(04-012) - Validation of an aquatic macroinvertebrate as a bioindicator of water toxicity in the Katari Basin, Bolivia.

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The existence of technical and economic limitations for the evaluation of water quality in the Katari Basin in Bolivia generates the need to develop instruments that can contribute to the efficient detection of the degree of water contamination. Bioindicators are key tools since they provide information about the toxicity associated with a body of water in a rapid and integrated manner. This research sought to validate a local aquatic macroinvertebrate as a bioindicator to determine water status in the rivers of the Katari Basin. To validate the macroinvertebrate, bioassays were carried out to determine the sensitivity of these organisms to toxic substances. The bioassays were carried out with local Daphnia longispina as test organisms. The sensitivity determination was based on an experimental design to determine the concentration of toxic compounds representative of the study area that generates a 50% effect on the population of organisms. The main result was the validation of the biological tool to understand and effectively manage water-associated toxicity. The impact of this research is to generate information on the risk of water quality in the Katari Basin to human health and aquatic ecosystems.

Keywords: Bioassays;Daphnia;ecotoxicology;heavy metals

Validación de un macroinvertebrado acuático como bioindicador de toxicidad de agua en la Cuenca Katari, Bolivia.

La existencia de limitaciones técnicas y económicas para la evaluación de la calidad del agua en la Cuenca Katari en Bolivia generan la necesidad de desarrollar instrumentos que puedan contribuir a una detección eficiente del grado de contaminación del agua. Los bioindicadores son herramientas clave, que proporcionan información acerca de la toxicidad asociada a un cuerpo de agua de manera rápida e integrada. Esta investigación buscó validar un macroinvertebrado acuático local como bioindicador para determinar el estado del agua en los ríos de la Cuenca Katari. Para la validación del macroinvertebrado se realizaron bioensayos que determinaron la sensibilidad de estos organismos a sustancias tóxicas. Los bioensayos se efectuaron con Daphnia longispina como organismos de prueba locales. La determinación de la sensibilidad se basó en un diseño experimental para determinar la concentración de compuestos tóxicos representativos del área de estudio que genera 50% de efecto sobre la población de organismos. El resultado principal fue la validación de la herramienta biológica para comprender y gestionar efectivamente la toxicidad asociada al agua. El impacto de esta investigación es generar información sobre el riesgo de la calidad del agua en la Cuenca Katari para la salud humana y a los ecosistemas acuáticos.

Palabras clave: Bioensayos;Daphnia;ecotoxicología;metales pesados

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1. Introduction

Anthropic activities generate an excessive amount of toxicological and microbiological pollutants that significantly affect aquatic ecosystems and human wealth. Due to these activities, water pollution and quality are a critical issue, especially in urban and peri-urban areas. This problem has been perceived and increased during the last decades, and today it affects the life quality of millions of people worldwide (Guadarrama et al., 2016; Martínez & Villalejo, 2018). Because of these problems, using alive organisms in bioassays to evaluate water toxicity is an increasingly widespread practice. A variety of living organisms have been used to detect the presence and assess the impact of chemical contaminants in water bodies (Wang & Wang, 2024).

Examples of bioindicators used can be found in different investigations carried out in different regions.The daphnia genus is one of the most widely used bioindicators in bioassays. The ecotoxicological effects of the insecticide noenicotinoid acetamiprid (Argelia) and ketoconazole (Brazil) were evaluated using these organisms (Achiles do Prado et al., 2021; Getahun, Mengistou & Sitotaw, 2023). Also, the ecological risk for some important rivers as the Aveiro (Portugal), the Tama and Shibukuri (Japan), and the sediments of the Suches River on the Peru-Bolivia border were evaluated as many others around the world (Mamani, Biamont & Calsin, 2021; Rocha & Rocha, 2023; Suwa, Takahashi & Horie, 2022). On the other hand, the toxicological threat was assessed in some industrial and agricultural effluents as the Modjo tannery (Ethiopia), the effluents from an aquaculture farm (Iran), and the risk of toxic metals in industrial wastewater (South Korea) (Gacem et al., 2022; Park et al., 2023; Rasti et al., 2020). Furthermore, some countries already use *Daphnias* as bioindicators in their monitoring processes. In Canada, acute and chronic toxicity was evaluated using *Daphnia magna* towards exposure to natural oil sands deposits (Cardoso et al., 2020) and freshwater biomonitoring in the Parma district (Italy) (Pellegri, Gorbi & Buschini, 2019)

