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EDITED BY
Natalie Elizabeth Wildermann,
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REVIEWED BY
Raquel Fernanda Salla,
Federal University of Sao Carlos, Brazil
Alain Pagano,
Université d'Angers, France

*CORRESPONDENCE
Mónica Jacinto-Maldonado
monica.jacinto@unison.mx

†These authors have contributed equally to this work and share first authorship

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Batrachochytrium dendrobatidis and *Hannemania* mite's relationships with Mexican amphibians in disturbed environments

Mónica Jacinto-Maldonado[1†*], David Lesbarrères[2†], Eria A. Rebollar[3], M. Delia Basanta[4,5], Belem González-Grijalva[1], Agustín Robles-Morúa[6], Osiris Álvarez-Bajo[7], Efraín Vizuete-Jaramillo[6], Ricardo Paredes-León[8] and Diana Meza-Figueroa[1]

[1]Departamento de Geología, Universidad de Sonora, Hermosillo, Mexico, [2]Environment and Climate Change Canada, National Wildlife Research Centre, Ottawa, ON, Canada, [3]Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico, [4]Department of Biology, University of Nevada Reno, Reno, NV, United States, [5]Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City, Mexico, [6]Departamento de Ciencias del Agua y Medio Ambiente, Instituto Tecnológico de Sonora, Ciudad Obregón, Sonora, Mexico, [7]Departamento de Investigación en Física, Universidad de Sonora, Hermosillo, Mexico, [8]Colección Nacional de Ácaros, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico

The rapid transformation and pollution of ecosystems have severely impacted biodiversity. Specifically, anthropogenic activities have imposed adverse effects on amphibians, with evidence suggesting that these activities alter parasite and pathogen interactions within their hosts. To investigate these interactions in areas affected by different anthropogenic activities, our study focused on analyzing a pathogen and a parasite known to interact within the amphibian skin (spongy epidermis layer) and both compromising amphibian health: *Batrachochytrium dendrobatidis* (*Bd*), a fungus responsible for chytridiomycosis, a disease associated with massive population declines in amphibians and the *Hannemania* sp. mite in Mexico. Four sampling areas along the Sonora River were selected, representing different human activities: mining, livestock, wastewater discharge, agriculture, and one in an urban zone. We analyzed 135 amphibians across 10 anuran species. Among these, the most abundant species (*Lithobates yavapaiensis*) exhibited the highest prevalence of both pathogen and parasite (90.1% and 27.3%, respectively) and was significantly associated with the intensity of *Bd*-infection. The prevalence of *Hannemania* mites varied significantly across sampling sites as did *Bd* prevalence and infection load, with the highest *Bd* load found at the wastewater discharge site. A significant association between the intensity of *Bd*-infection and both mite abundance and amphibian species was observed when the sampling site was considered. Additionally, sites with *Bd*-positive individuals and *Hannemania* parasitism coincide with refractory elements characterized by mechanical or corrosion resistance. The persistence of these elements in the environment, along with the small particle size (<850 nm) found in sediments, poses a potential risk of internalization, bioaccumulation (e.g., Fe, Co, and Ti), and their transfer through the food chain. It is thus essential to consider monitoring environmental and biotic factors that modulate the relationships between parasites, pathogens, and amphibians if we are to propose conservation strategies adapted to disturbed environments.

KEYWORDS

***Batrachochytrium dendrobatidis*, *Hannemania* mites, amphibians, anthropogenic activities, land use change, pollution**

1 Introduction

Anthropogenic activities have had detrimental effects on amphibians (Da Rocha et al., 2020; Yang et al., 2022), and there is compelling evidence that anthropogenic factors drive disease dynamics for these taxa (Becker et al., 2016; Bienentreu and Lesbarrères, 2020; De Andrade Serrano et al., 2022; Haver et al., 2022). Land use changes due to anthropogenic activities (e.g., urbanization, mining, industrialization, and agriculture) have intensified, leading to the over-exploitation, deterioration, and pollution of ecosystems (Archer and Stokes, 2000; Rashid and Romshoo, 2013; Kija et al., 2020). These anthropogenic threats have affected many species and biological processes (Thushari and Senevirathna, 2017; Ukaogo et al., 2020). Among vertebrates, amphibians present the greatest population decline and the greatest risk of extinction (Green et al., 2020; Button and Borzée, 2021). With permeable skin through which they exchange gases, pollutants, or substances, amphibians are more susceptible to changes or diseases than other vertebrate groups (Kaufmann and Dohmen, 2016) and are considered indicators of ecosystem health.

