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Review of biomonitoring metals in rivers using bullfrog tadpoles: an applicability study of exposed animals to Sorocaba River

Revisão do biomonitoramento de metais em rios utilizando girinos de rã-touro: um estudo de aplicabilidade de animais expostos ao rio Sorocaba

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Abstract: Aim: This study reviewed recent literature on the use of the bullfrog species (*Aquarana catesbeiana*, Shaw, 1802) as an environmental bioindicator of pollution. Additionally, the total concentration of metals in the skin of bullfrog tadpoles exposed to the Sorocaba River was evaluated to explore the potential of this tissue as a biomonitoring tool and assess the bioavailability of metals in the river to aquatic organisms. **Methods:** A bibliographic review was conducted using the Scopus and PubMed platforms with the search terms "*Lithobates catesbeianus* biomarkers," "*Aquarana catesbeiana* biomarkers," and "*Rana catesbeiana* biomarkers." Tadpoles were exposed for 96 hours to water from two points along the Sorocaba River: *Point Ibiúna (PI)*, representing the river's source, and *Point Itupararanga Reservoir (PIR)*, a key water supply location for the Sorocaba-SP region. Deionized, contaminant-free water served as the control. Metal concentrations (Ba, Cu, Mn, Sr, and Zn) in skin samples (n=30) were analyzed after sample digestion using HNO3 and HCl, with determination via Microwave Plasma Atomic Emission Spectrometer (MP-AES). **Results:** A review of the last 10 years of bibliographic production revealed 35 articles, where metals were the second most studied contaminant using this species (approximately 34% of articles), following agricultural pesticides (43%). Metal concentrations of Ba and Zn in the PI group varied compared to the control group. For the PIR group, Mn concentrations varied significantly relative to both the control and PI groups. No significant variation in Cu and Sr concentrations was observed across the groups. **Conclusions:** Existing literature supports the use of various bullfrog tissues as bioindicators of environmental pollution. In this study, Ba concentrations increased by 12% and Mn by 54% in the PIR group compared to the PI group. No differences were observed for Cu, Sr, and Zn across groups. Metals such as As, Cd, Co, Mo, Ni, and Pb were below the quantification limit in all groups. The increased Mn concentration in PIR-exposed tadpoles suggests metal accumulation, pointing to a potential decline in water quality downstream from the river's source.

Keywords: environmental monitoring; metallic pollution; amphibians.

Resumo: Objetivo: Neste estudo foi avaliada a bibliografia recente sobre o uso da espécie de rã-touro *Aquarana catesbeiana* (Shaw, 1802) como bioindicador ambiental e a concentração total de metais na pele de girinos de rã-touro expostos às águas do rio Sorocaba, a fim de avaliar o potencial uso do tecido como ferramenta de biomonitoramento ambiental e a disponibilidade de metais no Rio Sorocaba para os animais. **Métodos:** A bibliografia recente foi avaliada através das plataformas *Scopus* e *Pubmed* utilizando os termos "*Lithobates catesbeianus biomarkers*", "*Aquarana catesbeiana biomarkers*" e "*Rana catesbeiana biomarkers*". Os girinos foram expostos por 96 horas às águas de 2 pontos do Rio Sorocaba, sendo o ponto Ibiúna (PI) representando a nascente do Rio Sorocaba e o ponto Represa de Itupararanga (PIR) representando um importante ponto de abastecimento de água para região da cidade de Sorocaba-SP. Foi utilizada água deionizada livre de contaminantes como controle. A avaliação da concentração dos metais Ba, Cu, Mn, Sr e Zn nas amostras de pele (n=30) foi realizada após digestão das amostras em sistema fechado utilizando HN_3 e HCl e determinação em Espectrômetro de Emissão Atômica por Plasma de Microondas (MP-AES). **Resultados:** A revisão da produção bibliográfica dos últimos 10 anos resultou em 35 artigos sobre o tema, onde os metais foram o segundo contaminante mais estudado utilizando esta espécie (cerca de 34% dos artigos), atrás apenas dos defensivos agrícolas (cerca de 43% dos artigos). A concentração dos metais Ba e Zn no grupo PI variou em relação ao grupo controle, e a concentração de Mn no grupo PIR variou em relação ao grupo controle e em relação ao grupo exposto às águas do grupo PI. Para os metais Cu e Sr não houve variação significativa entre os grupos. **Conclusões:** A bibliografia existente demonstra a capacidade de utilização de diversos tecidos de girinos de rã-touro como bioindicadores ambientais. Foi identificado o aumento de Ba (12%) e Mn (54%) no grupo PIR em relação ao grupo PI na pele dos girinos. Para os metais Cu, Sr e Zn não houve diferença entre os grupos. As, Cd, Co, Mo, Ni e Pb tiveram resultados abaixo do limite de quantificação nos grupos estudados. O aumento da concentração de Mn na pele dos girinos de rã-touro expostos às águas do reservatório de Itupararanga em relação ao grupo exposto às águas de Ibiúna pode indicar o acúmulo deste, apontando para piora na qualidade da água em relação ao ponto inicial do curso do rio.

