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# Environmental monitoring of trace metals in edible snails *Cornu aspersum* (Müller, 1774) from Morocco: Human health risk assessment

Coumba Daga Touré Fall[1], Mourad El Youssfi[1], Othmane Hammani[2], Taha El Kamli[3], Hassan Ouahidi[4], Asmae Benabbou[1], Rachid Ben Aakame[5], Aicha Sifou[1], Rachida Fegrouche[1*]

[1] Faculty of Sciences, Mohammed V University in Rabat, Avenue Ibn Battouta, BP 1014, Rabat 10000, Morocco
[2] National Center for Scientific and Technical Research (CNRST), Technical Support Units for Scientific Research, Angles Avenue des FAR and Allal El Fassi, Hay Ryad, B.P. 8027 N.U, Rabat 10102, Morocco
[3] Department of Veterinary Biological and Pharmaceutical Sciences, Hassan II Institute of Agronomy and Veterinary, Madinat Al Ifrane, P.O. Box 6202 Rabat, Morocco
[4] National Laboratory for Pollution Studies and Monitoring (LNESP). Sustainable Development Department, Minister of Energy Transition and Sustainable Development, Av Mohammed Ben Abdelah Erregragui, Madinat Al Irfane, Rabat, Morocco
[5] Laboratory of Food Toxicology, National Institute of Hygiene (INH), BP 769 Agdal, 27, Avenue Ibn Batouta, Rabat, Morocco
* Corresponding author's e-mail: r.fegrouche@um5r.ac.ma

## ABSTRACT

The continuous release of various chemicals into the environment is a major concern worldwide, as some persist in the ecosystem, leading to severe health issues. This study assessed the accumulation levels of selected trace metals (TMs), namely lead (Pb), cadmium (Cd), zinc (Zn), iron (Fe), copper (Cu), and nickel (Ni) in the edible tissues of the terrestrial snail *Cornu aspersum* (Müller, 1774) using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Sampling was carried out at three stations: S1T, S2P, and S3A, located in the Mechra Bel Ksiri area, the province of Sidi Kacem. The obtained results revealed that station S3A (characterized by the highest snail density and a more intensive use of chemical products to protect artichoke crops) is more TMs-polluted than the other stations S1T and S2P. High average concentrations of the most toxic TMs examined in this study were recorded in the flesh of the snails from S3A: Pb (2.31 mg $kg^{-1}$ dry weight) and Cd (2.03 mg $kg^{-1}$ dry weight) exceeded the maximum admissible level recommended by the European Union (2023) for Pb (1.5 mg $kg^{-1}$) and Cd (1 mg $kg^{-1}$). The assessment of risks to human health in combination with dietary exposure was carried out, the calculated hazard index (HI) remained below the safety threshold of 1, indicating no significant non-carcinogenic effects linked to snail consumption. In contrast, the total cancer risk (TCR) reached a peak value of $1.02 \times 10^{-4}$ at station S3A, slightly exceeding the recommended limit ($1 \times 10^{-4}$), suggesting a potential carcinogenic concern associated with the chronic exposure to TMs through dietary intake of contaminated snails. Therefore, regular monitoring of toxic metal levels in snails is needed to protect consumers against potential carcinogenic risks.

**Keywords:** *Cornus aspersum,* trace metals, bioaccumulation, risk assessment, Morocco.

## INTRODUCTION

The use of chemicals in agriculture presents considerable environmental risks, primarily due to their long-term persistence and their ability to accumulate rapidly. This situation can have harmful consequences for human health, disrupt non-target species, and compromise overall ecosystem stability (Fegrouche et al., 2023; Katagi, 2010; Katagi and Tanaka, 2016).

Among these organisms, mollusks are highly exposed and serve as key indicators for ecological risk assessment in both aquatic and terrestrial environments (Eijsackers, 2010). Terrestrial gastropods, particularly *Cornu aspersum,* commonly known as *Helix aspersa* snails, are sedentary or semi-sessile and possess unique characteristics such as a wide geographical distribution and the ability to accumulate various pollutants, especially TMs. Due to their tolerance to xenobiotics (e.g., pesticides and toxic substances), their ecological role in food webs, and the simplicity of their collection, *Cornu aspersum* snails are frequently used as bioindicators in environmental assessment studies (Pauget et al., 2015; Baroudi et al., 2020; Louzon et al., 2021). Furthermore, elevated concentrations of TMs in the environment can compromise the vital functions of mollusks (Joseph et al., 2021a).

In addition to their ecological role, edible snails also offer nutritional benefits (as a primary source of protein) and therapeutic properties (Maćkowiak-Dryka et al., 2020). Given that snails are a prominent food source, while pollutants that are virtually indestructible in the environment (Onuoha et al., 2016) can infiltrate the food chain and bioaccumulate in specific organisms, the evaluation of contaminant levels in snail tissues is a critical step in assessing the potential health risks for human consumers (Messina et al., 2025).

The growing global concern over TMs in the environment is largely driven by their potential adverse effects on human health (Ogwu et al., 2025). In response to these serious health risks, public authorities routinely monitor the concentration of various metals to mitigate the potential threats to public health. Thus, protecting the environment and human health requires a thorough assessment of the food consumed.

Currently, the accumulation of TMs in food represents a significant public health concern (Orisakwe et al., 2012; Onuoha et al., 2016). To evaluate the ecological and human health risks associated with trace metal exposure, several well-established assessment tools are commonly employed, including the Heavy metal pollution index (HPI), hazard quotient (HQ), hazard index (HI), and carcinogenic risk (CR) (Eid et al., 2024).

This study assessed the bioaccumulation capacity of the *Cornu aspersum* snails for various environmental contaminants. The primary objective was to estimate the potential health risks associated with human consumption of these snails, with particular focus on the concentrations of TMs, such as lead (Pb) and cadmium (Cd), in their edible tissues. This assessment provides a rapid and reliable approach for estimating the health risks associated with the exposure to TMs (Joseph et al., 2021).

## MATERIALS AND METHODS

### Description of study site

The study area is located in the Rabat-Salé-Kénitra region (Figure 1) along the Sebou River, specifically in Mechra Bel Ksiri (Lat. 34°34’ N; Long. 5°57’ WO). The site consists of an agricultural farm with three sampling stations: S1T tomato station, S2P: pepper station S3A: artichoke station. Mechra Bel Ksiri is known for its high agricultural potential (Foudeil et al., 2013) and is characterized by a temperate to warm climate.