The Katari River Basin in Bolivia is one of the areas with the highest population growth and economic activity in the region. Due to the ineffective waste management, the main waterbodies within the basin are the receptors of solid wastes, and domestic, industrial, and agricultural wastewater, that flow into Lake Titicaca. This pollution of the natural water in the river basin is directly linked to public health problems and severely damaged aquatic ecosystems (Molina et al., 2017; Revilla, 2021).

The few and unadapted monitoring campaigns of water pollution in the study area do not allow risk assessment in the area. The early detection of contaminants and the evaluation of their impact on the aquatic ecosystem and human health are needed (Revilla, 2021). In this context, aquatic bioindicators are an alternative and complement to the evaluation and monitoring of water toxicity. The use of Aquatic Macroinvertebrates (AMs) as water toxicity test organisms offers significant advantages in water quality management strategies. AMs provide a quick integrated biological response to water toxicity considering mono and multiple contaminant events. Moreover, regarding the socioeconomic situation of the area, this biological tool is a more cost-effective option compared to traditional physicochemical and microbiological methods (Prat & Munné, 2014; Vásquez et al., 2006).

This study proposes the use of a local AM as a test organism to evaluate the water toxicity level in the Katari River Basin. For this purpose, the capture, breeding, and laboratory adaptation of local macroinvertebrate species was needed. Then, the local AM was exposed to different metals representative of the main economic activities in the study area. The evaluation of the organism’s response to different ranges of pollutant concentrations allowed

to determine the mean lethal concentration (LC50). Thus, the sensitivity of the MA to the metal exposure was linked to the toxic response level.

2. Goal

Validate a local aquatic macroinvertebrate as a toxicity bioindicator in the Katari River Basin through the evaluation of its sensitivity to metal exposure.

3. Methods

This section is divided into two parts, the description of the study area and the methodology used.

