QUALITY PARAMETERS AND ASSESSMENT OF HEALTH RISKS IN PLUM ORCHARDS UNDER VARIOUS MANAGEMENT SYSTEMS IN NORTHEAST ROMANIA

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Abstract

The presence of heavy metals in fruits has become a major concern due to their potential impact on human health. This study aimed to assess the concentration of heavy metals (Ni, As, Cd, Cu, Zn) in plum fruits (Prunus domestica L.) varieties (Tuleu Gras and Carpatin), from "Adamachi" Farm district - Iaşi University of Life Sciences (IULS). The samples of ripe plum fruits were collected from the orchards ecological and conventional management systems from August 2023. The concentrations of metals in plum samples were determined using Flame Atomic Absorption Spectroscopy (fAAS) after wet digestion (HNO3 65%, H2O2, > 30% w/v). Specifically, the concentration (mg/kg fresh weight) range of Cd (0.005-0.01), As (nd), Ni (0.13-0.23), Zn (0.41-0.57) and Cu (0.76- 1.23) did not exceed the safe limits. The results indicated that the order of accumulated elements in plum fruits followed Cu>Zn>Ni>Cd>As. The potential health risks associated with consuming these fruits were evaluated DIM and HRI using the USEPA probabilistic risk assessment model. However, the calculated HRI values for heavy metals were all found <1, indicating that consuming plums from this area does not pose health risks to local consumers. Overall, the findings of this study demonstrate that plum fruit consumption in the study area does not present any health hazard associated with any of *the selected heavy metals.*

Key words: quality plum fruits, heavy metals, health risk assessment, pollution.

INTRODUCTION

The current state of food security and assurance is deeply intertwined with the rising incidence of illnesses in people. Plums, with a production of 12.25 million tons, are ranked as the second most produced stone fruit globally (after peaches and nectarines) (Zezulová et al., 2022), play a significant role in human nutrition, offering essential nutrients that promote health and help prevent various diseases (obesity, diabetes, cancer, cardiovascular, and other chronic diseases) (Nistor et al., 2022).

Plum fruits, are rich in phenolic compounds, and boast higher levels of anthocyanins and antioxidants compared to apples, oranges, and strawberries (Leong and Shui, 2002; Kim et al., 2003; Nisar et al., 2015).

These bioactive components, particularly anthocyanins, not only provide the vibrangt hues of plums but also contribute to a positive impact on human health due to phenolic compounds with their antioxidant properties (Kayano et al., 2002; Scutarașu et al., 2024).

However, concerns arise regarding heavy metal contamination in the environment, posing risks to public health due to its adverse effects on organs and the potential for accumulation in the food chain. Sources of heavy metal pollution encompass agricultural and industrial activities, vehicular emissions, and agrochemical usage, necessitating stringent measures to mitigate these risks and safeguard food safety (Jadaa and Mohammed, 2023).

Acknowledging the significance of understanding pollution levels, quality status, and health risks posed by heavy metals in plum fruits, the primary objectives of this study were to (1) assess plum fruit quality parameters in ecological and conventional systems, (2) determine the levels of heavy metals (Ni, As, Cd, Cu, Zn) in plum fruits, and (3) evaluating potential human health risks in terms of daily intake of metals (DIM) and health risk index (HRI) for adults.

In conclusion, the relationship between heavy metals and the proprieties phytochemical of plum fruit is essential for maintaining human health.

MATERIALS AND METHODS

The study was conducted on plums (*Prunus domestica* L.) orchard at Horticultural "Adamachi" Farm - IULS. Geographically, the city is located in northeastern Romania, at 47°10'24"north latitude and 25°15'3" east longitude. "Adamachi" Farm benefits from a favourable climate and fertile soil classified as aric-cambic chernozem with texture loamy-clay (80%). During Apr.-Sept. 2023, environmental conditions were favourable for the growth of *Prunus domestica* L. Compared to the data of 2022, the average temperature showed a slight increase from 18.54˚C to 18.9˚C. As for precipitation, it decreased significantly from 340.4 mm in 2022 to 246.1 mm in 2023.