### Snail collection site

To assess pollutant contamination, it is essential to use a biological model that accurately represents the studied environment. According to (Aude et al., 2019), *Cornu aspersum* is a terrestrial snail living at the air-soil interface, making it an ideal bioindicator candidate for assessing environmental pollution. Due to its ease of acclimatization and handling in the laboratory, as well as its sensitivity to contaminants, several studies have demonstrated that *Cornu aspersum* can accumulate various types of chemical contaminants and serves as a suitable species for monitoring: pesticides (Druart, 2011; Grara et al., 2012); TMs (Roma et al., 2017; Louzon et al., 2021; Al-Alam et al., 2022). Consequently, it is essential to investigate whether this species contributes to the transfer of pollutants commonly applied in agricultural environments through the various food chains in which it is involved (Al-Alam et al., 2022; Grara et al., 2012).

### Sampling and collecting snails

Sampling was conducted in July 2021, during the snail’s aestivation period (a fasting state). Specimens were collected randomly, without the use of a specific prospection method. A total of 150 individuals (n=150) were gathered, with 50 snails sampled from each of the three stations, found hidden under grasses, branches, leaves, and among

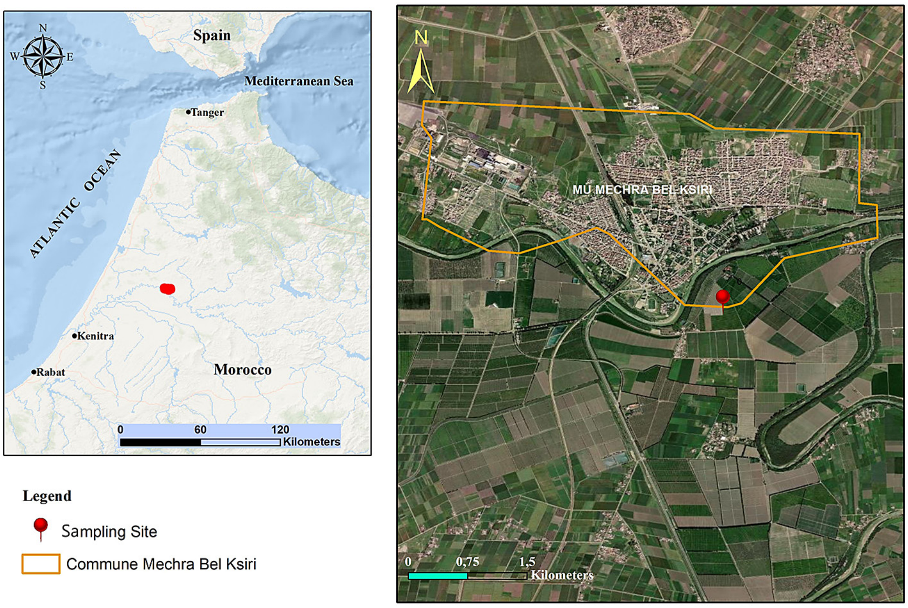


**Figure 1.** Snail collection site

plant roots and soil. The collected specimens were individually placed in labeled plastic bags and temporarily stored in a cooled container to maintain their condition during transport to the laboratory.

## Experimental protocol

The snails weighing between 3.5 and 3.6 g were first fasted to allow their digestive tracts to empty naturally. Each snail was then weighed to determine its total body mass (including shell and soft tissue), and the shell dimensions were recorded. The shells were gently removed using forceps, and the soft tissues were weighed to obtain their wet mass. To reflect what is typically eaten by consumers, the entire edible portion including the foot and other soft tissues, was homogenized. This approach aimed at providing a realistic estimation of the potential TMs exposure through human consumption (Roma et al., 2017). At each sampling site, a group of 20 individuals (n = 20 per station) was used for TMs analysis.

## Sample preparation and digestion

TMs, including Pb, Cd, Fe, Cu, Ni, and Zn, were quantified in snail tissues using a validated method developed by the Moroccan National Laboratory for Pollution Studies and Monitoring (LNESP). Soft tissues were carefully dissected, placed in sterile containers, and dried at 60 °C for 72 hours. The dried samples were then finely ground using a mortar and pestle. A mass of 0.2 g from each sample was transferred into Teflon digestion vessels (ETHOS One). Each sample was treated with 5 mL of 65% $HNO_3$ and left at room temperature for 1 hour before the addition of 2 mL of hydrogen peroxide $H_2O_2$. The samples were then digested using a microwave-assisted procedure described in Table 1. After cooling, the digests were filtered and diluted to a final volume of 50 ml using ultrapure deionized water (18.2 MΩ·cm). Elemental analysis was performed using inductively coupled plasma atomic emission spectrometry (ICP-AES) at the National Centre for Scientific and Technical Research (CNRST, Rabat).

## Instrumentation

TMs were determined using ICP-AES (HORIBA JOBIN Yvon Ultima 2). The instrument operated at a frequency of 27.12 MHz with a radio frequency (RF) power of 1200 W. Argon gas flow rates were set at 12 L/min for the plasma, 1.5 mL/min for the nebulizer, and 0.8 L/min for sample uptake. The nebulization chamber was maintained at a constant temperature of 2 °C to ensure analytical stability. Before analysis, the instrument underwent a preliminary verification following a 20-minute warm-up period.

## Quality control

Calibration was carried out using a multi-element standard solution (JYICP-MIX 23) with certified concentrations of 1000 mg $L^{-1}$ for 23

**Table 1.** Microwave oven temperature program

| Step | Power (W) | Power % | Ramp time (min sec) | PSI | °C | Hold time (min sec) |
|---|---|---|---|---|---|---|
| 1 | 1200 | 100 | 10.00 | 600 | 170 | 12.00 |

elements. The working solutions for calibration were obtained by serial dilution of a primary stock solution (1 g/L) through appropriate concentration ranges optimized for each TMs. Ultrapure deionized water (18.2 MΩ·cm), obtained from a Millipore Milli-Q system, was used to prepare all solutions.

Limits of detection (LOD) and quantification (LOQ) were calculated using the following equations: LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of ten blank measurements and S is the slope of the calibration curve (El Youssfi et al., 2025). To ensure quality control, each analytical batch included sample blanks, standard reference.

## Potential health risk assessment

### *Estimated daily intake*

The estimated daily intake (EDI) of the studied elements through snail consumption was determined using Equation 1, in accordance with the guidelines of the US Environmental Protection Agency (USEPA) ) (U.S. EPA, 2001).

$$EDI = (C \times IR \times EF \times ED) / (BW \times AT) \quad (1)$$

where: *C* represents the concentration of each element in snails (mg/kg), and *IR* is the intake rate of snails (kg/person/day). In Morocco, the average annual per capita consumption of snails is estimated at 174 g (IndexBox, 2018). *EF* denotes the exposure frequency (365 days/year), *ED* is the exposure duration, assumed to be 76.6 years (HCP, 2020), *AT* is the average exposure time calculated as 76.6 years × 365 days/year, and *BW* corresponds to the average body weight of Moroccan adults, estimated at 70.7 kg (Mahjoub et al., 2021).

