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Anurans as bioindicators of the effect of hazardous waste discharge in the waters of the Gurjaú state reserve, northeastern Brazil

Frogs use different kinds of environment in their life cycle; hence, they may be indicators of pollution and environmental degradation. In the present study, the frogs are relevants to evaluate the harmful effects of waste disposal. We carried out weekly sampling by active search from January to December 2004 in a 1-km stretch of the Gurjaú River, at the Gurjaú State Reserve (SRSG), located in the south of the city of Recife, which is managed by Pernambuco Sanitation Company (COMPESA), maintaining a water treatment plant in the reserve since the damming of the Gurjaú River. COMPESA discharges quarterly the chemical waste from the cleaning of its settling tanks directly into the Gurjaú River, downstream of the water uptake. The study displayed that among 40 species, six were observed near the river: Hypsiboas semilineatus (Spix, 1824), Leptodactylus gr. marmoratus (Steindachner, 1867), Scinax x-signatus (Spix, 1824), Litobathes palmipes (Spix, 1824), Leptodactylus vastus (Spix, 1824), and Pseudopaludicola gr. falcipes (Hensel, 1867). The site located upstream of the waste discharge had much more captures than the site located downstream of it. Based on the physicochemical analysis of the water, we conclude that the activities of COMPESA have high environmental impact.

Keywords: Amphibians; Pollution indicators; Gurjaú River; Environmental degradation.

Anuros como bioindicadores do efeito do lançamento de resíduos perigosos nas águas da reserva estadual do Gurjaú, nordeste do Brasil

Anuros, durante o seu ciclo de vida são expostos a diversos ambientes, podendo atuar como importantes indicadores de poluição e degradação ambiental. O presente trabalho utilizou os anuros como bioindicadores, com o objetivo de confirmar o efeito nocivo da eliminação dos dejetos no presente domínio morfoclimático. Foram realizadas coletas semanais por busca ativa entre janeiro e dezembro de 2004, à margem do rio Gurjaúna Reserva Estadual de Gurjaú (RESG). Os resultados revelararam que das 25 espécies listadas para a RESG, seis foram observadas nas proximidades do rio: Hypsiboas semilineatus (Spix, 1824), Leptodactylus gr. marmoratus (Steindachner, 1867), Scinax x-signatus (Spix, 1824), Lithobates palmipes (Spix, 1824), Leptodactylus vastus (Spix, 1824) e Pseudopaludicola gr. falcipes (Hensel, 1867). O ponto anterior ao despejo dos resíduos, quando comparado ao número total de espécimes capturados, foi muito superior ao ponto após a eliminação dos resíduos e através da análise físico-química da água pode-se concluir que a ação da COMPESA se mostrou altamente impactante ao meio ambiente.

Palavras-chave: Anfíbios; Degradação ambiental; Indicadores de poluição; Rio Gurjaú.

Topic: **Conservação da Biodiversidade**

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INTRODUCTION

The Atlantic Forest is considered one of the world's biodiversity hotspots, with its high endemism rates and large number of animal and plant species (MYERS et al., 2000; PALMEIRA et al., 2015; OITAVEN et al., 2021). In the beginning of the 16^{th} century, this ecosystem covered approximately 17.4% of the Brazilian territory (1,481,946 km^2), including coastal and inland areas (METZGER et al., 2009; FREITAS et al., 2019). Currently, the Atlantic Forest covers approximately 8% of its original area and is classified as one of most threatened ecosystems on Earth (COLOMBO et al., 2010; PALMEIRA et al., 2015; VIEIRA et al., 2023).

Due the high degree of threat, it is highly necessary to assess the quality of these domains. Anuran amphibians are considered excellent bioindicators of environmental quality (WELSH et al., 1998; DIXON et al., 2011), since they are particularly sensitive to environmental degradation (BEEBEE et al., 2005; BRAGA et al., 2022) and have exclusive characteristics, among them an ectothermic physiology with limited activity; skin permeability, which makes them vulnerable to several biological or chemical agents; and eggs and larvae that depend on water or humid environments (KLARK et al., 1985; HENRY, 2000; WELLS, 2007).

