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Biomonitoring of soil quality in the Souk Ahras region (Northeast Algeria) using *Cornu aspersum* as a bioindicator: Trace metals and sulfates analysis

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Graphical abstract

Abstract

The present study aims to assess the impact of physicochemical parameters on the availability of trace metal elements (TME) in soil by analyzing the accumulation of copper (Cu), zinc (Zn) and sulfates in both soil and edible tissues of *Cornu aspersum* using a passive biomonitoring approach at three sites in Souk Ahras province: Zaarouria, Mechrouha and ENAP (National Paint Enterprise). Statistical analysis of the data indicated that adsorption of TME and sulfates on soil particles inhibits their uptake by other terrestrial organisms such as snails. In addition, physicochemical parameters significantly influenced the distribution of TME in the soil, revealing marked spatial differences in the accumulation of Cu in the foot and viscera as well as Zn especially in the viscera. Furthermore, a significantly higher accumulation of sulfates was observed in the foot compared to the viscera especially at the ENAP site. These observations could confirm the potential of *C. aspersum* species as an effective bioindicator of metal pollution in soil.

Keywords: *Cornu aspersum*, bioindicator, bioavailability, accumulation, metal pollution, soil

1. Introduction

One of the main areas of current research is environmental pollution caused by the release of different types of pollutants into water, air and soil (Baroudi 2020, N'Doua *et al.* 2024). These different pollutants originate from the expansion of human activities and chemical emissions, notably trace metal elements (TME) associated with industrial and agricultural needs (Dossou *et al.* 2022, Amlan *et al.* 2023; Hidouri *et al.* 2024). TME occur naturally in the environment and are considered among the most harmful pollutants, as they are non-biodegradable and their concentration is constantly increasing in different ecosystem compartments (Ibrahim *et al.* 2019, Ning *et al.* 2024). Due to their excessive presence in the ecosystem, heavy metals can also have a direct impact on the life cycles of animal and plant organisms, both terrestrial and aquatic. This dynamic could lead to a reduction in their density and abundance. This creates an imbalance in the food chain and poses a threat to biodiversity. As a result, the ecosystem becomes more vulnerable to environmental changes (Ibrahim *et al.* 2023, Shaffique *et al.* 2024). High concentrations of TME can cause serious problems for organisms, particularly due to the increased sensitivity of invertebrates to TME compared to vertebrates (Monchanin *et al.* 2021, Benhamdoun *et al.* 2024), such as disruption of the physiological balance in earthworms, as well as in snails and beetles resulting in an increase or decrease of certain oxidative stress biomarkers such as AchE, GST, MDA and antioxidant enzymes. This is due to the accumulation of TME in their tissues, which is directly caused by the presence of these metals in their natural environment, be it water, plants or soil (Du *et al.* 2019, Bennour and Soltani 2020, Larba *et al.* 2023, Bouzahouane *et al.* 2024; Ogbeide and Amayanbo 2024).

Currently, assessing the bioavailability of metals in soil and studying the multiple effects and hazards of polluted soils are a crucial issue in ecotoxicology (Chen *et al.* 2023; Selvanarayanan *et al.* 2024a) in order to protect potentially exposed populations, such as living fauna and flora organisms (Alsherif *et al.* 2022, El Mageed *et al.* 2023, Bici *et al.* 2024). Recent developments in real-time environmental monitoring using IoT-based systems have shown promise in improving pollution management and water quality assessment (Maruthai *et al.* 2025; Periasamy *et al.* 2024). as well as in predicting and classifying water quality using advanced deep learning models such as Attention-based Deep Differential RecurFlowNet (Enkatraman *et al.* 2025).

Soil is considered to be a habitat for numerous microorganisms, plants and terrestrial fauna and their interactions within which diverse life communities develop and reproduce. It also provides a capacity to retain pollutants to prevent their transport especially by water, and subsequent transfer into food chains (Owojori *et al.* 2022, Soliman *et al.* 2022, Li *et al.* 2023, Mwelwa *et al.* 2023). Different species are able to indicate soil quality, among which we find terrestrial gastropods, due to their direct contact with the soil and their high capacity to accumulate TME, they are considered the most reliable bioindicators (Guessasma *et al.* 2020, Salih *et al.* 2021, Cheriti 2022, Nwagu *et al.* 2022, Owojori *et al.* 2022, Sargsyan *et al.* 2022, Ajayi and Oyewole 2023, Louzon *et al.* 2023). Due to its sedentary lifestyle and diet, it is easily contaminated by various pollutants present in the ecosystem (Al-Alam *et al.* 2024). The assessment of metal bioavailability in soils can be performed using this indicator in situ by active bioindication (by confining sentinel snail species from in vitro breeding, thus making them with a known biological history) or passive bioindication (based on direct sampling of wild snails from their habitats, which therefore have an unknown biological history) (Al-Alam *et al.* 2022, Sahraoui 2022). In a context where environmental conservation is a global concern, it is crucial to understand the mechanisms of metal contamination in ecosystems and the main reasons for it (Selvanarayanan *et al.* 2024b). How does this contamination affect human health, ecosystems and local biodiversity?

