

COMPARATIVE ANALYSIS OF DIGESTION METHODS FOR QUANTIFYING HEAVY METALS IN PLUM ORCHARDS

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ABSTRACT. Increasing interest in healthy food among the population raises concerns about heavy metals in fruit and their impact on public health. To assess this issue, this study presents a comparative analysis of digestion methods for quantifying heavy metals in plum orchards managed under conventional and ecological practices in the "Adamachi" Farm district of Iasi University of Life Sciences (IULS). We evaluated and optimised two wet digestion methods - in an open system and microwave-assisted - to determine the concentrations of heavy metals, such as copper (Cu), zinc (Zn), nickel (Ni), lead (Pb), and cadmium (Cd), which pose environmental and health risks. The metal concentrations were measured using an atomic absorption spectrophotometer, according to standard methods. Microwave-assisted digestion was more efficient and faster than the conventional method (in an

open system). The ecologically managed orchards showed a lower heavy metal content overall, with the exception of Cu levels, due to the Cu-based treatments. By determining the estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) for both children and adults, the potential health risks from heavy metals were determined. There were no related associated risks to human health (THQ and HI < 1), and the accumulated metals in plum fruit samples showed that the EDI values followed the descending order of Cu > Zn > Ni > Pb > Cd. The analysis revealed non-significant differences for most data obtained after processing using the two methods. These results highlight the importance of selecting the optimal digestion methods for heavy metal analysis in plums and sustainable agricultural practices to safeguard the



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environment and consumer health from heavy metal contamination.

Keywords: health risk; heavy metals; plum orchard; wet digestion.

INTRODUCTION

Prunus domestica L., a fruit tree belonging to the Rosaceae family, native to Western Asia and Europe, is extensively cultivated in the temperate regions of the Northern Hemisphere (Zhang *et al.*, 2024) and widely consumed globally, with a considerable presence in Europe and Romania (Figure 1). Its increasing economic and nutritional importance is reflected in its contribution to the global annual production of about 12.6 million tonnes of plums, securing a substantial position in the world market. In recent years, Romania has enhanced its plum yields, spurred by the expansion of ecologically managed orchards in response to the rising global demand for sustainable products, which have been recognised for their health and environmental benefits (Karadag, 2024).

Plum fruits are an integral component of human diets due to their rich nutritional profile, which is characterised by high levels of vitamins, minerals, and antioxidants. The World Health Organization (WHO) guidelines recommend that 400 g of fruit be consumed daily for human health (Harmankaya *et al.*, 2012). Regular plum consumption offers a range of health benefits, such as cardioprotective effects, bone health enhancement, and anticancer effects, specifically against colon cancer, among other nutritional benefits that contribute to its popularity among consumers (Ma *et al.*, 2022).

However, despite these recognised health benefits, plums can be contaminated with agricultural pollutants, including heavy metals and mycotoxins (Einolghozati *et al.*, 2022). The contamination of fruit with heavy metals is influenced by anthropogenic activities, such as urbanisation, industrialisation, and improper pesticide use, in addition to natural processes and water pollution (Heshmati, 2020). These changes affect food quality and contribute to the disappearance of many plant species, which have become rare or even threatened by extinction (Chirilă, 2023; Chirilă and Vassilev, 2024a, b). In recent decades, environmental pollution in the air (Yin *et al.*, 2023), soil (Li *et al.*, 2022), water resources (Chen *et al.*, 2022), and food (Assad *et al.*, 2023; Bai *et al.*, 2023) has intensified, further complicating food safety (Khaneghah, 2021).

Anthropogenic landscape modifications have led to ongoing challenges in food security, which was a pressing issue in 2024 that was further exacerbated by heavy metal accumulation in plums, posing risks to both human health and ecological systems (Gysbrechts, 2024). Essential metals, such as iron (Fe), zinc (Zn), and copper (Cu), are necessary for human metabolic processes, and non-essential metals, such as cadmium (Cd), lead (Pb), nickel (Ni), and mercury (Hg), are toxic even at low concentrations, highlighting the necessity for precise monitoring (Zhou *et al.*, 2024). High cadmium (Cd) concentrations lead to memory loss, cardiovascular disorders, cancer, renal dysfunction, and even death. Lead (Pb) can have adverse effects on the central nervous system. Arsenic (As) is known to

induce diabetes and neurologic and neurobehavioral disorders. Cu concentrations above the permissible limit cause skin infections, hair loss, and respiratory illnesses, while an excess of Zn leads to soft stools, obesity, autism, and reduced immune function (Mehri *et al.*, 2024). The increasing risk of ingestion of these metal ions through vegetables and fruit has underscored the importance of accurate contamination assessment to ensure food safety and to protect consumer health.

The quantification of heavy metals in fruit samples can be effectively achieved by applying advanced spectroscopic techniques. Recent developments in analytical chemistry have facilitated the utilisation of inductively coupled plasma atomic emission spectrometry (ICP-AES), atomic absorption spectrometry (AAS), and inductively coupled plasma mass spectrometry (ICP-MS) (Scutaraşu and Trincă, 2023; Wu *et al.*, 2024). These methodologies provide high sensitivity and precision in measuring metal concentrations among crops. These analytical techniques involve transforming solid samples into liquid solutions to determine the metal concentrations. Heavy metals in fruit samples are usually converted into a solution by acid digestion methods. Effective digestion techniques are a fundamental step in reliable heavy metal determination in various samples, providing reassurance about the safety of our food (Wale, 2024).

The most commonly used methods for the digestion of fruit samples include open and closed vessel digestion, which are microwave assisted.