The Gurjaú State Reserve (SRSG) is located in the south of Recife, within the municipalities of Jaboatão dos Guararapes, Cabo de Santo Agostinho, and Moreno (SANTOS et al., 2016; SILVA et al., 2017). The reserve is managed by Pernambuco Sanitation Company (COMPESA), which maintains a water treatment plant in the reserve since the damming of the Gurjaú River. COMPESA discharges quarterly chemical waste in the reserve, which comes from the cleaning of its settling tanks. The waste, which was already identified at preliminary phases of the present study, is discharged in the Gurjaú River, probably affecting the local riparian community as well as the aquatic fauna and flora. Through the use of socio-economic-environmental questionnaires given to the local community, we detected that the riparian population uses the Gurjaú River as a source of drinking water, leisure, hygiene, food, and goods, such as fish and shrimp, among them the species that is locally known as pitú (*Macrobrachium carcinus*) (Linnaeus, 1758) (LARRAZÁBAL, 2003).

Waste discharge in the Gurjaú River requires an assessment from the perspective of conservation biology, a multidisciplinary science developed as a response to the current biodiversity crisis (SOULÉ, 1985; JOFRÉ et al., 2012). An ecological diagnosis of the situation is required, as only by evidencing the ongoing environmental pollution it will be possible to demand adequate measures from the authorities. In order to obtain this kind of evidence, it is necessary to analyze how the biota responds to pollution. We chose anuran amphibians as bioindicators, because in their life cycle they use different kinds of environment and are vulnerable to environmental change, acting as important indicators of contamination (DUELLMAN et al., 1994; BRAGA et al., 2022).

Hence, the present study have the aim to assess the effect of the hazardous waste discharged in the waters of the Gurjaú State Reserve on the ecology of anurans, as well as the influence of abiotic factors, such as climatic conditions and temperature.

MATERIALS AND METHODS

Study site

The study was carried out in an Atlantic Forest fragment in the Gurjaú State Reserve (08º13’45’’S and 35º03'35”W) (Figure 1), Pernambuco, northeastern Brazil. The reserve is located within the crystalline complex of the Cabo Formation, with altitudes varying between 80 and 130 m a.s.l. The climate is classified, according to the Köppen classification, warm and humid, typical of the eastern part of the Northeastern Region of Brazil (SANTOS et al., 2016; SILVA et al., 2017). The rainy season occurs in autumn and winter, as is typical in the Zona da Mata Pluvial, with an annual rainfall of 1,900 mm. The rainy season occurs from March to August and the dry season from September to February (FADURPE, 2003). Regarding anthropic activities, the Gurjaú State Reserve is located in an Atlantic Forest remnant, surrounded by the sugar cane farms Contra-Açude and Novo da Conceição (FADURPE, 2003).

The vegetation of the forest surrounding the Gurjaú River has a height between 20 and 40 m and is composed of a large number of species of the regional flora, many of them endemic to the Atlantic Forest. The fauna is typical of the Atlantic Forest, with endemic, endangered, and hunted species (FADURPE, 2003). Regarding amphibians, forty anuran species were already listed for the Gurjaú State Reserve (CARnaval et al., 2003; SANTOS et al., 2016), of which six species were found in the present study: *Hypsiboas semilineatus* (Spix, 1824), *Leptodactylus* gr. *marmoratus* (Steindachner, 1867), *Scinax x-signatus* (Spix, 1824), *Litobathes palmipes* (Spix, 1824), *Leptodactylus vastus* (Spix, 1824), and *Pseudopaludicola* gr. *falcipes* (Hensel, 1867).

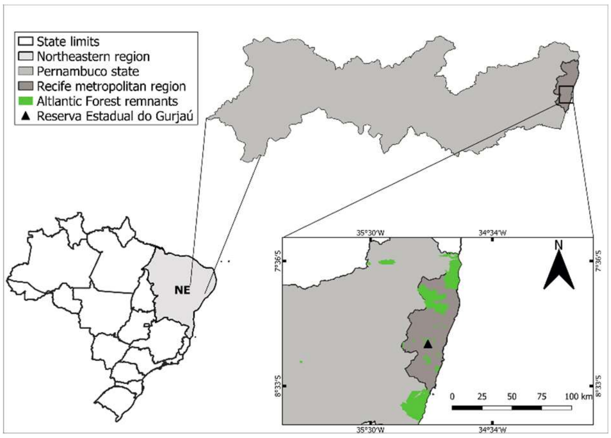

Figure 1: Reserva estadual do Gurjaú, Pernambuco state, Brazil, located at Metropolitan region of Recife (in gray; adapted from IBGE, 2021) remnants cover in Pernambuco state (in green; Adapted from INPE, 2019) of Atlantic Forest.

