [CrossRef]
37. Dimitrijevic, M.V.; Mitic, V.D.; Cvetkovic, J.S.; Stankov Jovanovic, V.P.; Mutic, J.J.; Nikolic Mandic, S.D. Update on Element Content Profiles in Eleven Wild Edible Mushrooms from Family Boletaceae. *Eur. Food Res. Technol.* **2016**, *242*, 1–10. [CrossRef]
38. Cvetkovic, J.S.; Mitic, V.D.; Stankov-Jovanovic, V.P.; Dimitrijevic, M.V.; Nikolic-Mandic, S.D. Elemental Composition of Wild Edible Mushrooms from Serbia. *Anal. Lett.* **2015**, *48*, 2107–2121. [CrossRef]
39. Moore, B.A.; Duncan, J.R.; Burgess, J.E. Fungal Bioaccumulation of Copper, Nickel, Gold and Platinum. *Miner. Eng.* **2008**, *21*, 55–60. [CrossRef]

40. Brown, P.H.; Dunemann, L.; Schulz, R.; Marschner, H. Influence of Redox Potential and Plant Species on the Uptake of Nickel and Cadmium from Soils. *Z. Für Pflanz. Und Bodenkd.* **1989**, *152*, 85–91. [CrossRef]
41. Mazurkiewicz, N.; Podlasińska, J. Bioaccumulation of Trace Elements in Wild-Growing Edible Mushrooms from Lubuskie Voivodeship, Poland. *Chem. Ecol.* **2014**, *30*, 110–117. [CrossRef]
42. Isildak, O.; Turkekul, I.; Elmastas, M.; Aboul-Enein, H.Y. Bioaccumulation of Heavy Metals in Some Wild-Grown Edible Mushrooms. *Anal. Lett.* **2007**, *40*, 1099–1116. [CrossRef]
43. Giannaccini, G.; Betti, L.; Palego, L.; Mascia, G.; Schmid, L.; Lanza, M.; Mela, A.; Fabbrini, L.; Biondi, L.; Lucacchini, A. The Trace Element Content of Top-Soil and Wild Edible Mushroom Samples Collected in Tuscany, Italy. *Environ. Monit. Assess* **2012**, *184*, 7579–7595. [CrossRef]
44. European Union Commission Regulation (EC) No 629/2008 of 2 July 2008 Amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. 2008. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:173:0006:0009:EN:PDF#:~:text=Commission%20Regulation%20(EC)%20No%201881,metals%20lead%2C%20cadmium%20and%20mercury.&text=It%20is%20essential%2C%20in%20order,do%20not%20cause%20health%20concerns (accessed on 10 March 2023).
45. U.S. EPA. Integrated Risk Information System US EPA. Available online: https://www.epa.gov/iris (accessed on 29 July 2022).
46. JECFA Zearalenone. *Safety Evaluation of Certain Food Additives and Contaminants*; Joint FAO/WHO Expert Committee on Food Additives, Ed.; World Health Organization: Geneva, Swizerland, 2000.
47. Sarikurkcu, C.; Sarikurkcu, R.T.; Akata, I.; Tepe, B. Metal Concentration and Health Risk Assessment of Fifteen Wild Mushrooms Collected from the Ankara University Campus (Turkey). *Environ. Sci. Pollut. Res.* **2020**, *27*, 32474–32480. [CrossRef]
48. U.S. EPA. A Review of the Reference Dose and Reference Concentration Processes. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, EPA/630/P-02/002F; 2002. Available online: https://www.epa.gov/osa/review-reference-dose-and-reference-concentration-processes (accessed on 10 March 2023).
49. Badshah, H.; Khan, M.U.; Mumtaz, A.S. Elucidating Heavy Metals Concentration and Distribution in Wild Edible Morels and the Associated Soil at Different Altitudinal Zones of Pakistan: A Health Risk Implications Study. *Biol. Trace Elem. Res.* **2022**, 1–14. [CrossRef]