The choice of the correct digestion method is essential in the analytical process, as it directly influences heavy metal recovery, sensitivity, and the accuracy of subsequent quantitative analysis. Open vessel digestion involves the use of pure acids under reflux at atmospheric pressure, facilitating the gradual decomposition of organic components. However, this method is prone to the loss of volatile analytes and requires longer processing times, which negatively affect performance in heavy metal analysis by reducing recovery and measurement accuracy (Ishak *et al.*, 2015). In contrast, microwave-assisted digestion accelerates chemical reactions in a sealed environment at controlled temperatures and pressures, ensuring complete and rapid sample decomposition. This method minimises contamination risks and analyte loss, enabling more efficient heavy metal extraction from the sample matrix, improving the sensitivity and accuracy of subsequent measurements (Kasahun, 2024). Although open vessel digestion is more economical and straightforward, it often requires additional post-digestion steps to fully recover all components, thus increasing the time and cost. In comparison, microwave-assisted digestion reduces contamination risks due to its closed-system design, is faster, and enhances reproducibility and yield in heavy metal extraction. Furthermore, precise control over temperature and pressure allows for the optimisation of digestion protocols tailored to different sample matrices, leading to improved overall analytical performance (Rantala *et al.*, 2023).

As different digestion procedures produce variable results, a validation process is essential for ensuring data accuracy and quality. The choice of the optimal digestion method is necessary to determine the total heavy metal content of the fruit correctly and thus to assess its impact on human health. Therefore, method selection must be carefully reasoned to provide relevant information on contamination levels (Alsaleh *et al.*, 2017).

Highlighting the importance of this widely consumed fruit, this study aimed to assess the Cu, Zn, Ni, Pb, and Cd concentrations in plum fruit cultivated under ecological and conventional management practices in northeastern Romania. Heavy metal quantification was achieved using flame atomic absorption spectrometry (fAAS), comparing two of the most frequently applied digestion methods - conventional (acid digestion in an open system) and microwave assisted. The aim was to identify the most efficient and

appropriate digestion method for this food matrix. The results were compared with the safety limits established by WHO/Food and Agriculture Organization (FAO). Furthermore, the risks associated with consuming heavy metals through plum fruits, both for adults and children, were assessed using the Monte Carlo simulation (MCS) technique.

MATERIALS AND METHODS

Research area and management practices

The orchard was established in 2014 in Iasi, northeastern Romania (47°15' N; 27°30' E), at the Horticultural Research Station “Adamachi” Farm, from which fresh fruit samples of the “Centenar” variety were collected (*Figure 2*).

The orchard’s climate is constant due to its location on the Moldavian Plateau, which has a humid temperate–subtropical climate.

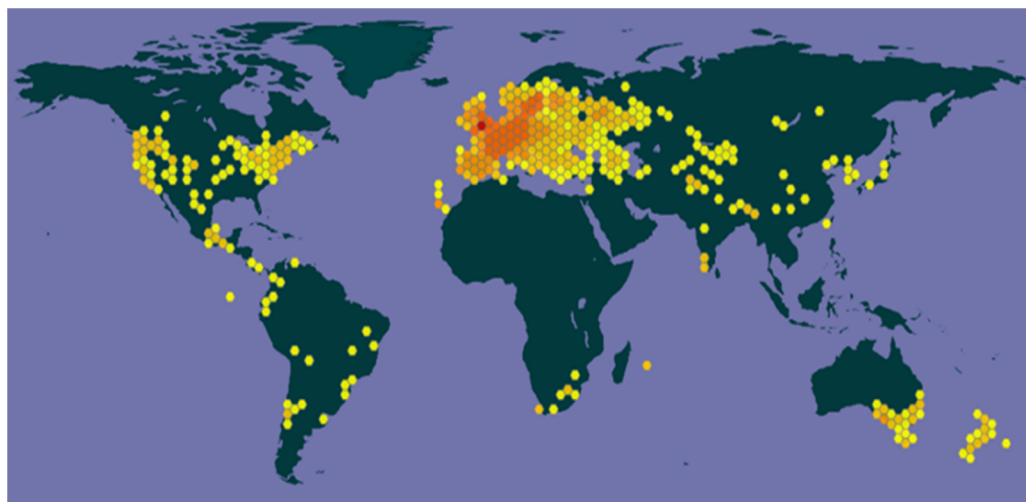


Figure 1 – Distribution of *Prunus domestica* L. throughout the world
(<https://www.gbif.org/species/7931731>)



Figure 2 – Map depicting the sampling location of the plum orchard in the Iasi region of Romania

The climate of the region is characterised by warm summers and moderate winters, with an average annual temperature of 10°C and annual precipitation of about 518 mm (WBG, 2024). The orchard's soil type is an aric-cambic chernozem with a loamy clay texture (Rusu *et al.*, 2024). The “Centenar” variety was chosen for its resilience and high yield potential under local climate conditions.

Ecological plum orchards received yearly fertilisation with 600 kg ha⁻¹ of organic fertilisers applied in two sessions per season as well as 2 kg of Cu, potassium sulphate (K₂SO₄), Fe, and Zn chelates. Integrated pest management used paraffinic oil and Cu-based foliar sprays, while manual weeding was used for weed control. The emphasis on organic fertilisers and the careful selection of micronutrients aim to build soil health and promote beneficial microbial activity. These practices contribute to improved nutrient uptake by trees, which is essential for optimal fruit development. The manual weeding method further supports the soil structure and reduces resource competition, enabling plum trees to thrive without the adverse effects of chemical herbicides. In

contrast to the conventional plum orchard, the horticultural techniques applied included integrated pest management (such as deltamethrin (0.250 g L⁻¹), Lamba-cyhalothrin (0.250 g L⁻¹), and Boscalid + pyraclostrobin (0.5 l ha⁻¹)) and 250 kg ha⁻¹ of 11-15-15 (N-P-K) fertilisers, with plant protection products applied 3 times per year. In autumn, soil tillage was performed by ploughing, incorporating fertilisers into the soil up to a depth of 20 - 30 cm. Although this method can result in higher immediate yields, it raises concerns about long-term soil health and the potential for chemical residues in fruit. Annual soil tillage also poses risks of erosion and disruption of soil microorganisms, which are vital for nutrient cycling. The management practices in both orchards are designed to enhance not only the yield but also the sustainability of the production system.