Data collection

We carried out weekly collection of adult anurans by active search, during 2004 along a 1-km stretch located on both banks of the Gurjaú River. Sampling sites comprised from the riverside to the first three meters of the riverbank, measured perpendicularly to the riverbed. Collection began in the late afternoon (18:00) and lasted 4 h, totaling a sampling effort of 400 h/men, using the same among of collectors: two men. Waste is discharged in the center of the study area (Figure 2), and the river flows in the northeast direction. Hence, a 500-m stretch of the river that is ‘not impacted’ flows to the northeast of the site of waste discharge, and a 500-m stretch of the river that is quarterly 'impacted' flows to the southeast of the site of waste discharge. Waste was discharged four times during the present study. The sampling site was marked at every five meters, in order to precisely record the location of the specimens that were sighted and heard.

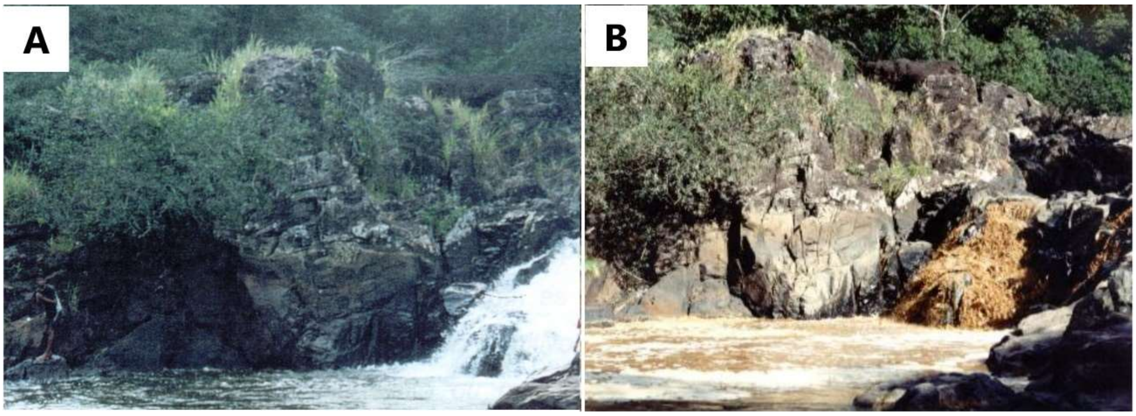

Figure 2: Landscapes conditions from Reserva estadual de Gurjaú at Gurjaú River, Pernambuco state, Brazil. (A) non-impacted area (02/01/2004); (B) impacted area (24/01/2004).

Simultaneously, we carried out monthly sampling of water and measured the depth of three sites of the studied river: 1. Precisely at the site of waste discharge (0); 2. 100 m upstream of the site of waste discharge (+100); and 3. 100 m downstream of the site of waste discharge (-100). Water samples were submitted to physicochemical tests, such as turbidity, pH, chloride, and COD, all performed in the Laboratory of Environmental Sanitation of the Department of Civil Engineering at UFPE. In addition, we monitored weekly some environmental characteristics, such as climatic conditions and temperature.

We used the method of Dajoz (1983) to calculate the constancy of occurrence, which expresses as a percentage the number of inventories in which the species occur in relation to the total number of inventories carried out. The species with constancy above 50% were considered constant, those recorded in 25% to 50% of the inventories were considered accessory, and those below 25% were considered occasional.

To compare whether the total number of captures and the number of captures per species varied between polluted and non-polluted areas we used the Mann-Whittney test. We used the Kruskal-Wallis test to assess a possible variation among locations (+100, 0, and -100 away from the site of waste discharge) in the abiotic factors and physicochemical parameters of the water. For a nonparametric multiple comparison, we used the Nemenyi test for the variable's chloride, turbidity, and COD, and the Dunn test for pH, because it has 'dependent' values. All tests were carried out considering significance level of 0.05 (ZAR, 1999), in StatSoft Statistica 8.0.