For instance, the pathogen *Batrachochytrium dendrobatidis* (*Bd*), the main causative agent of the disease chytridiomycosis, has resulted in mass mortalities among amphibians globally (Fisher and Garner, 2020). Additionally, the incidence of parasites like *Hannemania* mites has been acknowledged for their impact on amphibians, leading to deformities, loss of chemosensory function, reduced foraging capacity, diminished survival, and decreased reproduction (Anthony et al., 1994; Maksimowich and Mathis, 2000; Jacinto-Maldonado et al., 2016). It is recognized that anthropogenic activities, land-use changes, habitat loss, synergistic effects with pollutants (e.g., high concentrations of heavy metals), and other environmental factors (e.g., climate, altitude) may alter parasite and *Bd* infection dynamics, affecting occurrence rates, spread, transmission, prevalence, infection intensity of *Bd*, and host mortality (DeAlto, 2020; Siddons et al., 2020; Deknock et al., 2022). Both *Bd* and *Hannemania* affect amphibians by persisting and developing within the spongy epidermis layer (Duszynski and Jones, 1973; Stice and Briggs, 2010). During the larval instar of *Hannemania*, mites might transport toxic nanoparticles to the amphibian body (e.g., cerium oxide nanoparticles), potentially altering the parasite-host relationship and the pathogen-host dynamic, thereby increasing mortality, inducing behavioral changes, and inhibiting amphibians' growth (Jacinto-Maldonado et al., 2022).

Mexico is a hotspot of diversity and endemism of amphibian species (Ochoa-Ochoa et al., 2014), and the presence of *Bd* and *Hannemania* parasites has been reported throughout the country including the Sonora state (Hoffmann, 1965; Loomis and Welbourn, 1969; Goldberg et al., 2002; Basanta et al., 2021; Jacinto-Maldonado et al., 2022). The Sonora state is situated at the northern edge of the country, hosting thirty-eight amphibian species (Lemos-Espinal et al., 2015, 2019). In 2014, a spill of 40,000 m^3 of copper sulfate occurred in the Sonora River, and the consequences for wildlife remain unknown (León-García et al., 2018; Molina-Freaner and Martínez-Rodríguez, 2022). Additionally, the Sonora River receives waste such as garbage and wastewater, alongside ongoing anthropogenic activities like livestock rearing, agriculture, and urbanization. The impacts of these activities on the presence and dynamics of pathogens and parasites in amphibians are still poorly understood. Both the fungus and the mite can potentially interact in the spongy stratum of the amphibians; however, the impact of each one may be different and this can be influenced by the biotic and abiotic variables that characterize various types of disturbance. We aimed to analyze parasite and pathogen presence in amphibians (*Hannemania* mites and *Bd*) and their potential interaction with environmental variables where different anthropogenic activities are being developed in this region. We hypothesized that the presence and infection levels of the fungal pathogen *Bd* and *Hannemania* mites will vary depending on host species, the type of disturbance and the environmental variables associated with each study area.

2 Materials and methods

2.1 Study area

Our sampling was carried out in March, April, and July of 2021. Five sampling sites were selected for their anthropogenic activity. Three of them were affected by the copper sulfate spill that occurred in 2014 in Sonora state, Mexico (Figure 1): Bacanuchi (Mining site, closest to the spill site), Bacoachi (Extensive Livestock farming site, the spill did not reach this site), Aconchi (Wastewater discharge

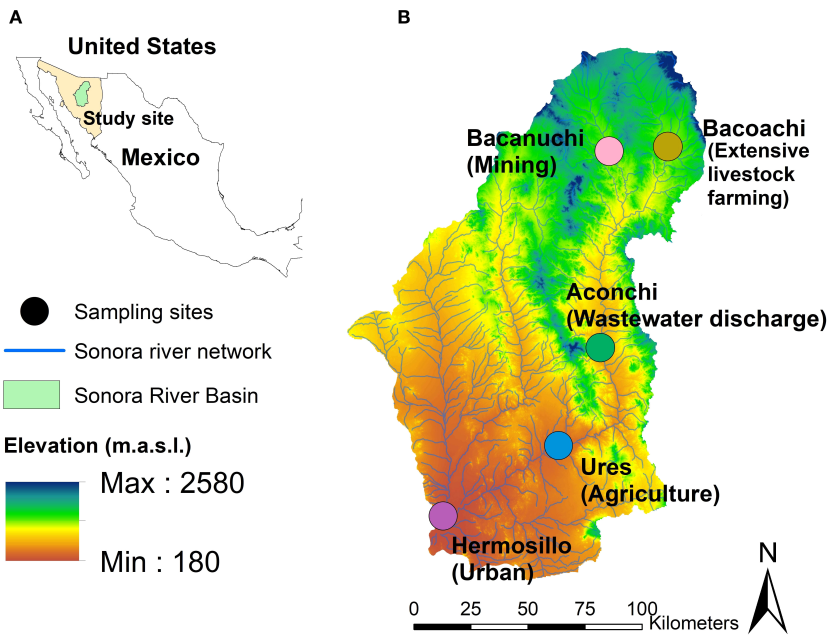

FIGURE 1
(A) Sonora state, Mexico. **(B)** Sampling sites where different anthropogenic activities are developing.

site, site affected by the spill), Ures (Agriculture site affected by the spill), and Hermosillo (Urban site, the spill did not reach this site). Sediment and water quality were analyzed at each sampling site.

