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Accumulation and interactions of trace elements in tadpoles of *Dendropsophus minutus* (Peters, 1872) (Anura: Hylidae) in natural and soybean monoculture areas from Central Brazil

Igor Gerolineto-Alves^{a,*}, Francisco Leonardo Tejerina-Garro^{a,b}, Nelson Jorge da Silva Jr.^a, Leonardo Luiz Borges^a, Darlan Tavares Feitosa^a, Matheus Godoy Pires^a, Fernanda Sousa Santos^a, Wilian Vaz-Silva^a

^a Programa de Pós-Graduação em Ciências Ambientais e Saúde, Pontifícia Universidade Católica de Goiás, CEP 74605-050, Goiânia, Goiás, Brazil.

^b Programa de Pós-Graduação em Sociedade, Tecnologia e Meio ambiente, Universidade Evangélica de Goiás, CEP 75083-515. Anápolis, Goiás, Brazil.

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Abstract

The contamination of natural ecosystems by trace elements represents a growing environmental concern, particularly in regions under agricultural expansion. In the Brazilian Cerrado, soybean monocultures contribute to soil and water contamination through agrochemicals containing potentially toxic elements. Amphibians, especially tadpoles, are highly sensitive to such disturbances and are thus recognized as reliable bioindicators of environmental quality. This study investigated the accumulation and interaction of eight metals (As, Ba, Cd, Cr, Cu, Hg, Pb, and Zn) in *Dendropsophus minutus* tadpoles collected from soybean cultivation areas and a preserved site on Cerrado biome, in Cezarina municipality, State of Goiás, Central Brazil. A total of 20 tadpoles were collected, equally divided between case and control areas, and analysed after the acid digestion of the whole tadpole, through inductively coupled plasma optical emission spectrometry (ICP OES). Tadpoles from soybean areas exhibited significantly higher concentrations of As, Ba, Cd, Cr, Cu, Pb, and Zn compared with those from the preserved site, while Hg did not differ significantly, suggesting low environmental mobility or absence in local inputs. The most critical increases were detected for Cd, Cu, and Pb, with values exceeding those of the control site by 6378%, 13842%, and 1108%, respectively. Statistical analyses revealed strong positive correlations, notably Pb with Ba, Cd, Cr, and Cu, in addition to interactions involving Cd–Cr, Cd–Cu, Cr–Cu, and Zn–As. These associations suggest synergistic accumulation, with potential to intensify physiological stress in amphibians, including oxidative damage and developmental impairments.

1. Introduction

The presence of trace elements in natural environments has become increasingly intense and concerning due to their accumulation within the food webs, leading to environmental damage and adverse effects on human health (Tundisi & Tundisi, 2008; Steffen *et al.*, 2011; Da Costa Benincá & Clemente, 2024). Although naturally present in soils at low concentrations, trace elements may undergo drastic alterations because of anthropogenic activities (Santos *et al.*, 2019). These substances accumulate in the tissues of living organisms and, through enzyme inhibition and modifications of biomolecules such as DNA, cause cellular damage and consequently impair organismal physiology (Klaassen & Watkins, 2012).

Cerrado biome has undergone drastic landscape changes in the face of advance of the agricultural frontier, where native vegetation is replaced by homogeneous landscapes, and monoculture becomes a common and degrading practice (Strassburg *et al.*, 2017). The use of agrochemicals in monocultures may increase levels of trace elements in soils,

which can subsequently accumulate in organisms inhabiting these environments (Defarge *et al.*, 2018). Brazil is the second largest producer of soya worldwide, surpassed only by the United States of America. Soya cultivation in the country began in the late 1960s and has since become essential for economic development, consolidating Brazil as the world's second largest producer (Gaboardi *et al.*, 2023; de Lima *et al.*, 2025). For instance, in the 2021 harvest, 3855277 hectares in the state of Goiás alone were allocated to soya cultivation, placing the state among the country's largest producers (IBGE, 2021). Currently, extensive areas of Cerrado are rarely found, given the transformation of the natural vegetation into agricultural land, primarily associated with soybean monoculture (Vilela *et al.*, 2021).

