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Evaluation of antioxidant activity, anti-lipid peroxidation effect and elemental impurity risk of some wild *Agaricus* species mushrooms

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Abstract

Background Mushrooms are natural antioxidant sources that have been consumed as food from past to present and have a nutraceutical effect thanks to the bioactive components they contain. The aim of this study is to comparatively evaluate the antioxidant activity, total phenolic content (TPC) and lipid peroxidation (LPO) inhibition effect of three mushroom species (*A. bernardii*, *A. bresadolanus and A. cupreobrunneus*) belonging to the *Agaricus* genus and to perform the carcinogenic and noncarcinogenic risk assessment of toxic elements such as cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) in mushrooms.

Results The highest antioxidant activity (12.85 mg/mL), TPC (993.04 mg GAE/100 g), and LPO inhibition effect (2.50 mg/mL) were detected in *A. bresadolanus* mushroom. The lowest content of bioactive compounds was measured in *A. cupreobrunneus* mushroom. The range of Cd, Pb, As, and Hg levels detected in the three mushroom species were 1775.54–7521.61 µg/kg, 1176.87–2377.37 µg/kg, 15201.26–3092.53 µg/kg and 147.86–576.53 µg/kg, respectively. The THQ value of As in *A. bresadolanus* was found to be higher than 1. The HI values of *A. bernardii*, *A. cupreobrunneus* and *A. bresadolanu* were 1.29, 0.98 and 5.57, respectively. The CR values of Cd, As, and Hg were found to be around 10^{-4} in *A. bernardii*, *A. cupreobrunneus*, and *A. bresadolanus*. Meanwhile, the CR levels of Pb were found to be around 10^{-6} in the three mushrooms. The HI value for non-carcinogenic risk assessment was higher than 1, and the CR for carcinogenic effect was around 10^{-4}, indicating that consumption of these mushrooms poses a risk to human health.

Conclusions It is thought that the elemental impurity levels in the analysed edible mushroom species were found to be at a risk potential level, and despite their antioxidant properties, uncontrolled consumption of wild edible mushrooms may cause serious risks. In order to minimize these risks, metal risk assessment studies should be continued in addition to the antioxidant effects and health-beneficial properties of mushrooms.

Keywords *Agaricus bernardii*, *Agaricus bresadolanus*, *Agaricus cupreobrunneus*, Antioxidant, Elemental analysis, Risk assessment

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Introduction

Mushrooms are an important group of living organisms with various shapes, behaviours, and life cycles. Mushrooms, which belong to the group of organisms with thallus, are eukaryotic, heterotrophic, and spore-producing creatures. Mushrooms can be found almost everywhere worldwide, but are most common in areas with high humidity [1]. Edible mushrooms are natural sources of bioactive compounds used in the treatment of some diseases in traditional medicine, as well as being consumed as food in human nutrition for centuries [2]. Edible and wild mushrooms are the agricultural product with the greatest economic value after grain, cotton, oil, vegetables and fruits [3]. Approximately 92% of fresh mushrooms consist of water, the remaining 8% consists of carbohydrates, protein, fat, various vitamins (B_2, B_3 and B_5) and minerals (potassium, selenium, copper, etc.) [4]. Thanks to the polyphenols and flavonoids in its structure, it has antioxidant properties and has various biological properties with various medical and pharmaceutical functions, including antimicrobial, antitumor, antiallergic and antidiabetic effects [4].

Agaricus L. genus, most of which are edible, is represented by more than 500 species worldwide [5, 6]. *Agaricus*, whose type specimen is *Agaricus campestris* L., is a well-known saprotrophic genus distributed worldwide. A large genus of the Agaricaceae family, this genus consists of small to large fleshy basidiomata with free lamellae that are white or pink when young but turn brown to dark brown when mature. They can be easily distinguished from other genera by their structures, such as the presence of the ring on the stem, brown basidiospores, and brown spore marks [5, 7]. Due to the many important species in the genus, it attracts the attention of scientists in fields such as medicine, biochemistry, and food sciences [5].

Agaricus bernardii Quél. is an edible mushroom species with high nutritional content. Pileus 50–145 mm broad, initially hemispherical, becoming broadly convex, margin incurved, smooth, disk frequently depressed at maturity, surface background white, becoming cracked or subscaly and sometimes developing brownish or brown, turning reddish-orange when cut. Lamellae free from the stem, close or crowded, pinkish-tan becoming chocolate brown, finally blackish brown, covered with a white partial veil when in the button stage. Stipe 40–100 × 23–37 mm, more or less equal, or narrower at the base, veil membranous, narrow sheathing ring with an upturned rim, whitish to brownish, bruising reddish. Spores: 5–8.2 × 4.5–6 µm; smooth, broadly ellipsoid, brown in KOH (Potassium hydroxide), with a pale, thick-walled, contrasting apiculus. Basidia 15–25 × 4–7.5 µm, hyaline, tetrasporic, clavate. Cheilocystidia 16–38 × 4.3–9 µm, broadly club-shaped to cylindrical, hyaline, abundant. Ethyl caproate, ethyl caprylate, hexyl acetate, butyl acetate, ethyl butanoate, and ethyl propanoate are the most dominant aroma compounds found in their structure [8, 9]. These ingredients provide a pleasant taste, increasing their potential to be preferred by consumers.

Agaricus bresadolanus Bohus pileus 30–130 mm, hemispherical, with involuted margin, thin and whitish, exceeding and decorated with minute traces of residual veil; pileus crossed by thin radial innate fibrils or fine fibrillose scales attached, more tightly packed in the disc and almost completely absent at the margin, with greyish brown tones. Lamellae at first pale pink, then salmon pink, and finally brownish, with a moderately lighter edge, dense, rich in lamellulae of all sizes, with a non-homogeneous and finely irregular edge, presenting rare interconnections, overall denoting a great structural and morphological variability. Stipe 30–110 × 10–22 mm, with thin cavity in the upper section, finally hollow, swollen at the base, featuring pronounced rhizoids, flaky in the section below the ring and moderately yellowing upon abrasion, minutely flaky and pink in the section above the ring. Spores 6.1–7.2 × 3.8–4.8 µm, ovoid with the distal pole attenuated, often a large guttulate is present; spore in dark brown mass. Basidia 16–28 × 4–7.5 µm, tetrasporic, clavate subcapitulates. Cheilocystidia, club-shaped, cylindrical, fusiform, absent, isolated, scarce, numerous or very abundant.