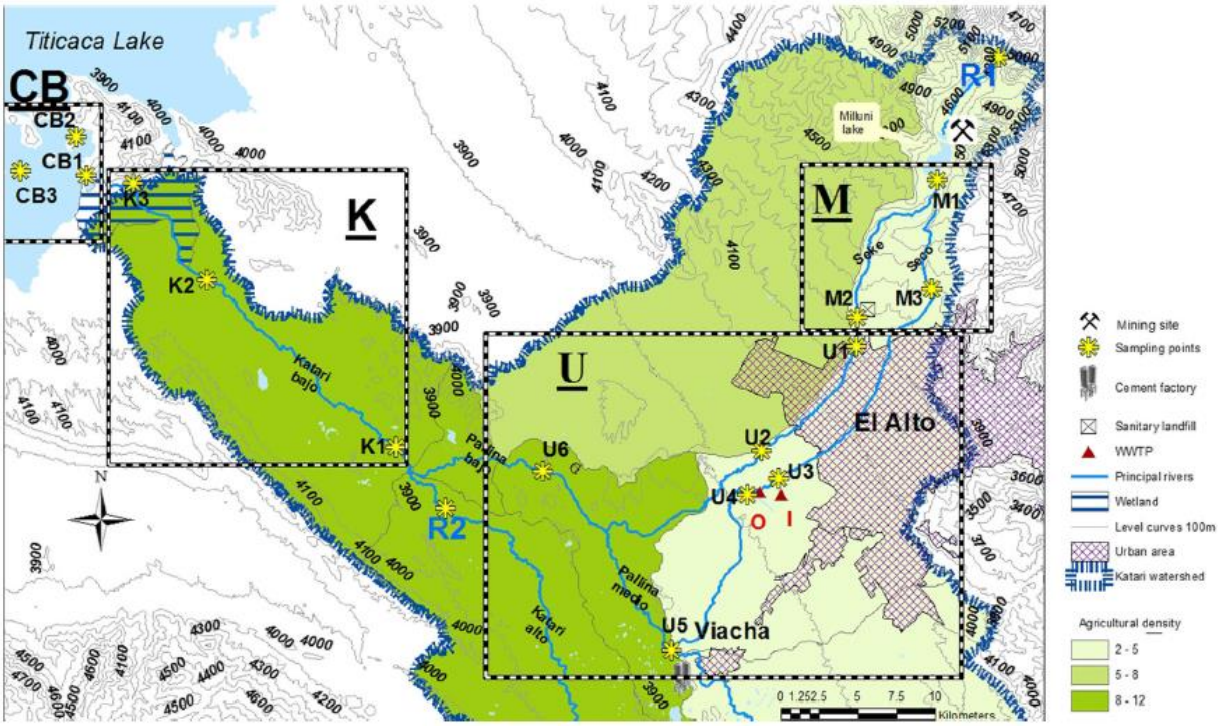
3.1. Study area

The Katari River basin is located on southeast of Lake Titicaca in the Department of La Paz, Bolivia. It extends from the snow-capped Huayna Potosí mountain, goes down through the Altiplano, and discharges its waters into Cohana Bay, which is Lake Titicaca. The watershed area has approximately 7,882 km^2, ranging from 5,200 m.a.s.l. to 3,800 m.a.s.l. It is located between latitudes 16°2' and 17°3' S, and longitudes 68°3' and 68°7' W (Ede & Fuentes, 2019; Ureña et al., 2018).

The water basin is made up of 7 provinces and 24 municipalities in the Department of La Paz. It represents approximately 11% of the Bolivian population (1,136,097 inhabitants in 2017). It is considered one of the most inhabited, pressured, and polluted water basins in Bolivia (MMAyA & VRHR., 2021). This area is also characterized by the largest urban centers of Bolivia, concentrated in the municipality of El Alto, Viacha, and around Titicaca Lake. Thus, the superficial water bodies that flow through these urban centers are poor-quality waters due to the pollution contribution linked to growing urbanization and the different economic activities of the region (Ede & Fuentes, 2019; MMAyA, 2014).

Thus, according to the anthropic influence, the basin is subdivided into 4 zones which are presented in Figure 1: Sector M: Mining area with environmental liabilities; Sector U: Great anthropic influence in the area (urban and industrial wastewater), Sector K and Sector CB: Areas with main agricultural influence (Archundia et al., 2017; Molina et al., 2017).

Figure 1: Zoning of the type of anthropogenic influence in the Katari River Basin



Source: Archundia et al., 2017.

3.2. **Methodology**

3.2.1. **Capture of local macroinvertebrates**

Following the recommendations of Darrigan et al. (2007), the local AM capture was carried out on the morning of 26 September 2023 on the shores of the Lake of Titicaca in the Cascachi local community. A plastic container with a rope was used to obtain deep samples at least 1 meter away from the shore. Next, 1-liter glass flasks were rinsed with water from the lake and filled to half their capacity to receive the captured AM. The organisms were carried out with Pasteur pipettes from the plastic container.

3.2.2. **Adaptation of macroinvertebrates to uncontaminated environments**

The recommendations of the United States Environmental Protection Agency [USEPA] (2002) were considered for the adaptation of AM to an uncontaminated environment. After the capture of the macroinvertebrates, the glass containers were transported to the Bolivian Catholic University [UCB] lab facilities in La Paz. The containers were stored in laboratory temperature and light conditions for 24 hours. The following day, 50% of the lake water in the glass flasks were diluted with distilled water. Finally, after 24 hours in diluted natural conditions, all

organisms in the flasks were transferred to 1-liter beakers with 100% distilled water enriched with Spirulina powder.