Sample collection

In August 2023, fruit samples from orchards managed with ecological and conventional practices were collected to assess the heavy metal content in plums. A total of 90 healthy fruit samples were collected from 30 selected. To eliminate sand particles, plant waste pieces, and

leftover chemicals from pest and disease treatments, the samples were cleansed using ultrapure water. After cleaning, the fruits were stored at -20°C until digestion.

Chemical materials and sample digestion

Sample digestion was performed to determine the heavy metal content using a 3:1v:v mixture of concentrated nitric acid (HNO_3 , 65%) and hydrogen peroxide (H_2O_2 , 30%). The stock metal standards Cd, Cu, Ni, Pb, and Zn at 1000 mg L^{-1} and the reagents were obtained from Merck (Darmstadt, Germany). All liquid dilutions were diluted using Milli-Q ultrapure water (Millipore, Bedford, MA, USA).

Method A (acid digestion in an open system). Fresh plum samples were homogenised, and 20 g were oven dried at 105°C (24 h). The dried samples were then calcined at 550°C (4 h) (Figure 3, a). After cooling, the ash was treated with 7 mL HNO_3 and 2 mL HClO_4 . Samples were heated on a hot plate for 2 h ($95 \pm 5^{\circ}\text{C}$) in beakers covered with watch glass (Figure 3, b) until complete digestion, as indicated by a limpid or pale solution. The resulting suspension was cooled before being filtered into 25 mL volumetric flasks with deionised water (Figure 3, c).

Method B (microwave acid digestion). A 1-g freeze-dried sample (Figure 3a) was digested with 5 mL HNO_3 and 1 mL H_2O_2 in a Teflon container at 170°C for 20 min (Figure 3b) for the microwave digestion system - Speedwave (Berghof Products Instruments GmbH, Eningen unter Achalm, Germany). The contents of each container were transferred and

quantitatively filtered into 50- cm^3 volumetric flasks with deionised water (Figure 3, c). The resulting solutions from Methods A and B were analysed and quantified using fAAS (Figure 3, d).

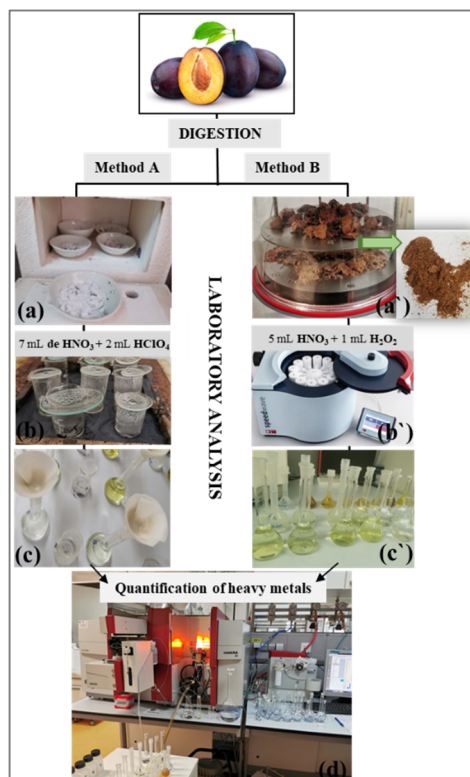


Figure 3 – Flowchart of the plum fruit digestion process for fAAS analysis

Measuring heavy metals in plum fruits

Metal analysis was performed using fAAS (ContraAA 700). An air-acetylene flame was employed to determine all metals.

Calibration instrument and quantification limits

Calibration was performed using stock metal standards of 1000 mg L^{-1} to prepare composite standards in 0.5% HNO_3 , with concentrations spanning

0.02–3 mg L⁻¹ for Cu and Zn, 0.05 - 1.5 mg L⁻¹ for Ni, and 0.05 - 2 mg L⁻¹ for Pb and Cd. The regression coefficient indicated strong linearity, $R^2 \geq 0.9996$. The instrument detection limit (LOD) for the analysed metals ranged from 0.11 mg L⁻¹ for Cu to 0.47 mg L⁻¹ for Cd, while the quantification limit (LOQ) varied from 0.38 mg L⁻¹ for Cu to 1.58 mg L⁻¹ for Cd. All calibration parameters are presented in *Table 1*. After instrument calibration, the sample solutions digested using methods A and B were aspirated into the fAAS to quantify the metal concentrations, with five replicates performed for each sample. The same analytical procedure was applied to the blank samples. Replicate analysis precision was defined as the relative standard deviation (RSD %), calculated by dividing the standard deviation (SD) by the mean.

Public health risk assessment

The human health risk assessment for both adults and children was performed by calculating various indicators: daily intake of metals (DIM), target hazard quotient (THQ), hazard index (HI), and target cancer risk factor (TCR).

The DIM was calculated using *Equation (1)*, based on the metal concentration in plum fruits consumed per person and normalised by the average body weight (Khan *et al.*, 2024).

$$\text{DIM} = (\text{FIR} \times C_{\text{fruit}} \times C_f) / \text{Bw} \quad (1)$$

where the fruit ingestion rate (FIR) for plums was 0.243 kg/person/day for children and 0.345 kg/person/day for adults; C_{fruit} is the metal concentration in fruits (mg/kg), applying a conversion factor (C_f) of 0.085; and the average body

weight (Bw) was 73 kg for adults and 32.7 kg for children in Romania.