Agaricus cupreobrunneus (Jul. Schäff. & Steer) Pilát pileus 40–110 mm diameter, convex when young, then flattening and finally curving outwards, flattened inwards, sometimes with a broad umbo in the centre, the surface covered with reddish-brown hairs on a white ground, the margin bearing white velum remnants. Stipe 30–80 × 13–22 mm, annulus close to the pileus, upper part smooth and cream-brown in colour, lower part of the annulus rough and white, cylindrical, even or slightly thickened downwards. Spores 6.8–10.3 × 4–6 µm, with a germ pore, dark brown, elliptical, and smooth. Basidia 22–27 × 7–8 µm, cylindrical to subclavate, tetrasporic, without basal clamp. Cheilocystidia 25–33 × 15–23 µm, clavate, brownish.

Oxidation is necessary for the production of energy that sustains biological processes in living cells. However, reactive oxygen species (ROS) are catalysts for oxidation, which are associated with numerous diseases and aging processes, such as cancer, cardiovascular diseases, Alzheimer's, rheumatoid arthritis, and atherosclerosis. Excessive accumulation of ROS damages lipids, proteins, carbohydrates and DNA, causing oxidative stress, cell dysfunction and cell death. Bioactive compounds show antioxidant properties, neutralizing free radicals in the body and helping to prevent cell damage. The health problems that most affect the world's population today

can be prevented or their effects can be minimized by the use of bioactive compounds along with traditional treatments [10]. The use of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) has been restricted due to their negative effects on health, and instead, research has been directed towards components and foods that have natural antioxidant properties [11].

Phenolic compounds and polyphenols are the main organic and antioxidant compounds found in mushrooms and plants. These compounds are important secondary metabolites that prevent the formation of free radicals and lipid peroxidation, chelate metals and protect the cell against oxidative stress [12]. The positive effects of some mushroom varieties on health are directly related to secondary metabolites such as antioxidants and phenolic compounds found in their structure [13]. Among the various secondary metabolites reported in mushrooms, polyphenols have been widely studied and found effective against various health complications [14]. Extracts of some mushroom species show good protective activity against DNA damage and free radical scavenging properties against H_2O_2-induced destruction [15]. This effect is due to the redox reaction that allows mushroom polyphenols to act as reducing agents or hydrogen atom donors [16]. In a study conducted by Erdoğan et al. [17] using 12 different mushroom species, it was emphasized that the phenolic compounds of mushroom species are in a very wide range and that mushrooms generally contain high phenolic compounds. Mushroom extracts have been reported to contain a variety of phenolic compounds, including caffeic, *p*-coumaric, gallic, cinnamic, protocatechuic, ferulic, chlorogenic, sinapic, *p*-hydroxybenzoic, vanillic, salicylic and syringic acids [18].

Lipid peroxidation is the fundamental reaction of ferroptosis, which occurs when oxidants attack lipids. Lipid peroxyl radicals cause changes in permeability and fluidity in cells, inhibiting metabolic processes and causing cell death. The anti-lipid peroxidation activity demonstrated by mushrooms in various studies is noteworthy. For example, Acharya et al. [19] reported that methanolic extracts of the medicinal mushroom *Antrodia camphorata* showed lipid peroxidation ranging from 5.32 to 5.78% at the concentration of 1.0 mg/mL, thereby exhibiting remarkable antioxidant effects. *Agaricus blazei* extract, another mushroom with medicinal properties, has a significantly high antioxidant potential, with lipid peroxidation up to 26% at a concentration of 1.0 mg/mL [19]. Gasecka et al. [14] reported that mushroom extracts exhibited relatively high antioxidant effects against lipid peroxidation, ranging from 57.7 to 71.5%. Although many studies have been conducted on the phenolic content of plant materials, there is still not enough research on the phenolic content and anti-lipid peroxidation effects of mushroom species [20].

Despite the many positive effects of mushroom consumption, toxic elements such as mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) that can accumulate in mushrooms can pose a health risk [21]. Elemental impurities in mushroom generally depend on the geochemical properties of the soil, its organic matter content, the presence of other mushroom or plant species, and genetic characteristics resulting from the physiology of each species. Considering these factors is vital to food safety and quality [22]. Various guidelines exist for risk assessment of metal toxicity in foods. Organizations such as the American Food and Drug Administration (US FDA) and the European Food Safety Authority (EFSA) take into account the toxic effects of metals on human health when determining the maximum allowable levels of metal elements in foods [23–25]. The metals that pose the greatest risk to human health include Cd, Pb, Hg and As. The detection of one or more of the elemental impurities such as Cd, Pb, Hg and As in mushrooms, which are widely consumed around the world, and the evaluation of their potential harmful effects on health have increased the importance of elemental impurity analyses and risk assessments [21].

Today, in many European and Asian countries, consumption of wild mushrooms as both food and medicine are preferred instead of cultivated mushrooms. This accelerates research on the determination of bioactive components, elemental content of naturally growing mushrooms and the daily intake amount. In this study, the antioxidant activity, total phenolic content and anti-lipid peroxidation effect of *A. bernardii*, *A. bresadolanus* and *A. cupreobrunneus* mushroom species were examined, and elemental impurity analyses (Cd, Pb, As and Hg) and carcinogenic and non-carcinogenic risk assessments of the mushrooms were performed.

