Mites as a Potential Path for Ce-Ti Exposure of Amphibians

Mónica Jacinto-Maldonado1, Diana Meza-Figueroa1*, Martin Pedroza-Montero2*, David Lesbarrères3, Agustin Robles-Morúa4, Sofía Navarro-Espinoza5, Belen González-Grijalva5, Efrén Pérez-Segura1, Erika Silva-Campa6, Aracely Angulo-Molina6 and Ricardo Paredes-León7

1Departamento de Geología, Universidad de Sonora, Hermosillo, Mexico, 2Departamento de Investigación en Física, Universidad de Sonora, Hermosillo, Mexico, 3Environment and Climate Change Canada, Sudbury, ON, Canada, 4Departamento de Ciencias del Agua y Medio Ambiente, Instituto Tecnológico de Sonora, Ciudad Obregón, Mexico, 5Programa de Doctorado en Nanotecnología, Universidad de Sonora, Hermosillo, Mexico, 6Departamento de Ciencias Químico-Biológicas, Universidad de Sonora, Hermosillo, Mexico, 7Colección Nacional de Ácaros, Instituto de Biología, Universidad Nacional Autónoma de, Mexico city, Mexico

Despite the documented effects on human and animal health, particles smaller than 0.1 µm in diameter found in soils, sediments, and the atmosphere remain unregulated. Yet, cerium and titanium oxide nanoparticles associated with traffic increase mortality, cause behavioral changes, and inhibit the growth in amphibians. Mites of the genus Hannemania spend their early stages in the soil before becoming exclusive parasites of amphibians. Unlike other mites, Hannemania is found inside the epidermis of amphibians, thus facilitating the intake of particles, and leading to direct and chronic exposure. To better understand this exposure path, we sampled amphibians hosting mites in a river potentially polluted by traffic sources. Particles collected from mites were studied by scanning electron microscopy and Raman spectroscopy while sediment samples were analyzed for total metal content by portable X-ray fluorescence. Our results indicate that sediment samples showed significant correlations between elements (Zr, Mn, Ti, Nb, Fe) often associated with components in catalytic converters and a level of Zr that exceeded the local geochemical background, thus suggesting an anthropic origin. Furthermore, particles adhered to mites exhibited the characteristic Raman vibrational modes of ceria (CeO2, 465 cm⁻¹), ceria-zirconia (CeO2-ZrO2, 149, 251, and 314 cm⁻¹), and rutile (TiO2, 602 cm⁻¹), pointing out to the deterioration of catalytic converters as the most likely source. This research highlights both the importance of unregulated catalytic converters as a source of ultrafine Ce-Ti particle pollution and the role of sub-cutaneous mites as a vector of these particles for amphibian exposure.

Keywords: mites, amphibian, vehicle emissions, ultrafine particles, cerium, titanium

1 INTRODUCTION

The global amphibian population decline is estimated at 3.79% per year (USGS, 2021). Together with freshwater fish, amphibians are the most endangered class among the vertebrate groups, with a functional loss of 27% (Toussaint et al., 2021). Causes for these declines are diverse including habitat destruction (Calderón et al., 2019; Borzée et al., 2021), disease (Kriger and Hero, 2009; Herczeg et al., 2021), exposure to pesticides and chemicals (Pinto-Vidal et al., 2021; Goessens et al., 2022), and
climate change (Velasco et al., 2021; Villamizar-Gomez et al., 2021). Even in natural protected areas, the steady decline of amphibian populations calls for more studies to be undertaken in pristine or rural areas to assess the extent of the threats worldwide (Green et al., 2020).

Amphibians are indicator species of ecosystem stress, as they are twice as sensitive to environmental factors due to their biphasic phases (Leduc et al., 2016; Velasco et al., 2021). Early life-history stages of semiaquatic or fully aquatic amphibians develop in water bodies where pollutants from urban runoff, vehicular traffic, agriculture, or mining effluents are common (Pounds et al., 2006; Brühl et al., 2013; Sievers et al., 2019). Under such environmental stress, amphibians may modify their behavior and physiology (Wong and Candolin, 2015), causing erratic swimming and altering their survival, growth, and reproduction (Sievers et al., 2018). For instance, in mining environments, high levels of copper (Cu) increase tadpole time spent swimming at the water surface (Hayden et al., 2015; Azizishirazi et al., 2021). Consequently, amphibians living in Cu-polluted environments show a higher-level of mortality (Azizishirazi et al., 2021) with disruptions in thyroid hormone metabolic pathways during metamorphosis (Thambirajah et al., 2019). In addition, Amphibians exposed to intensive vehicle traffic have a significant reduction in body size, body condition, and lower corticosterone concentrations compared to unexposed individuals (Cayuela et al., 2017). Yet, our understanding of the indirect effects associated with toxic exposure resulting from traffic on Amphibian life stages is still limited.