3.2.3. **Macroinvertebrate identification**

The identification of the captured MA was made through anatomically detailed observations with TetraView LCD digital biological microscope, which provided magnifications of 40X, 100X, and 200X. Subsequently, images of the organisms were taken through a function on the microscope. The images obtained were the full body and the most relevant parts of the organism for identification.

The taxonomic identification of the AMs was based on the following guides "Introduction to aquatic macroinvertebrate groups" (Hanson, Springer & Ramírez, 2010), "The Genus *Daphnia*" (Benzie, 2002), "Identification of the *Daphnia* species (*Crustacea: Cladocera*) in the lakes of the Ob and Yenisei River basins: morphological and molecular phylogenetic approaches" (Zuykova, Bochkarev & Katokhin, 2013). The iNaturalist platform (https://www.inaturalist.org/) was also used to identify the AM. These resources vary in complexity and range from family and genus-level identification to species-level identification.

To carry out the taxonomic characterization and species determination of the organisms, the captured images were compared with the illustrations and descriptions detailed in the reference guides. A morphological and morphometric comparison was performed. On the other hand, the iNaturalist platform was also used to have greater precision and another point of view regarding the identification of the AM.

3.2.4. **Macroinvertebrates breeding and preserving**

To obtain a stable and homogeneous population of organisms until at least the fifth generation, the review of different protocols for the culturing of macroinvertebrates was done. However, due to the conditions and resources of the university, a laboratory preservation protocol was adapted to local conditions. The adapted protocol considered optimal conditions of temperature, pH, dissolved oxygen, and feeding. These parameters were fundamental to guarantee successful survival and reproduction rates. Details of the protocol are shown in Table 1.

Table 1: Breeding and growing protocol

Temperature	19 ± 2°C
Light quality	Natural light according to the environmental conditions of the municipality of La Paz between October and March.
Photoperiod	Natural light, approximately 12 hours light and 12 hours dark.
Oxygenation	Every Monday, Wednesday, and Friday the water in the containers should be oxygenated with a fish tank oxygenator for 5 minutes.
Growing recipients	Fleas should be cultured in beakers depending on their age. Adults in 500 mL and 1L beakers, juveniles in 300 mL beakers and neonates in 150 mL beakers. Likewise, so that there is no contamination of the environment that could harm the organisms, the beakers should be covered almost entirely with Parafilm paper, leaving small openings for ventilation.
Feeding	3 g of spirulina should be prepared in 500 mL of distilled water and stored at room temperature. For the feeding of the AMs, this preparation was applied in the form of drops in the containers containing them, varying between 5 and 10 drops according to the number of individuals present.
Reconstituted hard water	The salts should be added to the beakers where the AMs are incubated in 1/5 of their capacity.
Cleaning	Every other day (Monday, Wednesday, and Friday) the water in the beakers should be changed by using a sponge and rinsing with plenty of water. Also, during these days the molts and remains of the AMs should be removed from the bottom of the beaker.
Newborn collection	The neonates should be separated from the adults daily with a Pasteur pipette and introduced into a new container (150 mL) with its respective label and culture medium.

Note: The salts used to reconstitute hard water were calcium chloride dihydrate, magnesium sulfate heptahydrate, sodium bicarbonate, and potassium chloride (Díaz Baez et al., 2008).