Soya production requires chemical inputs for the control of invertebrates, fungi, bacteria, and other plant organisms (Ribeiro *et al.*, 2002). Excessive use of these chemicals increases environmental contamination by trace elements, which can be assessed through studies of water and soil quality, as well as bioindicator organisms (Santos, 2014). The presence

*Corresponding authors: igor.herpeto@gmail.com

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of trace elements such as arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn) in agrochemicals has raised growing concern due to their direct impacts on ecosystems and exposed organisms (**de Lima et al., 2025**). Although most of these metals are prohibited in the formulation of agrochemicals (**Brazil, 2021**), many are still illegally used (**da Silva & dos Reis, 2024**). Cadmium, for example, is highly toxic, persisting in soils and aquatic environments, where it particularly damaging the kidneys and nervous system in tadpoles (**Defarge et al., 2018**). Copper, although essential in small amounts for metabolism, becomes toxic at elevated concentrations, disrupting biochemical processes and damaging the nervous system, liver, and kidneys of several species (**Klaassen & Watkins, 2012**).

Anuran amphibians, owing to physiological traits such as skin and egg permeability (**Gonçalves et al., 2014**), and a biphasic life cycle that exposes them directly to contaminants through water during embryonic and larval stages and through air and soil during juvenile and adult stages, are considered excellent indicators of environmental quality (**Trindade & Hartmann, 2017**). The use of tadpoles as indicators of trace elements accumulation in water and sediments relates to their feeding habits and occupation of different ecological niches (**Degarady & Halbrook, 2006**). They may be classified as benthic, inhabiting the bottoms of water bodies, or nektonic, associated with the water column (**McDiarmid & Altig, 1999**). Tadpoles also act as contaminant vectors within the food web. Even if lethal effects are not immediate, bioaccumulation over time may generate chronic impacts (**Unrine et al., 2007; Lam & Gray, 2003**). Given their abundance, they also represent a significant food resource for higher trophic levels (**Mann, 2005**).

The species *Dendropsophus minutus* has become a model in bioindicator studies due to its wide distribution and adaptability to diverse aquatic and terrestrial environments. Commonly found in Cerrado habitats, it is often associated with environments subject to variations in water and soil quality, particularly those influenced by agricultural activities (**Sotero et al., 2024**). This species has a high reproductive rate, increasing its availability for bioaccumulation studies, making it an efficient tool for monitoring environmental contamination in areas impacted by soya cultivation. Furthermore, its sensitivity to pollutants provides an early indication of ecological impacts caused by trace elements in aquatic ecosystems (**Leivas et al., 2018**). Therefore, the use of tadpoles as bioindicators of trace elements accumulation in the environment proves effective given the large numbers available for sampling and analysis, as hundreds of individuals may hatch from a single clutch (**Wells, 2007**).

This study aimed to quantify eight trace elements (As, Ba, Cd, Cr, Cu, Hg, Pb, and Zn) in *Dendropsophus minutus* tadpoles collected from areas directly affected by soybean cultivation and to compare them with those from native vegetation with less soybean cultivation influence.

2. Material and methods

2.1 Study area and sampling sites

This study was conducted in areas located in the municipality of Cezarina, southwestern Goiás, Central Brazil, where the original vegetation cover is Cerrado type. The climate is AW accordingly the Köppen-Geiger classification characterized by an annual temperature mean of 24.6 °C and 1,336 mm of precipitation (**Köppen-Geiger, 1936**) with two seasons: rainy (September to March) and dry (April to August). The southwestern region is among the state's leading soya-producing areas (Arruda & Teixeira, 2010), with an annual yield of 13,305 tonnes (**IBGE, 2017**).

Sampling was conducted at three sites in January 2024. The evaluation of land use in 2023 of the sampled sites at MapBiomias Brasil© platform (**Collection 9; MapBiomias, 2025**) using a 1000 m buffer indicated that the predominant categories at the first sampling point (PS1; 17°08'46.0"S, 49°41'43.0"W) were mosaic of uses (43.7%), pasture (23.4%) and soybean cultivation (22.4%), whereas at the second (PS2; 17°11'19.0"S, 49°41'51.0"W) the predominant were soybean (46.1%) and pasture and mosaic of uses (both of them with 18.9%). These sites were considered under influence of soybean cultivation. A third site (PC; 17°07'04.0"S, 49°44'58.0"W) was established in a fragment of Cerrado featuring native forest formations (28,6%) and savanna formation (0,7%), but including pasture (40,9%) and mosaic of uses (26,9%) and less soybean cultivation (1,6%; Figure 1). This was considered a reference site.