Catalytic converters have exhaustive and non-exhaustive emissions, the latter emissions associated with traffic are released from the catalytic converters, leading to contamination into soils and sediments (Meza-Figueroa et al., 2021). Chemical elements (Zr and Ce) used to manufacture three-way catalytic converters (TWCC) have low crustal abundances making the associated compounds valuable tracers of such sources in the environment (Meza-Figueroa et al., 2021; Navarro-Espinoza et al., 2021). A TWCC contains a honeycomb structure cordierite (2MgO·2Al₂O₃·5SiO₂) monolith, with a coating made of cerium oxide (CeO₂ or nanoceria) and zirconium oxide (ZrO₂ or zirconia). A layer of platinoid nanoparticles, generally platinum or palladium, covers the Ce-Zr’s refractory washcoat (Aruguete et al., 2020). A typical TWCC operates at temperatures up to 800°C that can cause its deterioration and the potential release of platinoids and ZrO₂·CeO₂ compounds into the environment (Rinkovec, 2019; Meza-Figueroa et al., 2021) leading to the TWCC deactivation. Catalytic converters can then expel ultrafine particles (UFP) of compounds (ZrO₂-CeO₂) that are less than 0.1 µm in diameter and may be deposited in soils and water sources which in turn become a direct

![FIGURE 1](A) Map of the study site, (B) the location of the Cananea mining area (Bacanuchi river), and (C) Bacoachi site and town.
exposure path for living organisms. While this issue is assessed by regulatory vehicle emission policies in major cities, such policies are lacking in rural areas making UFP released from the TWCC refractory washcoat impossible to prevent.

Mites are a good model for assessing the pollution of both aquatic and terrestrial ecosystems due to their close relationship with environment, high abundance, high diversity of species, their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabit (Walter and Proctor, 2013; Goldschmidt, 2016). Oribatid mites have often been used as bioindicators of soil contamination and toxicity (Eeva and Goldschmidt, 2016). This characteristic is attributed to their diet made of fungal hyphae (primary food resource), which accumulates heavy metals, and to their ability to regulate concentrations of metals through low intestinal absorption and rapid excretion (Skubala et al., 2019; Azizishirazi et al., 2021). Information about their cosmopolitan distribution, and the wide range of environments they inhabi...
point). All samples were collected with a stainless-steel shovel and kept in high-density plastic bags. Samples were homogenized and dried in an oven at 37°C for 24 h, then sieved through #18, 35, 60, and 120 mesh to obtain particulate matter with diameters of 51 mm, 500 μm, 250 μm, and 20 μm, respectively, for analysis by a portable X-ray fluorescence equipment.

Seven individuals of Lowland leopard frog, *Lithobates yavapaiensis* (Platz and Frost, 1984) were collected from the riverbank near Bacoachi town but we did not find any amphibians at the Bacanuchi site. Lowland leopard frogs are common in our study area and could thus represent a sentinel species for this pilot study. Amphibians were manually collected wearing vinyl gloves. Each specimen was weighed, measured, and inspected for mites. Specimens hosting mites (*n* = 5) were anesthetized with isoflurane and immersion baths of gel and water before removing the mites following procedures described in Doss et al. (2021). Mites were carefully extracted using a microscope and a small needle by opening the intradermal cation and extracting the parasites. Mites were counted and preserved in 100 and 70% ethanol. They were further cleared with lactophenol and then mounted with PVA medium in a semi-permanent microscope slide for taxonomic identification (Hoffmann, 1990; Krantz and Walter, 2009). Following removal of the mite(s), the area was disinfected with hyper oxidation solution, and Lowland leopard frogs remained in observation for 10 min until released at the same location they were found. No individuals were lost or injured in this procedure.

All animal capture and handling protocols for the scientific purpose at national territory were approved by the Secretariat of the Environment and Natural Resources in Mexico (SEMARNAT Permit No: FAUT-0027). The experimental procedure for mite’s collection permit was approved under permit: SGPA/DGVS/04418/21. All specimens were deposited in the National Mite Collection (CNAC) at the Biology Institute of the National Autonomous University of Mexico with access number CNAC012393-CNAC012402.