This study aims to monitor the distribution of TME in the soil of three different sites in the Souk Ahras province which are Zaarouria, Mechrouha and the paint factory (ENAP) under the influence of soil physicochemical parameters. This work is driven by growing environmental concerns over heavy metal contamination in regions affected by industrial and agricultural activities, and the need for effective biomonitoring tools to assess soil quality. We focused our

survey on measuring rates of two metals, copper (Cu) and Zinc (Zn) in the foot and viscera of Cornu aspersum as accumulation organs. In addition, a comparative analysis of sulfate concentrations was carried out between sites and snails' tissues of both foot and viscera using a passive biomonitoring approach which allowed us to assess the level of snail contamination by TME at the investigated sites and thereby evaluate soil quality.

2. Materials and methods

2.1. Sampling sites

This study was carried out in the geographical region of the extreme northeast of Algeria, which includes three different sampling sites in Souk Ahras province, bounded to the northwest by the cities of El Tarf and Guelma and to the southwest by Oum El Bouaghi city, with Tébessa to the southeast and Tunisia to the east. Our sampling area is characterized by its mountainous terrain, it is also characterized by its topographical diversity, with altitudes ranging from 1000 m in the north to 650 m in the south. The area is divided into two geographical regions: in the north, mountainous and forests are distributed, characterized by a subhumid climate and siliceous or limestone soils, with an average annual rainfall of approximately 730 mm. On the other hand, the south is composed of high plains and pastures, with a semi-arid climate and brownish agricultural soils, often covered with a layer of limestone, with an average annual rainfall of 350 mm per year (Boudy 1955).

In addition, the sampling sites were selected according to a gradient of soil contamination compared to their proximity (distances) to factories of different industries. An area without any industrial activity was also selected, taking into account their accessibility, the ease of sampling and the abundance of *C. aspersum* species. Three monitoring sites were selected, as illustrated in **Figure 1**:

ENAP: National Enterprise of Paints of Souk Ahras located near the national road N° 16 (36° 17′ 21″ N, 7° 56′ 19″ E), the factory is responsible for manufacturing paints by using several raw materials: binders, pigments, solvents and glues.

Mechrouha: is located 21 km northwest of Souk Ahras city and 58 km southeast of Guelma (36° 21′ 0″ N, 7° 49′ 60″ E), this region is characterized by its rugged terrain, it consists mainly of mountains, contains stunning natural sites and is known for its dense forest cover.

Zaarouria: is located 10 km west of Souk Ahras city (36° 13′ 38″ N, 7° 57′ 28″ E), it comprises a natural mountainous area, an urban and an industrial area characterized by the presence of a brick factory. It is also distinguished by its variety of trees, including vineyards.

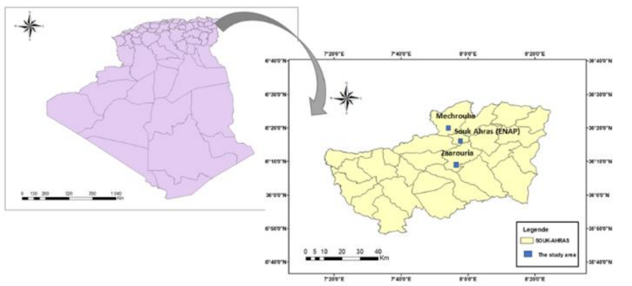

Figure 1. Map showing the sampling sites of *C. aspersum* in Souk Ahras province (northeastern Algeria).

2.2. Experimental approach

The use of sentinel bioindicator species has become a widely recognized phenomenon for assessing soil quality in recent decades. The bioindication method used in this study is passive bioindication. This approach is based on the principle that terrestrial gastropods due to their sensitivity to environmental changes can serve as reliable indicators of soil quality, thus allowing the monitoring of. concentrations of various analyses including metals (Dupont *et al.* 2023, Larba *et al.* 2023)

2.3. Biological model

The biological material used is a terrestrial gastropod, the *C. aspersum* snail (Müller 1774) randomly collected from three sites in the Souk Ahras province (northeastern Algeria). The snails (with an average weight of 8 ± 0.5 g) were placed in aestivation under dry conditions, then they were woken up by spraying with demineralized water and fed with fresh lettuce for two days before being deprived of food for another two days to ensure that their intestinal tract was empty.

2.4. Soil physicochemical characteristics

A physicochemical analysis was carried out on three soil samples collected from three different sites to determine common soil characteristics, in particular: hydrogen potential (pH) according to Baize and Jabiole (1995), electrical conductivity (EC) according to (Okalebo *et al.* 2002), salinity (Rhoades *et al.* 1982), nitrogen (N) based on Kjeldahl method (1883), phosphorus (P) according to Olsen *et al.* (1954), carbon (Walkley and Black 1934), total limestone content (Drouineau 1942), organic matter (OM) (Bonnefont *et al.* 1980), cation exchange capacity (CEC) according to Thomas (1982) and soil texture(Lag *et al.* 2008). This analysis was carried out at the Horizon private laboratory in Annaba city.

2.5. TME extraction by hot acid mineralization

After snails' dissection and drying of the viscera and feet of *C. aspersum* in an oven at 65 °C for 48 to 72 hours, the dry organic matter was ground to a fine powder using a ceramic mortar and pestle. 100 mg of dry matter was digested in 3 ml of an acid mixture consisting of: HNO3: H2SO4: HCLO4 in a volume ratio of 10: 1: 0.5 at 110 °C for 2 h in Teflon bombs.