2.2 Data collection

2.2.1 Tadpole sampling

Tadpoles were collected using one 55 cm diameter sieves swept through water bodies. The sampling effort was 1 person/40 minutes/sampling point site area. Afterwards, the individuals were maintained alive in plastic bags containing water from the sampling site and euthanized. This last procedure was performed by immersing the tadpoles in a 2% lidocaine hydrochloride solution (0.5–1 mL lidocaine/1 mL water).

After this procedure, 200 tadpoles collected were identified to species level using identification keys available for the region (**Santos et al., 2023**), where only 20 *Dendropsophus minutus* tadpoles were selected considering equivalent body sizes and stored individually in 2 mL cryogenic tubes. It was selected 10 specimens from sampling points under soybean cultivation influence (PS1 and PS2), and 10 from the reference site (PS).

Collections were conducted under the license for zoological material collection (SISBIO no. 74397) and approval of the Ethics Committee on Animal Use (protocol no. 6338170823 ID 026277 CEUA/PUC Goiás).

2.3 Laboratory procedures

The trace element quantification analysis was performed using and Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-OES 9000 Shimadzu). This method is appropriated to identification and quantification of meta trace in samples, including biological ones (**Houk et al., 1980**).

Previously, it was performed a chemical digestion of tadpoles. Specimens were individually placed in beakers containing a 5 mL solution of hydrochloric acid (HCl) and nitric acid (HNO₃). The material was then heated on an analogue hot plate at 100 °C (**Jesus et al., 2015**). After digestion, the samples were cooled and transferred to volumetric flasks, where the digested material was diluted with ultrapure water to a final volume of 15 mL. Subsequently, all samples were centrifuged for six minutes at 500 rpm.

2.4 Statistical analyses

A data matrix was organized with trace element concentration variations in the columns and specimens in the rows. A normality test indicated that data did not display normality (Shapiro–Wilk test, $p = 0.05$).

Thus a Kruskal–Wallis test ($p = 0.05$) was performed followed by a post-hoc test of Mann–Whitney in order to verify if tadpoles of *Dendropsophus minutus* from soybean cultivation areas display differences of metal trace concentrations (As, Ba, Cd, Cr, Cu, Hg, Pb, and Zn) from those sampled in an area of native vegetation with less soybean cultivation influence (reference area).

To test interactions among metals, a Spearman correlation analysis was performed. In addition, the percentage increase in the medians of trace element concentrations in the areas affected by soybean cultivation relative to the reference area was calculated.

All statistical analyses were conducted in R software version 4.4.2, using the packages ggplot2 (Wickham, 2016) to generate figures and rstatix (Kassambra, 2021) to facilitate statistical testing.