The *Prunus domestica* L. orchard, spanning 5500 m² , was established in autumn 2014 and is separated into buffer strips that are 5 m wide. Plots under study are spaced 200 m apart to prevent interference caused by synthetic inputs. Methods. In August 2023, soil and mature fruit samples (varieties Tuleu Gras and Carpatin) were collected with precision. Each conventional (Cv) and ecological management system (Eco) was represented by three areas, with five designated points each, with each area comprising 30 trees, creating composite samples that were meticulously stored in polyethylene bags. Soil samples (90) were collected using a shovel and hand drill, and plant matter and stones were removed. Ninety fruit samples were collected, washed with ultrapure water to remove contaminants and dried at room temperature. Fruits were stored at -20°C until analysis. Conventional methods involve the rationale of pesticides and approved fungicides, adhering to regulatory thresholds. Simultaneously, ecological practices, such as manually removing weeds and shallow tillage around tree trunks, enhance soil and plum fruit health. Disease control includes the incorporation of copper-based fungicides in

compliance with ecological production guidelines.

The soil's chemical properties were evaluated employing standard techniques. pH measurement was conducted with a pH meter using a glass electrode with a soil-to-water ratio of 1:2.5 w/v, while the total organic carbon (TOC) content was determined utilizing the Walkley-Black method (Calistru et al., 2024). Soil organic matter (SOM) was calculated by multiplying the TOC percentage by the globally recognized Van Bemmelen factor of 1.724 (Heaton et al., 2016). The extraction of available potassium (K) and phosphorus (P) was performed using neutral 1N NH4OAc, and their quantities were determined following the analytical procedures outlined by ICPA Bucharest (Țopa et al., 2021).

Acid digestion of soil and fruit samples for heavy metals follows defined protocols, such as USEPA method 3052. Soil samples are treated with a blend of nitric acid (HNO3) and hydrochloric acid (HCl), whereas fruit samples undergo treatment with HNO₃ and hydrogen peroxide (H_2O_2) . Post-digestion in the microwave, solutions are filtered to a final volume (50 mL) using ultrapure water. Concentrations of heavy metals are analysed through Atomic Absorption Spectrometry (AAS), adhering to specific parameters in Table 1.

Table 1. Operating parameters AAS for analysis

Parameters	Setting		
Elements	Cu, Zn, Ni, Cd, As		
Replicates			
Measurement	Absorbance		
Flame	Acetylene (C_2H_2) - Air		
Burner height	$5-9$ (nm)		
Fuel Flow (L/h)	$25-40$ (L/h)		

Physical-chemical and phytochemical determinations for plum quality parameters. Fruit weight was ascertained through the weighing of all sampled fruits using an analytical balance $(d = 0.1$ mg), followed by the calculation of the average fruit weight (g/fruit). Fruit firmness was evaluated employing a Qualitest HPE non-destructive penetrometer. The colour of the samples was measured using a Konica Minolta colorimeter (model CR-410, Tokyo, Japan) following the method described by Pinto et al. (2024). The

uniform colour space CIEL*a*b* coordinates were obtained through this device. To measure the pH of the fruits, the pH metre (WTW InoLab, Xylem Analytics GmbH, Weilheim, Germany) was used, which was calibrated before the pH measurement using buffer solutions with pH values of 4 and 7 (Rațu et al., 2023). The acidity was measured using a titrimetric method with phenolphthalein as an indicator. Homogenized fruit mixed with water was filtered, and the filtrate was titrated with NaOH (0.1 mol/L) to determine acidity.

Using BIOBASE BK-FD10T equipment from Jinan, China, plum fruits were freeze-dried for 50 hours at a temperature of -42°C and a pressure of 0.10 mBar to analyse the total content of anthocyanins, flavonoids, polyphenols and the antioxidant activity. After this process the samples were ground using MC 12 equipment (Stephan, Germany) to a fine powder. Before being analysed, these samples were stored in glass jars at room temperature and in the dark. A UV lamp was used to sterilize the finished powder to eliminate contaminants.