Materials and methods

Chemicals and reagents

Methanol (≥ 99.8%), ethanol (99.9%), Folin-Ciocalteu reagent, sodium hydroxide (97.0%), and sodium carbonate (Na_2CO_3) were provided by Merck (Darmstadt, Germany). Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid and 2-Thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Mushroom samples

The samples of *A. bernardii*, *A. bresadolanus*, and *A. cupreobrunneus*, which constitute the study samples, were collected in Çanakkale province in 2023 by Van Yüzüncü Yıl University faculty member Dr. İsmail Acar (Fig. 1). The collection place, collection date, fungarium

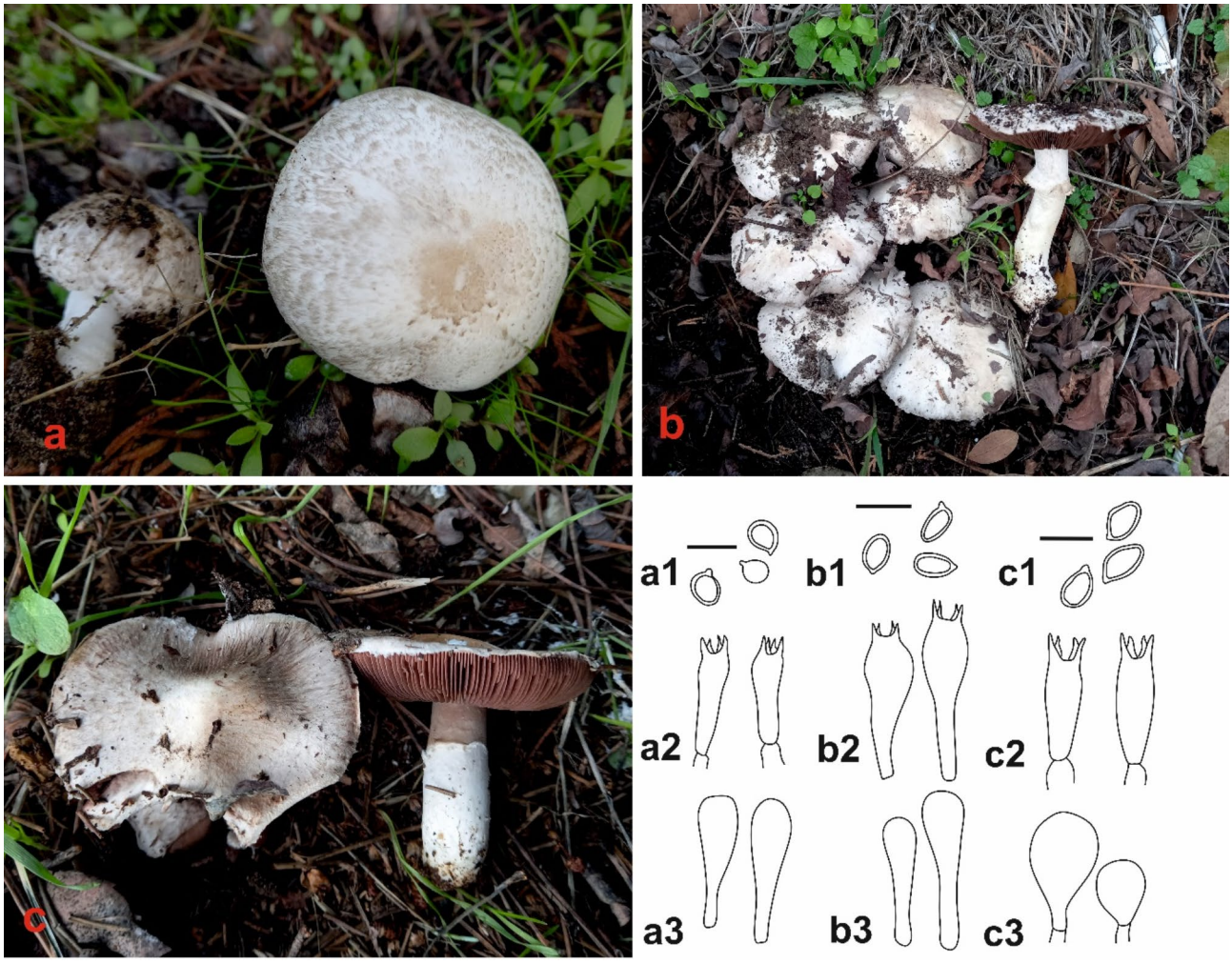

Fig. 1 **a** *Agaricus bernardii*, **b** *A. bresadolanus* **c** *A. cupreobrunneus*, a1, b1, c1. spores, a2, b2, c2 basidia, a3, b3, c3. cheilocystidia **a** Specimen examined: Çanakkale, ÇOMÜ, Terzioğlu campus, around the rectorate, under *Cupressus* sp., in meadow, 40° 06′43"N, 26° 24′48"E, 60 m, 24.11.2023, Acar 1320 **b** Specimen examined: Çanakkale, ÇOMÜ, Terzioğlu campus, Faculty of Science Garden, in meadow, 40° 06′35"N, 26° 25′02"E, 92 m, 19.12.2023, Acar 1536 **c** Specimen examined: Çanakkale, between Orhaniye redoubt and Kumkale village, in meadow, 39° 59′55"N, 26° 12′03"E, 7 m, 21.11.2023, Acar 1312

number, etc., characteristics of the examined samples are given below. *Agaricus* specimens were identified with the help of relevant literature [26–30]. The collected samples were transported to Çanakkale Onsekiz Mart University, Vocational School of Health Services Mycology Laboratory and were properly cleaned from all kinds of dust and contaminants. Then, the samples were placed on blotting paper out of sunlight and dried at 22 ± 2 °C to become fungarium material. Dried samples were ground in a laboratory type mill.

a. Specimen examined: Çanakkale, ÇOMÜ, Terzioğlu campus, around the rectorate, under *Cupressus* sp., in meadow, 40° 06′43"N, 26° 24′48"E, 60 m, 24.11.2023, Acar 1320.
b. Specimen examined: Çanakkale, ÇOMÜ, Terzioğlu campus, Faculty of Science Garden, in meadow, 40° 06′35"N, 26° 25′02"E, 92 m, 19.12.2023, Acar 1536.
c. Specimen examined: Çanakkale, between Orhaniye redoubt and Kumkale village, in meadow, 39° 59′55"N, 26° 12′03"E, 7 m, 21.11.2023, Acar 1312.