RESULTS

The number of captures downstream and upstream of the site of waste discharge differed significantly from each other, considering all species pooled ($U_{150,150} = 8.15$; $P < 0.0001$) (Figure 3A) and each species separately: *Scinax x-signatus* ($U_{25,25} = 88.00$; $P < 0.0001$), *Leptodactylus vastus* ($U_{25,25} = 167.50$; $P < 0.01$), *Lithobates palmipes* ($U_{25,25} = 173.000$; $P < 0.001$), *Pseudopaludicola falcipes* ($U_{25,25} = 48.00$; $P < 0.0001$), *Leptodactylus* gr. *marmoratus* ($U_{25,25} = 156.50$; $P < 0.01$), and *Hypsiboas semilineatus* ($U_{25,25}$=130.50; P <

0.001). The physicochemical parameters (Table 1a and b) of the water differed among locations (+100, 0, and -100) (Table 2) for all factors analyzed: turbidity (H = 10.24; df = 2; n = 18; P < 0.01), pH (H = 7.46; df = 2; N = 18; P = 0.02), chloride (H = 5.49; df = 2; N = 18; P = 0.05), and COD (H = 13.37; df = 2; N = 18; P = 0.001) (Table 3).

Table 1A: Anuran fauna recorded weekly in the Gurjaú State Reserve from January to December 2004, upstream of the site of waste discharge (Abbreviations: *OCC*, Occasional; *ACC*, Accessory; *CO*, Constant). Months of waste discharge: January, May, August, and December.

MONTHS/2004	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	Total per Species	Constancy of Occurrence	Categories	Relative Abundance
Nº de COLLECTIONS	3	4	5	4	4	4	4	5	4	4	5	4				
1- Hylidae																
1.1- *S. x-signatus*	20	13	20	44	40	40	45	92	36	33	31	11	425	90%	CO	20.14%
1.2- *H. Semilineatus*	0	11	9	24	21	21	26	54	22	19	16	0	233	66%	CO	11.04%
2- Leptodactylidae																
2.1- *P. falcipes*	57	7	57	119	120	79	114	188	116	83	145	103	1188	96%	CO	56.30%
2.2- *L. vastus*	2	6	6	3	6	4	6	9	6	9	12	3	72	78%	CO	3.41%
2.3- *L. marmoratus*	1	4	2	7	2	4	5	9	5	3	6	1	49	56%	CO	2.32%
3- Ranidae																
3.1- *L. palmipes*	3	12	9	13	14	13	21	30	11	7	8	2	143	94%	CO	6.78%
Total	86	57	108	214	207	165	221	387	200	158	223	124	2110	-	-	100%
	DRY SEASON		RAINY SEASON						DRY SEASON							

Table 1B: Anuran fauna recorded weekly in the Gurjaú State Reserve from January to December 2004, downstream of the site of waste discharge (Abbreviations: OCC, Occasional; ACC, Accessory; CO, Constant). Months of waste discharge: January, May, August, and December.

MONTHS/2004	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	Total per Species	Constancy of Occurrence	Categories	Relative Abundance
Nº de COLLECTIONS	3	4	5	4	4	4	4	5	4	4	5	4				
1- Hylidae																
1.1- *S. x-signatus*	2	1	5	6	2	8	2	10	1	0	3	0	40	48%	ACC	23.95%
1.2- *H. semilineatus*	0	1	0	0	0	2	3	5	0	0	0	0	11	22%	OCC	6.59%
2- Leptodactylidae																
2.1- *P. falcipes*	0	0	2	9	3	3	9	11	1	1	2	0	41	40%	ACC	24.55%
2.2- *L. vastus*	1	1	1	3	0	1	1	5	0	1	1	0	15	30%	ACC	8.98%
2.3- *L. marmoratus*	1	1	0	0	0	0	1	2	0	1	1	0	7	14%	OCC	4.19%
3- Ranidae																
3.1- *L. palmipes*	3	6	7	10	5	3	7	9	0	2	0	1	53	94%	CO	31.74%
Total	7	10	15	28	10	17	23	42	2	5	7	1	167	-	-	100%
	DRY SEASON		RAINY SEASON						DRY SEASON							

Table 2: Physicochemical data of the water and environmental data of the Gurjaú State Reserve, collected from January to December 2004.

