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Evaluation of the toxicity of microplastic-like particles extracted from the Rocha River on planarians as bioindicator organisms

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Due to deficient liquid and solid waste management, the Rocha River (Cochabamba, Bolivia) suffers from alarming environmental degradation, characterized by high concentrations of microplastic-like particles in both water and sediments. This situation represents a potential threat to aquatic fauna that must be analyzed by determining the effects of these pollutants within the study area. In the first stage, microparticles from river water samples were extracted and characterized using standard microplastic techniques. These isolated particles were subsequently used in toxicological assays with the planarians *Dugesia* sp. and *Schmidtea mediterranea*. Two exposure concentrations were utilized, representing conditions during the wet and dry seasons. After 7 days of exposure, fragments and fibers (primarily smaller than 100 µm) were found within the tissues of the exposed planarians. In certain treatments, exposure was associated with behavioral alterations, cutaneous depigmentation, a significant delay in blastema formation, or a reduction in survival observed in the higher concentration groups. In conclusion, microplastics extracted from specific areas of the Rocha River possess toxic potential, and their presence in the ecosystem represents an ecotoxicological risk to living organisms within the study region.

Keywords: Microplastics; Planarians; Ecotoxicology; Rocha river; Regeneration; Microplastic-like particles

Evaluación de la toxicidad de partículas microplastic-like extraídas del río Rocha en planarias como organismos bioindicadores

Debido a la mala gestión de residuos líquidos y sólidos, el río Rocha (Cochabamba, Bolivia) sufre una degradación ambiental preocupante, con altas concentraciones de partículas microplastic-like en el agua y los sedimentos. Esta situación representa una potencial amenaza para la fauna acuática que debe ser analizada determinando los efectos de estos contaminantes en la zona de estudio. En una primera etapa, las micropartículas de las muestras de agua del río fueron extraídas y caracterizadas con técnicas usuales para microplásticos. Estas partículas aisladas fueron después utilizadas en pruebas toxicológicas con planarias *Dugesia* sp. y *Schmidtea mediterranea*. Se utilizaron 2 concentraciones de exposición representando las condiciones en época húmeda y seca. Tras 7 días de exposición, se encontraron fragmentos y fibras (principalmente menores a 100 µm) en el tejido de las planarias expuestas. En algunos tratamientos, la exposición fue asociada a alteraciones comportamentales, despigmentación cutánea, un retraso significativo en la formación del blastema o una reducción de la supervivencia observada en los tratamientos de concentraciones más altas. En conclusión, los microplásticos extraídos de ciertas zonas del río Rocha poseen un potencial tóxico y su presencia en el ecosistema representa un riesgo ecotoxicológico para los organismos vivos en la zona de estudio.

Palabras clave: Microplásticos; Planarias; Ecotoxicología; Río Rocha; Regeneración; Partículas microplastic-like

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1. Introduction

Since 2020, global annual plastic production has exceeded 380 Tg, with a continuous increase reaching 413.8 Tg in 2023 (Plastics Europe, 2024). The increase in plastic waste is directly proportional to the growth in its production (Fu et al., 2020), leading to an environmental problem due to its mismanagement and difficulty in decomposing. Year after year, more than 11 Tg of plastic end up in the oceans (United Nations Environment Programme (UNEP), 2022). Once in the environment, plastics progressively fragment due to environmental factors. This process generates microplastics (MP), which comprise particles ranging from 1 μm to 5 mm in size, with varying dimensions, shapes, colors, and densities. Primary MP (industrially manufactured as microspheres in cosmetics and cleaning products) are differentiated from secondary MP, which result from the degradation of larger plastics in the environment (Cole et al., 2011; Thompson et al., 2004). Secondary sources constitute the largest proportion of MP in the environment and are generated through the degradation and fragmentation of macroplastics from packaging and synthetic textiles via physical, chemical, and biological weathering processes (Brahney et al., 2021; Kye et al., 2023).

These pollutants represent a threat because they are continuously generated in the environment and easily dispersed in aquatic and terrestrial ecosystems. These characteristics allow MP to interact with a wide variety of aquatic organisms, as they can enter water bodies directly as manufactured particles or indirectly through the fragmentation of larger plastics by processes such as ultraviolet (UV) radiation, abrasion, the action of microorganisms, and water dynamics. MP have been recognized by the United Nations as emerging pollutants due to their persistence in aquatic environments (Bhardwaj et al., 2024; Frank et al., 2022; Gao et al., 2024; Tan et al., 2023).