Extraction

Mushroom extraction was carried out by making some modifications to the method reported by Afrin et al. [31]. For this purpose, 5 g of powdered mushrooms were extracted in 100 mL of ethanol: water (60:40, v/v) with the help of a magnetic stirrer (at 500 rpm and 50 °C) for 2 h. At the end of the period, the homogenate obtained was centrifuged at 8000 rpm for 15 min. The same extraction procedures were applied to the remaining pulp a second time and the filtrates were combined. The supernatants were concentrated in a rotary evaporator to a total volume of 20 mL and stored at -18 °C until the analysis period.

Table 1 Microwave digestion program

Time (min)	Power (W)	Temperature (°C)
15	0–1800	30–200
15	1800	200

Antioxidant activity

Antioxidant activity of mushroom samples was measured by bleaching DPPH purple methanol solution [32]. In the principle of the DPPH method, antioxidant compounds present in the medium convert the DPPH radical into a more stable DPPH molecular product by donating an electron or a hydrogen atom. The conversion of the radical to the reduced form results in a colour change from purple to pale yellow, allowing the antioxidant activity to be determined spectrophotometrically. Briefly, in the analysis, 100 µL of each mushroom extract prepared in different concentrations was taken and mixed with 3.9 mL DPPH solution. The tubes were incubated in the dark and at room temperature for 60 min and the absorbance was read at 517 nm. Results were expressed as IC_{50} (mg/mL) after calculating % inhibition.

Results were expressed as IC_{50} (mg/mL) after calculating % inhibition according to the formula in Eq. 1.

$$\%\ Inhibition = \left[\frac{(Acontrol - Asample)}{Acontrol}\right] \times 100 \quad (1)$$

Total phenolic substance (TPC)

TPC of mushroom samples was measured spectrophotometrically using the Folin-Ciocalteu reagent [33]. Extracts (150 µL) were mixed with 2 mL of Folin-Ciocalteu reagent (2 N) and 600 µL of sodium carbonate (7.5%) in test tubes. Vortexed samples were incubated at room temperature and in the dark for 60 min. The absorbance was read at 760 nm and the results were expressed as mg GAE/100 g dw.

Lipid peroxidation (LPO) inhibition activity

TBA method was used in lipid peroxidation analysis. 30% TCA was added to the mushroom samples prepared at different concentrations and centrifuged at 8000 rpm for 10 min. Then, 1.5 ml TBA was added to the supernatants and the reaction was terminated by boiling the tubes at 100 °C for 10 min. Absorbance measurement was made at 532 nm. The results were calculated based on % inhibition values and expressed as IC_{50}. Ascorbic acid was used as a positive control in the analysis [34].

Cd, Pb, Hg and as analysis in mushrooms

0.3 g of dry mushroom samples were weighed into polytetrafluoroethylene tubes and then 8 mL nitric acid (60% HNO_3) and 2 mL hydrogen peroxide (H_2O_2) were added. After waiting for gas release in the fume hood for a while, the Teflon tubes were closed and the samples were shredded in the microwave shredding system (Ethos Easy, Milestone Srl., IT) for 30 min (Table 1). After microwave treatment, sample solutions were diluted to 30 ml with ultrapure water. The prepared sample solutions were analysed on the Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) device according to the conditions in Table 2.

Table 2 ICP–MS operating conditions

Parameter	Value
RF power	1500 W
RF voltage	1.80 V
S/C temperature	2 °C
Sample depth	10 mm
Nebulizer gas	1.00 L/min
Nebulizer pump	0.10 rps
Internal standards	^{6}Li, ^{45}Sc, ^{72}Ge, ^{89}Y, ^{115}In, ^{159}Tb, ^{209}Bi
Tune solution	^{7}Li, ^{89}Y, ^{205}Tl

Risk calculation

Carcinogenic and non-carcinogenic human health risk calculations developed by the American Environmental Protection Agency (US EPA) have been successful and accepted worldwide. This study used US EPA calculations and threshold values to evaluate the potential human health risks posed by heavy metal pollution [35, 36]. Risk calculations were performed for adult exposure.

Estimated daily intake (EDI)

The estimated daily intake (EDI) of the relevant elements (Cd, Pb, Hg and As) is based on the daily intake of the relevant food substances, the average exposure time and the average concentration of each type of food sample. Calculations were made according to the formula in Eq. 2.

$$EDI\ (\text{mg kg}^{-1}\text{day}^{-1}) = \left(\frac{(MC \times IR \times ED \times EF)}{(BW \times AT)}\right) \quad (2)$$

EDI represents estimated daily intake (mg kg^{-1} day^{-1}); MC, heavy metal concentration in wild edible mushroom (mg/kg); IR, intake rate (6.6×10^{-3} kg/person/day); EF, exposure frequency (350 days/year); ED, duration of exposure (adults = 26 years); BW, body weight (adults = 70 kg); AT is the averaging period a person is exposed to a contaminant during their lifespan time, which for non-carcinogenic risk, is 9100 days (ED × EF), and for carcinogenic risk is 25,550 days in adults (365 days/year × 70 years). All of the following metrics' values have been modified in accordance with the most recent US EPA guidelines [35–38].

Target hazard quotient (THQ)

The target hazard quotient (THQ) is used to assess the potential health risks of human consumption, which is used to assess the level of non-carcinogenic risks from ingestion of elemental impurities.

$$\mathrm{THQ} = \mathrm{EDI}_{\text{non-carcinogenic risk}}/\mathrm{RfD} \quad (3)$$

Here, EDI for non-carcinogenic risk; EF, frequency of exposure (350 days/year); ED, duration of exposure (adults = 26 years); BW, body weight (adults = 70 kg); AT, the averaging period a person (ED × EF; 9100 days) were used in the calculation. RfD is the oral reference dose of the element in mg/kg/day. RfD values for As, Cd, Hg and Pb are 3×10^{-4}, 10^{-3}, 3×10^{-4} and 3.5×10^{-3} mg kg^{-1} day^{-1}, respectively [21, 35].