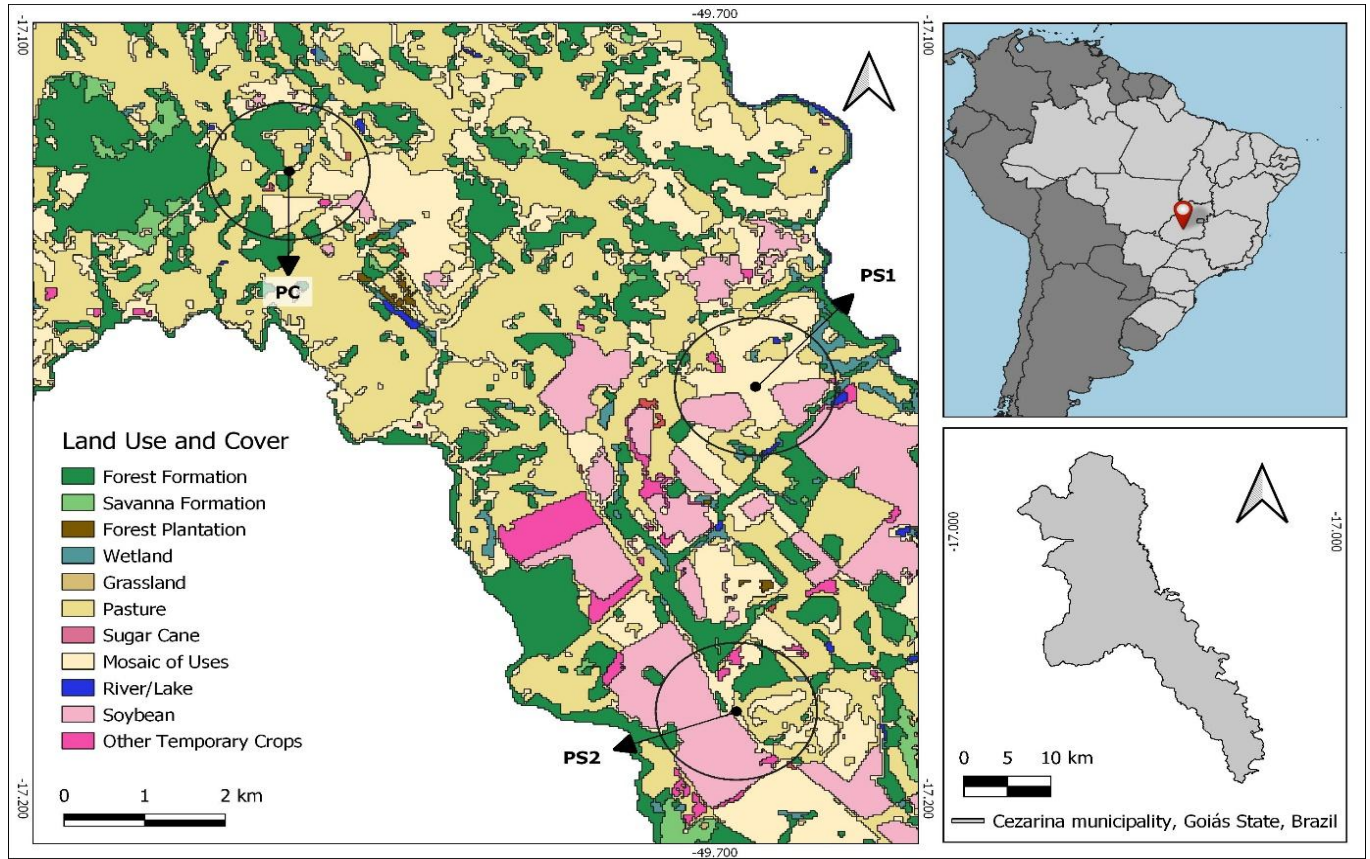


Figure 1 Geographical location of the study area in the Cezarina municipality, Goiás State, Central Brazil. The circles represents the 1000 m buffer around sampling points (blck dots) used to chacterize land use in 2023.

3. Results

3.1 Tadpoles' trace elements concentration

The Kruskal–Wallis test indicated that there are differences between trace elements concentration of tadpoles collected from the reference site of those under influence of soybean cultivation (Table 2A). These differences were stated by the Mann-Whitney test for seven trace elements (As, Ba, Cd, Cr, Cu, Pb and Zn; Table 2B). No significant difference was observed for Hg, where negative values were recorded, falling below the detection limits (Table 2B).

Table 2 Statistics of the Kruskal-Wallis analysis (A) and Mann-Whitney U test comparing tadpoles' trace element concentrations between areas influenced by soybean cultivation and the reference area (B). * indicates statistically significant differences ($p < 0.05$).

(A)	χ^2	df	p-value
	14.294	1	0.0001562*
(B)		U	p-value
As		7	0.0013150*
Ba		0	0.0001827*
Cd		0	0.0001827*
Cr		0	0.0001817*
Cu		0	0.0001817*
Hg		55	0.7394000
Pb		0	0.0001817*
Zi		0	0.0001817*

The most notable differences were observed for Ba, Cd, Cr, Cu, Pb and Zn with median concentrations values higher at the soybean-affected sites compared to the reference site (Figure 2). Median concentrations of Cd, Cu and Pb increased by 6372%, 13842% and 1108%, respectively (Table 3, Figure 2).

Table 3 Median trace element concentrations (mg/kg) per sampling point. Arrows indicate tendency.