The Rocha River, located in Cochabamba, Bolivia, originates from the Maylanco River in Sacaba and exhibits severe environmental degradation attributed to anthropogenic pressure (Servicio Departamental de Cuencas (SDC) Dirección de Planificación y Gestión Integral del Agua (DGIA).2014). The river's course flows through urban, industrial, and agricultural areas, receiving discharges of domestic wastewater, contributions from the Tamborada River, and pollutants from clandestine activities such as car washing, unplanned urban growth, and agricultural runoff containing pesticides and fertilizers (Toledo & Amurrio, 2006).

This work is part of the monitoring and diagnostic phase of environmental engineering projects, providing low-cost biological indicators for decision-making in watershed management. In the context of integrated management of Maylanco watershed, it is pertinent to evaluate the ecotoxicological risk associated to the presence of MP particles in urban water bodies such as the Rocha River, where studies have shown exceptionally high concentrations of MP in both water and sediments (Linerá Canedo, 2021).

Given this scenario, it is necessary to evaluate the potential effects of the aforementioned particles on aquatic organisms. Planarians are used as model organisms in this ecotoxicology study due to their high sensitivity to pollutants, which makes them early bioindicators of environmental stress. Furthermore, their regenerative capacity, supported by pluripotent neoblasts (Cebrià, 2007), makes them valuable. They also possess a nervous system with molecular homology to vertebrates, useful for studying neurotoxicity and behavioral alterations, and their permeable epidermis facilitates the absorption of pollutants (Buttarelli et al., 2008).

2. Objectives

The main objective of this research is to evaluate the toxic potential of microplastic-like (MP-like) particles extracted from different points in the Rocha River on the development of planarians in order to classify the ecotoxicological risk linked to MP-like particles in the different areas. To achieve this objective, the following specific objectives were established: (a) To characterize the MP-like particles in terms of concentration, size, and shape at the different sampling points in the Rocha River; (b) To determine the effects of the MP-like

particles extracted from the Rocha River on the survival, regeneration rate, and behavior of the planarians; (c) To analyze the relationship between the physical properties of the MP-like particles extracted at each point in the Rocha River and the effects observed in the planarians.

3. Methodology

First, it is important to define the term MP-like. MP-like particles are obtained using protocols designed for MP extraction, but whose chemical composition has not been verified by spectroscopic analysis (Strand et al., 2019). Because plastic debris fragments to microscopic and nanometric dimensions, its visual differentiation from natural debris is complex (Ríos and Balcer, 2019). Consequently, basing classification only on microscopy techniques can lead to an overestimation of MP abundance, as the extract may include non-polymeric particles with similar physical properties (Sanamiento, n.d.). This analytical limitation, common in the context of Global South countries (such as Bolivia), restricts definitively determining the precise origin and characteristics of the particles, but is adapted to determine their dynamics, toxicity, and real effects on organisms in aquatic ecosystems. This protocol is an efficient and low-cost screening method for managing local projects in countries with similar characteristics.

This section describes the methodological steps for evaluating the effects of MP-like particles extracted from the Rocha River on planarians. This methodology is a transferable engineering tool to other rivers with similar emerging pollutant problems in Global South countries. The work is divided into three parts:

- Stage 1: Sample Collection and Characterization

This stage focused on collecting water samples at three key points along the Rocha River and then implementing a standardized procedure for the extraction and analysis of MP-like particles. This procedure facilitated obtaining quantitative and qualitative information regarding the occurrence and categories of microplastics in the water flow.

- Stage 2: Exposure of test organisms to MP-like particles

The experimental design employed two concentration levels of MP-like particles at each sampling point: the actual concentration, corresponding to the amount measured in the Rocha River samples, and a concentration ten times higher (10X), used to simulate drought conditions, since the samples were taken during the rainy season, when the presence of microplastics in the water is more dilute (Lebreton et al., 2017).

- Stage 3: Evaluation of effects and data analysis

The ecotoxicological effects of MP-like particles on planarian development were evaluated through the systematic recording of survival, morphology, behavior, and regeneration rate.

3.1. Water sampling

The Rocha River is a crucial tributary in the central valley of Cochabamba, Bolivia. Its course, approximately 81.2 kilometers long, flows through the Cochabamba metropolitan region and connects municipalities such as Sacaba, Colcapirhua, Quillacollo, Vinto, and Sipe Sipe, making it an important artery for the region's economic and social activities. Its basin, covering nearly 700 km², is essential for agricultural development, as its waters are used primarily for irrigation and, in some rural areas, for human consumption (Servicio Departamental de Cuencas (SDC) Dirección de Planificación y Gestión Integral del Agua (DGIA).2014; Toledo & Amurrio, 2006).