2.2 Environmental samples

At each site, soil samples were collected in three areas (two in the flood zone and one in the river). Sediments were analyzed using a portableX-rayfluorescence (PXRF) Niton FXL analyzer (ThermoScientificInc, MA, USA) and PXRF analyses were performed according to the procedures described in US EPA Method 6200. Three measures were implemented to ensure quality control and precision of PXRF measurements (Supplementary Material 1).

Water physicochemical characteristics were analyzed with a multi-parameter (Oakton PCSTestr 35 Impermeable) and the following variables were registered: pH, conductivity, total dissolved solids, and salinity (Supplementary Material 1).

2.3 Amphibian species sampling

The sampling effort for each site equaled 18 person-hours. The amphibians were manually collected wearing new vinyl gloves per each individual. Each specimen was swabbed, weighed, measured, and individuals were identified to species (Lemos-Espinal et al., 2015; Rorabaugh and Lemos-Espinal, 2016). Swabs were taken following Hyatt et al. (2007) protocol, the drink path, thighs, and toes were swabbed (5 times), and then the swab was preserved in liquid nitrogen and stored at −80° in the laboratory. Morphological variables such as malformations, injuries, or erythema (skin reddening) were also recorded (Supplementary Material 2). All individuals were released after sampling. Amphibians were collected under a scientific collector permit (SPARN/DGVS/02985/23) from the Secretaría de Medio Ambiente y Recursos Naturales.

2.4 *Bd* detection

DNA extraction was conducted with a Qiagen DNA extraction kit and real-time PCR was conducted as described in Boyle et al. (2004). All samples were analyzed in duplicate. Standards of DNA synthetic fragments (gBlocks, Integrated DNA Technologies) of 1, 10, 100, and 10,000 internal transcribed spacer (ITS) *Bd* equivalent copies were estimated to know ITS copies of *Bd* in each swab. Samples were considered positive if an exponential amplification curve was generated in both replicates. When one replicate was negative, a third replicate was run to determine the infection status of the sample.

2.5 Mite detection and taxonomic identification

After amphibian sampling (swab, weight, identification), amphibians with mites were anesthetized using an immersion bath of isoflurane (100%) (Doss et al., 2021) before mite removal. Subsequently, the area was disinfected with an hyper-oxidase solution, and individuals remained in disinfected containers until release (max. 5 mins). No individual died during this procedure. Once removed, mites were counted and preserved in 70% and 100% ethanol. Mites were cleared with lactophenol and then mounted them with PVA medium in semi-permanent microscope slides. Using the keys by Brennan and Goff (1977) and Hoffmann (1990), taxonomic identification was made. The mites were collected under a scientific collector permit (SGPA/DGVS/05384/22) from the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT) and were deposited at the Colección Nacional de Ácaros (CNAC), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico.

2.6 Statistical analysis

The amount of sediment composition variance within and among sampling sites was determined using boxplots and a linear Discriminant Analysis (LDA) to model the environmental data (sediment composition and water quality). Five classes based on the environmental variables of each sampling site were delimitated and 20% of samples in each category were chosen to conduct a cross-validation test (Balakrishnama and Ganapathiraju, 1998; Izenman, 2013).

A Canonical Correspondence Analysis (CCA) was used to investigate the relationships among matrices of amphibian species, amphibians positive for *Bd*, and amphibians parasitized by *Hannemania* mites and a set of concomitant sediment and water variables. The CCA provided a direct gradient analysis of amphibian species, amphibians parasitized by *Hannemania* mites, and amphibians positive for *Bd* relative to the underlying gradients within the measured environmental variables. The derived axes are linear combinations of environmental variables so that amphibian species, amphibian species parasitized or *Bd* positive were directly related to these axes under the assumption of unimodal amphibian species, amphibian parasitized or *Bd* positive response to environmental variables. The significance of the relationships between the parameters and the canonical axes was tested by permutational multivariate analysis of variance (PermANOVA).

To estimate and analyze the differences in *Bd* and *Hannemania* prevalences (as the proportion of infected individuals per population with 95% confidence intervals) among sampling sites, the prop.test function was used. Additionally, a Kruskal-Wallis test was carried out to analyze the differences in *Bd*-infection load (log_{10} transformation was made) among sampling sites. Both analyses were done through RStudio version 4.1.3 and in the R Stats Package (R Core Team, 2021; Basanta et al., 2022) (www.R-project.org). Additionally, a multiple regression analysis was used to identify associations between the intensity of *Bd*-infection (average of ITS copies of *Bd* in each swab of each individual analyzed), and the sampling site, the abundance of mites, and amphibian species. All analyses were done in the statistical environment R version 4.1.3 (www.R-project.org) using the vegan package (Oksanen et al., 2019).