## Non-carcinogenic risk

The hazard quotient (HQ), established by the U.S. EPA, was used to assess the non-carcinogenic risks from snails' consumption and is calculated using Equation 2 (U.S. EPA, 2001).

$$HQ_i = EDI_i / RfD_i \quad (2)$$

The oral reference dose (RfD), expressed in mg/kg b.w./day, represents the estimated daily exposure to an element that is not expected to cause adverse health effects over a lifetime (U.S. EPA, 2001). The RfD values for Fe, Cu, Zn, Ni, and Cd were 0.7, 0.04, 0.3, 0.02, and 0.0001 mg/kg b.w./day, respectively (U.S. EPA, 2015, 2018, 2023). The U.S. EPA stated that it is difficult to establish a reliable threshold for Pb. For this reason, a RfD value of 0.0035 mg/kg b.w./day for Pb, as suggested by other studies in the literature, was adopted in this study (Agbor et al., 2024; Christophoridis et al., 2019; Scivicco et al., 2022). An HQ value below 1 indicates no significant health risk, whereas an HQ equal to or greater than 1 suggests a potential for non-carcinogenic effects from snail consumption (U.S. EPA, 2001).

The HI was employed to assess the cumulative non-carcinogenic health risk resulting from the exposure to multiple metals (U.S. EPA, 2001), and was calculated using Equation 3, as shown below.

$$HI = \sum_{i=1}^{n} HQi \quad (3)$$

HI = HQ (Pb) + HQ (Cd) + HQ (Ni) + HQ (Cu) + HQ (Zn) + HQ (Fe)

The HI value exceeding 1 indicates significant health risk due to the combined effects of the investigated elements.

## Carcinogenic risk

TMs such as Pb, Cd, and Ni are considered to be carcinogenic to humans (El Youssfi et al., 2022). The carcinogenic risk from the exposure to these TMs was evaluated by calculating the cancer risk (CR) using Equation 4 (U.S. EPA, 2001):

$$CR = EDI \times CSF \quad (4)$$

where CSF represents the cancer slope factor, expressed in $(mg/kg\ b.w./day)^{-1}$. The CSFs for Pb, Cd, and Ni are 0.0085, 6.3, and 1.7 $(mg/kg_{bw}/day)^{-1}$ (OEHHA, 2009a, 2009b; U.S. EPA, 1998).

The cumulative cancer risk is calculated using Equation 5:

$$TCR = \sum_{i=1}^{n} CRi \quad (5)$$

TCR = CR (Pb) + CR (Cd) + CR (Ni), where TCR is the total cancer risk.

In general, the carcinogenic risk of metals is considered negligible when CR and TCR are below $10^{-6}$, acceptable within the range of $10^{-6}$–$10^{-4}$, and unacceptable when exceeding $10^{-4}$ (U.S. EPA, 2001). Meanwhile, Health Canada typically considers a cancer risk of $10^{-4}$ as the maximum acceptable threshold for the risk of developing cancer due to the exposure to carcinogenic substances (Health Canada, 2010).

### Statistical analysis

Metal concentrations were individually measured in each snail sample. To explore the potential co-variation patterns among elements within the tissues, Pearson correlation analysis was performed. The results were visualized using a heatmap correlogram to illustrate the direction and strength of linear relationships between elements. Principal component analysis (PCA) was applied to reduce the dimensionality of the dataset and to identify the variables contributing most significantly to contamination variability (Jolliffe and Cadima, 2016). The suitability of the dataset for PCA was evaluated using the Kaiser–Meyer–Olkin (KMO) test (Kaiser, 1974) and Bartlett's test of sphericity. Only the datasets with KMO values exceeding 0.5 and statistically significant Bartlett results were retained for analysis. All statistical analyses and data visualizations were conducted in R (version 4.3.2) an open-source environment for statistical analysis and graphical representation (Posit team, 2023).

## RESULTS AND DISCUSSION

### Concentrations of TMs in snail samples

Table 2 presents the average concentrations (mg $kg^{-1}$ dry weight) of TMs Zn, Fe, Cu, Ni, Cd, and Pb measured in the soft tissues of snails collected from the three sampling sites (S1T, S2P, and S3A). Among the elements analyzed, Fe exhibited the highest levels, followed by Zn and Cu, whereas Pb, Ni, and Cd were present in comparatively lower amounts. Notably, the snail samples from station S3A showed the greatest accumulation of trace metals overall.

The mean Zn concentrations in snails ranged from (24.41 to 26.98 mg $kg^{-1}$ dry weight), with no significant difference between stations (F = 1.014; P = 0.376).

The mean Zn concentrations in snails ranged from (24.41 to 26.98 mg $kg^{-1}$ dry weight), with no significant difference between stations (F = 1.014; P = 0.376).

Iron concentrations ranged from 30.38 mg $kg^{-1}$ (dry weight) at S2P to a maximum of 35.42 mg $kg^{-1}$ (dry weight) at S3A.

Despite this variation, statistical analysis revealed no meaningful differences between the sampling sites (F = 2.027, p = 0.151).

Regarding Pb content, the mean concentrations in snail flesh were for S1T (1.54 mg $kg^{-1}$ dry weight), S2P (1.09 mg $kg^{-1}$ dry weight), and S3A (2.31 mg $kg^{-1}$ dry weight), with a highly significant difference between snails from S2P and S3A (F = 12.726; P = 0.000).

Among the three stations, the Cd concentrations were highest at S3A (2.03 mg $kg^{-1}$ dry weight), markedly exceeding the values found at S1T (0.29 mg $kg^{-1}$) and S2P (0.23 mg $kg^{-1}$). Statistical analysis revealed a highly significant difference between S3A and the other two stations (F = 154.629, $p < 0.001$).

The Ni concentrations in snail tissue varied between 1.04 and 1.33 mg $kg^{-1}$ (dry weight) across the sampled stations. In the case of Cu, average values were 4.21, 5.34, and 6.29 mg $kg^{-1}$ (dry weight) at stations S1T, S2P, and S3A, respectively. Statistical testing revealed a notable variation in the Cu levels among the stations (F = 7.4, p = 0.003). Accordingly, Table 3 provides a comparative overview between the present findings and previously published data.