Throughout its course, the Rocha River experiences variations in its flow, especially during the rainy season, when the drainage capacity of water from sub-basins and smaller

tributaries increases. However, urban and industrial growth in the region has generated a high pollution load, resulting from the discharge of solid waste and wastewater from domestic, industrial, and agricultural sources. These threats impact both water quality and its availability for diverse uses, negatively affecting ecosystems and the quality of life of communities that depend on this resource.

Sampling points along the Rocha River were selected using a stratified approach, considering different zones with contrasting land use and human activity characteristics. Each point was defined to represent a specific type of human intervention in the riverbed, thus encompassing an environmental gradient relevant to the study (Table 1).

- Chiñata bridge: This point is located in a semi-urban area, characterized by low population density and moderate anthropogenic activity (Linera Canedo, 2021).
- Cobija bridge: The Cobija Bridge, located over the Rocha River in Cochabamba, receives domestic, industrial, and agricultural wastewater, as well as potential contaminants associated with the nearby Jorge Wilstermann Airport, such as oils and fuels (Linera Canedo, 2021).
- La Maica: Situated in a predominantly agricultural area, this point is representative of the nutrient and pesticide load derived from agricultural practices. The waters in this area may present high concentrations of fertilizers and pesticides (Linera Canedo, 2021).

Table 1: Characterization of sampling points

Sampling point	Name of the sampling point	Coordinates		Area
		Latitude	Longitude	
P1	Chiñata bridge	17° 24'16,8"	65° 58'46,6"	Semi-urban
P2	Cobija bridge	17° 23'18,4"	66° 09'54,2"	Urban / Industrial
P3	La Maica	17° 25'42,6"	66° 12'56,0"	Agrícola

Samples were carried out during the rainy season. A 6 L sample was collected at each sampling point, ensuring an adequate baseline for characterizing MP-like particles and establishing initial experimental conditions. At the Cobija Bridge and Chiñata Bridge, the sample was taken midway across the river at a medium depth to avoid disturbing sediments. At La Maica, due to the higher flow rate from the upstream confluence of the Tamborada River, the sampling container was fully submerged using a rope to position it as far out as possible and avoid drawing water near the riverbank.

3.2. MP-like extraction and characterization

This step aims to characterize the physical properties of extracted particles, considering their concentration, size, and shape. Samples collected from three strategic locations and a mix of the samples were used. Additionally, a blank sample was prepared to assess cross-contamination and verify the possible presence of microplastics in the reagents used.

The water from each sampling point was sieved through a 5 mm sieve to separate the larger elements that could affect the experiments, respecting the size ranges of the MP.

The extraction and quantification of MP-like particles were carried out in several consecutive stages. First, chemical digestion was performed to remove organic matter that could interfere with particle identification. For this purpose, 23 mL of 30% H₂O₂ was added to 20 mL of each water sample (reaching a final concentration of 16% H₂O₂), and the mixture was maintained for five days at 40 °C until bubbling was minimal (Coral et al., 2024). Subsequently, the samples were vacuum-filtered using 0.45 µm nitrocellulose filters and washed with 10 mL of distilled water. Zinc chloride (ZnCl₂) was used for density separation. The zinc salt solution was adjusted to a density of 1.32 g/mL (36.20% w/v) (Maes et al., 2017). The filtered material was suspended in the zinc chloride solution for two hours in a separatory funnel.

For identification by fluorescence microscopy, three drops of Nile Red dye at a concentration of 1 µg/mL were added. The upper fraction of the samples in the separatory funnel. was collected and filtered again through 0.45 µm nitrocellulose membranes.

Identification and counting were performed using a fluorescence microscope (Optika, B1000LD4) at 10X magnification. The particles were morphologically characterized as spheres, fragments, films, fibers, or sponges (Crawford and Quinn, 2017) and classified by size into ranges of 1–100 µm, 100–350 µm, and 350 µm–5 mm (Duis and Coors, 2016).

3.3. Bioassay: planarian exposition

The planarians (*Girardia sp.*) used in this study were collected from the aquariums housing frog specimens at the Alcides d'Orbigny Natural History Museum in Cochabamba, Bolivia. After collection, the organisms were transferred to the UTOX laboratory and subjected to a two-week acclimatization period before multiplication and the performance of bioassays. This ensured the physiological stabilization of the organisms before the start of the experimental procedures (Rock et al., 2023).

The preparation and conditioning of the culture medium for the conservation, feeding, and reproduction of the planarians was standardized according to protocols established for *Schmidtea mediterranea* (Plusquin et al., 2012), with local adaptations at UTOX. This process involves two stages: the preparation of the stock solution and subsequently the final solution in 2.5 L of MilliQ water.