The *non-cancerogenic risk* associated with consuming heavy metals from plum fruit was expressed by THQ and HI, calculated according to *Equation (2)* and *Equation (3)*, respectively. HI is formulated based on the USEPA (2000) guidelines for health risk assessment.

$$\text{THQ} = \text{DIM} / (R_f D) \quad (2)$$

where HI is the sum of different poisonous metal hazards; and $R_f D$ is the oral reference dose (mg/kg/day), with values of 0.0001, 0.04, 0.02, 0.004, and 0.3 for Cd, Cu, Ni, Pb, and Zn, respectively (Khan *et al.*, 2024).

$$\begin{aligned} \text{HI} &= \sum_{i=1}^n \text{THQ} = \\ &= \text{THQ}_{\text{Zn}} + \text{THQ}_{\text{Cu}} + \text{THQ}_{\text{Pb}} + \\ &+ \text{THQ}_{\text{Cd}} + \text{THQ}_{\text{Ni}} \end{aligned} \quad (3)$$

According to Hassan *et al.* (2024), $\text{HI} < 1$ indicates no obvious health consequence, $\text{HI} > 1.0$ indicates a health risk, and $\text{HI} > 10$ results in serious long-term health issues.

TCR expresses the *carcinogenic risk*, representing the lifetime likelihood of developing cancer. The TCR was calculated using *Equation (4)* (Aksouh *et al.*, 2024).

$$\text{TCR} = (E_f \times E_d \times \text{FIR} \times C_{\text{fruit}} \times C_f) / (\text{Bw} \times \text{AT}_c) \quad (4)$$

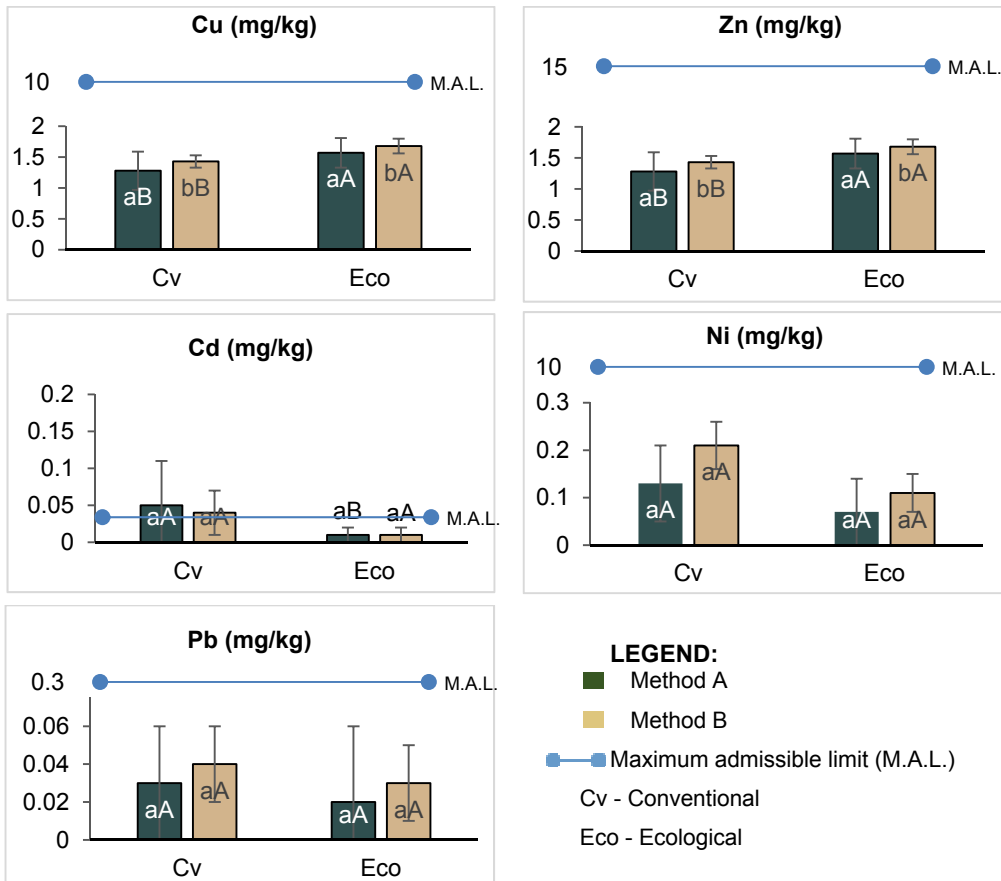
where E_f is the exposure frequency (365 days/year); E_d is the duration of exposure (62 years); C_f is the cancer slope factor attributed to the carcinogenic metal equal to 0.38 and 0.0085 (mg/kg/day) for Cd and Pb, respectively (Onoyima, 2021); and AT_c is the average time for carcinogens (22.630 days/years).

Statistical analysis

Following one-way analysis of variance (ANOVA), the Tukey test assessed statistically significant differences in the metal content under the same management system and between methods. IBM SPSS v20 software was used for statistical data analysis, with all samples processed in triplicate and results presented as the mean $\pm$ standard deviation (SD).

RESULTS

The application of open vessel digestion and microwave-assisted digestion in fruit samples showed different results achieved by both digestion methods. The heavy metal (Cu, Cd, Zn, Pb, and Ni) contents in samples mineralised with a mixture of pure acids from the two procedures are presented in *Figure 4*.



M.A.L – Maximum admissible limit according to the Romanian Ministry of Agriculture Food; Statistically significant differences between the metal contents with the same management system and different methods are marked with different lowercase letters based on Tukey's test ($p < 0.05$). Statistically significant differences between the metal contents with the same method and different management systems are marked with different uppercase letters based on Tukey's test ($p < 0.05$).