A recent investigation by (Messina et al., 2025), on the *Cornu aspersum* specimens collected in southern Italy reported mean concentrations of Fe (37.529 mg $kg^{-1}$), Zn (18.472 mg $kg^{-1}$), and Cu (5.641 mg $kg^{-1}$) that are comparable to those found in the presented study. However, their measured values for Ni (0.096 mg $kg^{-1}$ dry weight), Pb (0.077 mg $kg^{-1}$), and Cd (0.123 mg $kg^{-1}$ dry weight) were considerably lower than those recorded in the studied samples. In Algeria, (Guessasma et al., 2020) observed substantial bioaccumulation of Fe and Pb by *C. aspersum*, particularly within the hepatopancreas, where concentrations reached 2795.78 mg $kg^{-1}$ for Fe and 7.47

**Table 2.** Average ± SD (mg kg-1), minimum, and maximum TMs concentrations for snail groups (n=20/group) at the three stations

| TMs | Stations | Mean value | SD | 95% confidence interval lower | upper | Min | Max | F (Stations) | Sig | LOD (ppb) | LOQ (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pb | S1T | 1.54 (ab) | 0.74 | 1.092 | 1.971 | 0.56 | 2.43 | 12.726 | **0.000 | 22.54 | 67.62 |
| | S2P | 1.09 (a) | 0.32 | 0.897 | 1.282 | 0.68 | 1.47 | | | | |
| | S3A | 2.31 (b) | 0.50 | 2.024 | 2.630 | 1.64 | 3.01 | | | | |
| Cd | S1T | 0.29 (a) | 0.74 | 0.246 | 0.333 | 0.19 | 0.44 | 154.629 | **0.000 | 3.66 | 10.98 |
| | S2P | 0.23 (a) | 0.05 | 0.198 | 0.258 | 0.13 | 0.28 | | | | |
| | S3A | 2.03 (b) | 0.44 | 0.746 | 2.293 | 1.22 | 2.58 | | | | |
| Fe | S1T | 31.46 (a) | 5.98 | 27.868 | 34.849 | 25.07 | 40.02 | 2.027 | 0.151 | 1.68 | 5.04 |
| | S2P | 30.38 (a) | 6.91 | 26.269 | 34.779 | 22.01 | 40.25 | | | | |
| | S3A | 35.42 (a) | 4.55 | 32.438 | 38.406 | 29.95 | 42.23 | | | | |
| Cu | S1T | 4.21 (a) | 0.52 | 3.888 | 4.564 | 3.34 | 5.25 | 7.420 | **0.003 | 9.57 | 28.71 |
| | S2P | 5.34 (ab) | 1.22 | 4.608 | 6.057 | 3.87 | 7.01 | | | | |
| | S3A | 6.29 (b) | 1.62 | 5.350 | 7.343 | 4.65 | 9.25 | | | | |
| Ni | S1T | 1.31 (a) | 0.65 | 0.962 | 1.754 | 0.66 | 2.49 | 0.632 | 0.539 | 6.68 | 20.04 |
| | S2P | 1.04 (a) | 0.56 | 0.721 | 1.431 | 0.35 | 2.29 | | | | |
| | S3A | 1.33 (a) | 0.71 | 0.932 | 1.828 | 0.47 | 2.72 | | | | |
| Zn | S1T | 24.41 (a) | 2.66 | 22.888 | 26.081 | 21.65 | 30.01 | 1.014 | 0.376 | 3.46 | 10.38 |
| | S2P | 25.08 (a) | 3.84 | 22.566 | 27.722 | 21.45 | 30.65 | | | | |
| | S3A | 26.98 (a) | 5.58 | 23.794 | 30.375 | 21.78 | 35.45 | | | | |

**Note:** Groups marked with the same letter do not differ significantly. SD: Standard deviations; Min: Minimum; Max: Maximum; **: a highly significant difference.

mg kg⁻¹ (dry weight) for Pb. Roma et al. (2017), reported cadmium levels in *C. aspersum* ranging from 0.50 to 0.92 mg kg⁻¹, which exceed those observed at sites S1T and S2P in the performed study but remain below the levels recorded at S3A. (Pauget et al., 2015) while examining *C. aspersum* in France, found notably elevated TMs concentrations, with Cd reaching 33 mg kg⁻¹ (dry weight), Pb levels unspecified, and Zn peaking at 2,422 mg kg⁻¹ (dry weight).

Several studies have highlighted considerably higher concentrations of TMs in land snails compared to those found in the conducted investigation, further illustrating their notable capacity to bioaccumulate diverse metal contaminants. For example, Tardugno et al. (2023) reported Fe and Zn concentrations of 349.73 ± 1.93 mg kg⁻¹ and 208.95 ± 1.74 mg kg⁻¹ dry weight, respectively, in *Helix aperta*. Similarly, (Benhamdoun et al., 2025a) identified substantial levels of Fe (261.01 ± 0.84 mg kg⁻¹), Zn (742.78 ± 227.67 mg kg⁻¹), and Cu (126.78 ± 0.98 mg kg⁻¹ dry weight) in *Theba pisana*, the values that exceed those obtained in the present study (Table 3).

Pb and Cd are of particular toxicological concern due to their detrimental effects on human health, warranting stringent monitoring of their concentrations in food products (El Youssfi et al., 2023; Eneji et al., 2016). Caetano et al. (2021) observed notably elevated concentrations of these elements in *Theba pisana*, reporting 23.9 ± 0.29 mg kg⁻¹ dry weight for Pb and 31.3 ± 0.45 mg kg⁻¹ dry weight for Cd. These two TMs are known for their toxic effects on human health: Pb can cause neurological disorders, developmental delays in children, and cardiovascular diseases, while Cd is a proven carcinogen that can Pb to kidney diseases and bone disorders (Collin et al., 2022).

Concerning Fe, the majority of previous studies have highlighted its high concentration in snail tissue, suggesting that snail meat could represent a valuable dietary source of this essential micronutrient.

