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Estimate bioaccumulation factors and antioxidative response in land snail rumina decollata exposed to pb and Cu in Diyala Province, Iraq

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Abstract: This work aimed to determine the concentration of heavy metals, their bioaccumulation factors, and glutathione levels in the terrestrial snail Rumina decolata collected from three districts: Khanaqin, Mandali, and Muqdadiya in Diyala Governorate. In these districts, soil and snail samples were collected and analyzed using standard methods to determine metal concentrations. The results indicated that the soil and snails of Khanaqin had maximum levels of lead and copper, with mean values of 8.38 ± 2.85 and 3.51 ± 0.03 mg/kg in the soil samples and 1.09 ± 0.13 and 2.13 ± 0.91 mg/kg in snails, respectively. On the other hand, snail and soil samples in Muqdadiya had the lowest levels of such metals. Pb was not detected in snail samples. Bioaccumulation factor analysis showed a different level of accumulation, with a higher copper BCF than lead in Khanaqin, at 1.64 and 0.13, respectively, while the value of lead BCF was higher in Mandali, at 3.2. Additionally, the highest GSH level was observed in snails from Khanaqin, at 24±3.07 μmol/g, followed by Muqdadiya, at 19±1.4 μmol/g, and Mandali, at 17±0.9 μmol/g, which may reflect oxidative stress responses against metals. The above results highlight the potential role of Rumina decolata as a bioindicator for heavy metal pollution and demonstrate its role in heavy metal environmental contamination in Diyala Province. Variations in GSH levels and in the bioaccumulation factor reflect the differential adaptive response to ecological stresses.

Keywords: *Antioxidant, Bioaccumulation factor, Glutathione, Heavy metals, Rumina decollate.*

1. Introduction

Heavy metal pollution seriously affects human health and ecosystems and is considered one of the major environmental problems in the world. Metals such as lead and copper are notorious for their toxicity and persistence in the environment. They accumulate through the food chain, increasing the risk to organisms, including humans. These metals are formed through natural processes such as rock weathering and human activities including industrial production, agriculture, and the use of chemical pesticides and fertilizers [1].

Heavy metals play a dual role in nature: some are essential for certain physiological processes, while others become toxic when their concentrations exceed the permitted natural limits [2]. Elements such as copper and lead are highly toxic and persistent in the environment, and have the ability to accumulate in the environment and integrate into the food chain after reaching a certain limit. Bioaccumulation refers to the pathway by which pollutants move from one trophic level to another in the food web, so some snails and other organisms are used as bioindicators [3].

Land snails are very sensitive to the accumulation of heavy metals, making them effective bioindicators for monitoring environmental pollution. These snails interact with heavy metals in soil and food and accumulate them to varying degrees in different parts such as shells and soft tissues [4]. This property depends on the surrounding environment and pollution conditions, providing scientific evidence for the type and level of pollution in the study area [5]. Because of their high sensitivity to

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changes of the surrounding environment, land snails are among the most important environmental indicators in the study of the effects of pollutants on terrestrial ecosystems. They are used as important tools for monitoring of the environmental pollution with heavy metals. These play a major role in harming living organisms when present in high concentration, since they cause damage to cells and tissues. Monitoring the activity of antioxidant enzymes in snail tissues is one of the most effective ways to assess the level of pollution and the degree of adaptation of organisms to changes in the environmental conditions. Glutathione is a tripeptide with antioxidant properties, as Brown and Kim [6] attests, and one of the main defensive elements which help snails adapt to these harsh environmental conditions. It plays a pivotal role in reducing the toxicity of heavy metals by forming complexes with them, which reduces their toxic effect and enhances cellular defense mechanisms [7]. For this reason, scientific studies have indeed shown that, in land snails, for example, an initial response mechanism to high exposure levels of heavy metals is increased GSH production to counter the aggression, while continuing exposure to those would lead to diminishment of levels due to the oxidative stress experienced and cellular burden placed on resources [8]. Whereas there is overwhelming evidence that asserts the close relationship between GSH and the regulation of heavy metal toxicity, further studies are urgently needed to understand this relationship more precisely, taking into consideration the factors that influence the response of snails to those stresses. The Diyala province in Iraq is of ecological importance; it represents an intense variety of agricultural use, which can be considered indicative of a probably future increase in the level of soil, water, and plant heavy metal contamination. Because of that, bioaccumulation of heavy metals in terrestrial snails is important to be studied within this area.