Concentration (mg/kg)	Reference site	Soybean sites
As	0,0128	↑ 0,0777 (78%)
Ba	1,3800	↑ 10,1250 (634%)
Cd	0,0027	↑ 0,1715 (6372%)
Cr	0,6550	↑ 1,7750 (171%)
Cu	0,0121	↑ 1,6800 (13842%)
Hg	-0,0922	↓ -0,0996 (8%)
Pb	-0,0865	↑ 0,8720 (1108%)
Zn	1,3300	↑ 3,4850 (162%)

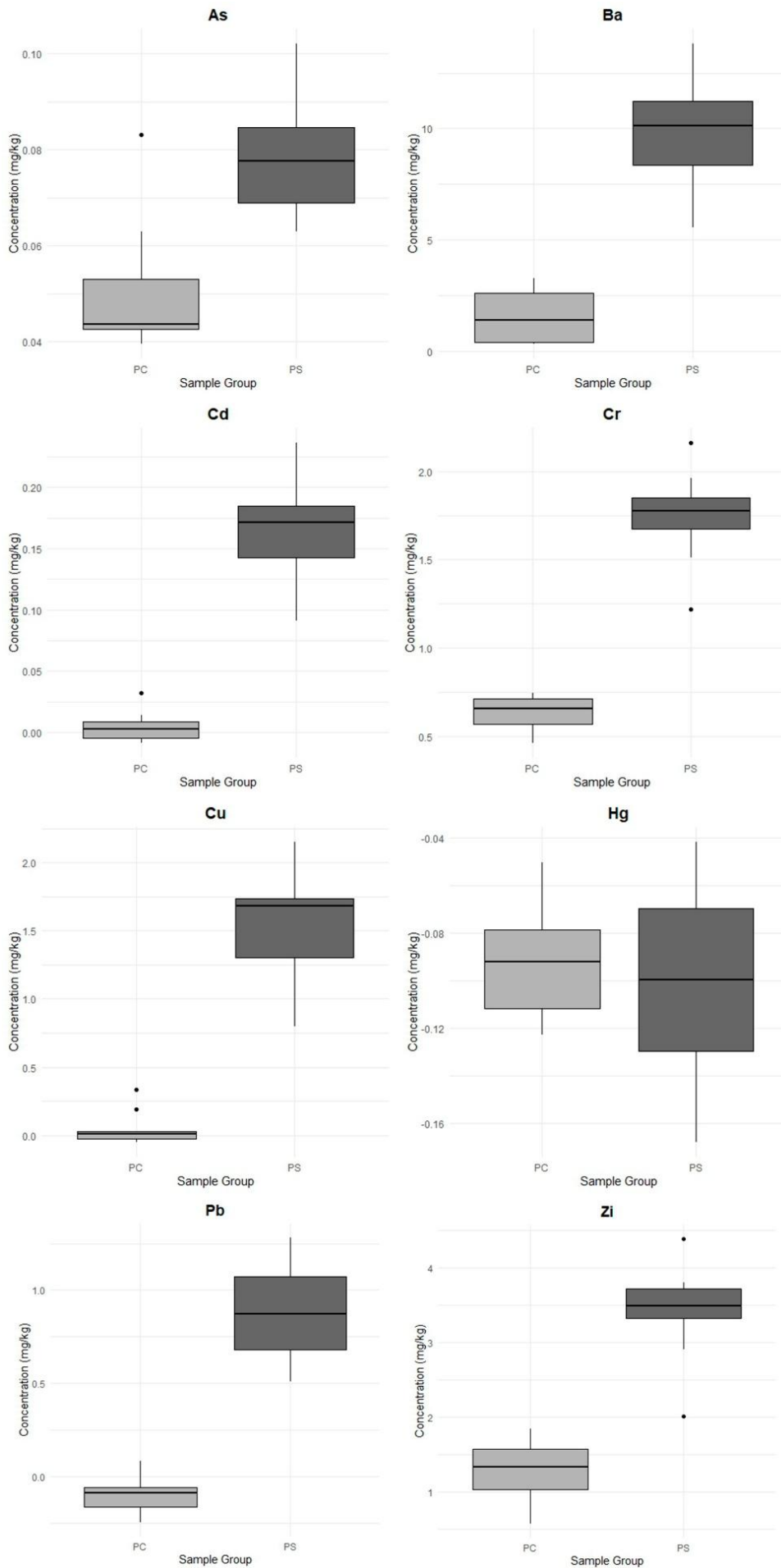


Figure 2 Boxplots showing the concentrations (mg/kg) of elements trace in tadpoles sampled from the reference site (PC) and soybean cultivation sites (PS) in Cezarina municipality, Goiás State, Brazil.