**Table 3.** Comparisons of TMs levels found in dried tissues of the snails (mg $kg^{-1}$ dry weight) from different areas in the world including Morocco with the present study results

| World regions (Species) | Fe (mg $kg^{-1}$) | Zn (mg $kg^{-1}$) | Ni (mg $kg^{-1}$) | Cu (mg $kg^{-1}$) | Pb (mg $kg^{-1}$) | Cd (mg $kg^{-1}$) | References |
|---|---|---|---|---|---|---|---|
| *Cornu aspersum* | | | | | | | |
| Average ± SD | 32.42 ± 2.02 | 25.49 ± 4.02 | 1.23 ± 0.64 | 5.28 ± 1.12 | 1.65 ± 0.52 | 0.85 ± 0.41 | Present study |
| Min - Max | (25.07-42.23) [b] | (21.45-35.45) [b] | (0.35 -2.69) [b] | (3.34 -9.25) [b] | (0.56 - 3.01) [b] | (0.13 - 2.58) [b] | |
| Italy | 37.53 ± 12.16 | 18.47 ± 4.69 | 0.096 ± 0.036 | 5.64 ± 1.26 | 0.077 ± 0.075 | 0.123 ± 0.084 | Messina et al. (2025) |
| Egypt | - | 56.91 ± 4.35 | 0.96 ± 0.56 | 18.01 ± 2.45 | 3.59 ± 1.09 | 1.03 ± 0.57 | Abdel-Halim et al. (2013) |
| Italy | - | - | 0.59 ± 0.81 | 77.46 ± 219.13 | 0.27 ± 0.79 | 1.9 ± 2.67 | Bongiorno et al. (2024) |
| Algeria | 102 [a] | 431 [a] | - | 12 [a] | - | - | Baghele et al. (2023) |
| Italy | 81.1 [a] (3.3–9730) [b] | 1.44 [a] (0.36–18.4) [b] | 87.8 [a] (10.8–3340) [b] | 3.66 [a] (0.072–391) [b] | 0.923 [a] (0.18–2.6) [b] | - | Roma et al. (2017) |
| Algeria | 2795.78 [a] | - | - | - | 7.59 [a] | - | Guessasma et al. (2020) |
| *Helix aperta* | | | | | | | |
| Italy | 349.73 ± 1.93 | 208.95 ± 1.74 | 0.14 ± 0.03 | 46.15 ± 1.71 | 0.02 ± 0.01 | 0.03 ± 0.01 | Tardugno et al. (2023) |
| *Theba pisana* | | | | | | | |
| Morocco | - | 852.17 ± 354.24 | - | 151.8 ± 21.6 | - | 17.4 ± 1.19 | Benhamdoun et al. (2025) |
| Italy | 253.91 ± 0.70 | 129,88 ± 1,05 | 0.09 ± 0.01 | 24.84 ± 0.59 | 0.003 * | 0.004 * | Tardugno et al. (2023) |
| Portugal | 5.40 ± 0.27 | 1.39 ± 0.15 | 8.93 ± 0.29 | 2.07 ± 0.06 | 23.9 ± 0.29 | 31.3 ± 0.45 | Caetano et al. (2021) |
| *Otala* spp. | | | | | | | |
| Morocco | - | 742.78 ± 227.67 | - | 371.89 ± 93.23 | - | 18.32 ± 1.44 | Benhamdoun et al. (2025) |
| Portugal | 4.12 ± 0.20 | 1.57 ± 0.12 | 11.4 ± 0.32 | 1.87 ± 0.05 | 13.1 ± 0.40 | 27.9 ± 0.60 | Caetano et al. (2021) |
| Recommended maximum limits | - | - | - | - | 1.5 [a] | 1 [b] | European union (2023) |

**Note:** –: not available; [a]: the mean value; [b]: the range; *: ET < 0.01.

Regarding Fe, most studies reported elevated Fe concentrations, indicating that snail meat is an excellent source of iron in the diet (Nontasan et al., 2023).

The ingestion of Cd and Pb through food may pose a toxicological risk to both animal and human health (Eneji et al., 2016). Given that snails are widely consumed (Caetano et al., 2021), the persistence of environmental contaminants represents a serious concern. These substances can enter the food chain and bioaccumulate in various organisms, thereby increasing the risk of chronic human exposure (Alengebawy et al., 2021). Therefore, the assessment of snail contamination is a crucial component of evaluating the health risks associated with their consumption.

Although land snails are commonly consumed in Mediterranean countries, no specific maximum residue limits (MRL) have been established for TMs in these snails. However, according to EU Regulation 2023/915, the MRLs set for bivalve mollusks are (1.5 mg $kg^{-1}$ dry weight) for Cd and (1mg $kg^{-1}$ dry weight) for Pb. Thus, the Pb concentrations recorded in the flesh of *Cornu aspersum* snails from all stations exceed these thresholds. Regarding Cd, only station S3A exceeds the allowed limit. However, this situation may compromise the safety of snail consumers, especially over the long term.

The application of Pearson correlation analysis (Figure 2a) and the correlogram (Figure 2b) allowed for the evaluation of relationships between Zn, Fe, Cu, Ni, Pb, and Cd in the studied agricultural stations: $S_{1T}$, $S_{2P}$, and $S_{3A}$. The correlation coefficients obtained helped identify significant

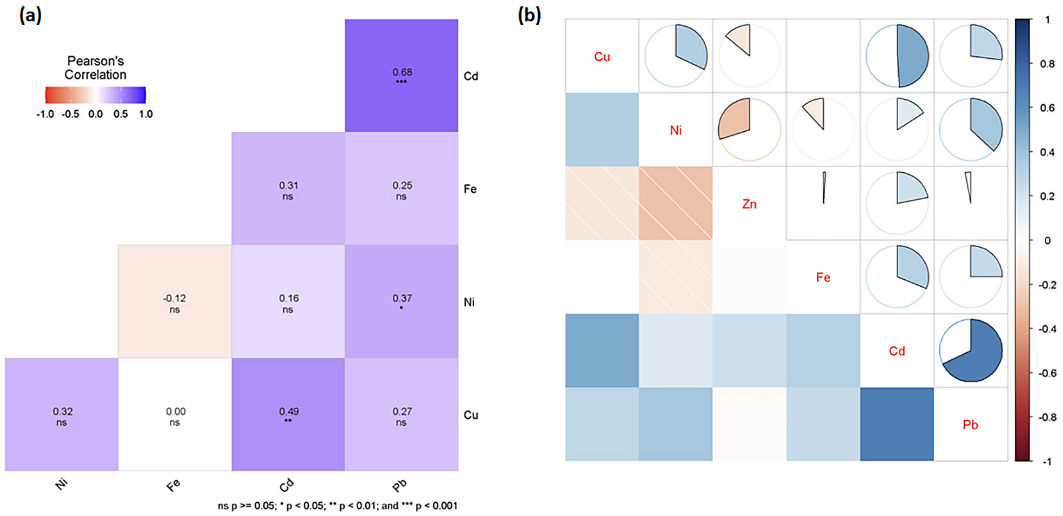


**Figure 2.** Pearson's correlation (a) and correlogram plot (b) on multi-element content in snails

trends in the distribution and association of these elements across the different stations.