The organisms were cultured under controlled conditions (18°C, total darkness) and fed twice a week (Abel et al., 2022). The food was dispensed directly onto the culture medium, and after two hours of exposure, the organisms were transferred to clean medium using a Pasteur pipette to avoid the accumulation of debris and microbial contamination (Bustillos Soria, 2024).

After the acclimation phase, the planarians were multiplied asexually (Abel et al., 2022), following standardized protocols for species such as *S. mediterranea*, *D. japonica*, and *G. dorocephala* (Abel et al., 2022; Hagstrom et al., 2016). The section was made transversely with three cuts corresponding to the cephalic, middle, and caudal regions, each of which has the capacity to regenerate a complete organism thanks to its pluripotent stem cells (neoblasts) (Cebrià et al., 2011). After sectioning, the organisms were kept in glass containers with the medium, which was changed twice a week to avoid the accumulation of metabolites and prevent infections (Accorsi et al., 2017; Gentile et al., 2011; Hanif et al., 2022; Oviedo et al., 2008).

To prepare the MP-like particle extracts and to ensure that the organisms are exposed only to these particles, the sieved fraction followed the extraction protocols of MP as mentioned above:

- H₂O₂ digestion
- Density separation (ZnCl₂)

After the last filtration, the MP-like material was preserved for bioassays by resuspension in 20–25 mL of Milli-Q water and stored in refrigerated glass bottles to ensure its stability and

preservation before exposure to the test organisms. This solution was diluted into the planarian culture medium to reach the exposition concentration of MP-like particles.

The bioassay experimental design employed two levels of MP-like particle concentration at each sampling point. Thus, the actual concentration corresponds to the amount measured in the samples from the Rocha River. A concentration ten times higher (10X) was also used to simulate drought conditions, as the samples were taken during the rainy season, when the presence of microplastics in the water is more dilute (Lebreton et al., 2017).

Each treatment consisted of three independent replicates, using a total of six planarians per treatment, a quantity frequently used in ecotoxicological bioassays with planarians and considered adequate for obtaining reliable biological responses in regeneration studies (Hagstrom et al., 2015; Morris et al., 2022). A control was also included per bioassay, with the same number of planarians exposed to the culture medium without MP-like particles.

The experiment was designed following methodological guidelines for planarian regeneration bioassays, with an experimental period of fourteen days (Hagstrom et al., 2015). Three photographs were taken of each whole planarian, which were then cut into cephalic (after the auricles and before the pharynx) and caudal fragments. Both segments are valid biological units for analyzing regeneration under chemical stress conditions (Zhang et al., 2019). These fragments were subsequently deposited in wells of actual concentration and a concentration 10 times higher, with two fragments per well and 1 mL of solution per fragment.

The ecotoxicological effects of MP-like particles on planarian development were evaluated by systematically recording survival, morphology, behavior, and regeneration rate. Observations were made on days 3, 5, 7, 11, and 14 of the experimental period, using a stereomicroscope equipped with a camera at 10x magnification, through photographic and video recordings of 120 seconds per fragment. All variables were quantified following the methodological parameters and observation criteria proposed by Hagstrom et al. (2015) and Leynen et al. (2019).

Survival was recorded daily from day 1 by counting live fragments per treatment. An organism was considered dead when it no longer exhibited a motor response after gentle stimulation with a Pasteur pipette and showed a whitish fiber around it. For each day and treatment repetition, survival was determined according to Equation (1).

$$Survival\ rate = \frac{N_{living\ organisms}}{N_{initial\ organisms}} \times 100 \quad (1)$$

The behavior of the fragments was evaluated by reviewing and analyzing the videos. The presence or absence of C-shaped and screw-shaped movements was recorded. To assess the difficulty of movement, the proportion of time each fragment remained in motion or inactive was determined, allowing for the objective quantification of motor capacity (Caronti et al., 1999).

The regeneration rate was assessed by measuring blastemas in planarian fragments on days 3, 5, and 7 using ImageJ software. The result was divided by the total area of the fragment and calculated (Equation 2) (Leynen et al., 2019):

$$Growth\ coefficient = \frac{Blastema\ area}{Total\ area\ of\ the\ organism} \quad (2)$$

To complete the regenerative characterization, the appearance of eyes in tail fragments was recorded, identifying their presence or absence on days 3, 5, and 7.

4. Results

This section presents the results of the different parts of the experimentation.