3 Results

3.1 Amphibian species found in the sampling sites

In total, 135 amphibians across 10 species and 7 families were sampled: *Anaxyrus woodhousii, A. punctatus, Gastrophryne mazatlanensis, Lithobates magnaocularis, L. yavapaiensis, Incilius alvarius, I. mazatlanensis, Spea multiplicata, Scaphiopus couchii*, and *Smilisca fodiens*. *Scaphiopus couchii* was observed in all sampling sites. *L. yavapaiensis* and *Smilisca fodiens* were present in three out of five sites with more individuals found in Aconchi. *L. magnaocularis* was present in three out of five sites. Four amphibian species were observed in only one sampling site: *A. woodhousii, A. punctatus, I. alvarius*, and *S. multiplicate* (Figure 2; Supplementary Material 2).

3.2 *Bd* prevalence and infection intensity

Three amphibian species were positive for *Bd*: *L. yavapaiensis, S. couchii*, and *G. mazatlanensis*, the former showing the highest prevalence of 90.1% among all individuals. *Bd* was recorded at three sites with the highest number of *Bd*-positive individuals observed in Aconchi where wastewater discharges were constant (Table 1). Three *Bd*-positive individuals of *L. yavapaiensis Bd*-positives had erythema in Aconchi while two negative individuals of *Anaxyrus woodhousii* had erythema in Bacoachi (Supplementary Material 2).

The prevalence of *Bd* among sampling sites was significantly different (X^2 = 30.937, df = 4, p-value = 3.154e-06) as was *Bd*-infection load (Kruskal-Wallis X^2 = 9.867, df = 2, p-value = 0.007). A higher number of individuals with high *Bd*-infection load were observed in Aconchi and Bacoachi. At Aconchi 75% of individuals had a higher *Bd*-infection load range as compared to Bacoachi where just a few individuals showed a high *Bd*-infection load. No infected individuals were observed at Ures and Hermosillo and only 2 individuals were *Bd*-positive at Bacanuchi (Figure 3).

3.3 *Hannemania* mites' presence and prevalence

Three amphibian species were positive for *Hannemania* mites: *L. yavapaiensis, S. couchii*, and *S. fodiens* with the former having the highest prevalence (Table 2). The prevalence of *Hannemania* mites

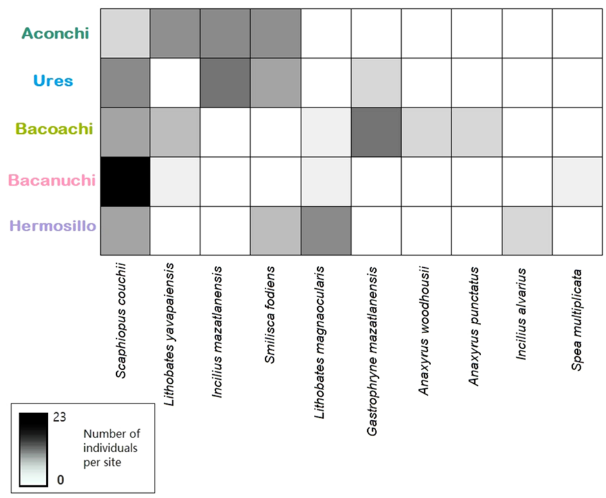

FIGURE 2
Amphibian species and the number of individuals per site.

TABLE 1 *Bd* prevalence per amphibian species at each sampling site.

Amphibian species	N	*Bd* prevalence (%) per species	Bacanuchi (Mining)	Bacoachi (Extensive Livestock Farming)	Aconchi (Wastewater discharge)	Ures (Agriculture)	Hermosillo (Urban)
A. punctatus	2	0	0	2 (0 +) 0%	0	0	0
A. woodhousii	2	0	0	2 (0 +) 0%	0	0	0
G. mazatlanensis	13	7.69	0	11 (1 +) 9.09%	0	2 (0 +) 0%	0
I. alvarius	2	0	0	0	0	0	2 (0 +) 0%
I. mazatlanensis	17	0	0	0	8 (0 +) 0%	9 (0+) 0%	0
L. magnaocularis	14	0	1 (0 +) 0%	1 (0 +) 0%	0	0	12 (0+) 0%
L. yavapaiensis	22	90.91	1 (0 +) 0%	6 (6 +) 100%	15 (14 +) 93.33%	0	0
S. couchii	47	4.26	23 (2 +) 8.70%	5 (0 +) 0%	2 (0 +) 0%	12 (0 +) 0%	5 (0 +) 0%
S. fodiens	15	0	0	0	6 (0 +) 0%	5 (0 +) 0%	4 (0 +) 0%
S. multiplicata	1	0	1 (0 +) 0%	0	0	0	0

Bd prevalence of each amphibian species is expressed as the number of hosts Bd-positive divided by the number of hosts examined. N = number of individuals analyzed for Bd, (+) the number of Bd-positive amphibians, %. prevalence in percentage.

was different among sampling sites ($X^2 = 16.682$, df = 4, $p = 0.002$) with the highest prevalence at Bacoachi (Table 2).