The extracts were recovered in 50 ml of 0.5% (v/v) HNO3, then filtered through Whatman N° 1 filter paper and injected into the atomic absorption spectrometer (PinAAcle 900T, PerkinElmer, Waltham, MA, USA) to determine the content of the two metals (Cu and Zn). The Cu and Zn standards used to calibrate the instrument were prepared using PerkinElmer solutions of 1000 mg L-1 (PerkinElmer, Waltham, MA, USA). The concentration range varied depending on the nature of element being analyzed and its expected abundance in the mineralized samples. The calibration ranges for Cu and Zn were 0-5 mg L-1 and 0-1 mg L-1 respectively (Sleimi *et al.* 2022, Bankaji *et al.* 2023, Bouzahouane *et al.* 2024).

2.6. Soil analysis by X-ray fluorescence spectrometry

The analyses were carried out at the common service unit for research "Atomic Absorption Spectrometer" and the Research Laboratory RME-Resources, Materials and Ecosystems, University of Carthage, Tunisia.

The studies presented here were carried out using an X-ray fluorescence (XRF) spectrometer. This analyzer offers the highest performance in XRF analysis, providing the lowest possible detection limits for nearly forty elements. It is specifically designed for the analysis of solid metals, alloys, ores and soils. This last feature was implemented for our study. The analysis time lasts 30 s per sample. The results are expressed in parts per million (ppm), and they are particularly positive, allowing the direct use of the cooper and zinc concentrations.

Soil samples (5 cm deep) were collected from the ENAP, Zaarouria and Mechrouha sites. Once collected, the soil to be analyzed must first be dried in an oven at 65 °C, then ground and sieved to a mesh size of 2 mm. They are then placed in capsules and covered with Mylar film. For analysis, the samples are placed in an automatic feeder of the XRF spectrometer (Téreygeol *et al.* 2010, Guessasma *et al.* 2020, Weindorf and Chakraborty 2020, Kim *et al.* 2023).

2.7. Sulfates analysis

For the quantification of sulfates, a photometric method was employed, sulfates are precipitated in a hydrochloric acid medium, forming barium sulfate. After mineralization of our samples and preparation of stock solutions, a precise dosing technique was followed. In test tubes, 2.5 ml of the solution (test sample) was combined with 17 ml of distilled water, 0.5 ml of HCL and 2.5 ml of the 20% BaCl2 + PVP solution. These mixtures were vigorously stirred at a constant speed for exactly 1 minute, followed by a resting period of 15 minutes. The spectrometer cuvettes were then filled and the turbidity measured 30 seconds later at a wavelength of 650 nm. The sulfates transformed into suspended barium sulfate in a range solution were distributed in 6 tubes: 0-15-21-27-30-90 mg L-1 of SO4-2. This detailed methodology ensures a meticulous analysis of the sulfates present in the sample (Gboko *et al.* 2022).

2.8. Statistical analysis

In the present study, all our statistical analyses were performed using R, version 4.2.2 (R Core Team 2022, Ihaka and Gentleman 1996) for MacOS (http://cran.r-project.org). Shapiro-Wilk test was used to test variables normality. Data were expressed as mean ± standard error (se). The comparisons between sites for the soil physico-chemical characteristics and between tissues for trace metal elements (TME) and sulfates were assessed by the nonparametric Kruskal-Wallis (KW) rank sum test. The KW test was followed by the nonparametric pairwise Dunn's test (with Bonferroni adjusted p-value) to find post-hoc statistical differences at α= 0.05 as significant level. Correlations between the analysed variables were also calculated by using Spearman's nonparametric correlation (with Bonferroni adjusted p-value). Finally, in order to characterise the soil samples according to their physicochemical characteristics and their possible impacts on the TME and sulfates concentrations in the snail's tissues; we carried out also a multivariate analysis by applying a principal component analysis (PCA) as ordination technique. Several R packages (libraries) were also used in our statistical analyses and to plot data results such as 'FactoMineR'(Lê *et al.* 2008) , 'ggplot2' (Wickham 2016), 'dunn.test' (Dinno 2017), 'factoextra' (Kassambara and Mundt 2020), 'Hmisc' (Harrell 2023), 'ggcorrplot' (Kassambara 2023a), 'ggpubr' (Kassambara 2023b), 'psych' (Revelle 2023) and 'PMCMRplus' (Pohlert 2023).

3. Results

3.1. TME and sulfates analysis across distinct sites

We used the Principal Component Analysis (PCA) because it is a real versatile statistical method. In our study we applied it as an ordination technique and it allowed us to reduce the dimensionality of our dataset (based on 21 variables) and to preserve the most important patterns or relationships between the analyzed variables according to the target sites. The PCA provided an approximation of our original data on *C. aspersum* snails' TME: copper (CuF and CuV) and zinc (ZnF and ZnV) as well as sulfates (Sulf. F and Sulf. V) within three distinct sites in the Souk Ahras province (Zaarouria, Mechrouha and ENAP). In our case, the PCA enabled us the assessment of correlations between snails' TME (blue variables on the biplot, **Figure 2**) and with other soil variables (TME and texture-physicochemical parameters): copper (Cu), zinc (Zn), sulfates (Sulf), sand, silt, clay, cation exchange capacity (CEC), organic matter (OM), total lime (T, Lime), carbon (C), phosphorus (P), nitrogen (N), salinity (Sal), electrical conductivity (EC) and pH. In a synthetic way, the PCA results indicate that the first two axes explain 100% of the total inertia (data variance) present in our dataset, indicating that the biplot represents the entirety of the variability (**Figure 2**).