3.2 Interactions of trace elements in the sites under soybean cultivation influence

Positive interactions among trace elements were indicated by the Spearman's correlation (Figure 3). Pb showed significant

interactions with Ba ($p = 0.002$), Cd ($p = 0.002$), Cr ($p = 0.002$), and Cu ($p = 0.001$). Additional interactions were detected between Cd and Cr ($p = 0.005$), Cd and Cu ($p < 0.001$), Cr and Cu ($p = 0.002$), and Zn and As ($p = 0.034$).

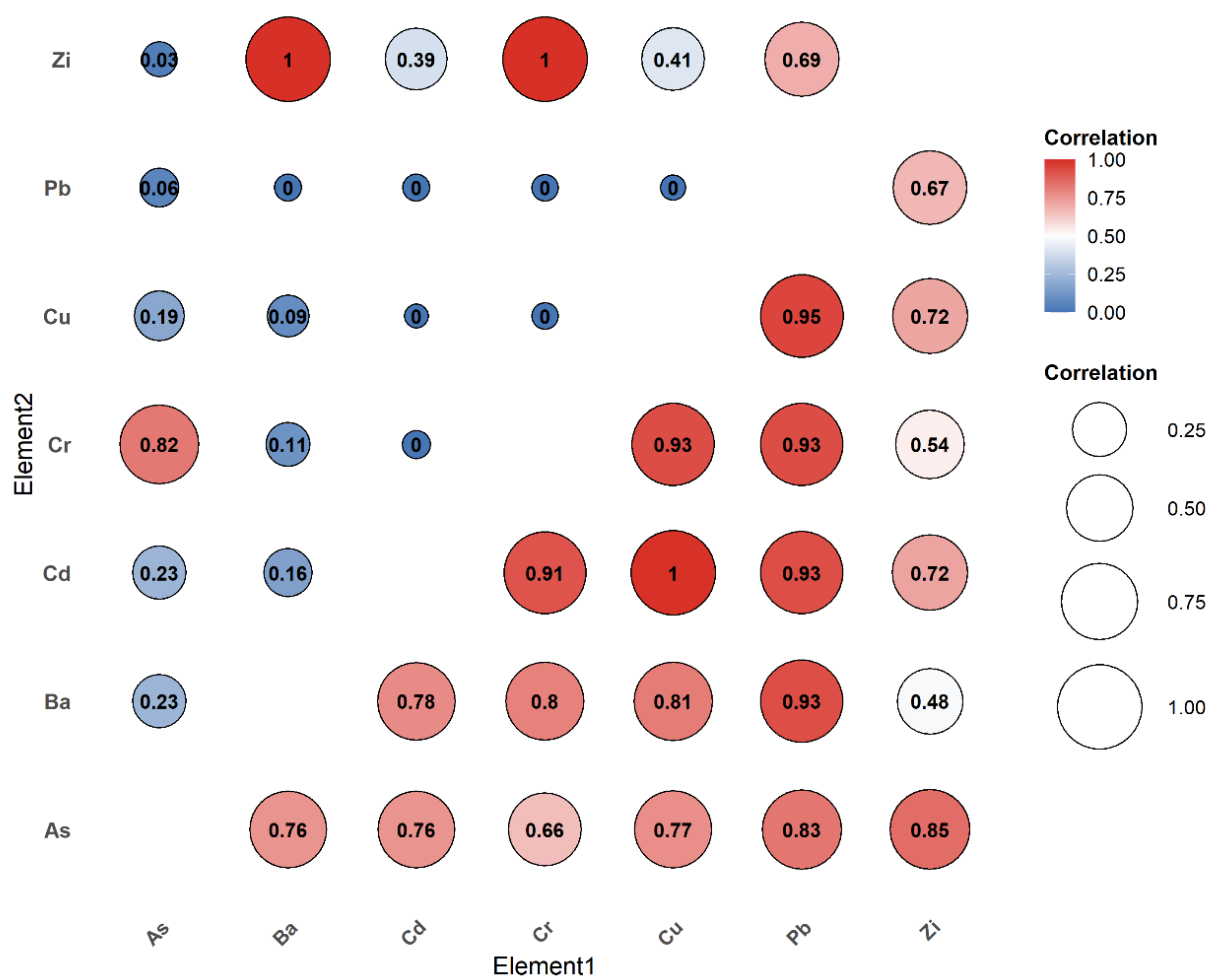


Figure 3 Spearman's rank correlation heatmap for trace elements concentrations in tadpoles collected at the sampling points under soybean influence (PS). Correlation values are indicated in the lower triangle and probabilities in the upper one ($p = 0.05$).