Reagents are sourced from Sigma Aldrich Steinheim (Darmstadt, Germany): Folin-Ciocalteu reagent, 2,2-diphenyl-1 picrylhydrazyl (DPPH), citric acid (1%), C2H5OH, Gallic acid, NaOH, KCl solution, NaNO₂, CH₃COONa, AlCl₃ and Na₂CO₃.

The method for extracting bioactive compounds from plum powders involves the use of ultrasound, as described by Lima et al. (2017), with minor adjustments. In this process, 1 g of plum powder was dissolved in 9 mL of 70% ethanol, acidified with a 1% citric acid solution (acid: solvent, 1: 8, v/v). The mixture was then subjected to treatment sonication (Elmasonic S 180 H) for 40 minutes at 40 kHz and 25°C. After sonication, samples were centrifuged at 6000 rpm and 4°C for 10 minutes, and the resulting supernatant was analysed for phytochemical content (Lipșa et al., 2024).

The total flavonoid content in plum powder extract was determined using the aluminium chloride method. Briefly, plum powder extract (250 µL) was mixed with sodium nitrite (75 μ L) and distilled water (2 mL), followed by the addition of aluminium chloride $(150 \mu L)$ after 5 minutes and sodium hydroxide (0.5 mL) after 6 minutes. The mixture was then measured at 510 nm using a UV–VIS spectrophotometer (Analytik Jena - Specord 210 Plus, Germany). Catechin was used as a standard for the calibration curve, and the results were reported as milligrams of catechin equivalents per 100 grams of dry weight (mg CE/g d.w.) (Horincar et al., 2019).

The total polyphenolic content of the plum powder extract was measured using the Folin-Ciocalteu method. In summary, 200 µL of the extract was mixed with 1 mL of the Folin-Ciocalteu reagent and 15.8 mL of distilled water. After 10 minutes, $3 \text{ mL of } Na_2CO_3 20\%$ was added, and the mixture was stored in the dark for 60 minutes at room temperature. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer. Results were calculated in mg of Gallic acid equivalents per gram of dry weight (mg GAE/g d.w.) using a standard curve for Gallic acid (Sadiq et al., 2015).

The total antioxidant activity of the samples was tested using the DPPH method. Diluted extract (100 μ L) was mixed with DPPH solution (3.9 mL), shaken for 30 seconds, then left for 30 minutes at room temperature. Absorbance at 515 nm was measured using a UV-Vis spectrophotometer. Methanol (100 μL) was used as a blank with DPPH. Results were expressed as µmol Trolox equivalent per gram of dry weight (μ mol TE/g d.w.), determined by a Trolox standard curve $(R^2 = 0.993)$ (Stoica et al., 2024).

The plum extract's anthocyanin content was analyzed using a UV-Vis spectrophotometer with diluted samples $(1: 10)$ following the pH differential method. Plum sample (200 μL) was mixed with buffer solutions at pH 1.0 and 4.5 (800 μL) in a spectrophotometer cuvette. After 15 minutes without light exposure, absorbance was measured at 520 and 700 nm wavelengths. Anthocyanin content was quantified as mg cyanidin 3-glucoside equivalents (C3G)/100 g dry weight (dw) (Meyers et al., 2003).

 The assessment of the potential health hazards posed to humans by the consumption of plum fruits contaminated with metals was conducted using dietary intake of metals (DIM) and health risk index (HRI). If the calculated HRI value is <1, the exposed population faces minimal risk, whereas a value ≥ 1 signifies a risk to human health (Einolghozati et al., 2023). DIM aids in assessing the daily exposure levels to heavy metals ingested by humans (AL-Huqail et al., 2023).), and is calculated using the formula: $DIM = (IR \times C_{hv} \times C_{factor})/B_w$, where [IR] refers to plum fruit ingestion rate (0.345 $kg/person/day)$, $[C_{hv}]$ represents the concentration of heavy metal in fruit sample (mg/kg) [Cfactor] signifies the conversion factor (0.085) and $[B_w]$ symbolizes the body mass (male - 67 kg; female - 62.3 kg) (Ezez et al., 2023).