Palavras-chave: monitoramento ambiental; poluição metálica; anfíbios.

1. Introduction

Freshwater bodies are crucial for sustaining human and animal communities and for supporting the productive processes that drive city operations (Frascarelli et al., 2016). Access to high-quality water is vital for a dignified life and plays a critical role in disease prevention. However, improper discharge of emissions into water bodies without adequate treatment can elevate water toxicity through the accumulation of metals and other pollutants. This contamination harms local fauna and flora, as well as the populations relying on these waters (Wang & Yang, 2016). Biological processes, species, or entire communities can be used as tools to assess environmental quality and track changes over time. This can be achieved by evaluating physical or chemical parameters in animals exposed to these environments or by monitoring shifts in the diversity of local fauna and flora (Holt & Miller, 2011).

Metal pollution in water stems from various human activities, primarily mining and industrial processes. These activities generate waste that, if not properly treated, can have significant environmental impacts (Sakakibara et al., 2011). The presence of metals poses risks to local communities, as metals can accumulate in the organs of animals and biomagnify at higher trophic levels (Holt & Miller, 2011). As described by Zhou et al. (2008), the use of bioindicators depends on the ability to analyze the effects and markers of chemical substances in organisms' tissues. These markers may include reaction products of foreign substances entering the organism, changes in the bioindicator itself, or the presence of the chemical substances within the organism.

Common bioindicators for metal pollution in water include plankton, insects, mollusks, fish, plants, and others (Stibilj et al., 2014; Gagnow & Rawson, 2017). Each bioindicator species or taxon possesses unique characteristics tailored to specific analytical needs. Several techniques exist for using organisms as bioindicators, including monitoring population dynamics in organisms exposed to polluted waters, determining metal concentrations in their tissues, conducting biomarker analyses, and performing histopathological observations (Zhou et al., 2008; Stibilj et al., 2014).

Amphibians are strong candidates as bioindicators of metal pollution due to their unique traits, such as their ability to respire through the skin and their susceptibility to pollutant absorption via epithelial tissue. These characteristics make them suitable for various research approaches, including morphological, behavioral, metabolic analyses, and measuring total metal or pollutant content in tissues (Zhou et al., 2008; Prokic et al., 2016; Fernandes et al., 2021).

Due to their low mobility and the fact that part of the amphibian larval cycle occurs in aquatic environments, these animals are particularly vulnerable to discharges and pollutants that may affect rivers and lakes. Current knowledge about amphibian species from tropical and subtropical regions remains limited, which could hinder the protection of species at greater risk of extinction. As noted by Ocaña-Fernández and Fuster-Guillén (2021), bibliographic review articles serve as valuable tools for assessing existing knowledge on specific topics, identifying problems, uncovering gaps, or answering questions about previously produced scientific content. Such reviews are fundamental for advancing various fields of knowledge.

Consequently, examining studies from the last 10 years on the use of bullfrogs as environmental bioindicators is crucial for evaluating their potential in biomonitoring efforts (Ossana et al., 2017; Fernandes et al., 2021; Vidal et al., 2021).

This study aims to review recent literature on the use of *Lithobates catesbeianus* (syn. *Rana catesbeiana*) as bioindicators of contaminant exposure and to assess the concentration of metals in the skin of bullfrog tadpoles exposed to water from two points along the Sorocaba River over a 96-hour period. The goal is to evaluate the feasibility of using bullfrog skin as a biomonitoring tool for detecting metal contamination in water. This research tests the hypothesis that tadpoles exposed to water from the Itupararanga Reservoir, an important water source for the population of the Sorocaba city region, São Paulo State, Brazil, will show higher concentrations of metals in their skin compared to tadpoles exposed to water collected at the river's source in Ibiúna or to the control group. Such findings would indicate a decline in water quality along the course of the Sorocaba River.

2. Material and Methods

2.1. Literature review

Using the key text search systems of major international scientific article platforms, the available scientific literature from the past 10 years was reviewed. Searches were conducted on PubMed and Scopus using the terms "*Lithobates catesbeianus biomarkers*," "*Aquarana catesbeiana biomarkers*," and "*Rana catesbeiana biomarkers*." The resulting scientific papers were categorized by contaminant, publication year, and country of origin. Articles not related to contaminant exposure in environmental monitoring or ecotoxicology studies were excluded. The searches were performed in 2023 and cover the period from 2013 to 2023.

2.2. Study area

The Sorocaba River is of great importance to the cities of Votorantim and the entire Metropolitan Region of Sorocaba in São Paulo, Brazil. It supplies the Itupararanga Reservoir, which provides water for approximately 1 million residents and serves as an environmental protection area (Oliveira & Amaral, 2022). Spanning over 929 km^2, the Sorocaba River flows through multiple regions where it faces significant anthropogenic impacts, including large agricultural zones and industrial facilities, such as cement and mining operations. These activities contribute to the transport of agricultural chemicals and mining waste into the river via leaching or improper waste disposal (Conceição et al., 2011; Fernandes et al., 2021; Oliveira & Amaral, 2022).