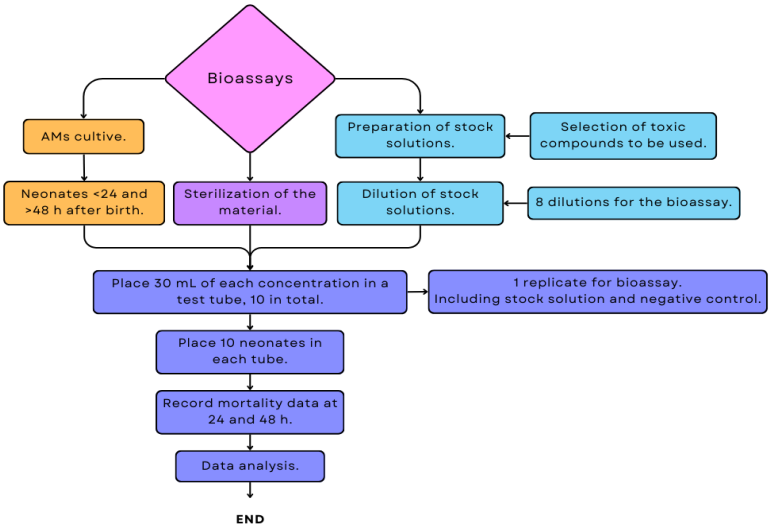
Source: Own elaboration based on Díaz et al., 2008 and ISO 6341.

3.2.5. Bioassay

To determine the LC50 of the metals, the local AM organisms were exposed to a wide range of concentrations, an existing experimental design was implemented. The adaptation of the bioassay protocol proposed in the "Acute toxicity test with *cladocerans* of the family

Daphnidae" (Martínez, 2008), was needed due to laboratory conditions. The procedure followed is presented in Figure 2.

Figure 2: Experimental design flowchart



Source: Own elaboration, 2024.

Initially, the presence, relevance, and bioavailability of the toxic compounds present in the natural water of the area were compared. Lead (Pb), aluminum (Al), and Hexavalent chromium (Cr(VI)) were selected. Stock solutions of the 3 metals were prepared from lead nitrate, aluminum sulfate, and potassium dichromate. Potassium dichromate is a toxic reference compound commonly used in toxicity bioassays (ISO 6341:2012; USEPA, 2002).

All solutions were prepared with distilled water in 250 mL volumetric flasks and subsequently diluted to 12 further concentrations, as shown in Table 2. Negative controls were also considered, which only consisted in distilled water, mineral salts, and food, representing the culture medium.

Table 2: Concentrations of the pilot tests with toxic compounds

	Pb mg/L	Al mg/L	Cr mg/L
1	10	10	10
2	5	5	5
3	1	1	1
4	0.5	0.5	0.5
5	0.1	0.1	0.1
6	0.05	0.05	0.05
7	0.01	0.01	0.01
8	0.005	0.005	0.005
9	0.001	0.001	0.001
10	0.0005	0.0005	0.0005
11	0.0001	0.0001	0.0001
12	0.00005	0.00005	0.00005

10 neonates from the fifth generation (<24 h to <48 h old) of local AM were exposed to 30 mL of prepared metal solutions (Figure 3). The incubation period was 48 h, and the mortality of the individuals was recorded at 24 h and 48 h.

Figure 3: Assembly of the tubes in the bioassay of Al.

To analyze the data obtained from the bioassays and determine the LC50, the drc (analysis of dose-response curves) package was used in the R studio statistical programming environment (Ritz & Strebig, 2022). This package provides tools for fitting regression models to dose-response data. Specifically, the drm function fit a log-logistic model to the experimental data. This model allows to estimate the mean lethal concentration (LC50) of the metals evaluated.

Once the model was fitted, dose-effect curves were generated, which show the relationship between the concentration of the toxic compound and the biological response (in this case, mortality). These curves were plotted using the plot function, which allows the relationship between the heavy metal dose and its effect on the model organisms to be visualized graphically. This approach makes it possible to understand the toxicity of the contaminants better and to determine their level of danger at specific concentrations.