A strong positive correlation was identified between Pb and Cd ($r = 0.68$, $p < 0.001$), indicating that these elements may share a common source, possibly linked to industrial emissions or agricultural inputs, such as fertilizers and pesticides. This relationship is particularly pronounced in station S3A, where Pb and Cd concentrations are the highest, likely due to the soil characteristics or specific agricultural practices related to artichoke cultivation.

Cu and Ni exhibit a positive correlation of $r = 0.32$ ($p \geq 0.05$), although not statistically significant. This trend may indicate a co-occurrence of these elements in certain type of soils or a similar assimilation by crops, particularly in station S2P, where Cu and Ni levels are relatively homogeneous. Cu, although essential for many biological functions, can become toxic in excess, leading to liver damage and neurological disorders (Gaetke et al., 2014; Gaier et al., 2013; Tóth et al., 2016). Ni, on the other hand, is associated with allergic reactions and, with prolonged exposure, an increased risk of respiratory cancers.

The observed correlation between Ni and Pb ($r = 0.37$, $p < 0.05$) points to a potential association between these metals in the soil, especially at station S1T. This connection may be influenced by the physicochemical characteristics of soil, which affect the bioavailability and uptake of these elements by tomatoes. Elevated exposure to Pb and Ni has been linked to adverse effects on the immune and respiratory systems, potentially contributing to chronic health conditions (Ebrahimi et al., 2020; Genchi et al., 2020).

Conversely, a moderate correlation between Cd and Cu ($r = 0.49$, $p < 0.01$) suggests that these two elements may be introduced simultaneously into agricultural soils, possibly through the use of fertilizers or organic amendments enriched with TMs. This relationship is more pronounced in station S3A, where artichoke crops may be more exposed to Cd and Cu accumulation (de Burbure et al., 2006). As it was mentioned earlier, Cd is particularly hazardous to human health, whereas excess Cu can be toxic to the liver (Lizaola-Mayo et al., 2021; Moini et al., 2021) and kidneys (Hoareau et al., 2019).

The observed correlations involving Fe exhibit varying patterns. The association between Fe and Pb–Cd ($r = 0.49$, $p > 0.05$) reflects a moderate but statistically insignificant relationship, which may imply that although these metals coexist in the same environments, their mobility and uptake in soils are governed by different local factors (Bouras et al., 2010). In contrast, the negative correlation between Ni and Fe ($r = -0.12$, $p > 0.05$) suggests distinct sources for these elements or Fe fixation mechanisms that limit the availability of Ni in the soil. This trend is particularly evident in station S1T, where the Fe levels are higher, which may inhibit the Ni accumulation in plant tissues. Fe is an essential element for the organism, but its excess can induce oxidative stress and liver diseases (Handa et al., 2016).

The findings reveal diverse interaction patterns among the analyzed TMs across the different agricultural stations. The strong

**Table 4.** Factor loadings for PCA

| Variables | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| Zn | -0,05 | 0,62 | 0.58 | 0.20 | -0.43 | 0.21 |
| Cd | 0.57 | 0.3 | 0.16 | 0.068 | 0.2 | -0.71 |
| Pb | 0.55 | 0.078 | -0.07 | 0.46 | 0.43 | 0.53 |
| Cu | 0.44 | -0.22 | 0.28 | -0.73 | -0.14 | 0.35 |
| Fe | 0.23 | 0.42 | -0.73 | -0.15 | -0.44 | 0.0 6 |
| Ni | 0.34 | -0.54 | 0.05 | 0.43 | 0.61 | -0.15 |
| Eigen value | 2.24 | 1.43 | 0.95 | 7.72 | 0.46 | 0.19 |
| Percentage of variance | 37.33 | 23.79 | 15.89 | 12.06 | 7.73 | 3.17 |
| Cumulative (%) | 37.33 | 61.13 | 77.03 | 89.9 | 96.82 | 100.00 |

correlations observed between Pb and Cd, as well as between Cd and Cu, suggest common sources, likely related to agricultural practices or environmental inputs (Xiao et al., 2017). In contrast, the weaker or negative relationships between certain elements, such as Ni and Fe, indicate specific distribution dynamics influenced by the pedoclimatic characteristics of the studied stations. A more detailed analysis of the soils and potential contamination sources could provide a better understanding of the underlying mechanisms behind these correlations as well as their impact on crops and human health.

Table 4 summarizes the PCA results, including eigenvalues, explained variance percentages, and cumulative contributions. On the basis of standard interpretation criteria, loading values below 0.5 suggest a weak association, values between 0.5 and 0.7 indicate a moderate relationship, and those exceeding 0.7 reflect a strong association thereby supporting the existence of meaningful inter-element correlations.

PCA identified six main components, of which the first two (PC1 and PC2) have eigenvalues exceeding 1 and together account for 61.13% of the total variance observed (Figure 3). The remaining components PC3 through PC6 with eigenvalues below 1, contribute the remaining 38.87% of the variance. PC1, which explains 37.33% of the variance, is chiefly influenced by moderate positive loadings from Cd (0.57), Pb (0.55), and Cu (0.44), suggesting a notable impact from anthropogenic sources, such as industrial processes and agricultural practices.

PC2, which accounts for 23.79% of the total variance, is marked by a moderate positive association with Zn (0.62) and Fe (0.42), alongside a negative contribution from Ni (-0.54). This contrast may reflect a differentiation between TMs of natural origin and those introduced through anthropogenic activities. Meanwhile, PC3 (15.89% of the variance) is defined by a positive loading for Zn (0.58) and a strong negative loading for Fe (-0.73), indicating possible antagonistic behavior or differing geochemical pathways between these two metals. PC4 (12.06% of the variance) highlights a strong negative loading for Cu (-0.73), and moderate positive loadings for Pb (0.46) and Ni (0.43), possibly indicating industrial contamination. PC5 (7.73% of the variance) shows moderate contributions from Ni (0.61) and Pb (0.43), suggesting common sources, potentially related to soils. PC6 (3.17% of the variance) is marked by a negative loading for Cd (-0.71), indicating a specific influence on this element.

These results demonstrate the diversity of sources of metal contamination, combining both natural and anthropogenic factors (Diop et al., 2015). The representation of individuals in the factor space (Figure 3a and 3b) reveals a structure into three distinct groups, corresponding to the sampling stations:

S3A is primarily positioned on PC1, indicating a strong presence of Cd, Pb, Cu, and Ni, suggesting contamination by TMs, likely related to agricultural activities.