### 2.3 Total Metal Content in Sediments

We used a portable X-ray fluorescence NitonTM FXL 950 instrument (ThermoFisher Scientific Inc., Boston, Massachusetts, U.S.) with an X-ray tube: silver anode, 50kV/200 μA/4W, and a geometrically optimized area drift detector (GOLDD). We analyzed soil samples using the TestAllGeo mode for a fixed period of 120 s. We used seven replicates of the reference material standard NIST 2710a and a blank made of pure SiO₂ for quality control following method 6200 (United States Environmental Protection Agency). Our pilot study was restricted to analyzing the elements: Zr, Sr, Rb, Pb, As, Ce, Zn, Cu, Ni, Co, Mn, Cr, V, Ti, K, Sb, Nb, Y, Fe, and Ca. Each sample was analyzed in triplicate, and we reported the recovery range from 100 to 110% for all analyzed elements, but Ce whose concentration was below the detection limit. The detection of Ce was obtained from Raman and SEM analysis.

### 2.4 SEM and Raman Analysis in Mites

Mites were analyzed both superficially and internally after the dissection. We obtained the particle size, semiquantitative chemical analysis, and morphology of UFP using scanning electron microscopy and energy dispersive spectroscopy (SEM-EDS) Phenom ProX desktop (ThermoFisher Scientific Inc., Boston, Massachusetts, U.S.). We used the analytical conditions of 5 kV for particle morphology and at 15kV for chemical compositions by EDS. We used an Alpha300 RA Raman confocal microspectrometer (WITec, Ulm, Germany) to identify crystalline particles in mites. The instrument has a Nd:YAG frequency-doubled laser excitation of 532 nm. We resuspended the samples using ethanol and placed them on a calcium fluoride substrate for 2h in a desiccator (CaF₂, 13 mm Ø × 1.0 mm, Crystan Ltd.,United Kingdom). We used a cubic zirconia solid standard (ASTIMEX MINM25-53 Serial 1Al) for checking the instrument performance of both Raman and SEM.

### 2.5 Multivariate Statistical Analysis

We used a Principal Component Analysis (PCA) to evaluate the chemical differences among sediments collected within the mining area and our study site where amphibians and mites were sampled. PCA has been employed for source apportionment of metals (Shi et al., 2022) and to assess chemical differences between used and new TWCC (Navarro-Espinoza et al., 2021). The suitability of the dataset was evaluated by the Kaiser-Meyer-

![FIGURE 2](image-url) Comparison of Ti and Zr contents in sediments from the study site of Bacochi (where amphibians were collected) and the mining area. Local geochemical background is shown for reference as the red dotted line (after Calmus et al., 2018). The box plots show the average, maximum, minimum, and median values (red cross) for each metal in the <20 μm fraction of sediments of our study.
Olkin (KMO) and Bartlett’s test of sphericity. The obtained KMO (0.732) value was >0.7 and we considered a Spearman correlation coefficient >0.5 as significant to determine a common source of metals (Liu et al., 2020). All statistical analyses were performed using XLSTAT 2021.4.1 (Addinsoft, 2022).

3 RESULTS

3.1 Total Metal Content in Sediments

We observed a range of total metal content in sediments across the mining and our study sites (Table 1). The elements that exceeded the LGB around mining activities were in the following order: Co > Sb > Cu > Zr > Nb > Zn > Ni > Sr > Pb while the ranking was slightly different in the study site: Co > Sb > Zr > Cu > Nb > Y > V > Zn > Fe. Due to its crustal values, and conservative behavior, Zr has been recommended as a reference element in environmental studies and it is commonly assumed to have a natural origin (i.e., geogenic; Calmus et al., 2018). However, the average content of Zr in our study area (838 mg kg⁻¹) was more than twice the mean content in sediments from the mining area (364.5 mg kg⁻¹), and more than four times higher than the LGB (193 mg kg⁻¹) suggesting an anthropic origin unrelated to mining.

The following elements occurred in higher concentrations at the study site than those found in the mining area: Zr-Cu-V-Sb-Nb-Y-Fe. The concentration of Ti and Zr is remarkably different in the study area compared to the mining site (Figure 2) indicating a source different to mineralization.