4.1. Physical characterization of MP-like particles

The characterization of MP-like particles (Table 2) shows that fragments represent the majority of particles at all sampling points, especially within the 1 to 100 μm range. The highest quantity was recorded at P2 (671,500 particles/L), followed by P2 (479,600 particles/L), while the lowest concentration was found at La Maica (276,200 particles/L), although larger fibers and fragments were also found. Studies in rivers similar to the Rocha River agree that the smallest MP are the most abundant, due to the weathering of plastics, which causes fragmentation during hydrodynamic transport (Horton et al., 2017; Lin et al., 2018). Thus, the most common form of microplastics in the Rocha River is fragments, followed to a lesser extent by fibers. The weathering of larger plastic debris, such as wrappers and bags, is the main source of MP in this watershed.

Table 2: Concentration of microplastic-like particles in water samples

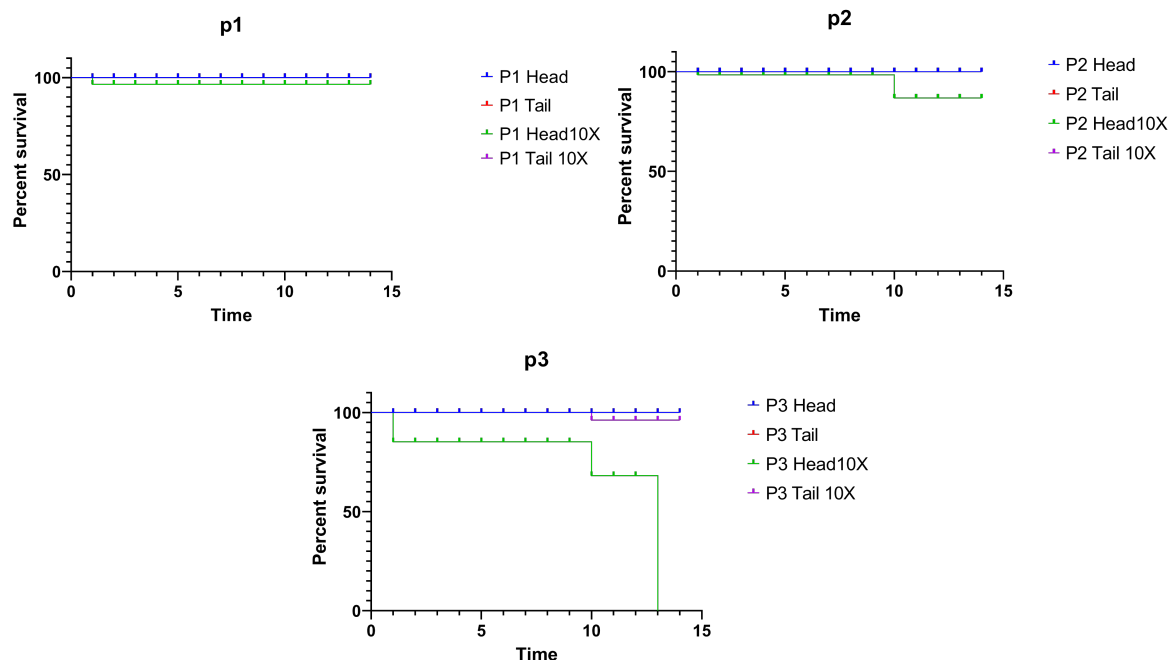
Sample point	Shape	MP-like concentration (particles/L) / Size		
		1–100 μm	100–350 μm	350 μm –5 mm
P1	Fragments	479,600	0	0
	Fibers	0	0	0
	Films	0	0	0
	Spheres	0	0	0
	Sponges	0	0	0
Total		479,600		
P2	Fragments	671,500	600	0
	Fibers	0	0	0
	Films	0	0	0
	Spheres	0	0	0
	Sponges	0	0	0
Total		672,100		
P3	Fragments	276,200	800	0
	Fibers	0	900	100
	Films	0	0	0
	Spheres	0	0	0
	Sponges	0	0	0
Total		278,000		

4.2. Effects of extracted microplastic-like particles on the development of planarians

Survival results in *Girardia* sp. exposed to MP-like particles from the Rocha River demonstrate a significant interaction between sample concentration, contaminant source, and body fragment type (Kruskal-Wallis; $p=0.020$) (Figure 1). The reduction in viability over time (Friedman; $p<0.001$) confirms a cumulative toxicity effect that compromises the organism's physiological stability.

A differential response was observed depending on the anatomical region. At normal concentrations, cephalic fragments maintained 100% survival, while caudal fragments showed declines starting on day 5. This vulnerability of the posterior region suggests that the persistent chemical environment of the Rocha River induces tissue stress that selectively affects cellular homeostasis in the tails (Almuedo-Castillo, 2014; Rink, 2013). This phenomenon indicates that cellular repair mechanisms in the caudal region may be more sensitive to the chemical interference of MP-like particles.