	Month	Location	Depth (cm)	Turbidity (uT)	pH	Chloride (mg/L)	COD (mg/L)	T(°C)	Rainfall
DRY SEASON		+100	42	8.71	6.7	67	180		
	JAN	0	21	8.5	6.9	48	192	24	249.9
		-100	30	8.2	6.9	46	190		
		+100	45	7.6	6.7	75	157		
	FEB	0	22	35.7	6.9	185	1080	23	226.0
		-100	40	35.4	6.5	172	960		
RAINY SEASON		+100	45	7.3	6.7	73	152		168.8
	MAR	0	22	22.8	6.8	178	1078	24	
		-100	44	22.5	6.6	171	957		
		+100	53	7.1	6.9	70	156		378.2
	APR	0	35	20.7	6.8	170	1064	22	
		-100	45	19.9	6.8	167	950		
		+100	60	7.0	6.8	70	154		327.7
	MAY	0	39	32.7	7.0	178	1077	25	
		-100	60	31.7	6.5	171	960		
		+100	98	6.2	6.7	66	157		537.3
	JUN	0	52	28.8	6.9	165	1062	22	
		-100	88	25.7	6.6	159	945		
	JUL	+100	90	5.9	6.7	69	152	18	359.8

		0	46	22.6	6.8	160	1078		
		-100	81	24.4	6.5	158	948		
		+100	92	6.0	6.9	69	157		
	AUG	0	49	98.0	6.9	192	1082	20	138.9
		-100	84	90.1	6.8	188	952		
		+100	95	6.1	6.9	68	154		
	SEP	0	51	35.2	6.7	166	1081	19	81.1
		-100	86	31.0	6.7	160	955		
		+100	91	6.5	6.8	71	152		
	OCT	0	49	22.8	6.6	158	1076	22	33.6
		-100	83	21.6	6.7	148	948		
		+100	65	7.3	6.9	71	156		
DRY SEASON	NOV	0	38	23.1	6.7	152	1066	23	18.2
		-100	51	22.5	6.7	149	941		
		+100	62	7.4	6.8	70	151		
	DEC	0	36	36.8	6.9	162	1061	25	13.2
		-100	49	34.0	6.6	159	952		

Table 3: Physicochemical data of the water and environmental data collected at the three sampling locations from January to December 2004 in Gurjaú River, in the Gurjaú State Reserve, state of Pernambuco, Brazil.

Location	Variable	Mean ± SD
Upstream (+100)	Turbidity	7.31 ± 0.82
	pH	6.75 ± 0.08
	Chloride	70.16 ± 3.43
	COD	159.33 ± 10.30
Waste discharge (0)	Turbidity	24.86 ± 9.38
	pH	6.88 ± 0.07
	Chloride	154.00 ± 52.39
	COD	925.50 ± 359.42
Downstream (-100)	Turbidity	23.90 ± 9.59
	pH	6.65 ± 0.16
	Chloride	147.66 ± 50.03
	COD	827.00 ± 312.12

The analysis showed that the variables turbidity (Figure 3b) and chloride (Figure 3c) varied only between the locations +100 and –100, and +100 and 0; the variable COD varied only between the locations +100 and 0; and the variable pH (Figure 3d) varied only between the locations 0 and -100 (Table 4). We noticed that the area downstream of the site of waste discharge has more turbid water with higher concentration of chloride, i.e., there is evidence that the water characteristics were modified by the waste discharge by COMPESA.