Figure 4 – Heavy metal content (mg/kg) in plum fruit obtained from digestion methods A and B relative to M.A.L.

Comparative analysis of digestion methods for quantifying heavy metals in plum orchards

Table 1 – Calibration parameters for the fAAS instrument

Metals	Standard concentration (mg/L)	Calibration equation	R ²	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)
Ni	0.05, 0.1, 0.5, 1, 1.5, 2	$A = 0.1344x + 0.0069$	0.9998	0.19	0.65
Cu	0.02, 0.05, 0.5, 1, 2, 3	$A = 0.0797x + 0.0052$	0.9999	0.11	0.38
Zn		$A = 0.2242x + 0.0133$		0.12	0.4
Pb	0.05, 0.1, 0.5, 1, 1.5, 2	$A = 0.0052x + 0.0005$	0.9996	0.39	1.3
Cd	0.05, 0.1, 0.5, 1, 1.5, 2	$A = 0.1473x + 0.0004$	0.9997	0.47	1.58

Methods A and B obtained similar results for Zn and Cu, with slightly greater differences observed for Cd, Ni, and Pb. Regardless of the digestion method, the metal concentrations followed the order: Zn > Cu > Ni > Cd > Pb. The high levels of Zn and Cu are beneficial, as they serve as essential micronutrients.

The maximum permissible limits for the analysed metals were 0.05 mg/kg for Cd, 0.3 mg/kg for Pb, 15 mg/kg for Zn, 10 mg/kg for Cu, and 10 mg/kg for Ni. The determination of the metal contents was determined within legally admitted limits by Order of the Ministry of Health No 756/1997 and Order of the Minister of Health No 276/1998, published in M.O. of Romania No 268/11.06.1999.

The influencing factors on the methods included the distinct chemical reactivity of each acid mixture used in methods A and B, as well as the effect of temperature and pressure on the microwave digestion process. For example, method B-optimised heating conditions may enhance the volatilisation of volatile metals, such as Cd and Pb, resulting in more precise recoveries and lower standard deviations, as noted in the statistical analysis (*Table 2*). This may explain the observed higher accuracy and lower RSD values for method B compared to method A.

The heavy metal concentrations determined by method A presented the highest SD values for Zn and Cu. In contrast, method B, by optimising the matrix decomposition conditions and the heating process, contributed to increasing measurement accuracy compared to method A. The RSD values for microwave digestion were lower and more uniform than the values obtained by open digestion, ranging from 5.96 to 100% for method B, compared to the range of 15.29 to 200% for method A. This validation process indicated that microwave-assisted digestion was more accurate. Additionally, open vessel digestion recoveries were between 84 and 97%, while microwave-assisted digestion achieved recoveries from 88 to 105% for all metals.

Table 3 presents the estimated daily metal intake from plum samples for children and adults. The DIM in plums was ranked as follows for both demographics: Cu > Zn > Ni > Pb > Cd.

Comparative analysis revealed the highest DIM value for Cu in the ecological system using method B with values of 1.08^{-3} mg/kg/day for children and 5.07^{-4} mg/kg/day for adults, while Cd recorded the lowest value (6.42^{-6} mg/kg/day). All health risk indices (THQ, TCR, and HI) for the analysed metals were below 1, indicating that exposure to these metals does not pose a health risk.

Table 2 – Statistical assessment (N=5) of evaluated metals by digested using open system (method A), and microwave-assisted digestion (method B)

Metal	Management system	SD (mg kg ⁻¹)		RSD (%)	
		Method A	Method B	Method A	Method B
Cu	Cv	0.31	0.1	24.22	6.99
	Eco	0.24	0.12	15.29	7.14
Zn	Cv	0.2	0.09	15.63	5.96
	Eco	0.27	0.13	18.00	7.39
Cd	Cv	0.06	0.03	120	20
	Eco	0.01	0.01	100	100
Ni	Cv	0.08	0.05	61.54	23.81
	Eco	0.07	0.04	100	36.36
Pb	Cv	0.03	0.02	100	50
	Eco	0.04	0.02	200	66.67

SD: standard deviation; RSD: relative standard deviation: Cv – conventional; Eco – ecological

Table 3 – Health risks of the heavy metal content in plum (*Prunus domestica*) samples digested using open system (A) and microwave-assisted methods (B)