3.4 Environmental variation

Bacanuchi and Aconchi showed the lowest water quality levels (higher conductivity values, salinity, and total dissolved solids). Additionally, the lowest pH value (<6.5) was recorded in Aconchi while the highest value was found in Ures (>8; Figure 4). There were also differences among sampling sites in sediment composition. Except for a few outliers, Sr, Cu, As, Zn, Mn, Sb, and Ca had the highest values in Bacanuchi (mining area). The highest concentrations of Cl, Rb, Fe, K, Nb, Y, Co, and Ti were found in Bacoachi (livestock area). In Aconchi (wastewater discharge area), V and Pb had the highest concentrations. By contrast, all elements showed low values In Ures as compared to other sites (Figure 4).

The LDA indicated a good fit of the data (accuracy, sensitivity, and specificity); 96% (n= 169) of all our environmental data analyzed (n= 176) were classified correctly in one of the five classes based on the environmental variables of each sampling site. Bacanuchi and Bacoachi were well segregated (sampling sites with less environmental similarities) while Hermosillo, Ures, and Aconchi showed some overlap (Figure 5).

3.5 Interaction among the intensity of *Bd*-infection versus the abundance of mites, the amphibian species, and the sampling site

While sampling site was not a significant predictor of *Bd*-intensity ($r^2 = 0.06$, $p = 0.99$, df = 4), we found a significant interaction between *Bd*-infection and the abundance of mites ($r^2 = 2.63$, $p = 0.03$, df = 4), and the amphibian species ($r^2 = 21.64$, $p = 0.01$, df = 3) with our model explaining 63.71% of the variation in the multiple regression analysis. In particular, the intensity of *Bd*-infection was associated with the presence of *L. yavapaiensis* ($p = 0.01$).

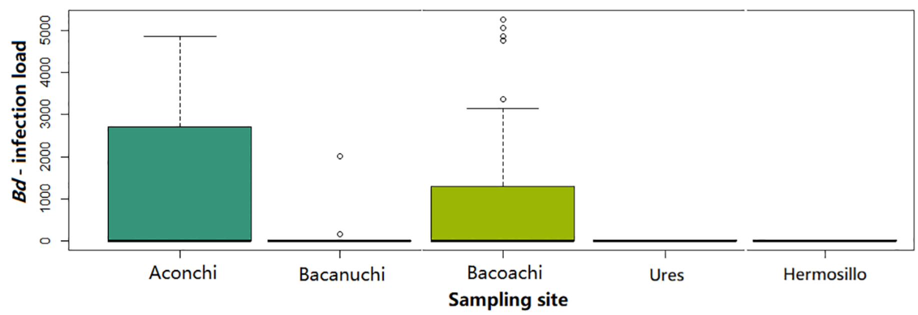

FIGURE 3
Bd-infection load (number of ITS copies of *Bd*) among sampling sites.

TABLE 2 *Hannemania* mites' prevalence per amphibian species at each sampling site.

Amphibian species	N	*Hannemania* prevalence % per species	Bacanuchi (Mining)	Bacoachi (Extensive Livestock Farming)	Aconchi (Wastewater discharge)	Ures (Agriculture)	Hermosillo (Urban)
A. punctatus	2	0	0	2 (0 +) 0%	0	0	0
A. woodhousii	2	0	0	2 (0 +) 0%	0	0	0
G. mazatlanensis	13	0	0	11 (0 +) 0%	0	2 (0 +) 0%	0
I. alvarius	2	0	0	0	0	0	2 (0 +) 0%
I. mazatlanensis	17	0	0	0	8 (0 +) 0%	9 (0+) 0%	0
L. magnaocularis	14	0	1 (0 +) 0%	1 (0 +) 0%	0	0	12 (0+) 0%
L. yavapaiensis	22	27.27	1 (1 +) 100%	6 (5 +) 83.33%	15 (0 +) 0%	0	0
S. couchii	47	2.13	23 (0 +) 0%	5 (1 +) 20%	2 (0 +) 0%	12 (0 +) 0%	5 (0 +) 0%
S. fodiens	15	6.67	0	0	6 (1 +) 16.67%	5 (0 +) 0%	4 (0 +) 0%
S. multiplicata	1	0	1 (0 +) 0%	0	0	0	0

Hannemania prevalence of each amphibian species is expressed as the number of hosts infected with one or more individuals of Hannemania mites divided by the number of hosts examined. N = number of individuals analyzed for mites, (+) the number of positive individuals.

3.6 Association of *Bd* and *Hannemania* mites with environmental variables

Both axes of the CCA explained 65.9% of the variance of environmental variables recorded in sediments and water (26.4% and 39.5% for axes 1 and 2 respectively). Environmental variables had significant effects on amphibian species, amphibians *Bd*-positive, and amphibians parasitized by *Hannemania* mites (all $p < 0.05$). In particular, both *Bd*-positive and *Hannemania*-parasitized amphibians were associated with two sampling sites (Bacoachi and Aconchi), the distribution of four amphibian species (*L. yavapaiensis*, *G. mazatlanensis*, *A. woodhousii*, and *A. punctatus*) and the signature of Ti, V, Y, Nb, Zr, Rb, Fe and Co (Figure 6).