The Health Risk Index (HRI) is determined through the division of the DIM for each specific heavy metal by its corresponding reference dose (RfD). The RfD values for Cd, Cu, Ni and Zn are 0.001, 0.040, 0.020, and 0.300 mg/kg/day, respectively (USEPA). The calculation of HRI is performed using Equation: $HRI = DIM/R_fD$.

The data presented in this study consist of mean values, obtained from triplicate analyses, along with the standard deviation of the mean. Statistical data processing was done using IBM SPSS v26 software and the Duncan test was used to determine significance at a level of $p \leq$ 0.05.

RESULTS AND DISCUSSIONS

Chemical soil properties. The results indicate that the soil within the Eco and Cv systems was characterized as possessing neutral pH levels with medium to high fertility and very good availability of macro elements (Table 2). *Prunus domestica* L. demonstrates excellent adaptability to the characteristics of this soil type.

Chemical properties	Management system				
	Cv	Eco			
Depth (cm)	$0-20$ cm				
pН	$7.10 \pm 0.21a$	$7.16 \pm 0.11a$			
$C_{\text{org}}(\%)$	$2.11 \pm 0.25a$	$2.43 \pm 0.19a$			
Humus $(\%)$	3.64 ± 0.17 a	$4.19 \pm 0.12 b$			
N_{t} (%)	$0.19 \pm 0.04a$	$0.22 \pm 0.06a$			
P (ppm)	$97.7 \pm 0.34a$	82.6 ± 0.41			
K (ppm)	$421.5 \pm 0.63a$	409.2 ± 0.54			
Zn (mg/kg)	$65.82 \pm 0.39a$	49.72±0.41b			
Cu (mg/kg)	88.13±0.36a	114.83±0.49b			
Ni (mg/kg)	$23.21 \pm 0.18a$	16.42±0.22b			
Cd (mg/kg)	$0.37 \pm 0.07a$	0.17 ± 0.05			
As (mg/kg)	$1.43 \pm 0.09a$	$1.47 \pm 0.10a$			

Table 2. Selective chemical soil properties in Aug.

¹Means associated with different letters exhibit significant differences, as determined by Tukey's test at a significance level of $p < 0.05$; ²Ecoecological; Cv-conventional;

The rank order of heavy metal concentrations (mg/kg) is: $Cu > Zn > Ni > As > Cd$, with values $88.13 > 65.82 > 23.21 > 1.43 > 0.37$ for Cv, $114.83 > 49.72 > 16.42 > 1.47 > 0.17$ for Eco (Table 2). The maximum permissible limits according to WHO/FAO, 2023 are: Zn (200 mg/kg), Cu (100 mg/kg), Ni (50 mg/kg), Cd (1 mg/kg), As (5 mg/kg). All values are below the maximum permissible limit except for Cu in the ecological system (114.83 mg/kg), but they did not exceed the intervention limit (200 mg/kg). This higher concentration of Cu in the ecological system results from using antifungal treatments with copper-based products, in recommended doses to maintain productivity and care for the orchard, and to minimize the risk of contaminating the ecosystem with contaminants. The bioavailability of heavy metals is mainly influenced by soil physicochemical properties. The higher content of organic matter (4.19%) at a 0-20 cm depth can essentially impact metal behaviour by binding with toxic metals, increasing metal toxicity. The availability and mobility of heavy metals are influenced by pH. Heavy metals in low-pH soil tend to be more mobile than in soil with higher pH (Sintorini et al., 2021). Numerous studies have investigated the impact of different cultivation practices on the physico-chemical properties of plum fruit, including plant nutrition, soil fertility, and sensory evaluation of the fruits (Walkowiak-Tomczak, 2008; Usenik, 2009; Rop, 2009; Akšić et al., 2023). Ecological orchards have been found to exhibit higher levels of soil organic matter and improved soil biological functions, providing superior support for ecosystem function regulation compared to conventional orchards (Ionica et al., 2013).