S2P is more strongly influenced by PC2, showing a greater correlation with Zn and Fe, elements often of natural origin or associated with agricultural inputs. S1T occupies an intermediate position, reflecting a mix of influences from the different elements studied. PCA highlights two major axes of interpretation: PC1 reflects contamination by TMs (Cd, Pb, Cu, Ni), possibly due to anthropogenic sources. PC2 distinguishes

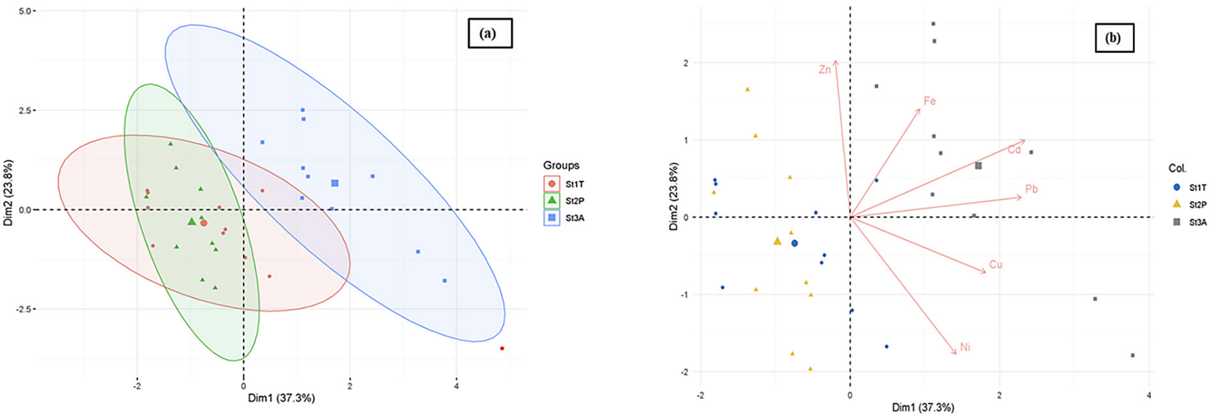


**Figure 3.** Graphical representation of PCA: (a) Score plot and (b) Biplot. Note: S1T: Tomato station, S2P: Pepper station, S3A: Artichoke station

natural elements (Zn, Fe) from those associated with human activities (Ni, Cu).

The analysis of the stations shows that S3A is the most affected by metal contamination, while S2P is more influenced by the naturally occurring elements. These results demonstrate the importance of PCA in identifying the potential anthropogenic sources of TMs and in understanding the distribution of elements in the environment.

## Evaluation of potential health risks

This study evaluated the health risks associated with toxic element exposure from date consumption among adults, based on the EDI values. Table 5 showed the EDI and HQ values of toxic elements in the snails from the three stations. As it was shown, Fe had the highest EDI value ($2.39 \times 10^{-4}$ mg/kg b.w. per day), while Cd showed the lowest EDI value ($1.55 \times 10^{-6}$ mg/kg b.w. per day). Recently, Benhamdoun et al. (2024) investigated the levels of TMs (Cd, Cr, Cu, Zn, and Co) in two edible snail species, *Otala* spp. and *Theba pisana*, collected from a dumpsite in Safi city, Morocco. Their findings revealed that among the analyzed metals, Zn and Co had the highest and lowest average EDI values of Zn and Co for children and men of $2.42 \times 10^{-2}$ and $1.06 \times 10^{-5}$ mg/kg b.w. per day, respectively. In Nigeria, the EDI of metals through the intake of *A. marginata* (African Giant Land Snail) from Ikot Ada Udo were $3.42 \times 10^{-3}$, $5.61 \times 10^{-3}$, $3.19 \times 10^{-3}$, and $1.52 \times 10^{-2}$ mg/kg b.w. per day for Pb, Cd, Ni, and Zn, respectively, which were higher than the reported EDI in the present study (Joseph et al., 2021c).

To assess the potential health risks relating to metal exposure, the HQ values were calculated using EDI and the RfD for each toxic metal. The HQ values ranged from $2.93 \times 10^{-4}$ for Fe (in station S2P) to $1.37 \times 10^{-1}$ for Cd (in station S3A) (Table 5). The HQ values for all analyzed TMs were well below 1 (Figure 4), indicating no significant non-carcinogenic risk to consumers from the ingestion of snails. Likewise, the HI values were also below the threshold of 1, confirming negligible risk. Benhamdoun et al. (2024) indicated that long-term consumption of

**Table 5.** Estimated daily intake (EDI) and hazard quotients (HQ) of selected TMs from snail consumption (mg $kg^{-1}$ b.w. per day)

| Sampling stations | Pb | | Cd | | Ni | | Cu | | Zn | | Fe | | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EDI | HQ | EDI | HQ | EDI | HQ | EDI | HQ | EDI | HQ | EDI | HQ | |
| S1T | 1.04E-05 | 2.97E-03 | 1.96E-06 | 1.96E-02 | 8.85E-06 | 4.43E-04 | 2.84E-05 | 7.10E-04 | 1.65E-04 | 5.49E-04 | 2.12E-04 | 3.03E-04 | 2.45E-02 |
| S2P | 7.34E-06 | 2.10E-03 | 1.55E-06 | 1.55E-02 | 7.04E-06 | 3.52E-04 | 3.60E-05 | 9.00E-04 | 1.69E-04 | 5.64E-04 | 2.05E-04 | 2.93E-04 | 1.97E-02 |
| S3A | 1.56E-05 | 4.45E-03 | 1.37E-05 | 1.37E-01 | 8.98E-06 | 4.49E-04 | 4.25E-05 | 1.06E-03 | 1.82E-04 | 6.07E-04 | 2.39E-04 | 3.41E-04 | 1.44E-01 |