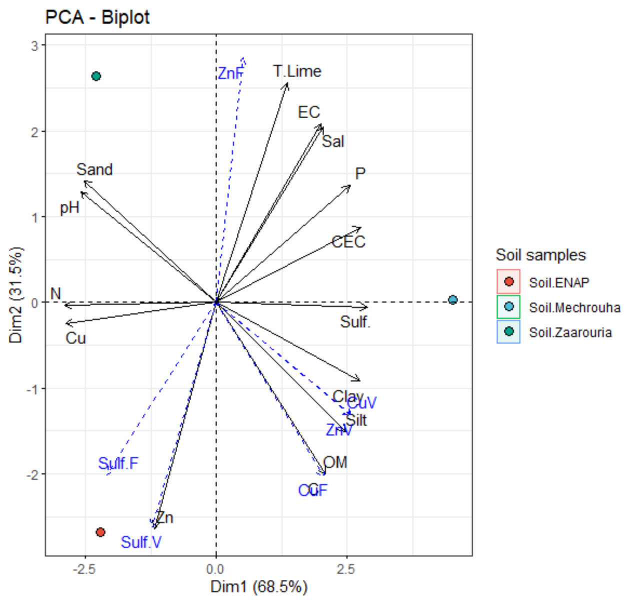

Figure 2. Principal component analysis (PCA) biplot of the three sampled site characterization (Dim 1: 68.5% and Dim 2 = 31.5%). The biplot shows the PCA scores of the fifteen explanatory variables as black arrows. Points on the same side as a given variable should be interpreted as having a high contribution on it. In this study, the six TME measured in snails' tissues of *C. aspersum* were treated as supplementary variables (the six blue arrows).

On the one hand, the 1st axis (Dim 1) alone, explained 68.5% of the total variation and showed strongly positive correlation especially with: Sulf ($r = 1$; $\cos^2 = 1$), CEC ($r = 0.95$; $\cos^2 = 0.91$), clay ($r = 0.95$; $\cos^2 = 0.90$), P ($r = 0.88$; $\cos^2 = 0.78$), silt ($r = 0.85$; $\cos^2 = 0.73$), OM ($r = 0.72$; $\cos^2 = 0.52$), C ($r = 0.72$; $\cos^2 = 0.52$) and Sal ($r = 0.71$; $\cos^2 = 0.71$). In addition, the 1st axis is also negatively correlated with the following variables: N ($r = -1$; $\cos^2 = 1$), Cu ($r = -1$; $\cos^2 = 0.99$), pH ($r = -0.90$; $\cos^2 = 0.80$) and sand ($r = -0.87$; $\cos^2 = 0.76$). On the other hand, the 2nd axis (Dim 2) just explained 31.5% of the total variation is and it was also positively correlated with the variables: T, Lime ($r = 0.88$; $\cos^2 = 0.78$), EC ($r = 0.72$; $\cos^2 = 0.52$) and Sal ($r = 0.71$; $\cos^2 = 0.50$) and as well as negative correlations with the variable Zn ($r = -0.91$; $\cos^2 = 0.83$).

Moreover, PCA showed different patterns of correlations between analyzed variables and the results revealed an important differentiation between sites due to fluctuations in the concentrations of various TME measured values from the soil as well as in the foot and viscera of *C. aspersum*.

The 1st axis generally distinguished Zaarouria and ENAP sites from the Mechrouha one which is characterized by silty-clay soil and high concentrations values, mostly of Sulf, CEC, OM, C, P, Sal, CuV, ZnV and CuF. By contrast, it exhibited low values for Cu, Sand, N and pH concentrations of Mechrouha in comparison with the two other sites. Furthermore, the 2nd axis mainly differentiated the Zaarouria site from the ENAP one. The Zaarouria site which is characterized by sandy texture and high values of pH, ZnF and T. Lime. By opposition, the ENAP site is marked by very high concentrations of Zn, Sulf. F and Sulf. V.

The PCA results also highlighted the relationship between the soil's physicochemical parameters and the distribution of sulfates as well as TME in the soil and the edible tissues of *C. aspersum*. The findings indicated that the soil Zn is completely independent of T.Lime, Sal and EC. Conversely, these latter physicochemical parameters could promote the accumulation of ZnF. Additionally, ZnV appeared negatively correlated with the soil pH.

Likewise, soil sulfates appeared positively correlated with OM, CEC, P, C and the silty-clay textures. In contrast, T. Lime, EC and Sal could inhibit the absorption of sulfates in the foot and viscera (Sulf. F and Sulf. V) in *C. aspersum species*. Copper exhibited a tendency to be present in higher concentrations in soils rich in N and sandy texture compared to silty-clay ones. Finally, the PCA biplot showed that copper absorption by snails' tissues (CuF and CuV) could be more pronounced in silty-clay soils rich in OM and C than in sandy soils.