4. Results and discussion

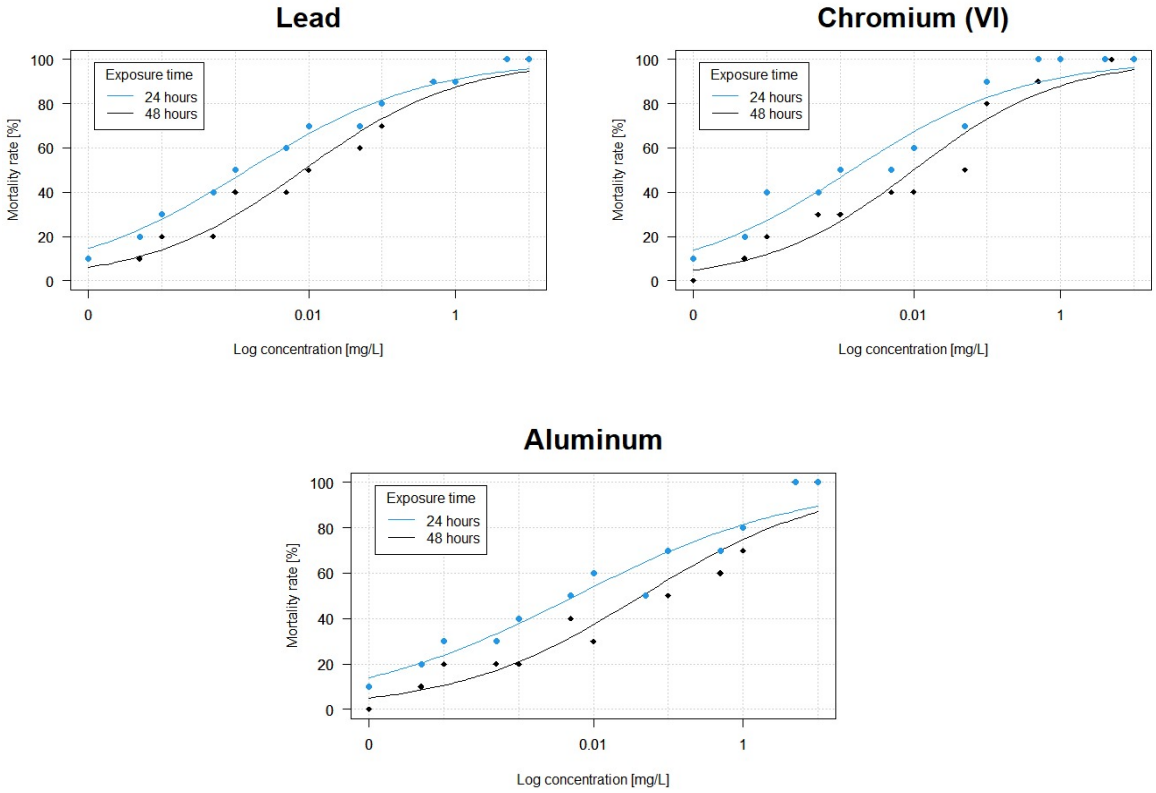
4.1. Macroinvertebrate identification

The identification guide of Hanson, Springer & Ramírez (2010) allowed the organisms to be classified in the phylum *Arthropoda*, subphylum *Crustacea*, and finally to the taxonomic level of "class", where they were identified as *Cladocerans*. Moreover, with morphological comparisons of the organisms with the papers of Benzie (2005) and Zuykova, Bochkarev & Katokhin (2013), it was possible to identify the *cladoceran* as *Daphnia longispina*. Likewise, also employing the "iNaturalist" platform, the identification of the macroinvertebrate was matched. By uploading the images of the organisms (provided by the biological microscope), the application facilitated a second identification, which matched the one previously identified.

4.2. Bioassay data analysis

Each bioassay was carried out with only one replicate and using 12 different concentrations of the selected pollutants and one negative control. Figure 4 below shows the Dosis-Effect curves generated from the bioassay results. These graphical representations illustrate the relationship between the increase in mortality rate and the concentrations of the metals evaluated. Despite the differences in the concentrations of the toxic contaminants and toxic compounds used, the preliminary results reveal a consistent and homogeneous response.