4 Discussion

To our knowledge, this is the first investigation of the presence and interaction of the fungal pathogen *Bd* and the *Hannemania* mites in disturbed environments in amphibians. Among sites with different types of perturbation, our results suggest that the prevalence of *Bd* and H*annemania* mites were associated with the presence of four amphibian species and coincided with refractory elements characterized by mechanical or corrosion resistance. Moreover, we observed a positive association between the intensity of *Bd*-infection and the abundance of *Hannemania* mites in *L. yavapaiensis*. Such co-infection highlights the importance of monitoring environmental and biotic factors that modulate the relationship between parasites, pathogens, and hosts in transformed and polluted environments for future conservation strategies.

Aconchi exhibited the highest *Bd* infection load, coinciding with the presence of wastewater discharge at that site, aligning with previous studies that highlighted the association between low water quality or water pollution, particularly wastewater discharges, and *Bd* presence, prevalence, and infection load (Battaglin et al., 2016; Congram et al., 2022; Jacinto-Maldonado et al., 2023). By contrast, Hale et al. (2005) did not detect *Bd* in specimens collected in Aconchi in 2000, suggesting that *Bd* arrived recently or that changes in environmental conditions, such as water pollution, might have influenced the presence and *Bd* infection load in the region. Moreover, *L. yavapaiensis* showed the highest intensity of infection of *Bd* among amphibian species, with 23.08% of positive individuals showing erythema. The lowland leopard frog is listed as special protection with a declining population (NOM-059-SEMARNAT, 2010; IUCN, 2023) and further investigation should test the hypothesis that *L. yavapaiensis* acts as a reservoir or carrier of *Bd* in these sites and a potential threat to other susceptible amphibians (Miaud et al., 2016).

Among all species, *Bd* was also observed in *G. mazatlanensis* and *S. couchii* for the first time. In Sonora state, *Bd* has been previously reported in seven amphibian species (*L. yavapaiensis, L. magnaocularis; Leptodactylus melanonotus; Agalychnis dacnicolor; Lithobates tarahumarae; Smilisca fodiens; Lithobates pustulosa*) based on museum (Hale et al., 2005; Basanta et al., 2021) and live specimens from the Northern Jaguar Reserve and the locality of Tesopaco (Zamora-Bárcenas et al., 2012). Previous reports also indicated the presence of *Bd* in *L. yavapaiensis* in nearby Arizona, United States. While we observed a prevalence of 90.1% in this species, other studies reported higher (93%) and lower (43% and 1.6%) prevalences in Sonora (Schlaepfer et al., 2007; Savage et al., 2011). In addition, there are studies of *L. yavapaiensis* in Sonora with no information on prevalence (Bradley et al., 2002; Hale et al., 2005). Variations in prevalence, susceptibility, and vulnerability of amphibians to *Bd* infection could be linked to skin microbiome richness and composition, host life-history traits, phylogeny, morphology, physiology, gene expression, immune response, and resistance (Ortiz-Santaliestra et al., 2013; Eskew et al., 2018; Varela et al., 2018; Zamudio et al., 2020).