Method	Management system	Heavy metals	Children			Adults				
			DIM	THQ	HI	TCR	DIM	THQ	HI	TCR
A	Cv	Cu	8.22 ⁻⁴	2.06 ⁻²	3.78 ⁻¹	9.51 ⁻³	5.08 ⁻⁴	1.27 ⁻²	2.17 ⁻¹	6.05 ⁻³
		Zn	8.21 ⁻⁴	2.74 ⁻²		9.51 ⁻³	5.07 ⁻⁴	1.69 ⁻³		6.04 ⁻³
		Cd	3.21 ⁻⁵	3.21 ⁻¹		1.41 ⁻⁴	1.97 ⁻⁵	1.97 ⁻¹		8.98 ⁻⁵
		Ni	8.35 ⁻⁵	4.18 ⁻³		2.23 ⁻⁴	5.16 ⁻⁵	2.58 ⁻³		1.42 ⁻⁴
		Pb	1.93 ⁻⁵	4.83 ⁻³		1.86 ⁻⁶	1.19 ⁻⁵	2.98 ⁻³		1.21 ⁻⁶
	Eco	Cu	9.92 ⁻⁴	2.48 ⁻²	9.77 ⁻²	1.17 ⁻³	6.24 ⁻⁴	1.61 ⁻²	6.11 ⁻²	7.42 ⁻³
		Zn	9.63 ⁻⁴	3.21 ⁻³		1.11 ⁻²	5.96 ⁻⁴	1.99 ⁻³		7.08 ⁻³
		Cd	6.42 ⁻⁶	6.42 ⁻²		2.82 ⁻⁵	3.97 ⁻⁶	3.97 ⁻²		1.79 ⁻⁵
		Ni	4.49 ⁻⁵	2.25 ⁻³		1.49 ⁻⁴	2.78 ⁻⁵	1.39 ⁻³		9.45 ⁻⁵
		Pb	1.28 ⁻⁵	3.20 ⁻³		1.26 ⁻⁶	7.90 ⁻⁶	1.98 ⁻³		8.03 ⁻⁷
B	Cv	Cu	9.18 ⁻⁴	2.29 ⁻²	9.99 ⁻¹	1.06 ⁻²	5.68 ⁻⁴	1.42 ⁻²	6.22 ⁻¹	6.76 ⁻³
		Zn	9.70 ⁻⁴	3.23 ⁻³		1.12 ⁻²	5.99 ⁻⁴	1.20 ⁻³		7.13 ⁻³
		Cd	9.63 ⁻⁵	9.63 ⁻¹		4.24 ⁻⁴	5.96 ⁻⁵	5.96 ⁻¹		2.69 ⁻⁴
		Ni	1.35 ⁻⁴	6.75 ⁻³		1.56 ⁻³	8.34 ⁻⁵	4.17 ⁻³		9.92 ⁻⁴
		Pb	2.97 ⁻⁴	7.43 ⁻³		2.52 ⁻⁶	1.89 ⁻⁴	4.72 ⁻³		1.61 ⁻⁶
	Eco	Cu	1.08 ⁻³	2.70 ⁻²	7.18 ⁻¹	1.25 ⁻²	6.67 ⁻⁴	1.67 ⁻²	5.95 ⁻¹	7.94 ⁻³
		Zn	1.13 ⁻³	3.76 ⁻³		1.31 ⁻²	6.99 ⁻⁴	2.33 ⁻³		8.32 ⁻³
		Cd	6.42 ⁻⁶	6.42 ⁻¹		2.82 ⁻⁵	3.97 ⁻⁶	3.97 ⁻¹		1.79 ⁻⁵
		Ni	8.17 ⁻⁴	4.09 ⁻²		8.17 ⁻⁴	5.19 ⁻⁴	2.59 ⁻²		5.19 ⁻⁴
		Pb	1.93 ⁻⁵	4.83 ⁻³		1.89 ⁻⁶	1.19 ⁻⁵	2.97 ⁻³		1.21 ⁻⁶

A - digested using open system; B - microwave-assisted methods; Cv – conventional; Eco – ecological; DIM - daily intake of metals, THQ - target hazard quotient, HI - hazard index, TCR - target cancer risk factor.

DISCUSSION

Nitric acid (HNO₃)-based mixtures are highly effective in decomposing organic matrices for metal determination, as supported by the literature (Abbruzzini et al., 2014; Akinyele and Shokunbi,

2015; Sadee and Ali, 2023; Siaka et al., 1998). However, no universal method exists for the digestion of plum fruit samples, as extraction yields vary based on the metal properties and techniques used. The high organic matter content in plum fruit significantly affects the

digestion efficiency (Oteef *et al.*, 2015). Organic substances are the main components complicating the digestion process, and studies on other plant samples have demonstrated that organic matter is a fundamental factor influencing the digestion efficiency (Adamczyk-Szabela *et al.*, 2017; Ghane *et al.*, 2021). Additionally, the chemical composition of the fruit, the type of acid, and digestion conditions impact mineralisation efficiency (Bacon *et al.*, 2019).

This study demonstrated that the $\text{HNO}_3/\text{H}_2\text{O}_2$ digestion method under high pressure was the most effective for decomposing plum fruit samples, ensuring optimal extraction and metal recovery. These results align with those of Turek *et al.* (2019), who found that microwave-assisted digestion was highly efficient for plant sample decomposition, offering better metal extraction compared to open digestion. Key findings include the higher efficiency of the microwave-assisted digestion method compared to the conventional approach. Both digestion methods applied to plum samples resulted in increased metal concentrations in the following order: $\text{Cd} < \text{Pb} < \text{Ni} < \text{Zn} < \text{Cu}$. However, the microwave-assisted method resulted in higher metal recoveries, indicating its superiority for accurate heavy metal analysis. High Cu and Zn levels provide nutritional benefits, as these metals are essential micronutrients. Cu is essential for physiological processes, including haemoglobin synthesis, connective tissue metabolism, and bone development (Tamene *et al.*, 2022). However, excessive Cu exposure leads to gastrointestinal issues (Faez *et al.*, 2018). Zn is indispensable for immune function

and cellular metabolism (Tamene *et al.*, 2022), but too much Zn can inhibit the absorption of other metals, such as Cu and Fe, leading to adverse effects (FAO/WHO, 2011; Onoyima *et al.*, 2021).

In this study, the Cu concentration varied from 1.28 (method A, Cv) to 1.68 mg/kg (method B, Eco), which are comparable with values found in previous studies on watermelon, orange, and banana, ranging from 1.22 to 2.13 mg/kg using classical HNO_3 digestion methods (Radwan and Salama, 2006; Onianwa *et al.*, 2000). Karasakal (2020) analysed kumquat (*Citrus japonica*) through different microwave digestion methods using HNO_3 , H_2O_2 , and HCl to assess the contents of multiple elements, showing Cu levels between 1.64 and 9.93 mg/kg.