Figure 4: Dosis-effect curves of the metals



The response of the organisms exposed to the 3 metals is a sigmoid curve. These curves reflect the different stages of toxic dynamics (de Santana & Rebello, 2020). First, a phase is observed where the exposure generates few effects on the organisms, which corresponds to a physiological compensation of the effects. Only the most sensitive organisms are affected by the treatment. Afterwards, there is the "suffering" phase where mortality increases linearly with the concentration of the metal. At this stage, most organisms are affected by the treatment, and the LC50 is found. Finally, a saturation of the response is reached where the response decreases depending on the increase in concentration. The most resistant organisms are those that survive. for the 3 metals, the toxic response is greater with prolonged exposure (48 hours). It is observed that the slope of the suffering phase is greater in the case of 24-h exposure. This reflects the acute toxicity of the metals. In the case of the 48h exposure, the slope is lower, and the effects are observed at lower concentrations, which is typical of the behavior of the response to chronic exposures (Gao et al., 2016; Sánchez-Bayo, van den Brick & Mann, 2011).

Table 3 shows the different LC50 values obtained for 24-h and 48-h exposures of the different toxic compounds used.

Table 3: LC50 values of metals in 24-h and 48-h exposure treatments

Toxic compound	Incubation time [h]	LC50 [mg/L]	Standard error
Cr(VI)	24	$9.96*10^{-3}$	$\pm 3.55*10^{-3}$
	48	$1.42*10^{-3}$	$\pm 5.76*10^{-3}$
Al	24	$4.44*10^{-2}$	$\pm 1.75*10^{-2}$
	48	$5.70*10^{-3}$	$\pm 2.49*10^{-3}$
Pb	24	$8.45*10^{-3}$	$\pm 3.32*10^{-3}$
	48	$1.46*10^{-3}$	$\pm 3.29*10^{-4}$

Sourse: Own elaboration, 2024.

In the case of the bioassays performed for Cr(VI), the value obtained for LC50-24 h is $9,96*10^{-3}$ mg/L, and for LC50-48 h is $1.42*10^{-3}$ mg/L. Other studies Martínez, Rodríguez & Martínez (2008), Núñez & Hurtado (2005), Silva et al., (2003) find higher LC50 values at 24 h, $8.02*10^{-2}$ mg/L; $4.04*10^{-1}$ mg/L; $1.45*10^{-1}$ mg/L, respectively. These variations may be due to several reasons, one of them being the use of other daphnia species: *Daphnia exilis, Daphnia magna* Straus, and *Daphnia pulex*, and the variety of regions and conditions in which the studies were carried out.

Al, on the other hand, is not considered a toxic reference compound, so its use in bioassays is limited. However, the value obtained for LC50-24 h is $4.44*10^{-2}$ mg/L and the one for LC50-48 h is $5.70*10^{-3}$ mg/L. These values obtained differ greatly from those obtained using *D. magna* by Satizábal, Andrade y Zúñiga (1999), where the LC50-48 is 10.85 mg/L. However, Satizábal, Andrade y Zúñiga (1999) y Vorobieva et al. (2021) indicate that the LC50 values for Al vary due to the hardness or the hydrochemical composition of the water used in the preparation of solutions and considers that Al is more toxic in soft water than in moderately hard or hard water. In this case, the presence of hard water in the bioassay is the reason for the higher sensitivity. On the other hand, Quiroz, Sigee & White (2010) indicate that a neutral pH also influences the solubility of this metal in water, resulting in less toxicity at neutral pH. These parameters should be considered in future bioassays.