The Sorocaba River is formed by the confluence of the Una, Sorocabuçu, and Sorocamirim Rivers in the cities of Cotia, Vargem Grande Paulista, and São Roque. It then flows into the Itupararanga Reservoir, which is managed by Companhia Brasileira de Alumínio (CBA) (Fernandes et al., 2021; Oliveira & Amaral, 2022).

Water sampling was conducted during the dry season (August 2019), using 50-liter capacity containers to collect a total of 120 liters of water. Samples were obtained from two points along the river. The first sampling site, near the city of Ibiúna (PI sample point) (23°37'27" S, 47°24'10.1" W), represents a location close to the river's source. The second site, the Itupararanga Reservoir (PIR sample point) (23°38'11.3" S, 47°13'22.6" W), was selected due to its strategic role as a major water supply source for the regional population and its distance from the PI sampling point. Sample points are represented in figure 1.

2.3. Animal collection

As described by Fernandes et al. (2021), bullfrog tadpoles (*Rana catesbeiana*, syn. *Lithobates catesbeianus*) were obtained from the Santa Rosa Frog Farm (Santa Barbara d'Oeste, SP, Brazil) (22°46'53.0"S, 47°24'17.7"W). The animals (n=108) at Gosner stage 25 (Gosner, 1960) were kept in 80-liter tanks filled with dechlorinated water for 10 days. During this acclimatization period, conditions included a continuous flow of water (1.2 $L.h^{-1}$), continuous aeration (6 mg $O_2.L^{-1}$), and a 12-hour light/12-hour dark photoperiod. The tadpoles were fed a commercial diet containing 40% protein, and feeding was halted 24 hours prior to exposure to the waters of the Sorocaba River.

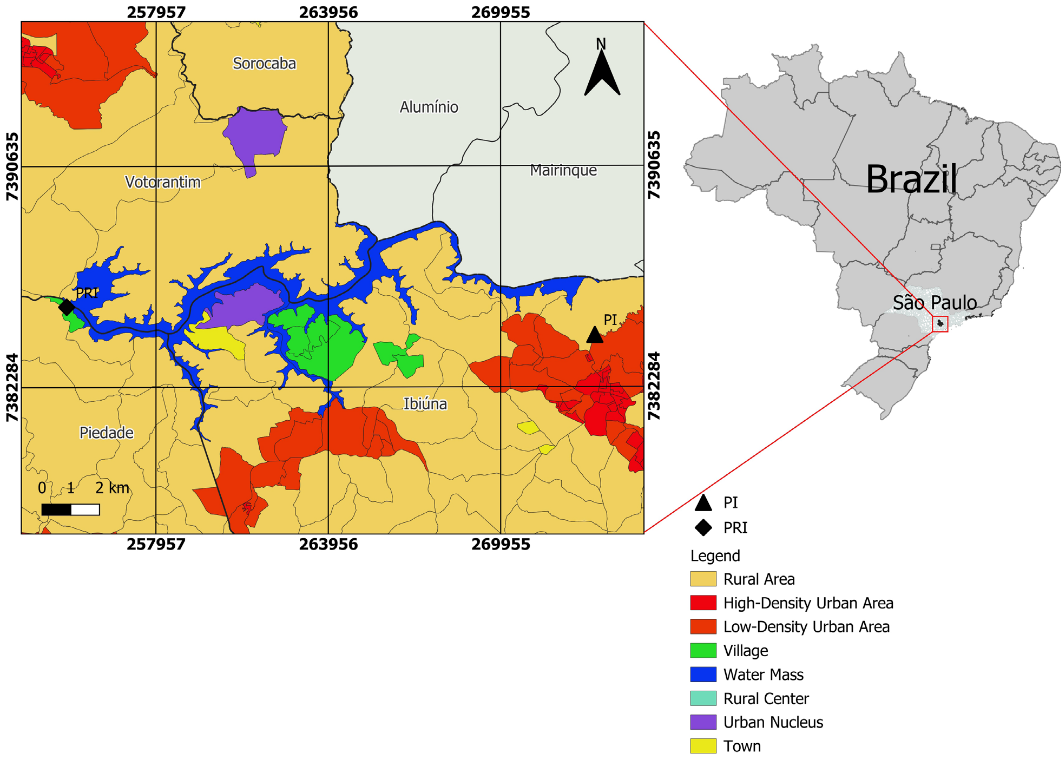

Figure 1. Map of sample points in São Paulo State, Brazil. Ibiúna sample point (PI) (23º37'27" South latitude – 47º24'10.1" West longitude) represented by the triangle marker and Itupararanga Reservoir (PRI) (23º38'11.3" South latitude - 47º13'22.6" West longitude) sample point represented by the diamond shape marker. Datum - SIRGAS 2000/UTM zone 23S (IBGE, 2024).

Source: The authors.