The hazard index (HI) is the sum of the THQ values using each food type assessment element. When the HI is less than 1, the risk for chronic systemic effects is acceptable. However, when the HI is greater than or equal to 1, there are potential adverse non-carcinogenic health risks with long-term consumption and the potential for adverse non-carcinogenic health effects may occur.

$$\mathrm{HI} = \mathrm{THQ}_{\mathrm{cd}} + \mathrm{THQ}_{\mathrm{Pb}} + \mathrm{THQ}_{\mathrm{Hg}} + \mathrm{THQ}_{\mathrm{As}} \quad (4)$$

Carcinogenic risk (CR) assessment

It is used to assess the potential risk of consumers' lifetime exposure to carcinogenic substances.

$$\mathrm{CR} = \mathrm{EDI}_{\text{carcinogenic risk}} \times \mathrm{CSF} \quad (5)$$

Here EDI carcinogenic risk; EF, frequency of exposure (365 days/year); ED, duration of exposure (adults = 70 years); BW, body weight (adults = 70 kg); AT, the averaging period of time a person (ED × EF; 25,550 days) were used in the calculation. CSF is the oral disposition factor of a carcinogen and is 1.5 mg kg^{-1} $day^{-1}$for As, 0.0085 mg kg^{-1} $day^{-1}$for Pb, 6.3 mg kg^{-1} day^{-1} for Cd and 6.177 mg kg^{-1} $day^{-1}$for Hg [21, 37].

Risks exceeding 10^{-4} are considered a potential carcinogenic risk, while a CR value below 10^{-6} is considered not to pose a health risk. A CR value in the range of 10^{-4}-10^{-6} is generally considered a tolerable degree of effect on the body [35].

Table 3 Antioxidant, TPC and LPO values of mushroom samples

	DPPH (IC_{50} mg/mL)	TPC (mg GAE/100 g)	LPO (IC_{50} mg/mL)
A. bernardii	14.53 ± 0.01^{c}	680.14 ± 6.14^{b}	6.46 ± 0.11^{c}
A. cupreobrunneus	15.51 ± 0.09^{d}	552.65 ± 5.27^{a}	9.77 ± 0.16^{d}
A. bresadolanus	12.85 ± 0.03^{b}	993.04 ± 8.20^{c}	2.50 ± 0.03^{b}
Ascorbic acid	10.81 ± 0.01^{a}		0.17 ± 0.01^{a}

[a, b,c, d] different superscript lowercase letters indicate differences between samples ($p < 0.05$)

Statistical analysis

Analyses were performed in triplicate. Statistical analysis was performed using IBM SPSS 27 software and a normality test was first applied to the data. Bioactive compounds and metal concentrations were compared between samples using One-Way ANOVA followed by Tukey's post-hoc test for all normally distributed data.

Results

Antioxidant activity and TPC

Antioxidant activity values and TPC amounts of the mushroom samples examined are given in Table 3. The highest antioxidant value was measured in *A. bresadolanus* mushroom with an IC_{50} value of 12.85 mg/mL ($p < 0.05$). This value was followed by *A. bernardii* and *A. cupreobrunneus* mushrooms, with values of 14.53 and 15.51 mg/mL, respectively. The antioxidant activity value of all samples was found to be lower compared to ascorbic acid ($p < 0.05$). In this case, the high antioxidant effect of ascorbic acid plays a big role.

Similar to the antioxidant results of the samples, the highest TPC value was measured in *A. bresadolanus* mushroom (993.04 mg GAE/100 g dw) ($p < 0.05$). The lowest TPC value belongs to the mushroom *A. cupreobrunneus* (552.65 mg GAE/100 g dw) ($p < 0.05$).

Anti-lipid peroxidation activity

Lipid peroxidation in cells results from oxidative stress, which causes disruption of membrane integrity and cell damage [39]. Table 3 shows the inhibition values of the examined mushroom samples against lipid peroxidation. The highest LPO effect was measured in *A. bresadolanus* mushroom with an IC_{50} value of 2.50 mg/mL ($p < 0.05$). The LPO values determined for *A. bernardii* and *A. cupreobrunneus* mushrooms are 6.46 and 9.77 mg/mL, respectively.

Risk assessment

Elemental impurities commonly found in nature can accumulate in mushroom through soil, water, air or other means. As a result of these elemental impurities causing various health problems in the human body, non-toxic limits for these elements are specified in the guidelines. In our study, the amounts of Cd, Pb, As and Hg heavy metals classified as Class 1 in USP < 232> [24] in three different mushroom samples were determined by ICP-MS and risk assessments were made according to the obtained values. Samples were analysed on the ICP-MS device using the microwave sample preparation method to determine four elemental impurities (Cd, Pb, As, Hg), which are classified as Class 1 elements in the USP < 232 > guide. Samples

were prepared according to the standards specified in USP < 232 > and USP < 233 > regulations [24].

The quantitative parameter data in Table 4 includes calibration curve details for ICP-MS and limit of detection (LOD) values obtained from calibration standards for four elements. In the quantification method, the calibration range for Cd, Pb, and As was 0.1–100 µg/kg, while for Hg, it was 0.1–20 µg/kg. The regression (R^2) value for each element was $R^2 \geq 0.9997$ (Table 4).

Heavy metal concentrations in edible wild mushrooms are presented in Table 5. Concentrations were compared to permissible limits for foods set by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) [40]. When Cd, Pb, As and Hg levels in mushroom samples were examined, it was seen that *A. bresadolanus* had the highest Cd level. *A. bresadolanus* is followed by *A. bernardii* and *A. cupreobrunneus* (Table 5). Statistically significant differences were observed between the Cd levels of each sample ($p < 0.05$). The highest Pb level in the mushroom samples analysed in our study was found in *A. cupreobrunneus*. *A. cupreobrunneus* is followed by *A. bernardii* and *A. bresadolanus* (Table 5). Statistically significant differences were observed between the Pb levels of each sample ($p < 0.05$). The highest As level in mushroom samples was found in *A. bresadolanus* species. *A. bresadolanus* is followed by *A. bernardii* and *A. cupreobrunneus* (Table 5). Statistically significant differences were observed between As levels of each sample ($p < 0.05$). The highest Hg level in mushroom samples was found in *A. cupreobrunneus* species. *A. cupreobrunneus* is followed by *A. bernardii* and *A. bresadolanus* (Table 5). Statistically significant differences were observed between the Hg levels of each sample ($p < 0.05$).