Figure 1: Survival rate of planarian fragments exposed to extracted MP-like particles



Increased exposure (10X) resulted in a progressive decrease in survival, with a statistically significant difference compared to the normal concentration (Mann-Whitney, $p<0.001$). The total mortality (100%) observed in head fragments from the La Maica site on day 14 suggests critical inhibition of neoblasts. Since these stem cells are responsible for the regeneration and maintenance of vital tissues, their dysfunction in the face of high MP-like concentrations prevents the body's recovery, resulting in acute lethality (Rink, 2013; Wagner et al., 2011).

Table 3: Behavior rate of live planarian fragments exposed for 7 days to MP-like particles

	C-shape (%)	Screw-shaped (%)	Contractions (%)	Difficulty moving (%)
Control Head	0,00	0,00	0,00	0,00
P1 Head	0,00	0,00	0,00	16,67
P1 Head 10X	0,00	0,00	0,00	75,00
P2 Head	0,00	0,00	0,00	33,33
P2 Head 10X	0,00	0,00	0,00	20,00
P3 Head	0,00	0,00	0,00	33,33
P3 Head 10X	50,00	0,00	0,00	100,00
Control Tail	0,00	0,00	0,00	33,33
P1 Tail	0,00	0,00	16,67	16,67
P1 Tail 10 X	0,00	16,67	0,00	33,33
P2 Tail	0,00	0,00	0,00	16,67
P2 Tail 10 X	0,00	16,67	33,33	66,67
P3 Tail	0,00	16,67	0,00	33,33
P3 Tail 10 X	16,67	16,67	33,33	83,33

Non-parametric statistical analysis of the behavioral responses (Table 3) of *Girardia sp.* to MP-like particles from the Rocha River indicates that the origin of the samples does not significantly influence the appearance of abnormalities (C-shape: $p=0.485$; screw-like shape: $p=0.181$; contraction: $p=0.879$). However, determining factors related to exposure concentration, body fragment differentiation, and contact time were identified.

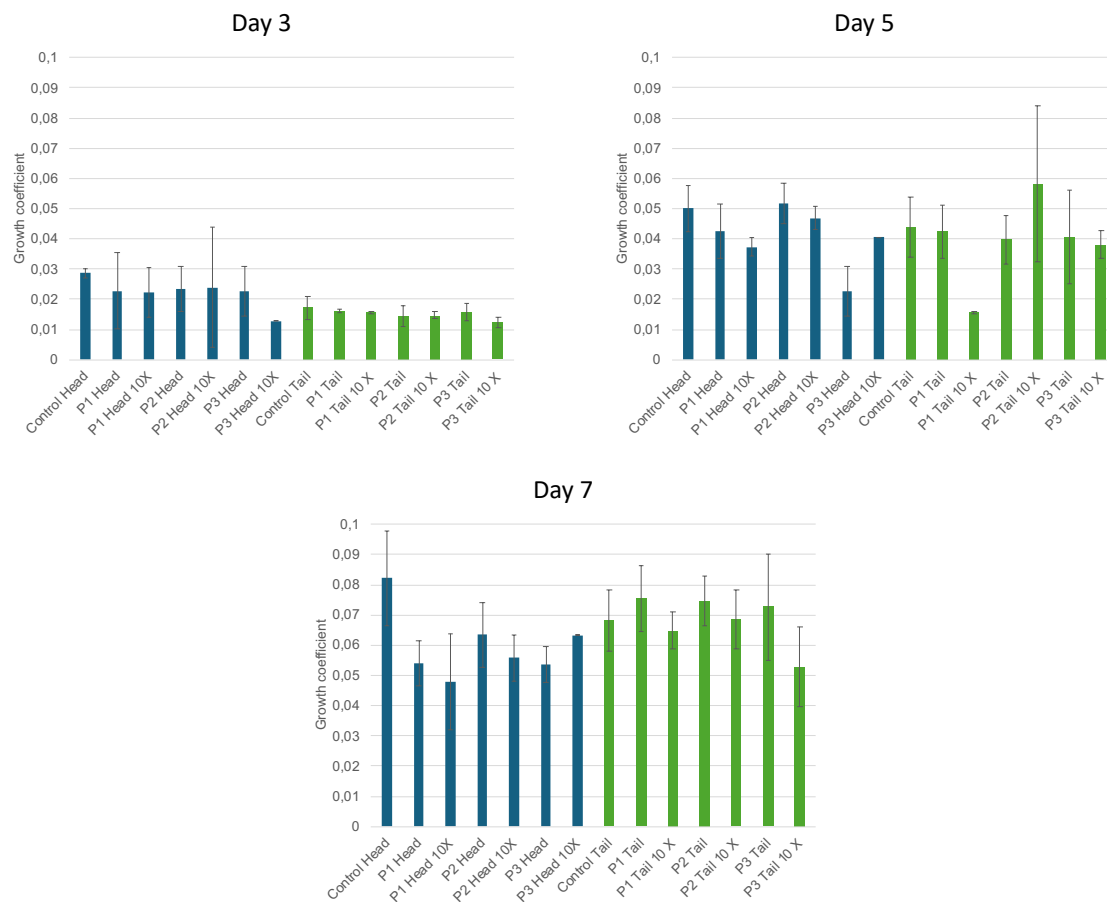
A positive correlation was observed between particle concentration and the frequency of abnormal behaviors. The incidence of the C-shape was significantly higher in treatment 10X compared to the normal concentration ($p=0.005$). Similarly, the frequency of contracted organisms increased significantly with the dose ($p=0.021$). Although the screw-shaped response did not reach the threshold of statistical significance ($p=0.076$), an increasing trend was observed in the condition of greater exposure, suggesting a neurotoxic or mechanical impact dependent on particle load.