3.2. Hierarchical cluster analysis (HCA)

The objective of HCA is to evaluate the similarities between soil samples according to the bioavailability of various TME in the soil as well as the physicochemical parameters analyzed across the three geographic sites, the cluster analysis dendrogram presented a plausible classification (in concordance with the PCA results). It clearly separated the Mechrouha site (cluster 1, high sulfates level according the PCA biplot), characterized by silty-clay soil, from the two others. The cluster 2 included two relatively homogeneous sites: Zaarouria and ENAP, mainly characterized by high N and Cu levels (according to the PCA biplot). Thus, the hierarchical analysis revealed heterogeneity levels among the soil samples, reflecting the north-south geographic transect. The observed heterogeneity could support the TME and sulfates distributions both in soil and in snails (**Figure 3**, see PCA biplot **Figure 2**).

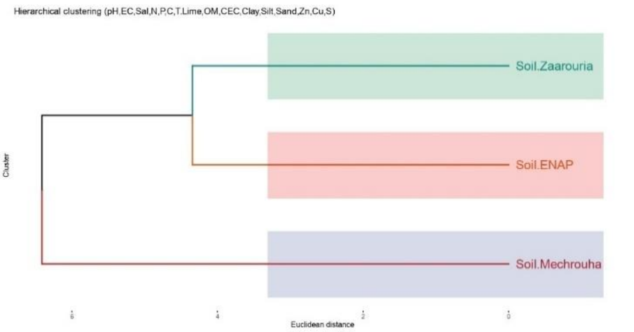

Figure 3. Hierarchical classification of sampling sites of *C. aspersum* (Zaarouria, Mechrouha and ENAP) based on soil physicochemical parameters and TME.

3.3. *TME and sulfates assessment in the snail's foot and viscera*

The mean of the CuF concentrations (in dry weight) ranged from 46.91 ± 2.83 µg/g to 78.58 ± 5.06 µg/g at Zaarouria and Mechrouha sites; respectively (**Figure 4**); with a minimum of 36.44 µg/g at Zaarouria site and a maximum of 140.33 µg/g at ENAP site. The mean of the CuV concentrations ranged from 49.52 ± 2.06 µg/g to 99.55 ± 8.04 µg/g at Zaarouria and Mechrouha sites, respectively; with a minimum of 37.45 µg/g at Zaarouria site and a maximum of 146.66 µg/g at Mechrouha site. The nonparametric Kruskal-Wallis rank sum test revealed significative differences between sites only in viscera tissues (CuV, p <0.05).

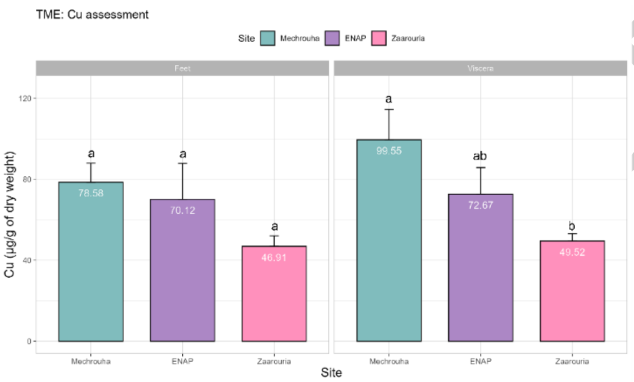

Figure 4. Variations in copper (Cu) concentrations measured in foot and viscera tissues of *C. aspersum*. Data are expressed as mean ± standard error (SE); the lowercase letters indicate significant differences (Dunn's test) among sites (n= 5).

Besides the Cu assessment, the mean of the ZnF concentrations ranged from 68.85 ± 1.21 µg/g to 73.51 ± 1.81 µg/g at ENAP and Zaarouria sites, respectively (**Figure 5**); with a minimum of 61.60 µg/g at Mechrouha site and a maximum of 84.10 µg/g at Zaarouria site. In addition, the mean ZnV concentrations ranged from 161.61 ± 8.38 µg/g and 327.11 ± 32 µg/g at Zaarouria and Mechrouha sites, respectively; with a minimum of 123.68 µg/g at Zaarouria site and a maximum of 472.41 µg/g at Mechrouha site. The Kruskal-Wallis rank sum test also revealed significative differences among sites only in viscera tissues (ZnV, p <0.05).

In this study we have also performed the nonparametric Mann-Whitney rank sum test to compare, within each site, TME and sulfates concentrations between viscera and feet tissues. The statistical results highlighted significant differences in sulfates concentrations between snails' viscera and feet tissues only for ENAP site (**Figure 6**). Regarding to the TME data, the Mann-Whitney test also revealed, in each site, no significant differences in Cu concentrations between viscera and feet tissues. In contrast, significant differences in Zn concentrations were highlighted among viscera and feet tissues within each site. All these results will be explained in the discussion section.