Although Pb is not a toxic reference compound, it is widely used in toxicity bioassays. The value obtained for LC50-24 h is $8.45*10^{-3}$ mg/L and the one for LC50-48 h is $1.46*10^{-3}$ mg/L. Like the previous metals evaluated, these present differences with other bioassays performed. Arambasic, Bjelic & Subakov (1998), using *D. magna*, determined a LC50-48 h of $2.68*10^{-1}$ mg/L, Cooper, Bidwell & Kumar (2009) with *D. carinata* found a LC50-48 h of 444 mg/L and, using *D. magna* too. Offem & Ayotunde (2008) for 24 h and 48 h determined, 2.51 and 1.88 mg/L, respectively. Likewise, Araujo et al. (2019) indicate that the effects of Pb are more long-term, where the organisms used present malformations and reproductive problems.

The LC50 obtained for the three metals evaluated (Pb, Cr, and Al) reveals significant differences both in absolute values and in the relative sensitivity of the organisms to each metal. Pb exhibits higher toxicity compared to Cr and Al, with LC50-24 h values of $8.45*10^{-3}$ mg/L and LC50-48 h of $1.46*10^{-3}$ mg/L, respectively. In contrast, Cr shows lower toxicity

compared to Pb, with LC50 - 24 h values of $4.44*10^{-2}$ mg/L and LC50 - 48 h of $5.7*10^{-3}$ mg/L. Finally, Al shows even lower toxicity compared to Pb and Cr, with LC50-24 h values of $9.96*10^{-3}$ mg/L and LC50 - 48 h of $1.46*10^{-3}$ mg/L, respectively. For the case study, the *daphnias* tested proved to be more sensitive to Pb and less sensitive to Al.

Variations of the LC50 results regarding the literature can be attributed to different factors as discussed in the previous paragraphs. Two of the main causes identified by Martínez, Rodríguez and Martínez (2008) are the effect of environmental temperature and the genetic characteristics of the test organisms. In the study by Martínez, Rodríguez & Martínez (2008), bioassays were performed at different temperatures and with different organisms under the same conditions. It was determined that the lower the temperature in the environment, the more sensitive the test organism is. Additionally, under the same bioassay conditions, different sensitivities of the organisms may occur due to their genetic differences. At the same time, the existence of variability in the sensitivities of the organisms is due to changing environmental conditions of the place where the bioassays are performed, the exposure conditions, and the methodology used (Jensen et al., 2022; Melnikov & de Freitas, 2011).

The results of the bioassays were evaluated with the permissible limits established by Bolivian regulations. According to the regulations, the limits allowed in bodies of water are 0.05 mg/L for Cr(VI), 0.1 mg/L for Pb, and 1 mg/L for Al. The high sensitivity obtained in the bioassays indicates that these *Daphnia longispina* strains would be useful for evaluating effluent samples with contamination of the metals under study since the LC50 values are well below the permissible limits. It is important to note that the evaluation of water quality should not be limited only to not exceeding the permissible limits established by regulations but should also consider the cumulative effects of multiple contaminants and the interaction between them. Therefore, the results of the bioassays must be interpreted in an integrated manner, considering both the limit values and other factors relevant to water management.

The findings of this study support the importance of continuing with the evaluation of the sensitivity of the AM organisms through additional bioassays, using a more complete experimental design, including multiple replicates and a better-established range of concentrations. Likewise, the experimental strategy implemented allowed to obtain a more specific range of concentrations, which facilitated an accurate evaluation of the sensitivity of the organisms to different toxic substances present in the Katari River basin.

5. Conclusions

In order to become using alive organisms as bioindicators as in other regions of the word, the results obtained in this study reflect a significant step towards the validation of a local aquatic macroinvertebrate, *Daphnia longispina*, as a bioindicator of water quality in the Katari River Basin. The determination of the LC50 of various toxic compounds represents a crucial advance in the evaluation of the sensitivity of these organisms to toxic pollutants such as Cr(VI), Pb, and Al. The data obtained indicate a consistent response of the organisms tested across the different concentrations evaluated, suggesting the potential feasibility of using these bioindicators in future water toxicity studies in the study area. This approach provides an effective and locally adapted tool for monitoring water quality in the Katari River Basin, thus contributing to the protection of human health and the preservation of aquatic ecosystems in the region.

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