The animals were divided into three groups: a control group (C), a group exposed to water from the city of Ibiúna (PI), and a group exposed to water from the Itupararanga Reservoir (PIR). Twelve animals were randomly assigned to each group and housed in 16-liter aquariums. They were exposed to water under static conditions for 96 hours, with monitored water parameters: pH (7.2–7.6), dissolved oxygen (7.0–7.5 mg.L^{-1}), hardness (50–58 mg $CaCO_3$.L^{-1}), conductivity (56–97 ± 0.02 µS/cm), and ammonia concentration (< 1 mg.L^{-1}). The experiment was conducted in triplicate.

At the conclusion of the exposure period, the animals were anesthetized using 0.1% benzocaine and euthanized via cranial concussion, following the recommendations of the American Veterinary Medical Association (AVMA, 2001). The skins were then separated and stored in a biofreezer at -80ºC until further analysis. All procedures adhered to the standards established by the American Society for Testing and Materials (ASTM, 2000) and were approved by the Ethics Committee of the Federal University of São Carlos (CEUA-UFSCar) under process #2578040219/2019.

2.4. Sample preparation

Arsenic (As), barium (Ba), cadmium (Cd), lead (Pb), cobalt (Co), copper (Cu), nickel (Ni), manganese (Mn), molybdenum (Mo), strontium (Sr), and zinc (Zn) were evaluated in skin samples of bullfrog tadpoles. The samples (n=30) were dried in a laboratory oven at 60°C for 7 days to eliminate moisture and determine total dry mass. This procedure significantly reduced tissue mass due to water loss. Each sample was weighed at 0.15 g (dry mass) in a pool of 10 samples per group (Fernandes et al., 2016; Laurin et al., 2019).

Sample preparation followed the fish tissue digestion procedure recommended by the Anton Paar® Multiwave Pro microwave digestion oven manufacturer. This involved acid digestion in a closed system with the following program: Temp/Power: 650/1400, Ramp: 5/10, Hold: 10/10/15, Fan: 1/1/3. Each sample was digested with 6 ml of 65% nitric acid (HNO_3) and 1 ml of 32% hydrochloric acid (HCl), both of analytical grade. After one hour in the microwave digestion oven, the samples were diluted to 25 ml in a glass volumetric flask using a

5% HNO_3 solution, prepared by mixing 50 ml of HNO_3 with 950 ml of ultrapure water. For the blank, 6 ml of HNO_3 and 1 ml of HCl were subjected to the same digestion process, then diluted to 25 ml with 5% HNO_3 solution in a volumetric flask.

2.5. Total metal concentration analysis

The microwave-induced plasma atomic emission spectrometry (MP-AES) technique was employed using an Agilent MP-AES 4200 instrument equipped with a cyclonic nebulizer. This setup was used to determine the concentrations of As, Ba, Cd, Co, Cu, Pb, Mn, Mo, Ni, Sr, and Zn. The results, initially obtained in $\mu g.ml^{-1}$ and automatically calculated by the Agilent MP Expert Software, were converted to $\mu g.g^{-1}$ to correlate the sample mass with the measured concentrations using the following equation 1.

$$\frac{\left[Sample\ conc. - blank\ average\ conc.\left(\ g\ ml^{-1}\right)\right]\ vollumetric\ flask\ volumn(ml)}{Sample\ mass(g)} \quad (1)$$

The values obtained represent concentrations of metals in dry weight and were expressed as mean ± standard deviation. The results were compared between groups using the Student's T test, with the significance level of $p<0.05$ using *SigmaStat for Windows version 3.5, Copyright© 2006 Systat Software, Inc.*

3. Results and Discussion

3.1. Literature review

The scientific literature from 2013 to 2023 indicates a growing interest in using the species *Rana catesbeiana* as a tool for environmental biomonitoring. Searches on the PubMed and Scopus platforms using the terms "*Lithobates catesbeianus biomarkers*," "*Aquarana catesbeiana biomarkers*," and "*Rana catesbeiana biomarkers*" reveal that bibliographies have been published annually since 2017. A total of 49 works were published in the last decade on topics involving *Aquarana catesbeiana*, but only 34 specifically address the use of this species as biomarkers for environmental monitoring. The number of published papers can be seen in figure 2.