The research was focused on the estimation of the following main items: the concentration of lead and copper in Rumina and soil from different sites in Diyala province, and the bioaccumulation coefficient (BCF) of these metals. It is important for understanding the dynamics of pollution and gives baseline data for effective environmental strategy development [9].

2. Material and Methods

2.1. Study sites

Samples were collected randomly from 3 different sites in Diyala Governorate (map 1), including : Khanaqin District, which is located on the latitude 34°20'00 " N, 45°23 '00 " E of Khanaqin City near the border, and is represented by mountainous areas and agricultural areas such as palm orchards and fruit trees. Mandali District Located on the latitude 33°746277 " N , 45°5508208 " E in the east of Diyala Governorate, 160 km northeast of Baghdad, near the Iraqi-Iranian border, and is represented by orchards with sandy soils characterized by the presence of fruit trees, most notably pomegranates. Lastly Al- Khalis District Located in the Khalis area, which is located north of Diyala Governorate and is about 55 km from the capital, Baghdad, on the latitude 33°2450348 N , 44°521980 " E and is represented by fields planted with types of vegetables and clover, and orchards planted with palm and fruit trees.

Soil samples were collected from the same areas from which the snail samples were collected, at a depth of 5-15 cm, with three replicates, and placed in polyethylene bags.

2.2. Heavy Metal analysis

After drying the snail tissues or soil, one gram of it was digested in 30 ml of a mixture of hydrochloric acid and nitric acid in a ratio of 3:1 for 5 hours. The solution was left on a hot plate for 5 hours to evaporate to about 1 ml after which it was left to cool and then distilled deionized water was added to it and filtered using filter paper quantitatively with distilled water and filtered using Whatman 42 filter paper without ash No. 0.45 into a 25 ml volumetric flask. The solutions were analyzed for heavy metals using atomic absorption spectrometry [9].

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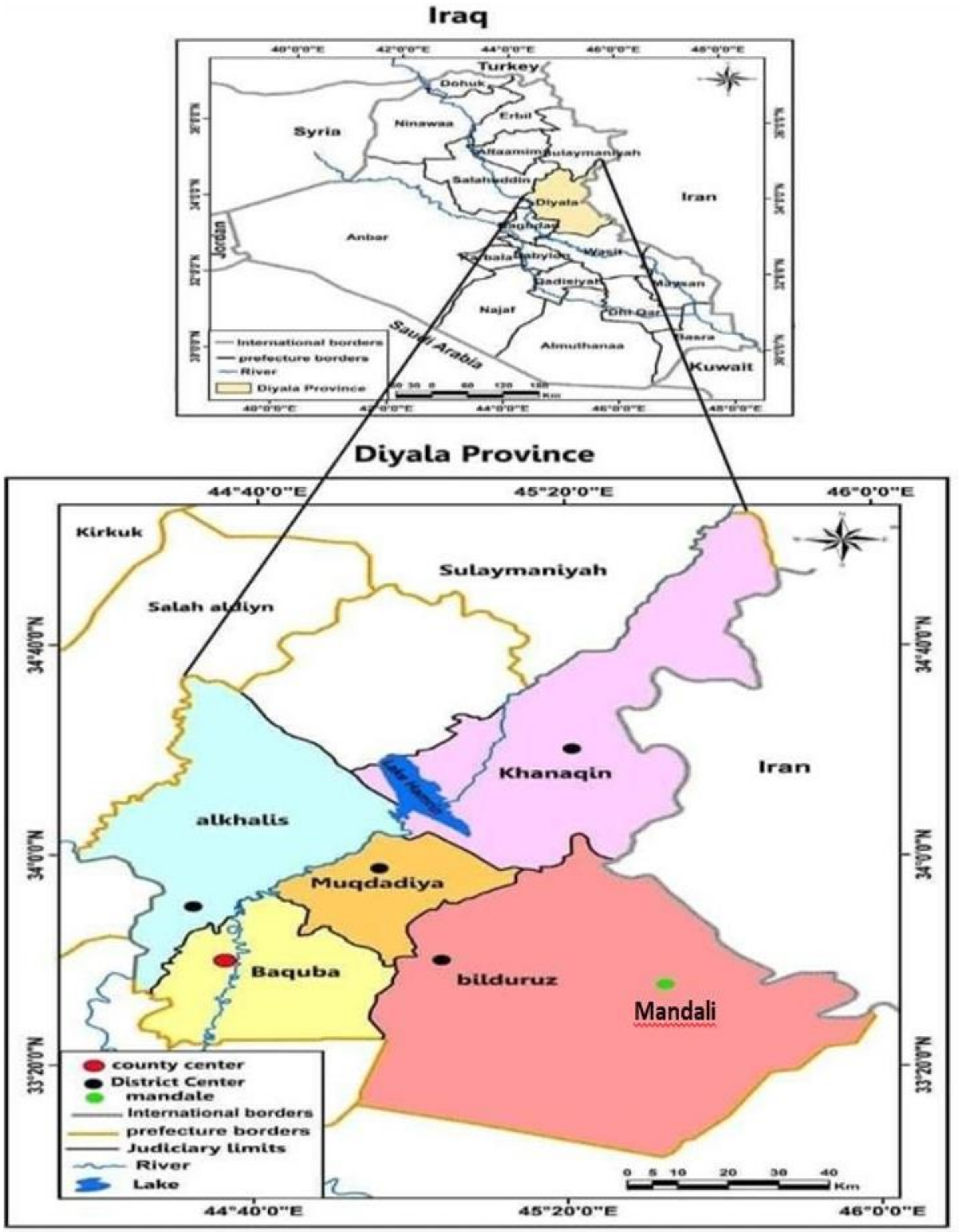