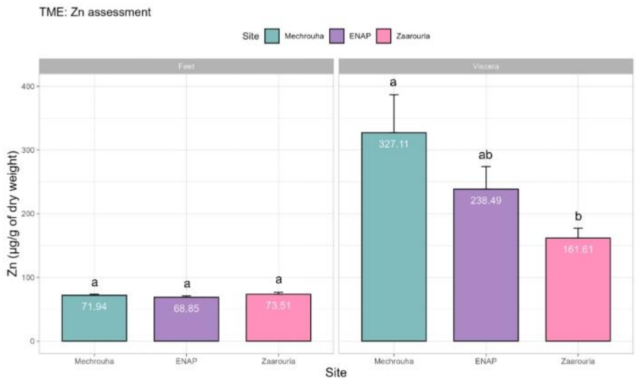

Figure 5. Variations in zinc (Zn) concentrations measured in foot and viscera tissues of *C. aspersum*. Data are expressed as mean ± standard error (SE); the lowercase letters indicate significant differences (Dunn's test) among sites (n= 5).

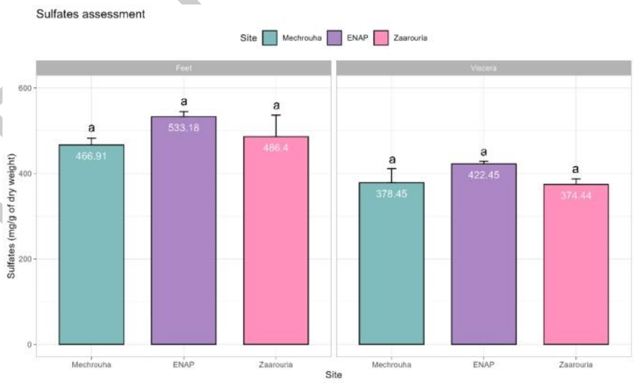

Figure 6. Variations in Sulfates (Sulf) concentrations measured in foot and viscera tissues of *C. aspersum*. Data are expressed as mean ± standard error (SE); the lowercase letters indicate significant differences (Dunn's test) among sites (n= 5).

3.4. *Correlation analysis*

The results of the Spearman's correlation analysis showed that 68% of the correlations, calculated between the twenty-one studied variables, were not statistically significant. All correlation coefficients are shown in **Figure 7** as a corrplot; the latter provided a visual exploratory tool on the correlation matrix relating to TME, sulfates and physicochemical parameters concentrations (measured in snails' tissues and soil samples) and it determined the statistical significance of the correlations.

The corrplot highlighted that only 67 correlation coefficients were statistically significant (p <0.05). As an example: the results clearly indicated highly significant positive correlations (r = 1) between several variables, such as Sulf. -CuV, ZnV-CuV, Zn-Sulf.V, Sal-EC, P-N and CuF-ZnV. On the other hand, the corrplot also highlighted highly significant negative correlations (r = -1) between many variables such as pH-CuF, Sulf.V-ZnF, EC-Sulf.F, C-pH, Zn-ZnF and Sulf. -Sand.

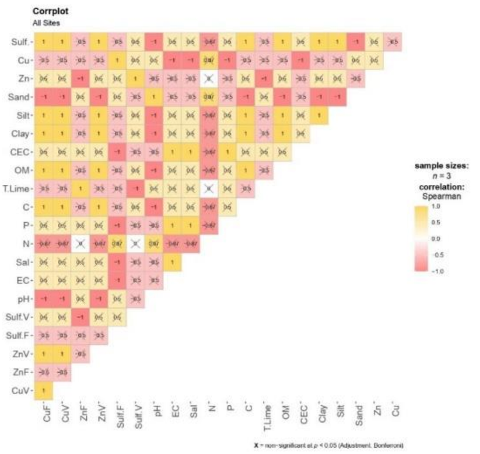

Figure 7. Spearman's correlation carried out on TME, sulfates and physicochemical parameters measured in both snails' tissues and soil samples in all sites.

4. Discussion

4.1. Impacts of soil physicochemical parameters on the bioavailability of TME and sulfates

Physicochemical parameters are among the main factors influencing the distribution and mobility of metals in soils. Several studies including those by van Gestel (2008), Laurent *et al.* (2020) and Kicińska *et al.* (2022) have demonstrated that an increase in pH reduces the concentrations of certain metals such as zinc, cadmium and lead. Włostowski *et al.* (2016) highlighted that under acidic soil conditions the concentration of cadmium tends to increase, which promotes its absorption by *Helix pomatia* and its accumulation in the intestinal gland. This accumulation results from various mechanisms, including ingestion of plants that have absorbed cadmium, direct ingestion of soil particles, and cutaneous diffusion through the snail's foot. According to Wei *et al.* (2021) acidic soils contribute significantly to the increased availability of Cu, Ni and Zn while simultaneously reducing the accumulation of Cd, Cu and Zn. These observations emphasize the influence of soil pH on the mobility and bioavailability of heavy metals as well as their uptake by terrestrial organisms. Our results are consistent with these findings showing that in alkaline soils with elevated pH levels the concentrations of ZnV, CuV and CuF tend to decrease. Heavy metals are generally insoluble and less available for plant uptake in alkaline soils, which could be explained by the fact that at high pH levels metals may form insoluble complexes or precipitate with other soil elements thereby reducing their mobility and bioavailability in soil (Yu *et al.* 2016; Sintorini *et al.* 2021). Shahid *et al.* (2017) demonstrated that soil pH plays a critical role in modulating the chemical forms of heavy metals by affecting various physicochemical processes, including adsorption /desorption, precipitation/dissolution and the formation of complexes with soil components. Conversely, the study by Li *et al.* (2014a) specifically mentions that soil acidity not only promotes the bioavailability of metals to terrestrial species such as *Eisenia fetida* but also increases their accumulation in soils.