Table 2 contains a correlation matrix for sediments collected at both sites. The element Zr is correlated at an alpha = 0.05 significance level with Pb-Zn-Cu-Ni-Mn-V-Ti-K, and Fe at the site where amphibians were collected. The geochemical signatures of riverbank sediments were different among sites (Figure 3) and the first two principal components explained 68.02% of the variability. The first principal component (PC1) explained 47.76% of total variance, defined by the contributions (%) of the following elements: Zr, Co, V, Ti, Nb, Y, Mn, and Fe, thus separating the group of sediments impacted by traffic sources in Bacoachi from the mining site. The second principal component (PC2) explained the 20.27% of the total variance and it is defined by the contributions of Pb, Zn, Cu, which follow the mineralization of the area. The third component (PC3) contributed 10.63% of the total variance and contained geogenic elements such as Sb, Cr, K, and Ca. Cerium, titanium, and zirconium content in riverbank sediments collected at the mining area are similar to those reported worldwide (Table 3).

3.2 Characterization of UFP Adhered to Mites

The mites had an average size of 270 by 530 µm (Figure 4A), and the UFP adhered to mites consisted of polycrystalline agglomerates with particle sizes varying from 850 nm to 5 µm (Figure 4B). The analysis of UFP revealed the presence of Zr, Ce, Sb, and Ti with traces of La, Mo, and Br. We also identified particles smaller than 1 µm composed of Zr, Sb, Ce, La, Br, and Ti.
FIGURE 3 | Principal component analysis (PCA) loadings of chemical composition of studied sediments.

FIGURE 4 | *Lithobates yavapaiensis* parasitized by *Hannemania* mites (A) Backscattered electron micrographs of a *Hannemania* mite, and (B) ultrafine particles containing Ce, Sb, and Zr, among other traffic-related elements.
The Raman spectra of the TWCC washcoat reported by Meza-Figueroa et al. (2021) and Navarro-Espinoza et al. (2021), and the particles in the mites showed a peak for the TWCC washcoat at 465 cm$^{-1}$ attributed to the symmetric stretching mode of the vibratory unit Ce-O (Kosacki et al., 2002). The peaks at 149, 251, and 314 cm$^{-1}$ were ascribed to the vibrational model Eg, A1g, and B1g of the O-Zr-O stretches. The particles on the mites showed peaks at 149 cm$^{-1}$, and 251 cm$^{-1}$ of tetragonal ZrO$_2$, and 339 cm$^{-1}$ of monoclinic ZrO$_2$ (Quintard et al., 2002). The peak at 465 cm$^{-1}$ in mite particles corresponds to CeO$_2$ and the peak at 602 cm$^{-1}$ is attributed to the A1g vibrational mode of tetragonal TiO$_2$ rutile (Frank et al., 2012; Gallego-Hernández et al., 2020).

Additionally, three well-defined peaks revealed the presence of albite mineral (Figure 6). Albite can arise from natural sources, such as dust from local soils. The peak at 83 cm$^{-1}$ is assigned to tetrahedral cage shear displacements in conjunction with Na environment breathing-rotation motions. The peak at 720 cm$^{-1}$ was a
signature of four fundamental modes: O-Al-O bend. Si-Al tetrahedral deformation, Na-OA1 and Na-OB(o) stretch. The peak at 1032 cm$^{-1}$ was created by internal tetrahedral vibrations dominated by Si-O stretch (McKeown, 2005).

### 3.3 Hannemania Mites

The prevalence of Hannemania infesting Lithobates yavapaiensis was 71.42% ($n = 7$). On macroscopic examination, mites were found within the skin of the ventral abdomen and femoral areas of the amphibians. The taxonomic identification of the mite genus was based on characters with taxonomic importance, such as the shape of the scutum, the cheliceral, multiple genualae on all legs, and the number of branched setae in palpal tarsus. These characteristics were evaluated in 10 specimens (Brennan and Goff, 1977; Hoffmann, 1990; Alvarado-Rybak et al., 2018). The average value for Hannemania mites among the infested individuals was 2.8 ± 1.11.

### 4 DISCUSSION

Our study highlights the importance of considering the study of endoparasitic mites (e.g., Hannemania mites) within the biotic factors as an alternative route of exposure of amphibian species to pollutants. Regarding this topic, Ferreira do Amaral and collaborators (2019) mention amphibians are able to absorb nanomaterials through their skin. Hagens et al. (2007) also mention the nanoparticles in the dermis can migrate to the central blood circulation and from there to the entire body.