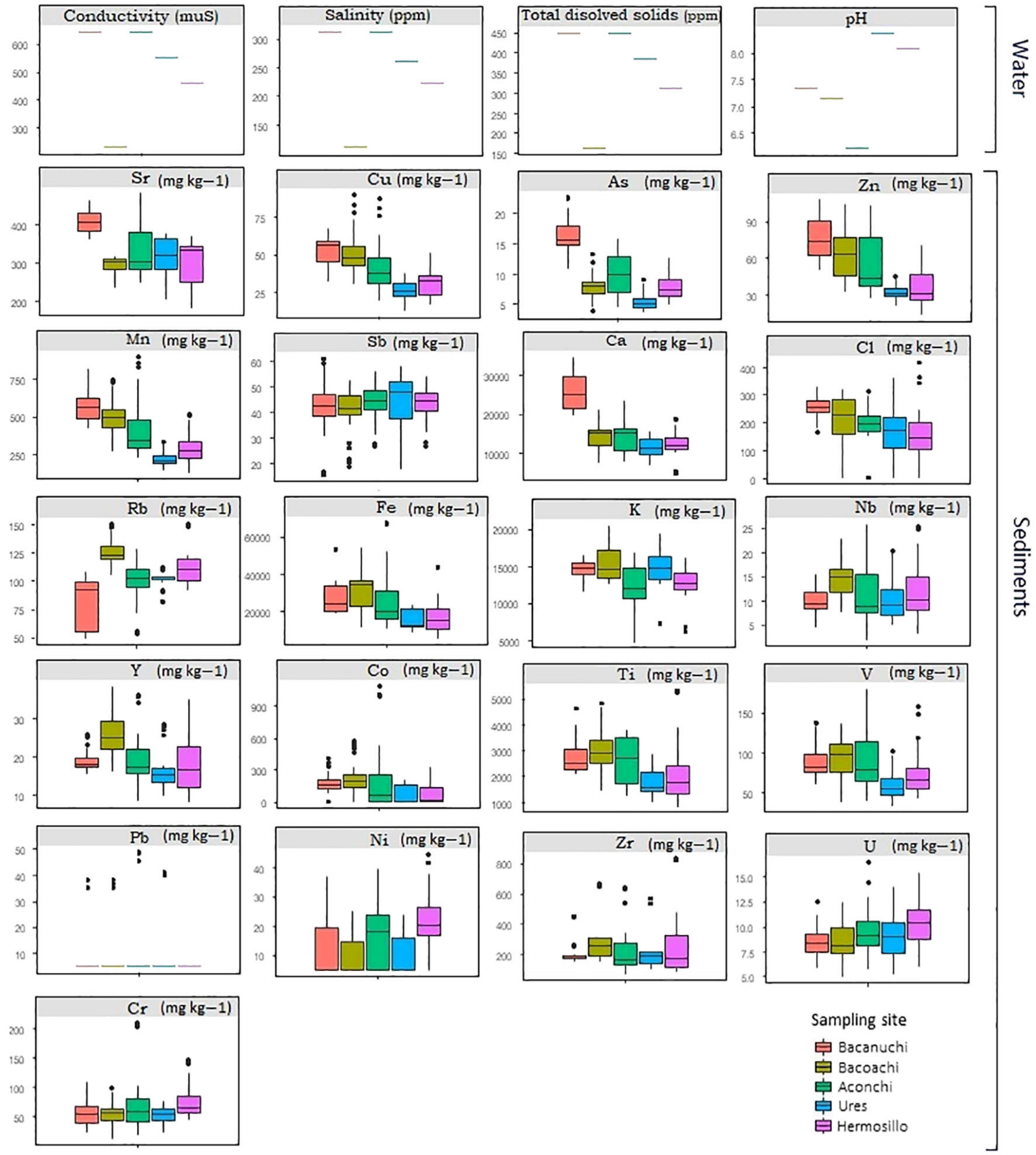

FIGURE 4
Water quality and sediment samples at each sampling site.

Yet chytridiomycosis-related symptoms were only observed in three *Bd*-positive lowland leopard frogs (erythema in the ventral region) so more fieldwork, and experimental studies are imperative to better understand infection in this species.

The lowland leopard frog also showed the highest prevalence of *Hannemania* mites (27.27%), followed by *S. fodiens* (6.67%) and *S. couchii* (2.13%). The susceptibility of amphibians to *Hannemania* mites and their infestation rates have been associated with host size, behavior, microhabitat use, sex, the exposure time to the chiggers as well as environmental variables such as high humidity, high air temperature, proximity to water bodies, neutral and alkaline-pH water and areas with low canopy cover (Rankin, 1937; Jung et al., 2001; Wohltmann et al., 2006; Hatano et al., 2007; Alvarado-Rybak et al., 2018; Jacinto-Maldonado et al., 2020). Previous studies reported a higher prevalence of mites in *L. yavapaiensis* in the area (71.42%; Jacinto-Maldonado et al., 2022) suggesting interannual variation associated with environmental factors such as precipitation or temperature. This species was also the only amphibian species coinfected with *Bd* and *Hannemania* mites, and the only species with a significant relationship with the intensity of *Bd*-infection, suggesting a possible association between parasite-pathogen in this amphibian species. For instance, *Hannemania* mites might more readily infiltrate the stratum corneum and granulosum of the skin of *Bd*-positive amphibians due to skin

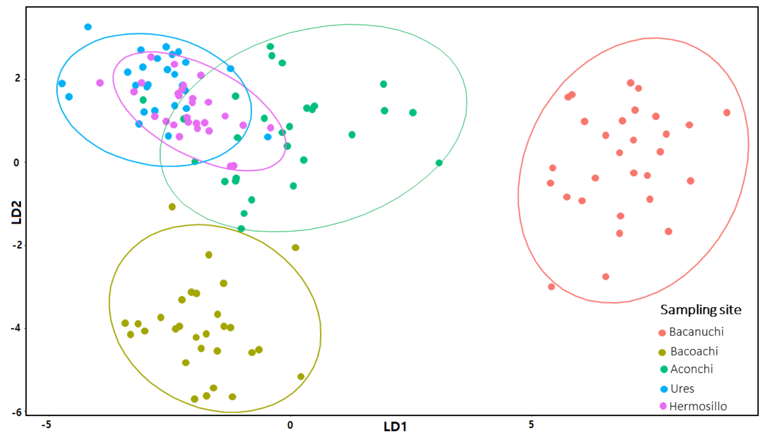

FIGURE 5
Linear Discriminant Analysis (LDA) of environmental variables at sampling sites. LDA1 = 39.5% and LDA2 = 26.4%.

damage, shedding, or ulcerations (Pessier, 2002; Berger et al., 2005). We also present the first report of *Hannemania* mites in *S. fodiens*. *S. couchii* has been recorded as the host of *Hannemania hylae* in Alamos, Sonora in 1943, but no information about its prevalence is available (Hoffmann, 1990). Among sites, Bacoachi presented the highest *Hannemania* prevalence, a cause for concern due to prior studies reporting toxic particles in sediments in this area. *Hannemania* mites have been identified as vectors of CeO_2 and TiO_2 particles (Jacinto-Maldonado et al., 2022). In amphibians, CeO_2 particles can result in high mortality, growth inhibition, and genotoxic effects, while TiO_2 particles may induce hormone disruption (thyroxine and triiodothyronine), cellular stress, decreased survival, altered growth, and cellular metabolism, as well as tissue damage (Zhang, 2011; Zhang et al., 2012; Hammond et al., 2013; Bour et al., 2015; Galdiero et al., 2017; Vijayaraj et al., 2018).