Plum quality parameters. Table 3 shows the attributes of two varieties of fruit (fruit mass, pulp firmness, titratable acidity (TA), pH, and colour parameters), as well as the phytochemical characteristics of the "Tuleu Gras" and "Carpatin" plum varieties in Eco and Cv systems. The variety "Carpatin" has larger fruit sizes than the variety "Tuleul Gras", but their weight was more significant in the Cv system for both varieties. According to Mditshwa et al. (2017), Cv-grown plums are larger and have a more uniform appearance. However, there are no significant differences in

colour between the two types of management, although eco plums show a more consistent and vigorous colour. "Tuleu Gras" variety in the Eco system management was distinguished by an intense reddish colour $(a*=10.27 \pm 0.08)$ which can be attributed to the pigments (anthocyanins) present in the plum pulp.

Means associated with different letters exhibit significant differences, as determined by Tukey's test at a significance level of $p < 0.05$.

Also, eco plums have firmer flesh than Cvgrown plums, and smaller sizes may contribute to this increased firmness through reduced water accumulation or a denser mesocarp (Hassan et al., 2021). Studies show that organic plums produce firmer flesh and a slightly different texture than Cv-grown plums. Compared to the variety 'Carpatin' (0.87 ± 1) 0.09%), TA in the variety 'Tuleu Gras' was significantly higher $(1.72 \pm 0.15\%)$. Regarding the variations between the management systems, the acidity levels were the highest for the "Tuleu Gras" variety in the conventional system and the lowest in the Eco system. Instead, the variety "Carpatin" recorded the lowest level of acidity $(0.81 \pm 0.08\%)$. The acidity of cultivars *Prunus salicina* L. revealed a wide range of acidity levels, from 0.31 to 0.55% (Rahimi et al., 2005), and even as high as 0.9% (Martínez-Esplá et al., 2014). The fruits of the "Stanley" *Prunus domestica* L. grafted on rootstocks of *Prunus cerasifera* L. showed acidity levels between 0.45% and 0.67% (Saridaş et al., 2016). The total acidity of plums (*Prunus cerasifera* Ehrh.) from the Mediterranean region varies significantly, between 0.72 and 1.81% (Ayanoğlu et al., 2007). These variations in acidity are influenced by factors such as fruit variety, level of maturity, and growing conditions. The preservation of food and products largely depends on titratable acidity and pH values (Da Silva et al., 2016). An inverse relationship was found between TA and pH values for all levels, as indicated in Table 3.

Eco plums displayed higher antioxidant capacity and polyphenol, flavonoid, and anthocyanin content than conventional plums (Table 3). The same results were obtained in a study of 30 Eco vs. Cv plum varieties by Cuevas et al. (2015). Another study is in agreement with our data on anthocyanin and polyphenol content (Cordova and Watson, 2010). Similar results were also found in the research by Arion et al. (2014), although they concluded that autumn compared to summer harvested plums contained higher phenolic content. However, in other fruit species, higher antioxidant contents were found in medium to late-ripening fruits compared to earlier ripening fruits, which were related to higher temperatures and light intensity (Drogoudi et al., 2017; Sarıdaș et al., 2022). Ecological systems of cultivation are linked to a certain level of stress (restricted and limited use of pesticides and fertilizers), which could lead to the accumulation of secondary metabolites responsible for plant defence (Raigón et al., 2010; Oliveira et al., 2013). The most important secondary metabolites in plums are polyphenols (Kim et al., 2003), whose content typically increases under stress conditions, as observed in the analysed samples.

The total polyphenol content of the two plum varieties ranged from 3.37-3.89 mg GAE/g dw, and the anthocyanin content ranged from 4.68- 5.0 mg C3G/100 g dw (Table 3). The total polyphenolic content determined in the present study was higher than the range of 174-375 mg GAE 100 g^{-1} fresh weight determined by Kim et al. (2003) for 6 plum varieties grown in New York. This may be due in part to the different extraction protocols used by Kim et al. (2003) (80% MeOH with ultrasound-assisted extraction), which can extract more phenols than stirring-based methods (Aboshora et al., 2014; Musa et al., 2011).