The PCA analysis from this study revealed that the soil at Mechrouha is richer in sulfates compared to other sites. It is well known that this site experienced several significant fires two years ago, which likely led to substantial changes in soil composition including pH, organic matter content, and nutrient availability. Among the constituents of ash is sulfuric acid, which can increase soil acidity as evidenced by our findings for this site. Clemente *et al.* (2003) emphasized that soil acidification resulting from the oxidation of sulfides is a key factor in increasing the bioavailability of metals as demonstrated by the concentrations of Cu and Zn observed in the tissues of *C. aspersum* (CuF, CuV and ZnV). Furthermore, soil texture as discussed by Angelaki *et al.* (2022); Sungur *et al.* (2023) influences the distribution and availability of metals. Clayey soils exhibit a high adsorption capacity for various metals except for lead, which is in agreement with our results. We observed that CuF, CuV and ZnV tend to accumulate in silty clay soils rather than in sandy soils. The presence of Cu and Zn in the tissues of *C. aspersum* can be attributed to the organic richness and acidity of silty clay soils. These conditions promote increased metal desorption enhancing their solubility and availability not only to plants but also to other soil organisms such as snails (He *et al.* 2020; (AbdelRahman *et al.* 2022) Zhu *et al.* 2023); . Our results suggest that an increase in certain physicochemical parameters such as T, Lime, EC and salinity reduces the presence of zinc and copper in the soil. This observation contradicts the findings of Benahmed *et al.* (2016) and Bartkowiak *et al.* (2020) who reported an increase in zinc concentration in response to salinity, EC and total lime. A possible explanation for our findings could be competition between sodium cations (Na^{+}) in saline soils and other cations such as calcium (Ca^{2+}) in calcareous soils with zinc (Zn^{2+}) and copper (Cu^{2+}) ions for sorption sites on soil particles. This competition could reduce the amount of zinc retained by the soil (Acosta *et al.* 2011) increasing its concentration in the soil solution, making it more accessible for uptake by plants and other terrestrial organisms. This mechanism could also explain the results for ZnF, whose accumulation is promoted by these same three parameters (T, Lime, Sal and EC). This observation aligns with studies by Zhang *et al.* (2020), Park *et al.* (2021) and which confirmed that salinity affects the mobility of metals particularly cadmium by increasing its mobility. Kadkhodaie A. (2012) also observed a significant increase in cadmium in different parts of sunflower and Sudan grass (roots, stems and leaves) in response to increased salinity.

In addition, our results also reveal that the parameters T, Lime, Sal and EC could influence the mobility and availability of sulfates inhibiting their uptake by the edible tissues of *C. aspersum* (Sulf.F and Sulf.V). This phenomenon could be explained by the fact that in calcium and sodium rich soils sulfates can form partially insoluble ionic complexes such as gypsum. These less soluble complexes tend to remain bound to the soil matrix. This hypothesis is supported by the study of Zunino and Scrivener (2020) which suggests that sulfates tend to form fewer mobile complexes in the presence of calcium. The study by Salao *et al.* (2017) confirmed that industrial emissions play a significant role in the concentration of nitrogen in the environment, which corroborates our results highlighting the high levels of nitrogen in the Zaarouria site (urban and industrial). This pollution could be due to the release of nitrogen compounds such as ammonia and nitrogen oxides during industrial processes. These emissions can be deposited in the soil by precipitation, thus increasing the concentration of nitrogen in the soil (Stevenson 1965). The application of nitrogen fertilizers can alter the mobility and bioavailability of metals in the soil as confirmed by Li *et al.* (2019) whose study suggests that increasing nitrogen inputs to the soil could reduce the bioavailability of certain metals thus limiting their uptake by plants and microorganisms. These findings are consistent with our results which show that the presence of nitrogen promotes Cu accumulation in the soil while reducing its transfer to the tissues of *C. aspersum*.