In a study using the open system method, Cu levels in Nigerian fruit ranged between 1.94 and 2.02 mg/kg (Chinazo *et al.*, 2020), while research in Pakistan reported values from 0.54 to 3.18 mg/kg (Khair *et al.*, 2020; Swati *et al.*, 2021). Both of these ranges were higher than those in this study. Cu levels in plum samples were below the WHO/FAO safe limit of 10 mg/kg.

Zn concentrations ranged from 1.28 (method A, Cv) to 1.76 mg/kg (method B, Eco). Previous studies indicated significantly higher Zn levels, between 33.48 and 40.13 mg/kg (Chinazo *et al.*, 2020) and between 3.40 and 5.13 mg/kg (Asuquo and Bate, 2020) as well as lower values of 0.625 mg/kg (Swati *et al.*, 2021) and 0.06 - 0.15 mg/100 g (Maria *et al.*, 2019). Bagdatlioglu (2010) reported Cu and Zn levels of 0.28 - 1.13 and 0.80 - 1.67 mg/kg, respectively, in strawberry, 0.84 - 0.87 and 0.97 - 1.66 mg/kg,

respectively, in cherry, and 0.40 - 0.71 and 0.26 - 0.58 mg/kg, respectively, in grape when determined through microwave-assisted digestion. The WHO safe limit for Zn is 15 mg/kg, which exceeds the values found in this study.

The Pb concentrations showed insignificant variations ($p > 0.05$) and remained below the WHO's permissible limit of 0.3 mg/kg. Pb is known to cause cognitive delays in children and increase the risk of hypertension and cardiovascular diseases in adults (Commission of the European Communities, 2002; Akinyele and Shokunbi, 2015). The Pb levels were comparable to those reported by Ezez *et al.* (2023) for mango fruit (0.035 - 0.097 mg/kg) and lower than those found in Nigeria and Bangladesh, where values ranged from 1.24 to 1.78 and 15.68 to 87.89 mg/kg, respectively (Ashraful *et al.*, 2022; Asuquo and Bate, 2020; Chinazo *et al.*, 2020; Swati *et al.*, 2021). The Pb level in Pakistan is 0.09 mg/kg (Muhammad *et al.*, 2020). The average metal levels in mango were 0.035 - 0.097 mg/kg for Pb and 0.193 mg/kg for Cd (Ezez *et al.*, 2023). According to research conducted in San Luis Potosi, Mexico, measuring the concentrations of 13 metal elements in the fruit and leaves of 3 tree species, Cd (0.15 mg/kg), Ni (0.577 mg/kg), Cu (3.34 mg/kg), and Zn (20.37 mg/kg) were found in fruit at phytotoxic levels (Alcalá Jáuregui *et al.*, 2022), exceeding concentrations found in the current study. The Cd, Zn, and Cu concentrations in apricot kernel peels (0.059, 1.6, and 18.59 mg/kg) were also higher than in apricot kernels (0.06, 1.3, and 16.19 mg/kg), according to studies conducted on a variety of vegetables, including *Prunus armeniaca*. Whole fruit

values were 0.058 mg/kg for Cd, 2 mg/kg for Zn, and 17.94 mg/kg for Cu. Fresh fruit and vegetables had metal concentrations of ND-1.12 for Cu, ND-0.91 for Cd, 0.74 - 1.51 for Ni, 0.02 - 1.74 for Zn, and 0.15 - 1.93 for Pb, according to a Nigerian study (Obi-Iyeke, 2019). Cd levels exceeded the maximum allowable concentration of 0.2 mg/kg (FAO/WHO 2021) (Uzakov *et al.*, 2023).

Cd, a heavy metal found in impurities of products, including petroleum, phosphate fertilisers, and detergents (Khair *et al.*, 2020), was detected at levels ranging from 0.01 to 0.05 mg/kg, all below the WHO/FAO maximum limit of 0.05 mg/kg. These findings are lower than those of Ezez *et al.* (2023), who reported 0.19 mg/kg in mango, Asuquo and Bate (2020) in Nigeria, with levels between 0.52 and 1.79 mg/kg, and Swati *et al.* (2021), who reported a value of 5.142 mg/kg. In Pakistan, Cd levels range from 0.01 to 0.33 mg/kg (Chinazo *et al.*, 2020; Khair *et al.*, 2020; Muhammad *et al.*, 2020). Ozcan and Haciseferogullari (2007) measured Cu, Zn, Pb, and Cd contents of at 1.65, 8.09, 0.51, and 0.19 mg/kg, respectively, in strawberry, while Radwan and Salama (2006) reported values of 2.17, 7.49, 0.87, and 0.02 mg/kg, respectively.

Excessive Ni exposure causes oxidative stress, disrupts enzyme function, and has toxic effects, including allergic reactions and heightened cancer risk (Genchi *et al.*, 2020). In this study, the Ni content ranged from 0.07 (method A, Eco) to 0.21 mg/kg (method B, conventional). These values are both lower than those recorded by Altundag (2011), who analysed fruit samples (*Prunus domestica* L., *Ficus carica* L.,

Morus alba L., *Vitis vinifera* L., *Prunus armeniaca* L., and *Malus domestica*) from Turkey using dry, open, and microwave digestion methods. The elemental concentrations measured ranged from 0.12 to 0.54 mg/kg for Cd, 0.43 to 2.74 mg/kg for Cu, 0.61 to 2.54 mg/kg for Ni, 0.40 to 2.14 mg/kg for Pb, and 2.16 to 6.54 mg/kg for Zn, with microwave digestion yielding superior results. Altarawneh (2019) analysed the heavy metal concentrations in fruit using a digestion procedure with a concentrated acid mixture (67% HNO₃ and 65% HClO₄, 2:1 ratio), revealing the Pb, Ni, and Cd contents. The concentrations measured were as follows: 0.37 mg/kg Pb, 1.50 mg/kg Ni, and 0.08 mg/kg Cd in banana; 0.33 mg/kg Pb, 1.81 mg/kg Ni, and 0.07 mg/kg Cd in apple; and 0.67 mg/kg Pb, 2.13 mg/kg Ni, and 0.08 mg/kg Cd in orange. Several factors influence the bioaccumulation of heavy metals in fruit, including the physiological properties of crops and atmospheric pollution from heavy metal deposition (Sun *et al.*, 2017; Yu *et al.*, 2006). Samples collected near highways and industrial areas show elevated levels of heavy metal contamination (Florea *et al.*, 2020).