Most of the plum varieties studied in this study had lower levels of anthocyanins compared to the average of 17 mg 100 g^{-1} found by González-Flores et al. (2011), who quantified anthocyanins using HPLC on the Japanese plum variety 'Crimson Globe' (*Prunus salicina*). Compared to common European plums, which were used in this study, Japanese plums typically have higher concentrations of anthocyanins (Fanning et al., 2014), which is the most likely explanation for this difference.

Table 4 presents the results of heavy metal contents: zinc (Zn), arsenic (As), copper (Cu), nickel (Ni), and cadmium (Cd), in plum fruit samples.

Table 4. The influence of plum variety on fruit heavy metals concentrations

Heavy	Tuleu Gras		Carpatin		M.A.L
metals	Cv	Eco	Cv	Eco	
Zn	$0.53+$	$0.41 \pm$	$0.57\pm$	$0.48+$	0.60
(mg/kg)	0.11a	0.14a	0.10a	0.19a	
Cп	$0.76\pm$	$1.23 \pm$	$0.87+$	$1.32 +$	10
(mg/kg)	0.13 _b	0.36a	0.48 _b	0.30a	
Ni	$0.23 \pm$	0.19±	$0.16\pm$	$0.13+$	10
(mg/kg)	0.08a	0.10a	0.11a	0.09a	
Cd	$0.009\pm$	$0.005\pm$	$0.01 \pm$	$0.007\pm$	0.02
(mg/kg)	0.01a	0.01a	0.01a	0.01a	
As (mg/kg)	nd	nd	nd	nd	0.50

M.A.L. - Maximum permissible limit; Means associated with different letters exhibit significant differences, as determined by Tukey's test at a significance level of p < 0.05.

Variations in metal concentrations are observed between the conventional system (Cv) and the ecological system (Eco) for both varieties. The heavy metals concentrations (mg/kg) in plum fruits collected were in the following range: 0.41-0.57 (Zn), 0.76-1.32 (Cu), 0.13-0.23 (Ni) and 0.005-0.01 (Cd). The specified ranges denote the bioaccumulation of heavy metals in the examined fruits, exhibiting the following descending sequence: copper > zinc > nickel > cadmium > arsenic. Similar to the soil results, higher Cu values are found in the ecological system for both types of plums. Noteworthy, all heavy metal concentrations were higher in the

"Tuleu Gras" cultivar than "Carpatin" 'Carpatin'. emphasizing its metal adsorption and accumulation capabilities. Cu and Zn were the most frequently detected metals in plum fruits, consistent with findings in soil samples.

Based on these metal concentrations, the potential health risks (DIM, and HRI) associated with the consumption of these fruits
were used to evaluate the orchard's orchard's management practices. The results of DIM and HRI studies given in Table 5 showed that plum fruit samples collected from Iasi showed no significant health concern related to their dietary consumption. Our study found that the DIM values for the heavy metals we examined suggest that the estimated amount of heavy metals consumed by individuals (RfD) is below acceptable daily limits, providing a safety net against potential hazardous exposure to heavy metals.

Moreover, the calculated HRI values for these heavy metals were consistently below 1, reaffirming that there are no significant health effects from the consumption of plum fruit. This means that in all samples, the level of exposure did not exceed the safe limit, making it highly unlikely to cause any adverse health impact.

CONCLUSIONS

The results of the present study demonstrated that plum fruit samples harvested from orchards in the "Adamachi" Farm - Iaşi University of Life Sciences (IULS), Romania, have benefits for human health due to their phytochemical content. According to the study results, the cultivar 'Tuleu Gras' in Ecological systems of cultivation exhibited elevated levels of total phenolics content, flavonoids, anthocyanins, and antioxidant capacity. The analysis revealed that concentrations of heavy metals (Cd, Cu, Ni and Zn) did not exceed safe limits, according to health risk studies. The decreasing order of heavy metal concentrations in plum fruit was observed as follows: Cu < Zn $\langle N_i \rangle \langle C_d \rangle \langle A_s \rangle$ are Eq. it was found that sampling sites in the ecological system showed higher levels of Cu than those in the conventional system due to the use of ecological-based Cu treatments.

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