The elements Zr-Cu-V-Sb-Nb-Y-Fe have been previously described by Navarro-Espinoza et al. (2021) as contaminant elements in TWCC that promote the detachment of the refractory washcoat made of Zr-Ce. This process is crucial for the release of UFP to the environment. An important finding of this pilot study is identifying the presence of Zr, Ce, and Ti UFP in the mites collected from amphibians. These elements are commonly considered as conservative, i.e., their concentrations have not been substantially modified by human activity and therefore, can be used as reference elements in the estimating of pollution indices (Calmus et al., 2018). However, our results show that Ti and Zr concentrations in sediments can be affected by traffic sources, even in pristine areas. The presence of traffic-related elements in levels above the LGB is relevant because of the toxicity of Ti to amphibians and the association of Zr with Ce in TWWC. Polycrystalline agglomerates identified in mites also contain particles with Al, Si, Mg, and K most likely derived from geogenic sources.

The presence of Zr at concentrations above LGB in the riverbank is relevant since there are no Zr-sources other than traffic in the area. Previous studies have shown the strong correlation of Ce and Zr in road dust collected at high traffic areas (Meza-Figueroa et al., 2021) and the Raman signature of ceria-zirconia particles (Navarro-Espinoza et al., 2021). Even though we did not obtain the total Ce composition of sediments, the UFP composed of Ce-Zr was identified by...
Raman spectroscopy and supported by the SEM-EDS analysis.

The Raman results of UFP analysis show peaks at 149, 251, and 314 cm\(^{-1}\) that result from the division of F\(^2\)g (465 cm\(^{-1}\)) because of the doping of cubic CeO\(_2\) with high concentrations of Zr (>20 mol\% Zr; Kuhn et al., 2013). The introduction of Zr generates the transition of cubic crystals of CeO\(_2\) to a tetragonal structure (Bolon and Gentleman, 2011; Kuhn et al., 2013). The distortion of this crystal lattice produces oxygen vacancies (V\(\text{O}\)) that are essential for the catalysis process (peak at 617 cm\(^{-1}\); Kosacki et al., 2002). ZrO\(_2\)-CeO\(_2\) compounds found in a TWCC can be released and incorporated into environmental matrices (Meza-Figueroa et al., 2021).

Bour et al. (2015) found that exposure, particle size, and the concentration of CeO\(_2\) could produce high mortality, growth inhibition, and genotoxicity in amphibians. CeO\(_2\) toxicity is species-dependent, and the route of exposure is a relevant variable influencing toxicity that should be further studied. Previous studies showed that bare CeO\(_2\) nanoparticles (mainly found in the water column) induced high genotoxicity on amphibian larvae (Bour et al., 2017). Furthermore, Keller et al. (2010) and Quik et al. (2010) found that CeO\(_2\) nanoparticles tend to form aggregates with consequent sedimentation, potentially entering the trophic chain through their integration to organisms such as amphibian. Most of the published research was performed under controlled conditions at the laboratory and this is one of the few studies reporting CeO\(_2\)-ZrO\(_2\) UFP in natural systems. Our results validate the data obtained by the elemental determination from the SEM-EDS, showing high concentrations of titanium (Ti) and oxygen (O) in the mite samples (Silva et al., 2021). On the other hand, the small size of TiO\(_2\) nanoparticles allows them to penetrate cells and accumulate therein (Nations, 2009) leading to alterations of the cellular metabolism or even apoptosis (Galdiero et al., 2017). Zhang et al. (2012), and Zhang (2011) reported that the increase of TiO\(_2\) (10 nm) and UVA light co-exposure decreased Xenopus laevis survival and the exposure to particles of different size (5,10, and 32 nm) with or without ultraviolet light and high concentrations of nano-TiO\(_2\), significantly affected tadpole growth. UV-A radiation can pass through the translucent skin of amphibians and interact with nano TiO\(_2\) particles damaging amphibian tissues and impacting growth (Nations et al., 2011; Zhang et al., 2012; Vijayaraj et al., 2018). Furthermore, Hammond et al. (2013) reported a risk of hormone disruption (thyroxine, and triiodothyronine) and cellular stress in amphibians exposed to different sizes and concentrations of TiO\(_2\) nanoparticles. Therefore, endoparasite mites such as Hannemania may indirectly increase the risk in juvenile amphibians or species with very light skin coloration (i.e., the family Centrolenidae).