Microwave-assisted acid digestion is faster and more efficient than open digestion due to the high pressure and temperature that enhance sample decomposition. Although microwave digestion systems require specialised equipment and safety devices, leading to higher initial laboratory costs, they also reduce reagent consumption, which lowers operating expenses. Using closed vessels minimises the loss of volatile analytes and decreases sample

contamination (Ishak *et al.*, 2015). Moreover, the controlled and uniform decomposition conditions improve the accuracy of heavy metal determinations in fruit. This method yields more reliable and reproducible results while optimising reagent use, thereby offsetting the initial costs of specialised equipment (Kasahun, 2024).

Consequently, the studied plum fruit served as a valuable dietary resource. Microwave digestion is a more accurate, simpler, and faster method than open system digestion for determining Pb, Cd, Ni, Cu, and Zn concentrations in plum samples using fAAS. The elemental recovery values were quantitative, with a RSD below 20%. The metal concentrations found in the analysed fruit samples are acceptable for human consumption in terms of both nutritional and toxic levels.

It is important to estimate exposure levels by quantifying how pollutants reach target organisms to determine the health hazards associated with heavy metals. Fundamental exposure pathways to humans include the food chain, dermal contact, and inhalation, but oral intake is the most significant. Food consumption accounts for over 90% of human exposure to environmental contaminants, far exceeding the contributions of inhalation and dermal routes (Wang *et al.*, 2021).

To evaluate potential health risks, the DIM and non-carcinogenic (THQ and HI) and carcinogenic (TCR) risks associated with heavy metals were assessed in plum. Table 3 presents the estimated daily metal intake for children and adults, showing the following ranking for both demographics: Cu > Zn

> Ni > Pb > Cd. Nie *et al.* (2016) found similar trends for Ni, Pb, and Cd in fruit, including peach, apple, and pear. Method B indicated the highest DIM for Cu at 1.08^{-3} mg/day for children and 5.07^{-4} mg/day for adults, while Cd showed the lowest intake at 6.42^{-6} mg/day. The calculated THQ for adults and children in the ecological system ranked as follows: Cd > Cu > Ni > Zn > Pb. In the conventional system, they consistently followed the order of Cd > Cu > Ni > Pb > Zn, which was consistent across both methods. THQ values for Cd were lower than those found in South Ethiopia (3.16×10^{-1} and 3.4×10^{-1} for adults) and Egypt (0.67–1.27). A study in China found heavy metal THQs ranked as Ni > Pb > Cd in pears and as Pb > Ni > Cd in apples, peaches, grapes, and jujubes. HI values for method A were 3.78^{-1} and 9.77^{-2} for conventional and ecological systems, while method B obtained values of 9.99^{-1} and 7.18^{-1} , which were both lower than those observed in Egypt, where HI ranged from 0.91 to 1.64. In another mango study, acceptable non-carcinogenic levels were indicated by HI values ranging from 0.007 to 0.35 (Ezez *et al.*, 2023). In Poland, HI averages for various fruit (pome and stone fruit) categories were below 1, suggesting no health hazards.

According to the New York State Department of Health Center for Environmental Health guidelines (NYSDH, 2011), TCR levels are categorised as follows: $TCR \leq 1 \times 10^{-6}$ is low, 1×10^{-4} to 1×10^{-3} is moderate, 1×10^{-3} to 1×10^{-1} is high, and $TCR \geq 1 \times 10^{-1}$ is very high. All health hazard indices (THQ, TCR, and HI) indicated that the heavy metals analysed do not pose risks to fruit consumers, as they

were below 1, implying minimal health effects (Wang *et al.*, 2005).

CONCLUSIONS

This study concludes that microwave-assisted digestion is an effective method for determining metal contents (Zn, Cu, Ni, Cd, and Pb) in plum fruit, offering a quicker and safer extraction process. This technique demonstrates exceptional accuracy, with recovery rates between 88 and 105% for all analysed metals, making it a reliable approach for assessing heavy metals in plums from the Moldavian region.

The heavy metal levels found were low and below FAO/WHO safety limits, indicating that these fruits can be safely consumed from both ecological and conventional systems. Analysis of plums from northeastern Romania revealed adequate heavy metal concentrations, with higher levels of Cu and Zn, which are essential micronutrients.

Microwave-assisted digestion yielded slightly higher heavy metal concentrations than the open system method, with the order of metals as follows: Zn > Cu > Ni > Cd > Pb. Compared to other methods, microwave-assisted digestion offers significant advantages, including greater efficiency, reduced contamination risk, faster digestion times, and higher analyte recoveries. Thus, it is the optimal method for analysing heavy metals in plum fruit, enhancing the assessment of food quality and safety. The health risk assessment indicates that consuming plums in Romania's main plum-growing region does not present public health concerns.

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DT; Validation: GJ; Formal analysis: MR, IGC and MF; Investigation: MR, IGC, MF and DT; Writing – original draft preparation: MR; Writing – review and editing: IGC, DT, and GJ; Visualisation: GJ, IGC and DT; Supervision: GJ. All authors declare that they have read and approved the publication of the manuscript in this present form.

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