**Note:** Abbreviations: HQ – hazard quotient; HI – hazard index; b.w. – body weight.

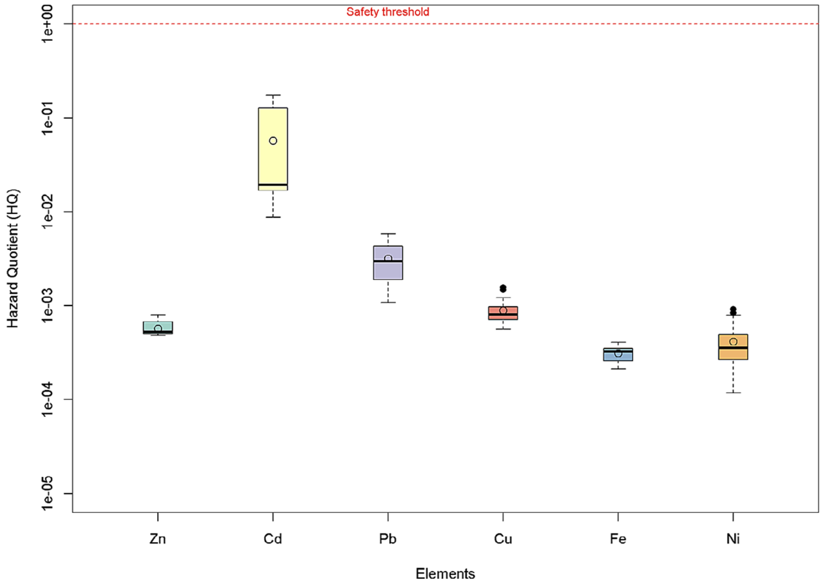


**Figure 4.** Estimated hazard quotient (HQ) values for TMs from snail consumption

contaminated snails was unlikely to pose a health risk to adults (HI < 1). However, children might be faced with non-carcinogenic risks (HI > 1). In another study, Joseph et al. (2021) showed that the HQ of Cd (5.61) and the HI of investigated metals (6.80) were greater than 1, indicating a possible risk to the inhabitants of Ikot Ada Udo (Nigeria) from long-term consumption of contaminated snails. Cheng and Yap (2015) reported that the calculated HQ for individual metals from consuming mangrove snails (*Nerita lineata*) in Peninsular Malaysia were all less than 1. However, the HI values from almost all sites were greater than 1 for high-level consumers, showing that the ingestion of contaminated mangrove snails may result in non-carcinogenic risks to the consumers.

Table 6 and Figure 5 showed the estimated CR and TCR values for potentially carcinogenic elements, including Pb, Cd, and Ni. For Cd

**Table 6.** Carcinogenic risk for potentially toxic elements

| Sampling stations | Cancer risk (CR) | | | Total cancer risk (TCR) |
|---|---|---|---|---|
| | Pb | Cd | Ni | |
| S1T | 8.81E-08 | 1.23E-05 | 1.50E-05 | 2.75E-05 |
| S2P | 6.24E-08 | 9.78E-06 | 1.20E-05 | 2.18E-05 |
| S3A | 1.32E-07 | 8.64E-05 | 1.53E-05 | 1.02E-04 |

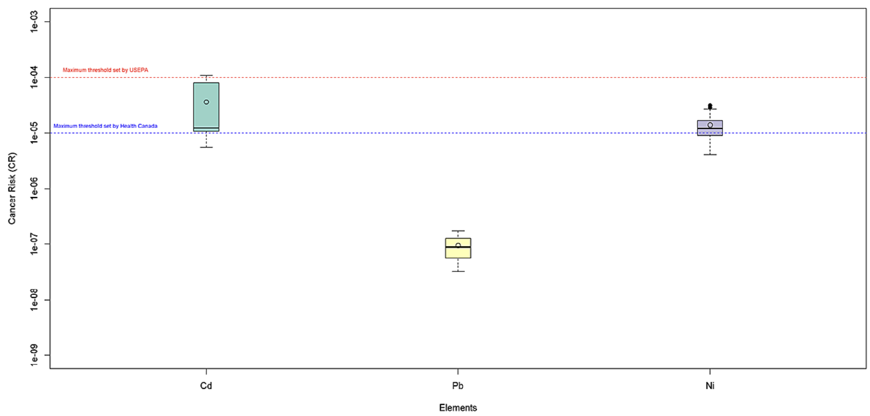


**Figure 5.** Values for different elements caused by consumption of snails

and Ni exposure, the threshold of $1 \times 10^{-5}$ set by Health Canada guidance was reached to the 25th percentile (Figure 5). Moreover, the maximum CR values for Cd exceeded the U.S. EPA threshold of $1 \times 10^{-4}$. Regarding Pb, the CR levels were below the threshold values. Accordingly, a potentially higher carcinogenic risk associated with cumulative TCR was noted for all stations (Table 6). The highest TCR value of $1.02 \times 10^{-4}$ was found in station S3A, which exceeded the threshold set by USEPA ($1 \times 10^{-4}$). These results suggest that snails from the investigated stations (especially station S3A) may accumulate significant levels of toxic elements, posing a potential lifetime cancer risk to consumers. Joseph et al. (2021) found that the CR of Ni ($5.43 \times 10^{-3}$) and Cd ($2.13 \times 10^{-3}$) from contaminated snails ingestion exceeded the safe range of $10^{-6}$ to $10^{-4}$, indicating the possibility of developing cancer over a long lifetime. (Benhamdoun et al., 2024)) reported that TCR was below $10^{-4}$ for adults and children, suggesting acceptable carcinogenic risks for all consumer groups.

## CONCLUSIONS

This study investigated the bioaccumulation of TMs (Pb, Cd, Zn, Fe, Cu, and Ni) by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) in the edible tissues of the terrestrial snail *Cornu aspersum* collected from three agricultural sites (S1T, S2P and S3A) in the Mechra Bel Ksiri region. The findings highlighted station S3A as a contamination hotspot due to the high density of snails present at this station and the more intensive use of chemicals to protect artichoke crops compared to other stations. The most toxic TMs examined in this study Pb and Cd presented concentrations exceeding the maximum admissible level recommended by the European Union (2023) for edible mollusks. Although the non-carcinogenic risk assessed via the Hazard Index (HI) remained below the safety threshold (HI < 1), the total carcinogenic risk (TCR) at S3A: surpassed the USEPA's critical limit ($1 \times 10^{-4}$), raising concerns about the long-term health implications of snail consumption in this area.

Multivariate statistical analyses, including Pearson correlations and Principal Component Analysis, revealed significant associations between certain metals, particularly Pb and Cd, suggesting a shared anthropogenic origin likely linked to agricultural practices. These insights underscore the utility of *C. aspersum* as a sentinel species for environmental metal contamination.

Given the absence of specific regulatory guidelines for terrestrial gastropods intended for human consumption, there is a pressing need to establish contamination benchmarks analogous to those used for bivalve mollusks. Moreover, the integration of snail biomonitoring into environmental surveillance programs could provide the early warnings of agro-environmental pollution.

Ongoing complementary studies in the region aim to further characterize soil properties, identify potential contamination sources, and evaluate the physiological impacts of metal exposure on *C. aspersum*. Such research is essential to refine health risk assessments and inform evidence-based mitigation strategies.

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