To our knowledge, this is the first record of Hannemania mites on Lithobates yavapaiensis although Hannemania mites have been reported in other species of juvenile and adults amphibians living in the Americas and New Caledonia (Silva-De la Fuente et al., 2016; Bassini-Silva et al., 2021). The prevalence reported in this study (71.42%) is within the range observed in other amphibians species parasitized by Hannemania mites in Sonora state with prevalences from 20 to 95% (Hoffmann, 1969; Loomis and Welbourn, 1969; Goldberg et al., 2002). While the number of mites per individual was low in this study, the infestation level is a key factor in the damage they can cause to their host, especially when abundances are as high as those observed in Bassini-Silva et al. (2021). Therefore, we suggest conducting further experimental research on the concentrations of metals that Hannemania mites may harbor and expose Amphibians to.

5 CONCLUSION

The UFP identified in mites were derived from polluted sediments impacted by traffic suggesting the likelihood for intradermal Hannemania mites to be a pathway for UFP exposure in Amphibians. The presence of Ce and Ti oxides on mites is thus potentially toxic to amphibians and further research should continue addressing the potential synergy of biotic and abiotic factors in threatening species (Carrasco et al., 2021). Given the prolonged period in which parasites remain within their host, the potentially detrimental effects of chronic exposure on amphibians are high (Welbourn and Loomis, 1975; Westfall et al., 2008). Therefore, we recommend future work to investigate different exposure times and assess the role of parasites load in increasing the toxicity in all life stages of the host. Ultimately, regulations should also be developed to reduce the threat of traffic derived UFP to amphibians and ecological consequences even in rural areas.

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

Ethical review and approval was not required for the animal study because all animal capture and handling protocols for the scientific purpose at national territory were approved by the Secretariat of the Environment and Natural Resources in Mexico (SEMAR Permit No: FAUT-0027). The experimental procedure for mite’s collection permit was approved under permit: SGPA/DGVS/0441821. All specimens were deposited in the National Mite Collection (CNAC) at the Biology Institute of the National Autonomous University of Mexico with access number CNAC012393-CNAC012402. No amphibians were lost or injured in this procedure, and after mite’s extraction, the specimens were placed in the exact place where we found them.

AUTHOR CONTRIBUTIONS

MJ-M: Investigation, conceptualization, formal analysis, Writing-original draft, Review and Editing. DM-F: Funding acquisition, Conceptualization, Investigation, Writing–original draft, Review and Editing. MP-M: Investigation, Methodology, Writing-review and Editing. DL: Investigation, Writing-review and Editing. AR-
ACKNOWLEDGMENTS

We gratefully acknowledge Iván Guillermo Souffle Lambar, Lourdes Gabriela Canizález Juárez, and Miguel Ernesto Rosas Morales from the Herpetology Club at the University of Sonora for field assistance and discussions. We also thank Daisy López, Jay Taylor, Enrique de la Re Vega, and Griselda Montiel Parra for their valuable comments and technical support.
Gallego-Hernández, A. L., Meza-Figueroa, D., Tanori, J., Acosta-Elias, M., González-Grijalva, B., Maldonado-Escalante, J. F., et al. (2020). Identification of Inhaletable Rutile and Poly cyclic Aromatic Hydrocarbons (PAHs) Nanoparticles in the Atmospheric Dust. Environ. Pollut. 260, 110406–110410. doi:10.1016/j.envpol.2020.110406

Godwyn-Paulson, P., Jonathan, M. P., Rodríguez-Espinosa, P. F., and Rodríguez-Figueroa, G. M. (2022). Rare Earth Element Enrichments in Beach Sediments from Santa Rosalia Mining Region, Mexico: An Index-Based Environmental Approach. Mar. Pollut. Bull. 174, 113271. doi:10.1016/j.marpolbul.2021.113271

Goessens, T., De Baere, S., Deknock, A., De Troyer, N., Van Leeuwenberg, R., Martel, A., et al. (2022). Agricultural Contaminants in Amphibian Breeding Ponds: Occurrence, Risk and Correlation with Agricultural Land Use. Sci. Total Environ. 806, 1–13. doi:10.1016/j.scitotenv.2021.150661

Goldberg, S. R., Wrenn, W. J., and Bursey, C. R. (2002). Bubo Mozatlanensis (Sinaloa Toad), Rana Tarahumarae (Tarahumara Frog). Ectoparasites. Herpetol. Rev. 33, 301–302.