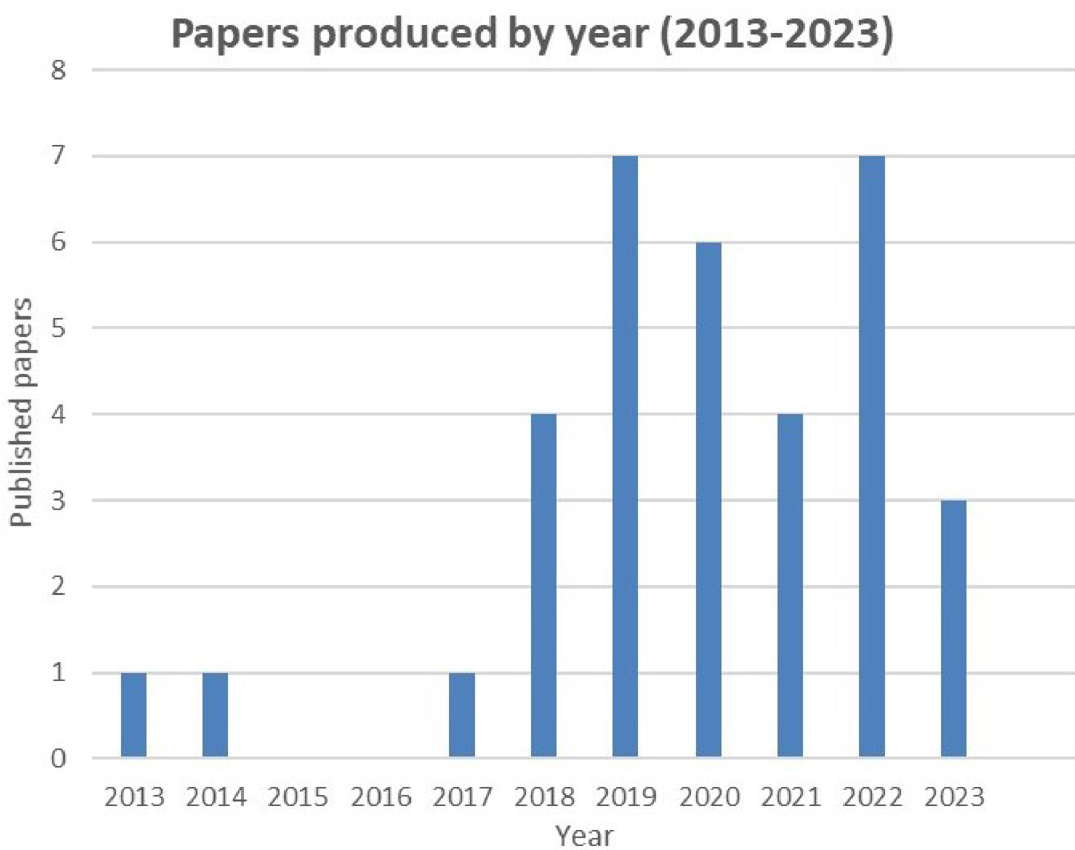

Figure 2. Number of papers published in the last decade with the term "Lithobates catesbeianus biomarkers", "*Aquarana catesbeiana*" and "*Rana catesbeiana* biomarkers" in PubMed and Scopus platforms. Fourty-nine papers were found and 15 papers were excluded from the survey by topic avoidance.

Source: The authors.

Some studies focused on the physiology, characteristics of the species, or veterinary and zoological approaches. However, 15 works were excluded from this survey after critical analysis, as they used the species for purposes beyond the scope of this research. The existing literature on this topic appears to be limited, highlighting both a potential lack of knowledge and opportunities for further exploration.

As can be seen in figure 3, of the 34 studies analyzed on this topic, 2 originated from Argentina (Ossana et al., 2013, 2017), 1 from Canada (Jackman et al., 2018), 1 from Mexico (Pérez-Alvarez et al., 2018), and 30 from Brazil. These findings indicate that the largest body of scientific research worldwide on the use of *Lithobates catesbeianus* tadpoles as tools for aquatic biomonitoring originates in Latin America. This research trend contrasts with most other topics, where the majority of studies are typically produced in countries such as China, the United States, and India. These countries often utilize species like *Rana zenhaiensis* (Wei et al., 2015), *Bufo gargarizans* (Yao et al., 2019; Zhao et al., 2020), *Xenopus laevis* (Yologlu & Ozmen, 2015), and others for amphibian biomonitoring studies.

As shown in figure 4, the majority of scientific articles evaluating biomarkers in bullfrog tadpoles focused on the effects of exposure to agricultural pesticides (42.90%), including herbicides (Dornelles & Oliveira, 2014; Wilkens et al., 2019; Grott et al., 2022), fungicides (Marcantonio et al., 2022), and insecticides (Amaral et al., 2018; Nimet et al., 2021; Santos et al., 2021). Another significant portion of the studies (34.30%) investigated the effects of metal exposure (Fernandes et al., 2021; Vidal et al., 2021; Motta et al., 2023).

Additionally, 17.10% of the studies examined the exposure of tadpoles to pharmaceutical drugs to assess the environmental impact of medicines introduced through treated and untreated sewage networks (Jackman et al., 2018; Amaral et al., 2019; Gregorio et al., 2019). The smallest fraction (5.70%) of the studies focused on the effects of surfactants on aquatic organisms, specifically through the exposure of bullfrog tadpoles to these compounds (Jones-Costa et al., 2018; Scaia et al., 2019).