Table 4 Quantification parameters for elemental impurities

Element	Isotope	LOD (µg/kg)	Calibration range (µg/kg)	Calibration curve regression (R^2) value
Cd	111	0.01	0.1–100	0.9999
Pb	208	0.03	0.1–100	1.0000
As	75	0.01	0.1–100	0.9998
Hg	201	0.03	0.1–20	0.9997

LOD; Limit of detection

Our study first found the non-carcinogenic EDI value to evaluate the non-carcinogenic risk calculation of elemental impurities. THQ and HI values were then calculated (Table 5). Equation 2 was used to calculate non-carcinogenic EDI (mg kg^{-1} day^{-1}) for an adult weighing an average of 70 kg. The findings are presented in Table 5. The RfD value and calculated non-carcinogenic EDI values of each element were used in Eq. 3 to calculate the THQ value. The HI for the combined risk for the examined metals was calculated by summing the THQs for Cd, Pb, As, and Hg in each mushroom sample (Eq. 4).

In Table 5, although the THQ value of all elements in *A. bernardii* was less than one, the HI value was found

Table 5 Levels of elemental impurities and carcinogenic and non-carcinogenic calculation results in three different mushrooms

Sample	MC (µg/kg) (mean ± std. dev.)			
	Cd	Pb	As	Hg
A. bernardii	1972.38 ± 0.53^a	2008.09 ± 4.25^a	3092.53 ± 1.98^a	250.09 ± 2.52^a
A. cupreobrunneus	1775.54 ± 1.37^b	2377.37 ± 2.56^b	1804.27 ± 0.76^b	576.53 ± 1.42^b
A. bresadolanus	7521.61 ± 2.84^c	1176.87 ± 1.21^c	15201.26 ± 2.34^c	147.86 ± 3.24^c

Risk assessment

Sample	Element	EDI (mg kg^{-1} day^{-1})	THQ	HI	CR
A. bernardii	Cd	1.86×10^{-4}	0.19	1.29	11.72×10^{-4}
	Pb	1.89×10^{-4}	0.05		1.61×10^{-6}
	As	2.92×10^{-4}	0.97		4.37×10^{-4}
	Hg	0.24×10^{-4}	0.08		1.46×10^{-4}
A.cupreobrunneus	Cd	1.67×10^{-4}	0.17	0.98	10.55×10^{-4}
	Pb	2.24×10^{-4}	0.06		1.91×10^{-6}
	As	1.70×10^{-4}	0.57		2.55×10^{-4}
	Hg	0.54×10^{-4}	0.18		3.36×10^{-4}
A. bresadolanus	Cd	7.09×10^{-4}	0.71	5.57	44.67×10^{-4}
	Pb	1.11×10^{-4}	0.03		0.94×10^{-6}
	As	14.33×10^{-4}	4.78		21.49×10^{-4}
	Hg	0.14×10^{-4}	0.05		8.61×10^{-4}

MC; Amount of elemental impurity in the mushrooms (µg/kg, calculations were made by converting the µg/kg elemental impurity level detected in mushrooms to mg/kg in the equation formulas), EDI; Estimated daily intake dose (mg kg^{-1} day^{-1}, non-carcinogenic and carcinogenic EDI values were calculated separately considering Eq. 2. Although the AT value was different for both cases, the EDI values were found to be numerically the same for both cases as the AT value in Eq. 3 was simplified by the EF*ED value), THQ; The target hazard quotient, HI; The hazard index, CR; Cancer risk, a, b, c different superscript lowercase letters indicate differences between results of samples MC values ($p < 0.05$)

to be greater than 1. It is seen that the element level that causes the high HI value among the four elements is due to As, which has a high THQ value. Although the THQ value of all elements in the *A. cupreobrunneus* species was less than one, the HI value was found to be close to 1. It is seen that the element level that causes the high HI value among the four elements is due to As, which has a high THQ value. The THQ value of the element As in *A. bresadolanus* was found to be greater than 1. The THQ value of the Cd element was found to be close to 1. THQ values of Pb and Hg elements were less than 1. The HI value of the elements in the mushroom was higher than 1. It is seen that the element levels that cause the high HI value among the four elements are due to As and Cd, which have high THQ values. While a THQ value below 1 indicates that the mushroom is safe for human consumption, a THQ value exceeding 1 is considered hazardous to human health.

For the carcinogenic risk assessment of elemental impurities in our study, the carcinogenic EDI value was recalculated considering the difference in exposure time. CR values were then calculated [37]. CR values for Cd, Pb, As, and Hg were found as 11.72×10^{-4}, 1.61×10^{-6}, 4.37×10^{-4}, 1.47×10^{-4} in *A. bernardii* mushroom, 10.55×10^{-4}, 1.91×10^{-6}, 2.55×10^{-4}, 3.36×10^{-4} in *A. cupreobrunneus mushroom*, 44.67×10^{-4}, 0.94×10^{-6} and 21.49×10^{-4}, 8.61×10^{-4} in *A. bresadolanus* mushroom, respectively.

Discussion

The antioxidant properties of mushrooms are due to the bioactive compounds and especially the phenolic fractions found in their structure [34]. Kalyoncu et al. [41] measured the antioxidant activity of *Agaricus bresadolanus* in different solvents and found it to be 12.28%, 18.89% and 24.70% for chloroform, ethanol and water, respectively. Huang [42] determined that methanolic extracts of *Agaricus blazei* had a DPPH radical scavenging effect that was 98.8% effective at 5 mg/ml. The IC_{50} value of *A. campestris* mushroom was found to be 1.18 mg/mL by Glamočlija et al. [43], and Woldegiorgis et al. [44] measured the antioxidant activity as 1.4 mg/mL in the same mushroom species. In a different study, methanol extracts obtained from mushroom species showed strong scavenging potential against DPPH free radicals and were reported as 77.5 mg/mL for *Agaricus bisporus* [45]. The antioxidant activity of *Agaricus bisporus* was measured as 83.93 µg/mL by Elhusseiny et al. [46] and 2.47 mg/mL for the same mushroom species by Iqbal et al. [47]. It is seen that the antioxidant activity values of the examined mushroom species vary from species to species and even between the same species when compared with literature data. The reason for this situation may vary depending on many factors such as genetic variation among mushroom species, growing geography, climate characteristics, altitude, soil structure, harvest period and storage conditions [48].