Goldschmidt, T. (2016). Water Mites (Acari, Hydrachnida): Powerful but Widely Neglected Biodicators - a Review. Neotropical Biodivers. 2, 12–25. doi:10.1080/23766808.2016.1144359

Green, D. M., Lannoo, M. J., Lesbiarreres, D., and Muths, E. (2020). Amphibian Toadlet Disease: A Perspective. Front. Ecol. Environ. 18, 99–105. doi:10.1002/fee.1507

Hagens, W. I., Oomen, A. G., de Jong, W. H., Cassee, F. R., and Sips, A. J. A. M. (2022). 137Cs and 134Cs in Atmospheric Particulate Matter and Sediment, and Host Mite Communities (Acari: Mesostigmata, Oribatida) as Bioindicators for Environmental Conditions from Polluted Soils. Sci. Rep. 9, 1–13. doi:10.1038/s41598-019-06700-8

McKeown, D. A. (2005). Raman spectroscopy and vibrational analyses of albite: From 25°C through the melting temperature. Am. Mineral. 90, 1506–1517. doi:10.2138/am.2005.1726

Mora-Moreno, D., Pedrosa-Montero, M., Barboza-Flores, M., Navarro-Espinosa, S., Ruíz-Torres, R., Robles-Moría, A., et al. (2021). Identification of Refractory Zirconia from Catalytic Converters in Dust: An Emerging Pollutant in Urban Environments. Sci. Total Environ. 11, 559–564. doi:10.1016/j.scitotenv.2020.143384

Nations, S. L. (2009). Acute and Developmental Toxicity of Metal Oxide Nanoparticles (ZnO, TiO2, Fe2O3, and CuO) in Xenopus laevis. [dissertation/ master's thesis]. [Texas]: Texas Tech University.

Nations, S., Wages, M., Cañas, J. E., Maul, J., Theodorakis, C., and Cobb, G. P. (2010). Copper and Temperature Modulate Predator-Prey Interactions in Amphibians: a Review. Appl. Geochem. 25, 251–267. doi:10.1016/j.apgeochem.2009.11.005

Papen, A. C. (1911). Acarologische Aanteekeningen VI. Herpetologica 7(1): 8–10.

Parks, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., et al. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439, 161–167. doi:10.1038/nature04246

Plata, J. E., and Frost, J. S. (1984). Rana yavapaiensis, a New Species of Leopard Frog (Rana pipsii Complex). Copeia 1984, 940–948. doi:10.2307/1445338

Quin, J. T. K., Lynch, I., Hoecke, K. V., Mierniers, C. J. H., Schampaehere, K. A. C. D., Janssen, C. R., et al. (2010). Effect of Natural Organic Matter on Cerium Dioxide Nanoparticles Settling in Model Fresh Water. Chemosphere 81, 711–715. doi:10.1016/j.chemosphere.2010.07.062