In all cases, the exposure of tadpoles to contaminants altered the physiological levels of the biomarkers analyzed in the exposed group compared to the control group to some extent, indicating that

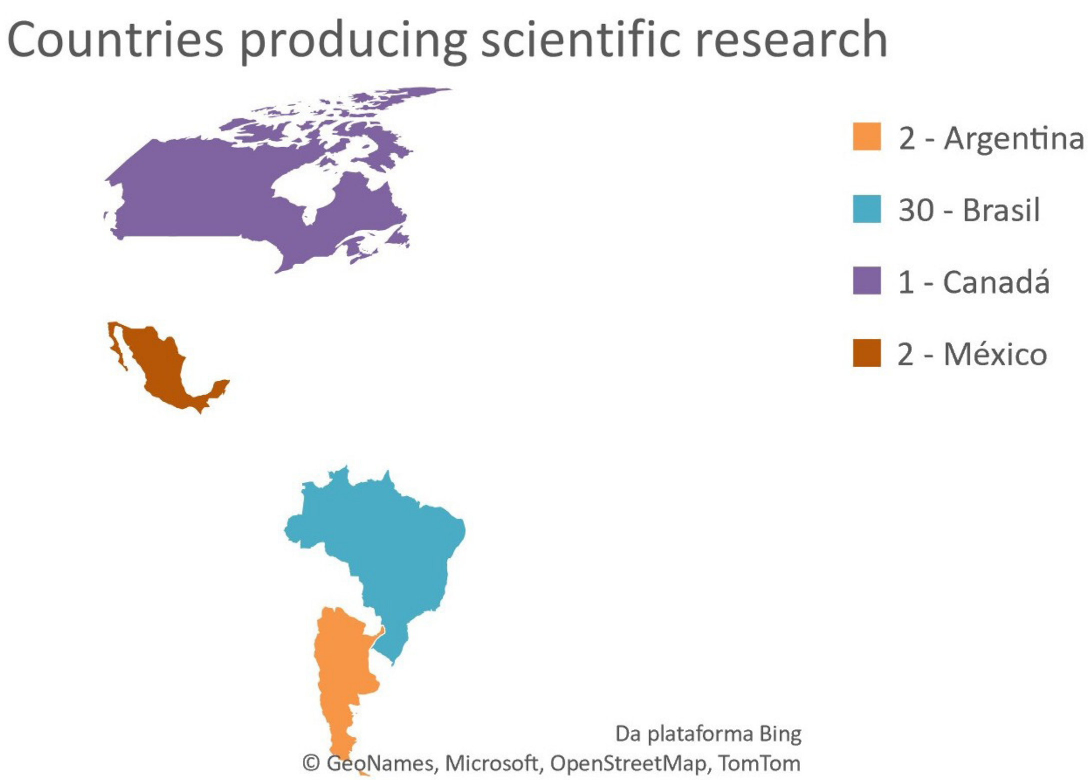

Figure 3. Scheme illustrating countries that had produced scientific papers about the theme "*Aquarana catesbeiana* biomarkers". The ilustration was created in Bing (2024).

Source: The authors.

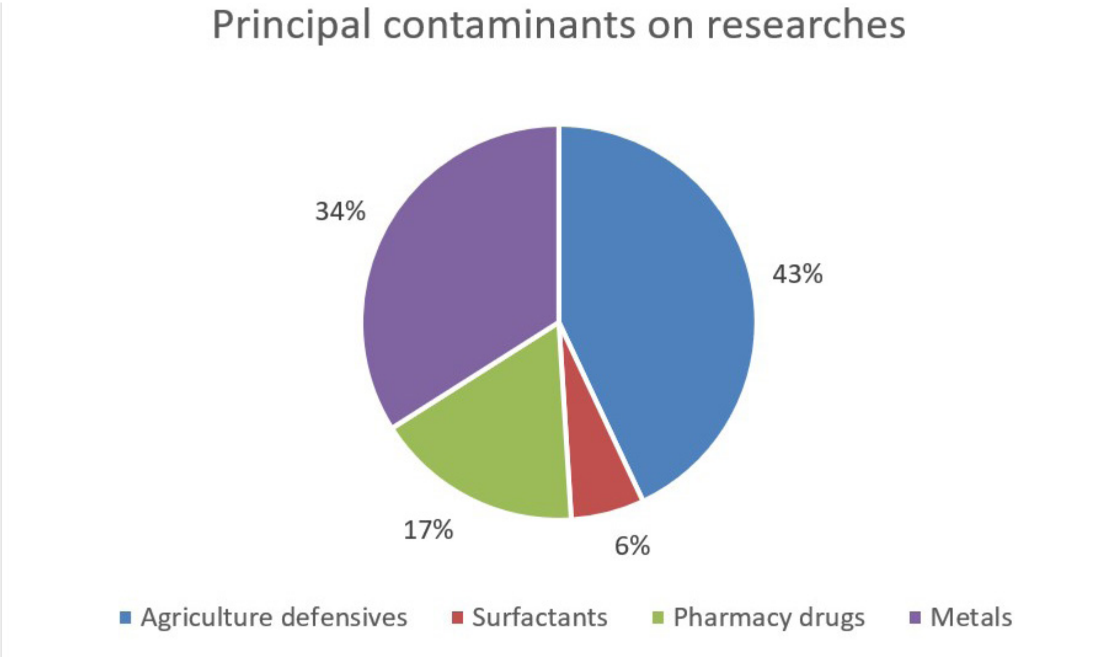

Figure 4. Scheme illustrating the proportions of principal contaminants on researches with *Aquarana catesbeiana* syn. *Lithobates catesbeianus* species.