4.2. TME (copper and zinc) in the foot and viscera of snails

The presence of TME in the soil can have a significant impact on organisms such as terrestrial gastropods which are considered bioindicators of pollution. Due to their sedentary lifestyle, these organisms have the ability to accumulate various pollutants including TME, thereby reflecting the contamination status of their environment (Mukhtorova *et al.* 2023; Rašković *et al.* 2023). Among the metals detected in the soils of the studied areas are zinc and copper which were also found in the edible tissues of the snails. Our analyses of the TME indicate that Cu and Zn were primarily accumulated in the foot and viscera of snails, revealing significant spatial variation among the three sampling sites for Cu in these parts, as well as for Zn in the viscera. This accumulation was particularly notable at the Mechrouha and ENAP sites compared to the Zaarouria site. The spatial variation of Cu can be explained by the different concentrations of this metal in the soils of three sites which may be influenced by the physicochemical parameters of the soil, leading to differences in absorption by the snail's foot and viscera. The spatial variation of Zn in the viscera only can be attributed to varying levels of Zn in the food sources of *C. aspersum* as well as the specific capacities of the viscera to absorb and accumulate this metal (Mitev *et al.* 2023) the absence of spatial variation of Zn in the snails' feet can be explained by similar or very close concentrations of Zn in the soils of the three sites. Our results also revealed a notable fluctuation in the distribution of Cu and Zn between the tissues of *C. aspersum*. The viscera exhibit a higher accumulation of metals compared to the foot which supports the findings of Gomot-de Vaufleury and Pihan (2002), Hoang *et al.* (2008) and Benhamdoun *et al.* (2024) who confirmed through their studies on metal bioaccumulation in land snails that the viscera accumulate significantly more metals than the foot. For example, Hoang *et al.* (2008) reported that copper was predominantly accumulated in the viscera, accounting for approximately 60% of the total burden, while the shell contained less than 4%. The foot represented about 40% of the total copper accumulation. This observation can be explained by the particularly active metabolism of the viscera which is responsible for the accumulation, processing and storage of nutrients, leading to higher metal accumulation. In contrast, the foot is primarily composed of muscles dedicated to locomotion. However, this does not exclude the accumulation of metals in the foot or other parts of the snail, as some metals tend to concentrate in the foot or the shell (Aleksander-Kwaterczak and Gołas-Siarzewska 2015; Ćirić *et al.* 2018; Huang *et al.* 2018). In addition, the mode of exposure to these contaminants (whether by dermal contact or ingestion) plays a significant role in the distribution of metals within various parts of the snail body. This observation aligns with the study by (De-Vaufleury & Bispo, 2000) who supported this theory through their experiments. Sebban *et al.* (2022) revealed that certain metals exhibit a preference for accumulation in specific tissues, with Zn tending to accumulate more heavily in the viscera, while Cu is found in abundance in the foot of snails. Our results regarding Zn are consistent with this study but they are different for Cu. Indeed, we observed that Cu accumulates almost equally in both the viscera and the foot of the snails with a slight predominance in the viscera in all three study areas. This can be explained by a significant presence of Cu in the snails' food sources rather than in the soils in which they live.

4.3. Sulfates concentration in the feet and viscera of snails

Although sulfur is an essential macronutrient (Maruyama-Nakashita and Ohkama-Ohtsu 2017; Li *et al.* 2020) high concentrations can cause issues in soil, water and microorganisms (Geurts *et al.* 2009; Björnerås *et al.* 2019). There are several potential sources of sulfates in the soil as we hypothesized in our study. We detected sulfates at all three distinct sites, the presence of sulfates at the Zaarouria site could be attributed to vineyards where sulfur-based treatments are commonly used (Hermes *et al.* 2022). Additionally, this presence could also be explained by household waste as this site is located in an urban area. The study by Malik *et al.* (2020) showed that organic matter promotes the presence of sulfur in the soil. Therefore, it is reasonable to conclude that household waste which often contains organic matter and nutrients could also be a source of sulfur. For the Mechrouha site, we hypothesize that the presence of sulfates is a result of the wildfires that occurred during the summer of 2022 both at this site and in other areas of Souk Ahras province. This is consistent with the study by Hermes *et al.* (2021) which shows that wildfire can introduce sulfur into the soil especially in agricultural areas by altering soil chemistry and facilitating the release of sulfur into the environment. Regarding the ENAP site (industrial activities), the presence of sulfates could be attributed to sulfur in raw materials used in paints particularly pigments based on barium sulfates. This site is the only one showing a significant difference in sulfate accumulation between the foot and the viscera of the snails (p-value = 0.0079), with accumulation being much higher in the foot (533.18 mg/g in dry weight). This contradicts the study by Lobo-da-Cunha (2019) which indicated that the digestive gland of mollusks is the primary site for nutrient absorption. Our result could be explained by a much higher concentration of sulfates in the soil compared to what is found in the snails' food sources.

Additionally, this difference may be due to a high concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions which compete with sulfates for absorption, resulting in different accumulation levels in the foot and viscera of the snails. In contrast, the accumulation of sulfates in both the foot and viscera of snails across the three study sites (inter-site) showed no significant variation. This could be due to consistent sulfate concentrations across the three study sites and a uniform absorption mechanism by the snails' feet and viscera.

5. Conclusion

The results of this research highlight the potential of utilizing metals concentrations as environmental indicators, while emphasizing the need to consider spatial variations as well as the physicochemical parameters of the soil that influence these indicators at our sites, which in turn affects the level of metal absorption by terrestrial gastropods. We also measured sulfate levels in the foot and viscera of *C. aspersum* based on preliminary soil analysis by X-ray fluorescence spectrometry which revealed the presence of sulfur both in the soil and our target snail species. The results demonstrate that physicochemical parameters significantly influence the availability of TME in the soil leading to differences in their accumulation within edible tissues. Specifically, it was observed that copper and zinc accumulate much more in the viscera than in the foot of *C. aspersum* while sulfate shows the opposite pattern with higher concentrations in the foot. These results confirm that *C. aspersum* is an excellent bioindicator of soil pollution. Accumulation in the different tissues of edible snails including our study species *C. aspersum* could have harmful effects on human health through the food chain. This study presents certain limitations, including the lack of seasonal monitoring, limited geographic scope, and a restricted range of analyzed contaminants. Future research should broaden the study area, incorporate a wider array of pollutants and bioindicator species, and include temporal monitoring to better understand bioaccumulation dynamics. However, such proposed work may also face constraints such as logistical challenges, environmental variability, and limited accessibility to target species.

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