4. Discussion

These results reflect the environmental conditions of a preserved ecosystem with low anthropogenic influence, emphasizing the importance of protected areas for maintaining environmental quality and serving as a reference for comparative studies (Lam & Gray, 2003; Wells, 2007), where the input of trace elements occurs primarily from natural sources such as rock weathering and soil leaching (Aguar *et al.*, 2022). In contrast, tadpoles from the case areas, which encompass regions under agricultural influence, exhibited significantly higher trace elements concentrations, reflecting anthropogenic contributions to environmental contamination. Metals analyzed in tadpoles from the control area were present at low concentrations, notably Cd, Cu, and Pb, which were either below or close to the detection limit. These results suggest that tadpoles from preserved areas are not exposed to the levels of these elements. However, the presence, albeit at low levels, of elements such as As and Ba may indicate natural processes transferring these metals into water bodies. Previous studies suggest that As bioaccumulation in aquatic organisms may be influenced by local soil characteristics and its interaction with water (Alves & Rietzler, 2015). Conversely, tadpoles from the case areas exhibited elevated concentrations of these elements,

with values significantly exceeding those recorded in the control area, indicating a direct influence of human activities, such as the use of fertilizers and agrochemicals contaminated with metals (Santos *et al.*, 2019).

Samples from both case and control points showed low Hg concentrations, supporting the hypothesis that this metal was not detected in either area due to the absence of point sources of contamination. Tadpoles collected from areas influenced by soybean cultivation exhibited significantly higher concentrations of trace elements such as Ba, Cd, Cu, and Pb compared to control areas. These results indicate a direct relationship between intensive agricultural practices and metal bioaccumulation in aquatic organisms. Fertilizers and pesticides containing traces of these elements are identified as major sources of contamination in agricultural regions. For example, pesticides containing barium sulphate (BaSO_4) and calcium arsenate ($\text{Ca}(\text{AsO}_4)_2$) are known sources of barium and arsenic, respectively, while fertilizers containing tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) often present cadmium contamination as a by-product of mineral processing (Magalhães *et al.*, 2024). Studies such as Defarge *et al.* (2018) and Santos *et al.* (2019) document the cumulative impact of these substances on the environment, highlighting the need for less impactful alternatives and increased monitoring in agricultural areas.

The concentration of Cd, which increased by over 6000% in soybean areas relative to control areas, is particularly concerning due to its high toxicity potential. Studies indicate that Cd can accumulate in the tissues of aquatic organisms, causing physiological and reproductive dysfunctions (Klaassen & Watkins, 2012; Huang et al., 2021). Furthermore, elevated Pb and Cu levels also indicate the influence of agricultural inputs, which frequently contain these metals as contaminants.

Exposure to trace elements can have severe ecological consequences, impacting amphibian populations and other organisms relying on the same aquatic environment (Lopes et al., 2023). High Zn concentrations, for instance, while essential at low levels, can be harmful at elevated concentrations, affecting fundamental metabolic processes in aquatic organisms (Gemaque et al., 2024).

Dendropsophus minutus has proven to be an effective bioindicator due to its high sensitivity to variations in environmental quality. Its permeable skin and biphasic life cycle facilitate the absorption of contaminants from both water and soil (Wells, 2007; Gonçalves et al., 2014; Lopes et al., 2023). Additionally, its abundance in the study areas allows for robust sampling and reliable comparative analyses (Sotero et al., 2024).

High trace elements concentrations in tadpoles from soybean areas reflect the species' efficiency in accumulating contaminants, providing a clear indication of environmental quality. The use of species such as *D. minutus* for environmental monitoring also offers logistical advantages, given their availability in both aquatic and terrestrial impacted environments (Leivas et al., 2018; Lopes et al., 2023).