Source: The authors.

the species can serve as an effective environmental bioindicator. Although no studies have been found that specifically evaluate the skin of this species as a matrix for biomonitoring, there are reports of the successful use of the edible frog (*Pelophylax esculentus*) skin (Prokic et al., 2016) as a tool for monitoring metal pollution in water.

Among the articles analyzing bullfrog tadpoles exposed to metallic contaminants, the liver was the most commonly used tissue for biomarker evaluation, utilized in 44.4% of the studies. This was followed by the kidney (22.2%), caudal muscle (22.2%), brain (5.55%), and assessments of the entire body (5.55%). The majority of the research (66.7%) was conducted by exposing animals to metallic solutions of known concentration. Other studies employed contaminated water from rivers and lakes, exposing the animals and subsequently assessing the effects on biomarkers after a designated period. In 100% of the studies reviewed, exposure to metals induced measurable changes in the biomarkers analyzed.

3.2. Metal concentration in skin

Calibration curves with good linearity were obtained ($r^2 > 0.99$) for all analytes. The detection and quantification limits can be seen in table 1.

The metal concentration for skin samples from bullfrog tadpoles (*Aquarana catesbeiana syn. Lithobates catesbeianus*) can be seen in table 2.

As shown in table 2, skin samples from the PIR group exhibited a manganese (Mn) concentration 54% higher than that of the PI group. The PIR group also had a 12.1% higher barium (Ba) concentration compared to the control group; however, there was no significant difference between the two sample points (PI vs. PIR). Zinc (Zn) concentrations in both the PI and PIR groups were at least 10% lower than those in the control group, a statistically significant reduction. No significant variation in copper (Cu) or strontium (Sr) concentrations was observed between the groups. The concentrations of arsenic (As), cadmium (Cd), cobalt (Co), molybdenum (Mo), nickel (Ni), and lead (Pb) were below the quantification limit of the method, defined at 0.1 mg/L.

Fernandes et al. (2021) detected metals at the start and end of a 96-hour exposure period, during which bullfrog tadpoles were exposed to water from the same points of the Sorocaba River. Their results showed a 67.19% reduction in Mn concentration at PI and an 80.49% reduction at PIR by the end of the exposure, indicating potential metal absorption and accumulation in the exposed animals (Simoncelli et al., 2015; Girotto et al., 2020). Although the PI group showed no significant difference from the control for Mn in skin samples, the observed reduction in waterborne Mn after exposure suggests that metals were absorbed by the animals. Considering the

Table 1. Shows the limit of detection and limit of quantification defined for each element in this study.

Elements	Limit of Detection (LOD)	Limit of Quantification (LOQ)
As	64.35 mg.L^{-1}	214.48 mg.L^{-1}
Ba	0.33 mg.L^{-1}	1.16 mg.L^{-1}
Cd	0.58 mg.L^{-1}	1.93 mg.L^{-1}
Co	0.47 mg.L^{-1}	1.55 mg.L^{-1}
Cu	0.17 mg.L^{-1}	0.53 mg.L^{-1}
Pb	5.67 mg.L^{-1}	185.58 mg.L^{-1}
Mn	0.03 mg.L^{-1}	0.10 mg.L^{-1}
Mo	0.03 mg.L^{-1}	0.13 mg.L^{-1}
Ni	0.57 mg.L^{-1}	1.90 mg.L^{-1}
Sr	0.03 mg.L^{-1}	0.10 mg.L^{-1}
Zn	0.23 mg.L^{-1}	0.78 mg.L^{-1}

Source: The authors.

Table 2. Metal concentration (µg.g^{-1}) in the skin of bullfrog tadpoles.

(µg.g^{-1})	SKIN		
	Control	PI	PIR
Ba	122.27 ± 0.79	133.21 ± 1.13	↑**137.02 ± 0.78** (12.1%)
Cu	2.18 ± 0.08	2.22 ± 0.02	2.25 ± 0.08
Mn	8.73 ± 0.10	8.88 ± 0.08	↑**13.48 ± 0.01*** (54%)
Sr	5.67 ± 0.10	50.33 ± 0.18	53.16 ± 0.34
Zn	574.96 ± 6.21	↓**520.28 ± 1.82** (10.5%)	↓**515.12 ± 4.89** (11.7%)

Note: The results are expressed in the format of mean ± standard error. Group control; PI: Ibiúna sample point. PIR: Itupararanga Reservoir sample point. In bold means difference in relation to the control and * means significant difference in relation to the points. N=10 per group.

Source: The authors.

permeable nature of amphibian skin, Mn could be absorbed and transported to other tissues. Additionally, Veronez et al. (2016) exposed *A. catesbeiana* to iron ore, iron (Fe), and manganese (Mn) during metamorphosis, observing increased iron concentrations in the group exposed to iron ore and Fe, as well as elevated whole-body Mn levels in tadpoles exposed to iron ore and Mn.