The most pronounced variability was observed between fragment types. Caudal segments exhibited a significantly higher frequency of screw-shaped movements and contraction states than cephalic fragments ($p<0.001$ in both cases). These findings are consistent with those reported by Hagstrom et al. (2015), who attribute the greater sensitivity of tails to organic contaminants and metals to an asymmetric tissue distribution and post-amputation physiological instability. Conversely, the low incidence of abnormalities in cephalic fragments is consistent with their neurophysiological robustness and superior regenerative capacity (Reddien, 2018).

The Friedman test confirmed significant temporal variations ($p<0.001$), demonstrating a cumulative effect of microparticle exposure. These alterations suggest a state of sublethal stress analogous to that caused by other pollutants such as ammonia, pesticides, or heavy metals (Hagstrom et al., 2016).

Analysis of blastema regeneration in *Girardia sp.* reveals a biphasic response dependent on exposure time and body fragment type (Figure 2). While the initial stages showed no alterations, the progression of the regenerative process revealed significant inhibitory effects linked to the presence of MP-like particles.

Figure 2: Blastema regeneration in planarians exposed to MP-like particles (Expressed as growth coefficient)



During the third day of evaluation, no statistically significant differences were detected between the treatments and the control group, in either cephalic (Kruskal-Wallis; $p=0.2957$) or caudal (ANOVA; $F=1.764$; $p=0.1123$) fragments. This absence of early effects suggests that the initial activation of neoblasts and the basic tissue repair mechanisms possess intrinsic resilience capable of initiating blastema development even in adverse environments, due to the high cellular plasticity of these organisms (Reddien & Sánchez Alvarado, 2004).

From day five onward, exposure to MP-like particles induced critical variations in the regeneration rate. In the cephalic fragments, highly significant differences were observed ($p<0.0001$), with treatment P3-CR (La Maica) showing greater inhibition compared to the control (Dunn post hoc test; $p<0.0001$). In the caudal segments, treatment P1 10X (Chiñatabridge) showed the lowest regeneration values ($p<0.0005$).

By day seven, the inhibitory trend persisted, especially in treatment P1 10X for cephalic fragments ($p=0.0165$) and in P3 10X for caudal fragments ($p=0.0033$). This delayed manifestation of the effects suggests that prolonged exposure to MP generates cumulative

physiological and biochemical damage that compromises the proliferation and migration of neoblasts (Prata et al., 2020). These findings are consistent with previous studies indicating that microplastics induce oxidative stress, interfering with complex biological processes such as invertebrate regeneration (Gao et al., 2022; Han et al., 2022).

The disparity in responses between the cephalic and caudal regions is attributed to the differential complexity of the tissues undergoing reconstruction; the regeneration of cephalic structures demands more stringent cellular and molecular coordination, which increases their susceptibility to neurotoxic or mechanical agents (Reddien & Sánchez Alvarado, 2004).

Finally, the constant stability observed in the control group throughout the experiment confirms that the variations in blastema formation are a direct consequence of exposure to MP-like particles extracted from the Rocha River and not of external environmental factors.

Although the sample size is consistent with standards for bioassays with *Girardia sp.*, it is recognized that expanding the population in future monitoring projects would increase statistical power. Nevertheless, the robustness of the significant differences found ($p < 0.001$) validates the model's sensitivity for local assessments.

5. Discussion

The biological impacts identified in *Girardia sp.* were determined by the physical properties (size, shape, and concentration) of the MP-like particles and the environmental context of each sampling site. While the organisms maintained physiological stability at normal concentrations, treatment with a concentration of 10X showed a transgression of tolerance thresholds, suggesting that toxicity manifests when exposure exceeds the homeostatic plasticity of the biological model.