Our results also highlight that sites with a high prevalence of *Bd* or *Hannemania* mites, exhibit elevated levels of Fe, Ti, Co, Zr, V, Rb, Y, and Nb in sediments, particularly if *L. yavapaiensis*, *G. mazatlanensis*, *A. woodhousii*, and *A. punctatus* are present at these sites. These aforementioned elements, characterized as refractory elements renowned for their mechanical or corrosion resistance, find application in various sectors like alloys, ceramics, paints, and coatings (Balazic et al., 2007; Lodhi et al., 2008; Karimzadeh et al., 2019; Meza-Figueroa et al., 2020). Their extended persistence in the environment and the observed particle size (e.g. <850 nm) increase the risk of internalization, bioaccumulation (e.g. Fe, Co, and Ti), and potential transfer through the food chain, thus impacting aquatic and terrestrial ecosystems, thereby necessitating a more detailed analysis of particles (e.g. chemical composition, charge, surface structure, aerodynamic size, morphology) as well as periodic studies in

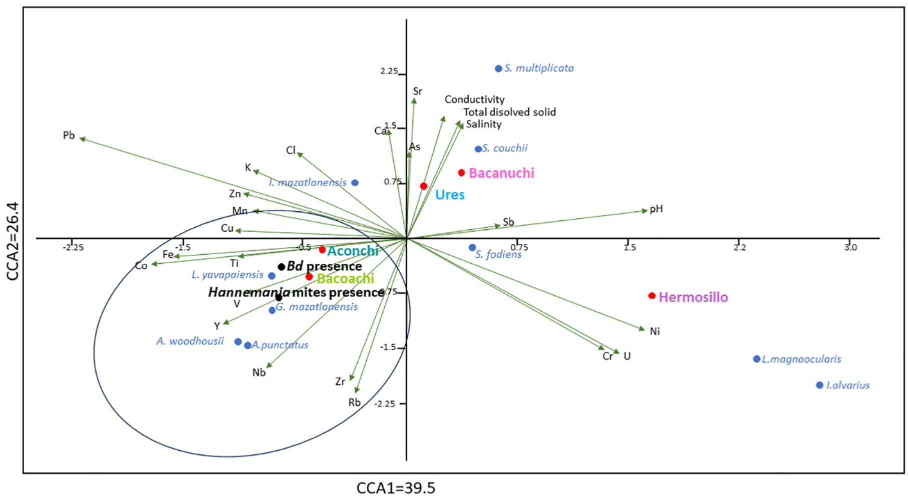

FIGURE 6
Canonical correspondence analysis of the variance of biotic variables (amphibian species, amphibians *Bd*-positive, and amphibians parasitized by *Hannemania* mites) due to environmental variables recorded in sediments and water. Sediments and water quality variables are in black, sampling site names are in color, and amphibian species are in blue. Amphibians positive for *Bd* and parasitized by *Hannemania* mites are in bold letters.

these areas (Gál et al., 2008; Fabrega et al., 2011; Hammond et al., 2013; Jacinto-Maldonado et al., 2022; Esteves-Aguilar et al., 2023). With 38 amphibian species reported in Sonora and 21 amphibian species, including six endemics and three under special protection in Aconchi and Bacoachi, the impact of pollution and its association with the disease should be considered (NOM-059-SEMARNAT, 2010; Lemos-Espinal et al., 2019; IUCN, 2023; Naturalista, 2023).

Anthropogenic activities negatively impact and put at risk ecosystems and the species that live in them. Given the richness of amphibian diversity and the impact of anthropogenic activities in Sonora state and specifically in our study sites, continuous monitoring of environmental conditions, particularly water and sediment pollution should be pursued to understand better parasite-pathogen coinfections as well as better protect amphibian diversity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Secretaría de Medio Ambiente y Recursos Naturales. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MJ-M: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. DL: Conceptualization, Investigation, Resources, Writing – review & editing. ER: Data curation, Formal analysis, Methodology, Writing – review & editing, Resources. MB: Conceptualization, Methodology, Resources, Writing – review & editing. BG-G: Data curation, Formal analysis, Resources, Writing – review & editing. AR-M: Formal analysis, Methodology, Resources, Writing – review & editing, Data curation. OÁ-B: Formal analysis, Methodology, Writing – review & editing. EV-J: Methodology, Writing – review & editing, Data curation, Investigation. RP-L: Writing – review & editing. DM-F: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/famrs.2024.1372993/full#supplementary-material

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