Figure 1.
map of Iraq showing Diyala province and an indication of study sites.

2.3. Glutathion Analysis

For prepare the tissue extract, we relied on what was mentioned in Sutariya, et al. [10]. The snail were washed, cut, weighed 20 g, added 10 ml of hydrochloric acid, stored in a glass vial, and placed in an aluminum heat sink at 50 °C for 5 hours to complete the decomposition process. Using a pipette, 100 microliters were drawn and pipetted into the evaporator to remove moisture with nitrogen gas. After drying, it was dissolved by adding 100 microliters of acetonitrile and exposed to ultrasonic waves for one minute. It was transferred to a heat sink at 50 °C for 30 minutes to complete the decomposition, and 100 microliters of it were injected into the HPLC column, noting that the mobile phase was a flow of an equal mixture of 50/50 (volume/volume) of water and acetonitrile at pH = 7 at 1 ml/min. Column 25 cm x 4.6 mm and detector = 465 nm

2.4. Bioconcentration Factor

Calculate the Bioconcentration factor (BCF) for snails using the following equation:

$$\mathrm{BCF}=\frac{C\ organism}{C\ \ soil}$$

C: the concentration of heavy metal

3. Results and Discussion

The results revealed significant differences in lead concentrations in the soil across the sites. The highest concentration was recorded in Khanaqin (8.38±2.85 mg/kg), followed by Mendeli (0.25±0.002 mg/kg) and Muqdadiya (0.12±0.012 mg/kg) (Table 1). Similarly, lead concentrations in snail tissues were highest in Khanaqin (1.09±0.13 mg/kg), undetectable in Muqdadiya (0 mg/kg), and low in Mendeli (0.08±0.01 mg/kg) (Table 2).

Table 1.
Shows the average concentration (mg/kg) of copper and lead mg/kg and the standard deviation values in soil samples at the study sites.

Site	Pb	Cu
Khanaqin	8.38±2.85	3.51±0.03
Mandali	0.25±0.002	4.36± 0.31
Muqdadiyah	0.12±0.012	4.88±0.71

Table 2.
Shows the average concentration of copper and lead (mg/kg) and the standard deviation values in *R. decollate* samples at the study sites.