The increased concentrations of barium (Ba) and manganese (Mn) in the skin of animals exposed to the waters of the Itupararanga reservoir indicate a deterioration in water quality compared to the starting point of one of the Sorocaba River's sources. This accumulation may induce oxidative stress in these animals. Pérez-Alvarez et al. (2018) and Fernandes et al. (2021) found in their experiments that oxidative stress can occur even when metal concentrations are within the limits established by legislation.

Oxidative stress in organisms can result in alterations in protein expression and concentration, such as metallothionein (Carvalho et al., 2016), as well as changes in enzymes including superoxide dismutase (SOD) (Fernandes et al., 2021), glutathione S-transferase (GST), and catalase (CAT) (Veronez et al., 2016). Other effects include DNA damage, increased micronuclei frequency (Veronez et al., 2016), and changes in behavior and growth (Pérez-Alvarez et al., 2018).

Additionally, studies emphasize the need to monitor the water quality of this reservoir. Martins et al. (2021) evaluated the presence of metals such as Mn and copper (Cu) in the Itupararanga reservoir and linked their occurrence to agricultural and mining activities. Animals inhabiting the region could face greater risks if they are more frequently exposed to these xenobiotics.

In their evaluation of metal concentrations in various tissues of *Pelophylax kl. esculentus*, a frog species widely consumed as food in Europe, Prokic et al. (2016) observed that high metal accumulation can occur even in environments where water metal concentrations are low—below the quantification limits. The liver and skin were identified as the tissues that accumulate the highest concentrations of metals, corroborating our findings.

Variations in metal accumulation across tissues are influenced by the method of exposure, which may occur through dermal absorption or oral ingestion via food (Girotto et al., 2020). The study also highlighted that elevated metal levels in the liver and skin are associated with increased activity in the antioxidant defense system. These molecules collectively protect organelles, cells, and tissues from damage caused by free radicals (Ighodaro & Akinloye, 2018), indicating the utility of these tissues as bioindicators for metal exposure.

This evidence underscores that, even with the physicochemical analyses of water samples from the Itupararanga Reservoir falling within levels permitted by Brazilian legislation, bioaccumulation of metals in aquatic organisms still occurs and can cause oxidative stress. This aligns with Fernandes et al. (2021), who observed oxidative stress through the analysis of metallothioneins and other biomarkers in liver and kidney tissues after 96 hours of exposing bullfrog tadpoles to water samples from the same sites analyzed in this study.

Monitoring metal concentrations in water is vital due to their significant impacts on aquatic organisms and human water usage. For instance, Wei et al. (2015) exposed Zenhai brown frog tadpoles (*Rana zenhaiensis*) to 1/10 of the acute toxicity concentration (LC50) of Cu^{2+}, Pb^{2+}, and Cd^{2+} for 18 days. They found that chronic toxicity caused notable differences between the control and exposed groups, including reduced size, mass, and survival rates, which could severely affect sensitive animal populations.

Exposure to metals such as cadmium (Cd) can elevate reactive oxygen species (ROS) levels, resulting in oxidative stress and impairing cellular and structural function (Simoncelli et al., 2015). The increased metal concentrations in the skin of animals exposed to waters from the Itupararanga Reservoir suggest that this region is subject to greater anthropogenic pressures—whether from industrial, agricultural, or community waste—compared to the Ibiúna source region. This highlights the degradation of water quality along the Sorocaba River, emphasizing the need for consistent monitoring and stronger public conservation policies.

4. Conclusion

Despite relatively low academic production over the last decade, the analyzed studies consistently highlight the success of using bioindicators for environmental monitoring, particularly African bullfrog tadpoles (*Aquarana catesbeiana*), for tracking metals and other xenobiotics in river and lake waters. These organisms are sensitive to environmental changes, enabling the correlation of exposure levels with observed biological responses across various methodologies.

The use of tadpole skin from this species has proven to be an effective tool for characterizing environmental conditions. It has also demonstrated a decline in water quality along the course of the Sorocaba River, with organisms exposed to waters from the PRI site showing significantly higher concentrations of barium (Ba) and manganese (Mn) compared to those from the PI source region. The detection of these metals in reservoir water, coupled with the findings of this research, underscores the urgent need for greater oversight in the area surrounding the Sorocaba River. Farms and factories in the region may be contributing to environmental degradation through the release of effluents and agricultural pesticides into the river.

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Data availability

All research data analyzed in the research is available in *Pubmed*, *Scopus* and *Google Drive* repository. Access is open. It can be accessed in https://www.scopus.com/home.uri (Scopus database); https://pubmed.ncbi.nlm.nih.gov/ (Pubmed database) and https://drive.google.com/drive/folders/1qu-qEE-hl-rdug7ORxYZQ796dOTPMhpb?usp=drive_link (directory of the data collected as results of research).

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