Quintard, P. E., Barbéris, P., Mirgorodsky, A. P., and Merle-Méjean, T. (2002). Comparative Lattice-Dynamical Study of the Raman Spectra of Monoclinic and Tetragonal Phases of Zirconia and Hafnia. J. Am. Ceram. Soc. 85, 1745–1749. doi:10.1111/j.1151-2916.2002.tb03466.x
Rinkovec, J. (2019). Platinum, Palladium, and Rhodium in Airborne Particulate Matter. Arch. Ind. Hyg. Toxicol. 70, 224–231. doi:10.2478/aiht-2019-70-3299
Sandu, M. C., Soroaga, L. V., Balaban, S. I., Chelaru, C., Chiscan, O., Iancu, G. O., et al. (2021). Trace Elements Distribution in Stream Sediments of an Abandoned U Mining Site in the Eastern Carpathians, Romania, with Particular Focus on REEs. Geochemistry 81, 125761. doi:10.1016/j.chemosphere.2021.125761
Schneider, L., Haberle, S. G., Maher, W. A., Krikowa, F., Zawadzki, A., and Heijnis, Sievers, M., Hale, R., Swearer, S. E., and Parris, K. M. (2018). Contaminant Mixtures Interact to Impair Predator-Avoidance Behaviors and Survival in a Larval Amphibian. Ecotoxicol. Environ. Saf. 161, 482–488. doi:10.1016/j.ecoenv.2018.06.028
Sievers, M., Hale, R., Parris, K. M., Melvin, S. D., Lancatót, C. M., and Swearer, S. E. (2019). Contaminant-induced Behavioural Changes in Amphibians: A Meta-Analysis. Sci. Total Environ. 693, 1–11. doi:10.1016/j.scitotenv.2019.07.376
Silva, L. F. O., Hower, J. C., Dotto, G. L., Oliveira, M. L. S., and Pinto, D. (2021). Titanium Nanoparticles in Sedimented Dust Aggregates from Urban Children’s Parks Around Coal Ashes Wastes. Fuel 285, 1–6. doi:10.1016/j.fuel.2020.119162
Skubala, P., and Zaleski, T. (2012). Heavy Metal Sensitivity and Bioconcentration in Oribatid Mites (Acari, Oribatida) Gradient Study in Meadow Ecosystems. Sci. Total Environ. 414, 364–372. doi:10.1016/j.scitotenv.2011.11.006
Slaby, S., Marin, M., Marchand, G., and Lemiere, S. (2019). Exposures to Chemical Contaminants: What Can We Learn from Reproduction and Development Endpoints in the Amphibian Toxicology Literature? Environ. Pollut. 248, 478–495. doi:10.1016/j.envpol.2019.02.014
Thambrirajah, A. A., Koide, E. M., Imbery, J. J., and Helbing, C. C. (2019). Contaminant and Environmental Influences on Thyroid Hormone Action in Amphibian Metamorphosis. Front. Endocrinol. 10, 1–29. doi:10.3389/fendo.2019.00276
Toussaint, A., Brosse, S., Bueno, C. G., Pärtem, M., Tamme, R., and Carmona, C. P. (2021). Extinction of Threatened Vertebrates Will Lead to Idiosyncratic Changes in Functional Diversity across the World. Nat. Commun. 12, 1–12. doi:10.1038/s41467-021-25293-0
USGS (2021). “Why Are Frog and Toad Populations Declining?,” in Science for a Changing World. Biology and Ecosystems. Available at: https://www.usgs.gov/faqs/why-are-frog-and-toad-populations-declining?gt-news_science_products=0&gt-news_science_products (Accessed February 03, 2022).
Velasco, J. A., Estrada, F., Calderón-Bustamante, O., Swingedouw, D., Ureta, C., Gay, C., et al. (2021). Synergistic Impacts of Global Warming and Thermohaline Circulation Collapse on Amphibians. Commun. Biol. 4, 141–147. doi:10.1038/s42003-021-01665-6
Vijayaraj, V., Liné, C., Cadarsi, S., Salvagnac, C., Baqué, D., Elger, A., et al. (2018). Transfer and Ecotoxicity of Titanium Dioxide Nanoparticles in Terrestrial and Aquatic Ecosystems: A Microcosm Study. Environ. Sci. Technol. 52, 12757–12764. doi:10.1021/acs.est.8b02979
Villamizar-Gomez, A., Wang, H., Peterson, M., Grant, W., and Forstner, M. (2021). Environmental Determinants of Batrachochytrium Dendrobatidis and the Likelihood of Further Dispersion in the Face of Climate Change in Texas, USA. Dis. Aquat. Org. 146, 29–39. doi:10.3354/dao03613
Walter, D. E., and Proctor, H. C. (2013). Mites: Ecology, Evolution and Behaviour. New York London: Springer Press.
Welbourn, W. C., and Loomis, R. B. (1975). Hannemannia (Acarina: Trombiculidae) and Their Anuran Hosts at Fortynine Palms Oasis, Joshua Tree National Monument, California. Bull. South. Calif. Acad. Sci. 74, 15–18.
Westfall, M. C., Ceca, K. K., Price, S. J., and Dorcas, M. E. (2008). Patterns of Trombiculid Mite (Hannemannia Dunnii) Parasitism Among Pothohodent Salamanders in the Western Piedmont of North Carolina. J. Parasitol. 94, 631–634. doi:10.1645/ge-1260r1.1
Wong, B. B. M., and Candolin, U. (2015). Behavioral Responses to Changing Environments. Behav. Ecol. 26, 665–673. doi:10.1093/beheco/aru183
Zhang, J., Wages, M., Cox, S. B., Mail, J. D., Li, Y., Barnes, M., et al. (2012). Effect of Titanium Dioxide Nanomaterials and Ultraviolet Light Coexposure on African Clawed Frogs (Xenopus laevis). Environ. Toxicol. Chem. 31, 176–183. doi:10.1002/etc.718
Zhang, Z.-Q. (2011). Animal Biodiversity: An Introduction to Higher-Level Classification and Taxonomic Richness. Zootaxa 3148, 7–12. doi:10.11646/zootaxa.3148.1.3

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.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Jacinto-Maldonado, Meza-Figueroa, Pedroza-Montero, Losbarreres, Robles-Moria, Navarro-Espinoza, Gonzalez-Grijalva, Perez-Segura, Silva-Campa, Angulo-Molina and Paredez-Leon. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided that the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.