The TPC value of *A. bernardii* was determined as 645.9 mg GAE/100 g by Kaya et al. [48]. TPC in *A. cupreobrunneus* mushroom samples was recorded as 12.25 mg GAE/g [49]. In a study investigating the TPC content of mushroom samples in different solvents, the TPC values of aqueous extracts of *Agaricus bisporus* and *Agaricus brasiliensis* species were found to be 21.47 mg GAE/g and 15.79 mg GAE/g, respectively. In the same study, it was reported that these values in ethanol extracts were 10.25 mg GAE/g and 12.50 mg GAE/g, respectively [50]. In a study conducted with *A. campestris*, the TPC value of methanol and ethanol extracts was given as 48.19 and 56.79 mg GAE/g, respectively [43]. TPC value of *Agaricus* sp. mushroom was found in the range of 569.9–1775 mg GAE/100 g [48]. TPC for *Agaricus bisporus* was reported as 9.25 mg GAE/g by Iqbal et al. [47]. Mushrooms have free radical scavenging activity and antioxidant activity thanks to the hydroxyl groups of the phenolic compounds found in their structures [51]. Geographical features such as climatic conditions, vegetation and altitude have significant effects on the bioactivities of mushroom. It is thought that the difference between the obtained results and literature data may be due to differences in the solvent used, extraction method and extraction conditions (temperature, time), as well as climate and geographical features, similar to antioxidant properties.

Edible and wild mushrooms are important natural resources in preventing diseases caused by oxidative stress, thanks to their high antioxidant activity and the total phenolic compounds they contain [52]. Compared to ascorbic acid, especially *Agaricus bresadolanus* mushroom has an effective LPO effect. Taofiq et al. [53] reported that the phenolic extract of *Agaricus bisporus* showed high activity against free radical formation by inhibiting nitric oxide production. Similarly, the high lipid peroxidation inhibition possessed by mushrooms has been highlighted by various studies [34, 54–56]. In most of the mushroom species showing free radical scavenging potential, there was a linear relationship between the total content of phenolic compounds in the mushroom extracts and their antioxidant potential [57]. This confirms that both wild and medicinal and edible mushrooms have a strong effect as naturally occurring antioxidants due to the capacity of their phenolic compounds to prevent lipid peroxidation [58].

Although mushrooms have antioxidant activity due to the phenolic substances they contain, their consumption may pose a health risk because they can accumulate elemental impurities. Mushrooms can be very rich in Cd [59]. The main reasons for this include water and air sources, as well as the ability of Cd to pass from the

soil to the mushroom. Cd levels in surface soils range from 0.01 to 2.7 mg/kg [60]. In addition to soil pH, the main factors that cause the Cd level to increase in the soil include speciation, soluble organic matter content, aqueous metal oxide content, clay content and type, the presence of organic and inorganic ligands and the presence of other metal ions [61]. The effect of different soil pHs (pH 5.5, pH 6.5) on Cd concentration was investigated by Singh and Myhr [62] between 1991 and 1994. Between 1991 and 1993, Cd levels were found to be higher when soil pH was 6.5. In 1994, Cd levels were found to be higher in soils with pH 5.5 [62]. In the study of Ateş and Turan [63], the pH measurement of a total of 29 soil samples taken from the agricultural lands of the Central district of Bingöl province was carried out. The average pH value of the soils was determined as 6.68 ± 0.32. The lowest pH was reported as 5.5 and the highest pH was 7.75 [63]. Since the mushroom samples analysed in our study were collected near the Central district of Bingöl, it is thought that the pH ranges that affect the Cd level in the literature may also have an effect on our samples. FAO/WHO states the maximum permissible level for Cd in mushroom as 0.20 mg/kg [40]. Cd levels in the mushroom samples analysed in our study were found to be above the allowed limit values (Table 5). However, similar data are available in the literature for some mushroom species. In the literature, it has been observed that the Cd level in mushrooms from the Agaricaceae family is in the range of 8–54,200 µg/kg [64]. In the study of Doğan and Şanda [65], the Cd level in *A. bresadolanus* was found to be 0.45 mg/kg, while the Cd level in *Agrocybe paludosa* in the same study was found to be above the permissible levels. Cd is a highly toxic metal found naturally in soil, but is also released into the environment due to human activities. Cd is a byproduct of the production of zinc (Zn) and Pb. Pyrometallurgical production of Zn is the most important anthropogenic source in the environment [59]. Excessive Cd exposure causes kidney, lung, liver and skeletal damage, as well as cancer. It has been reported that Cd accumulates mainly in the kidneys, spleen and liver, and a significant increase in blood serum levels follows mushroom consumption [66]. Therefore, Cd appears to be the most harmful of the heavy metals in mushroom.

Pb exposure can occur through air, water and food [66]. Pb is distributed into the environment as a component of pesticides and industrial wastes released into the environment, such as used car batteries, leaded fuels, alloys, solder, broken ceramics and plastics. The target organs where it can accumulate in the body are bones, brain, blood, kidneys and thyroid gland. Gradual accumulation can lead to lead poisoning. This can lead to conditions such as high blood pressure, muscle weakness and headaches [67]. In the literature, the Pb level in mushroom species from the *Agaricaceae* family has been reported as 2–5 mg/kg [68]. When the Pb levels detected in our study (Table 5) were compared with the allowed Pb concentration of 12–6000 µg/kg [68], it was seen that the lead value range in mushrooms was within the normal range and could be considered safe for consumption.

Inorganic As is acutely toxic and intake in large amounts causes peripheral vascular diseases, serious disorders of the central nervous systems and cardiovascular diseases. Long-term As exposure increases the risk of lung, bladder and kidney cancer [35]. In nature, As is the most dangerous inorganic element due to its carcinogenic risk and there are no safe levels of As [68]. In the literature, As content of *A. campestris*, which is from the *Agaricaceae* family, has been reported as 2.60 mg/kg. In the study of Doğan and Şanda [65], the As level in *A. bresadolanus* mushroom collected in Sakarya province (Turkey) was determined as 3.2 mg/kg. In our study, the As level of three mushroom species from the *Agaricaceae* family was found to be approximately 1804.27–15201.26 µg/kg (Table 5). It is reported in the literature that the As level in wild mushrooms is higher than the As level in cultivated mushrooms [69]. As levels of the wild edible mushrooms analysed were found to be high, similar to the literature.