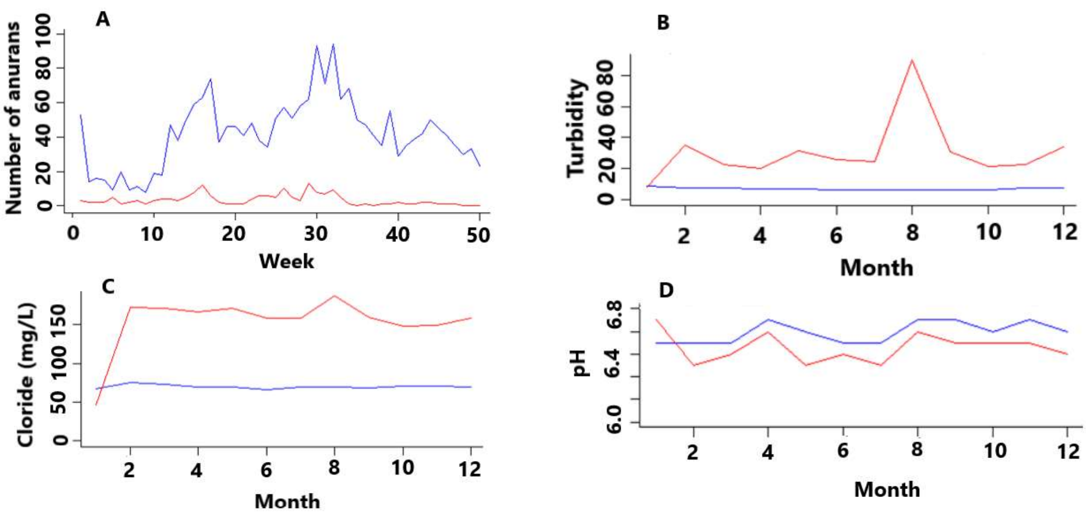

Figure 3: Weekly and monthly variation of anurans and analyzed patterns of water bodies, founded upstream (blue) and downstream (red), in Reserva estadual de Gurjaú at Gurjaú River, Pernambuco state, Brazil: (A) Weekly variation

in the number of anurans (B) Monthly variation in the value of turbidity (uT) r; (C) Monthly variation in the value of chloride (mg/L); (D) Monthly variation in the value of pH.

Table 4: Variation among sampling locations in physicochemical and environmental parameters, from January to December 2004, using Nemenyi and Dunn test, in Gurjaú River, Gurjaú State Reserve, state of Pernambuco, Brazil.

	Turbidity	Chloride	COD	pH
+100 Vs -100	q=624; $q_{0.05(\infty)\ 6}$=4.03	q=2.30; $q_{0.05(\infty)\ 6}$=4.03	-	-
+100 Vs 0	q=720; $q_{0.05(\infty)\ 6}$=4.03	q=3.38; $q_{0.05(\infty)\ 6}$=4.03	q=0.46; $q_{0.05(\infty)\ 6}$=4.03	-
0 Vs -100	-	-	-	q=2.683; $q_{0.05,3}$=2.394

DISCUSSION

The exposure to high levels of contaminants may lead to a decrease in body size in the metamorphosis and a reduction in the egg hatchling rate of anurans (KARASOV et al., 2005). In addition, anurans may develop higher susceptibility to disease and infection (JOHNSON et al., 2002; JOFRÉ et al., 2012). Our results evidenced marked population decreases in the locations downstream of the site of waste discharge, showing that the anurans are excellent bioindicators and that the activities of COMPESA have a strong environmental impact.

Since the waste gets in contact with the environment at the location 0, we can infer that it only influences turbidity, chloride, and pH. These are physicochemical parameters of the water that strongly affect anurans, because they alter physicochemically the micro-habitats used by these amphibians. The physiological vulnerability of anurans to these parameters does not allow adaptation (HENRY, 2000; DIXON et al., 2011; BRAGA et al., 2022). As a result of their early adaptation to the terrestrial environment, anurans do not have lungs able to fulfill their metabolic needs, and so they have developed auxiliary techniques, such as cutaneous respiration. Changes in water pH may also decrease the survival rate, body size, and time until the metamorphosis of some amphibians (ANDRÉN et al., 1988; WARNER et al., 1991; ROWE et al., 1992; KUTKA, 1994), which directly affect the size and population dynamics of anurans (JOFRÉ et al., 2012; BRAGA et al., 2022).

Gas exchange in the epidermis requires high peripheral vascularization and makes anurans dependent on humid environments for respiration; thus, they are extremely vulnerable to variations in abiotic factors, such as those measured in the present study (HENRY, 2000; DEGARADY et al., 2006). Among the substances used in water treatment that could alter biotic and abiotic parameters, we can highlight aluminum sulfate (flocculation), calcium oxide (lime), and chlorine, which potentially occur as ions in the water and are bioavailable to anurans (DEGARADY et al., 2006; BRAGA et al., 2022). The chemical pollution of the environment may also be responsible for the decline of anurans.

CONCLUSIONS

Based on the results of the present study we conclude that the activities of COMPESA directly affect the local low abundance of anurans downstream of the site of waste discharge. Therefore, these activities are highly harmful to the environment, which evidences the need for a change in the method of water treatment used by COMPESA in the Water Treatment Station of Gurjaú, Pernambuco. We need to emphasize that the current method is not only highly impacting, but also brings loss to COMPESA, since using chemical techniques that allow re-using the material used in water treatment (aluminum sulfate, calcium oxide, calcium carbonate, and chlorine) would greatly reduce the operational costs of the company and avoid disposing these compounds in the environment. Our study showed that anurans are excellent bioindicators because their sensitivity may give us important information on water pollution in some sites.

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