Bioaccumulation of trace elements in tadpoles not only poses direct risks to these organisms but also has serious ecological and human health implications. Contaminated aquatic organisms can transfer metals along the food chain, affecting predatory species including birds, fish, and mammals (Steffen et al., 2011; Da Costa Benincá & Clemente, 2024). For example, studies by Unrine et al. (2007) reported that cadmium bioaccumulation in fish from agricultural regions can lead to reproductive deficits in piscivorous birds. Similarly, Mann (2005) noted that lead contamination in aquatic environments can cause behavioural and neurotoxic effects in predatory mammals. These examples illustrate how trace elements accumulated in aquatic organisms can have cascading effects across ecosystems, exacerbating risks to biodiversity.

Pb, which exceeded permissible concentrations in soybean areas, is particularly concerning due to its association with severe neurotoxic and hematological impacts in exposed organisms (Vieira et al., 2022). Additionally, Cd contamination can affect the reproductive productivity of aquatic and terrestrial species, threatening long-term biodiversity (Mohammad et al., 2021).

For human health, risks are linked to the consumption of water and food from contaminated areas, emphasizing the need for rigorous monitoring and regulation in regions of intensive agricultural production (Brandão et al., 2024). Recent regulations, such as Brazilian Law No. 14785/2023, aim to limit the use of these metals in agricultural inputs due to ecological and human health risks. Nevertheless, these data reinforce the need for increased oversight and the substitution of these inputs with less impactful alternatives to protect the environment and food webs. Barium, with increases of over 600%, is frequently associated with pesticides containing barium sulphate, while Cd and Pb, with increases of thousands of times relative to control areas, represent the greatest toxicological risks due to their persistence in the environment and high toxicity (Defarge et al., 2018).

Such accumulation in amphibians reflects proportional environmental levels and often follows a synergistic pattern

(notably Pb and Cd), which consequently exacerbates oxidative damage in kidneys and livers, a process further facilitated in aquatic environments (Jofré et al., 2012). In turn, this triggers joint biomolecular responses, including increased superoxide dismutase (SOD) and malondialdehyde (MDA), alterations in glutathione (GSH), and the induction of metallothioneins, thereby intensifying the physiological impacts on growth, metamorphosis, and immunocompetence (Sobrinho-Figueroa et al., 2007; Flora et al., 2012). The Pb–Ba interaction, although less studied in amphibians, deserves attention as Ba, when bioavailable, can disrupt ionic homeostasis and compromise tegumentary/gastric permeability (Aziz et al., 2017), potentially facilitating Pb co-absorption.

Cd, on the other hand, is particularly detrimental to larval development, as it delays metamorphosis, impairs ossification, and alters thyroid function (Sun et al., 2018). Similarly, Cr and Cu negatively affect intestinal physiology, growth, and larval development; when combined, they may exert additive or synergistic effects on mortality and developmental delays (Huang et al., 2020). Accordingly, the Cd–Cr, Cd–Cu, and Cr–Cu correlations observed here suggest not only co-exposure but also an increased risk of disrupted developmental timing, reduced body size at metamorphosis, and histopathological alterations (Yao et al., 2019; Huang et al., 2020).

Zn, although essential, becomes toxic at elevated concentrations, whereas As is a well-established toxic and teratogenic metalloid. Interactions between Zn and As may emerge from competition in ionic transport pathways or from effects on the cutaneous and intestinal microbiome, which can modulate toxicity (Brodeur et al., 2009). Therefore, the detected Zn–As correlations imply co-exposure and the possibility of reciprocal modulation of toxicity, whereby increased Zn levels may alter As availability and flux, or vice versa, with potential consequences for larval survival, growth rates, and skin health (Brodeur et al., 2009; Cordeiro et al., 2024).

5. Conclusion

The results indicate that trace element concentrations differ between the sampled sites, with a significant increase observed for As, Ba, Cd, Cr, Cu, Pb, and Zn, except for Hg. Furthermore, statistical analyses revealed strong positive correlations among the trace elements analyzed, notably between Pb and Ba, Cd, Cr, and Cu, as well as interactions involving Cd–Cr, Cd–Cu, Cr–Cu, and Zn–As. Overall, this study provides evidence for ecological risk assessment and highlights the use of amphibians as sentinel species for monitoring environmental contamination in agroecosystems.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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