In terms of health, especially considering the dangers of ingesting toxic pollutants such as Hg, it is very important to determine the amount of Hg taken from food and foodstuffs. The amount of Hg allowed in foods by FAO/WHO has been determined as 0.05 mg/kg [40]. A high amount of Hg at 5978 µg/kg was detected in *A. bresadolanus* mushroom by Doğan and Şanda [65]. Hg levels in our study were low compared to the literature (Table 5).

In the study, carcinogenic and non-carcinogenic risk assessments of elemental impurities were carried out by considering the levels of elemental impurities detected in mushrooms in order to evaluate the toxic effects of consumption of mushroom species with elemental impurities on human health. In this study, carcinogenic and non-carcinogenic EDI values were calculated separately considering the exposure durations in the literature. While noncarcinogenic exposures produce damaging effects in the short term, prolonged exposure allows carcinogenic effects to accumulate over the long term. The noncarcinogenic EDI usually assesses the short-term effects of a particular chemical or pollutant on human health. Short-term exposure (26 years) and fewer days (350 days/year) are used. Carcinogenic EDI assesses the risk of cancer in the long term, generally. It uses a longer exposure period (70 years) and a higher number of days per year (365 days/year) [37]. This difference is due to the different accumulation time of the chemical in the organism. Carcinogenic substances usually show their effects in the long term, so a long exposure period and a high number of days of annual

exposure are used. For noncarcinogenic substances, a shorter-term effect is considered. Although the numerical value of AT in Eq. 2 is numerically different for noncarcinogenic and carcinogenic conditions, simplifying the EF × ED multipliers by AT in both cases may cause the calculated EDI values to be mathematically close to each other. However, the meaning of carcinogenic and noncarcinogenic conditions is different. Carcinogenic substances usually show long-term effects, whereas noncarcinogenic substances may have toxic effects in the short term. Therefore, the two calculation results have different meanings but can be numerically close due to mathematical simplification. In the study of Nowakowski et al. [21], the levels of Cd, Pb, As and Hg elements in the caps and stems of some mushroom species were examined and the carcinogenic and non-carcinogenic risk calculations of these elements were carried out. In the study, it was determined that THQ values of some mushroom species were greater than one for Hg and As analyses [21]. Considering the THQs calculated in the three mushroom samples in the present study, THQ_{As} was found to be higher than THQ_{Cd}, THQ_{Pb} and THQ_{Hg}. In *A. bernardii* the THQ_{As} value was 0.97, in *A. cupreobrunneus* the THQ_{As} were 0.57 and in *A. bresadolanus* the THQ_{As} were 4.78. In addition, THQ_{Cd} value in *A. bresadolanus* was higher than THQ_{Pb} and THQ_{Hg} (Table 5). The THQ value for As in *A. bresadolanus* mushroom was found to be greater than one. Therefore, consumption of *A. bresadolanus* is considered harmful to human health. In addition, the fact that the HI value of four elements in the analysed *A. bernardii* and *A. bresadolanus* mushrooms is greater than one suggests that the consumption of these mushrooms may be harmful to human health. Although *A. bresadolanus* has the highest phenolic substance content among the three mushroom species, it is thought that consumption of this mushroom will pose a non-carcinogenic health risk because its HI value is greater than 1. However, although the phenolic content of *A. cupreobrunneus* mushroom is lower than others, it is estimated that consumption of this mushroom species will not pose a non-carcinogenic health risk since its HI value is less than one.

Carcinogenic risks may occur due to exposure to Cd, Pb, As, and Hg elementel impurities through food consumption. Consumption of food with a CR value greater than 10 – 4 poses a potential carcinogenic hazard, whereas consumption with a CR value less than 10^{-6} is not considered a health hazard as there are tolerable risks. A CR value between 10^{-4} and 10^{-6} generally indicates an acceptable level of exposure and does not pose a health hazard [70]. The CR values of Cd, Pb, As, and Hg elemental impurities with carcinogenic risk potential, in mushrooms were analyzed separately for each elemental impurity in our study. In this study, the CR value of other elements except Pb was determined to be around 10^{-4}. In the wild edible mushroom samples examined in this study, especially Cd, As and Hg levels were found close to this threshold. Therefore, it is thought that direct consumption of the wild edible mushroom species included in our study may cause negative effects on human health. In order to minimize these risks, it is necessary to first raise awareness of local people about toxic substances that can pass from soil to food, and to reduce the health risks that may arise from the use of wild edible mushrooms in kitchens without going through the quality control process. Providing soil and water reclamation with good agricultural practices, monitoring the process with authorities in terms of food consumption, and encouraging the consumption of organic/cultured mushrooms are considered among the corrective actions.

Conclusions

This study revealed the potential of *A. bernardii*, *A. bresadolanus* and *A. cupreobrunneus* mushroom species as natural antioxidant sources. In particular, the effective lipid peroxidation inhibition activity shown by these mushroom species was determined for the first time in this study. In addition to all these positive effects, it is thought that the uncontrolled consumption of wild edible mushrooms may cause serious risks, especially since the Cd, As and Hg levels in the analysed edible mushroom species are at a level that poses a risk potential. In order to minimize these risks, metal risk assessment studies on mushrooms need to be continued in addition to their antioxidant effects and health-beneficial properties. In this way, the importance of sharing the analysis results for the detection of bioactive properties and risky components on the necessary platforms, supporting field-based experiments and tests, and developing risk management strategies becomes evident.

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Author contributions
Emine Okumus: Contributed to the concept and design of the study, completed the experimental analysis, data analysis and the original writing and formatting of the article. Fadime Canbolat: Contributed to the experimental analysis, data analysis, and the original writing and formatting of the article. İsmail Acar: Contributed to the concept and design of the study, completed the experimental analysis, data analysis and the original writing and formatting of the article. All authors read and approved the final manuscript.

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