Site	Pb	Cu
Khanaqin	1.09±0.13	2.13±0.91
Mandali	0.08±0.01	1.86±0.03
Muqdadiyah	0	2.08±0.16

Table 3.
Bioaccumulation factor of lead and copper in the land snail *R. decollate.*

Site	Pb	Cu
Khanaqin	0.13	1.64
Mandali	3.2	0.42
Muqdadiyah	0	0.42

Copper concentrations in the soil were more uniform across the sites, with the highest levels recorded in Muqdadiya (4.88±0.71 mg/kg) and Mendeli (4.36±0.31 mg/kg), followed by Khanaqin (3.51±0.03 mg/kg) (Table 1). In snail tissues, the highest copper concentrations were observed in Khanaqin (2.13±0.91 mg/kg), followed by Muqdadiya (2.08±0.16 mg/kg) , and Mendeli (1.86±0.03 mg/kg) (Table 2). Results revealed that the lead levels in snail tissues closely reflected its concentration in the soils. Several of these differences might be because of environmental factors such as soil pH and organic matter content, which are known to interact with lead to affect its bioavailability [4]. For instance, the relatively low bioavailability of lead at Khanaqin manifests as a lower value of its BCF as 0.13, in comparison to Mendeli, with a BCF of 3.2 (Table3).

For copper, the higher BCF in Khanaqin of 1.64 compared to Mendeli and Muqdadiya of 0.42 shows that copper would be highly available in Khanaqin soil. It could be due to some differences in speciation of metal ion or competitive ion interactions in soil matrix [9].

The concentration of glutathione (GSH) in snail tissues was measured to evaluate the biological response to heavy metal exposure. The highest GSH concentration was observed in Khanaqin (24±3.07), followed by Muqdadiya (19±1.4) and Mendeli (11±0.9) (Fig.1).

The higher levels of GSH in Khanaqin reflect protective responses to oxidative stress due to the higher concentrations of lead and copper. GSH is an important antioxidant involved in detoxification processes against heavy metals and cellular damage. Such findings are in agreement with previous studies indicating that increased heavy metal concentration is associated with increased production of GSH [11, 12].

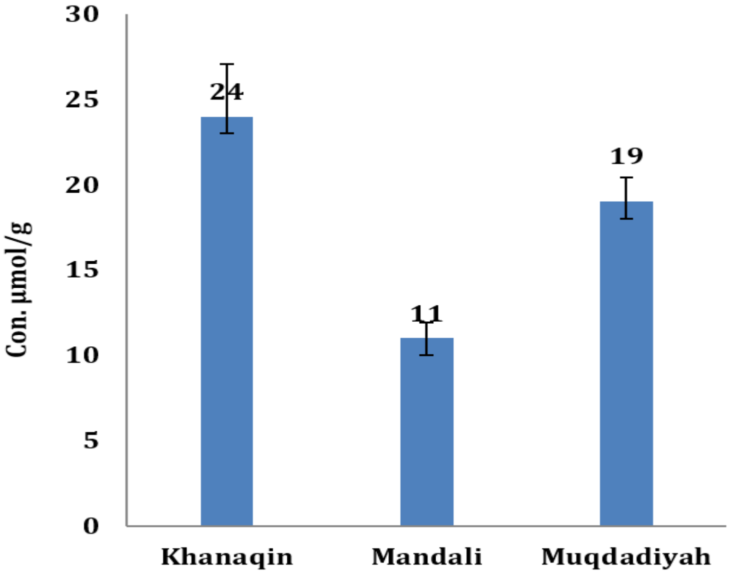

Figure 2.
Activity of GSH (µmol/g) in *R. decollate* in study sites.

The observed differences in the accumulation of lead and copper indicate the impacts of human activities and soil conditions at each sampling site. Khanaqin, being an agricultural and industrial center, may contribute more to heavy metal pollution through runoff and using metal-containing fertilizers [13]. On the contrary, the Mendeli and Muqdadiya samples had lower readings, which are possibly due to less anthropogenic activities.

4. Conclusion

This study highlights the bioaccumulation of heavy metals and the long-term antioxidant response of the land snail *Rumina decollata*. The concentration of metals varied across the study sites, with the highest levels recorded in Khanaqin, indicating that it is considered a hotspot for pollution in the governorate due to human activities. The bioaccumulation factors also indicated the preference for copper in terms of availability. With reference to the results recorded by the levels of glutathione (GSH) in the tissues of the snail *Rumina decollata* and its increase in samples collected from highly polluted sites, it can be considered a bioindicator for monitoring the region's pollution with heavy metals.

These results constitute important data for developing environmental management strategies to reduce heavy metal pollution in the region. Further studies are recommended to explore the long-term effects of exposure to metals and other pollutants on the health of biodiversity and ecosystem.

Transparency:

The author confirms that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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