The most severe impact was recorded at the La Maica site (agricultural area), where the 10X condition induced the highest mortality rate and the most significant delay in regeneration. Despite having the lowest total numerical concentration, this site stood out for its high morphological diversity and the presence of larger particles. Biologically, exposure to microparticles can induce oxidative stress, generating an excessive production of reactive oxygen species that overwhelms the enzymatic defenses of superoxide dismutase and catalase, resulting in damage to cell membranes and metabolic proteins (Livingstone, 2001; Lushchak, 2011).

In this context, fibers could exacerbate the damage because their elongated structure hinders tissue clearance. Furthermore, the ability of these particles to act as vectors for agrochemical compounds (Trojan horse effect) increases physiological stress even at low particle densities (Hüffer et al., 2019; Bhagat et al., 2020).

At Cobija bridge, characterized by a high density of small fragments (1–100 μm), sublethal alterations predominated in regeneration, without reaching the lethality levels observed at La Maica. This pattern suggests that smaller particles interfere with delicate cellular processes such as neoblast proliferation and differentiation. These particles may disrupt highly conserved signaling pathways, such as the Wnt/ β -catenin pathway, essential for the organization of the anteroposterior axis, affecting tissue architecture without compromising the immediate viability of the specimen (Gentile et al., 2011; Reddien, 2018).

The Chiñata bridge showed intermediate responses, indicating that, despite reduced morphological diversity (absence of fibers), planarians can partially maintain their metabolic equilibrium. The behavioral alterations observed at concentration 10X are interpreted as early indicators of neurophysiological dysregulation; the accumulation of oxidative damage can interfere with nerve transmission and muscle coordination before lethal effects occur.

The relative stability of regeneration at normal concentrations, compared to localized alterations at concentration 10X (especially on days 5 and 7), confirms that planarian regenerative mechanisms possess remarkable tolerance to environmental stress. However,

this functionality is locally compromised depending on the polymer load, aligning with studies reporting that prolonged exposure to microplastics progressively reduces the effectiveness of tissue repair in aquatic invertebrates (Gao et al., 2022; Han et al., 2022).

In summary, the results enable the ecotoxicological risk of the Rocha River to be classified within a technical prioritization framework for watershed management. Two critical scenarios were determined. Acute risk scenarios are linked to La Maica area, in which the morphological diversity of particles and their interaction with the agricultural environment exacerbate mortality. A chronic sublethal risk scenarios was identified in Puente Cobija area, where the high density of small fragments compromises long-term regenerative capacity of planarians. This differentiation of risk constitutes a fundamental engineering tool for the design of mitigation strategies, suggesting that remediation projects should prioritize the selective removal of fibers in agricultural areas to preserve the cellular viability of aquatic fauna. Whereas in urban areas the focus should be directed toward the filtration of microfragments that disrupt neurophysiological stability. Accordingly, the risk classification presented herein not only validates *Girardia sp.* as a sensitive bioindicator, but also provides technical benchmark parameters for the life-cycle assessment of future environmental remediation projects in similar fluvial ecosystems.

6. Conclusions

This research confirmed that MP-like particles present in the Rocha River basin exert adverse and differentiated ecotoxicological effects on aquatic fauna, using the flatworm *Girardia sp.* as a highly sensitive biological model.

First, it is concluded that the toxicity of MP is not determined solely by their numerical abundance, but by a complex interaction between their morphology and the environmental context. The La Maica site, despite having a lower concentration of particles, induced the highest mortality and regenerative delay due to the predominance of fibers and the probable presence of adsorbed agrochemicals, validating the risk of the "Trojan horse effect" in agricultural areas.

Secondly, the study demonstrated that the regenerative capacity and nervous system of planarians are the primary targets of sublethal toxicity. High concentrations (10X) caused neoblast proliferation failures and behavioral alterations (C-shaped and screw-like forms), indicating physiological dysregulation that precedes the organism's death.

Thus, the MP-like particles from the Rocha River represent a latent threat to the biodiversity of Cochabamba's lotic ecosystems. The proposed methodology allows for the standardization of impact assessment protocols within the life cycle of sanitation projects, facilitating the prioritization of investments in areas of greater ecotoxicological risk (such as La Maica). The urgent implementation of waste management policies and continuous biological monitoring is recommended to mitigate the entry of these emerging contaminants into the food chain.

7. Bibliography

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Use of Generative Artificial Intelligence

AI tools were used for language improvement

Communication aligned with the Sustainable Development Goals

