Microcrustaceans inhabiting tropical freshwater contaminated and non contaminated with arsenic: identification of regional suitable bioindicators.

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Abstract

We investigated microcustraceans inhabiting arsenic contaminated and non contaminated freshwater in order to identify those absent in arsenic contaminated water as suitable bioindicators of arsenic contamination in tropical freshwater in mining and metallurgical areas in northern Mexico. For doing so, we collected zooplankton, water and sediment samples, at five sampling points in two sampling campaigns, to determine water temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), alkalinity (Alk), salinity (Sal), and total arsenic concentration in water and sediments samples. We additionally determine arsenic mobility from sediments and its speciation. We also identified microcrustacean species and determined abundance, richness and Shannon Index. Results showed that the maximum arsenic concentration in freshwater was 53.23 mg/L, while the minimum arsenic concentration was < 0.01 mg/L. Arsenic concentration in sediments was between 10.37 mg/kg and 2472.84 mg/kg, with high arsenic mobility (up to 100%) where the arsenic leached was As(V). Nine species of microcrustaceans were found. *Simocephalus punctatus, Alona glabra, Eucyclops leptacanthus*, *Macrocyclops albidus* and *Pleuroxus (Picripleuroxus) quasidenticulatus*, inhabiting arsenic-free water, *Latonopsis australis, Eucyclops chihuahuensis*, *Acanthocyclops americanus, Pleuroxus (Picripleuroxus) quasidenticulatus*, *Macrocyclops albidus* and *Paracyclops chiltoni*, inhabiting moderately and highly contaminated water. *Simocephalus punctatus* (Orlova-Bienkowskaja, 1998) was absent in contaminated water bodies in study area, and therefore we proposed this microcustracean could serve as a bioindicator of water quality in waterbodies contaminated with arsenic in study area and northern Mexico, where arsenic contamination is common ground.

Introduction

Arsenic (As) is a chemical element present in the earth's crust and groundwater. In aqueous media, As speciation strongly depends on the redox condition of the aqueous system (De and Maiti, 2012). Under oxidizing conditions, inorganic As exists as arsenate (AsO_4^{3-}), with acidity constants (pKa) of 2.2, 6.9 and 11.5 for the species $H_3AsO_4^0$, $H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-}. On the other hand, in more reducing environments, inorganic As exists as arsenite (AsO_3^{3-}) with pKa values of 9.2, 12.1 and 13.4 where the uncharged ion $H_3AsO_3^0$ prevails at pH below 9.2 (Petrusevski et al., 2007; Ravenscroft et al., 2009; Samadzadeh Yazdi and Khodadadi Darban, 2010). The toxicity of As varies widely with its oxidation states. Arsenite is approximately 60 times more toxic than arsenate (Ventura-Lima et al., 2011). Arsenate is, in turn, 70 times more toxic than organic methylated species, such as monomethylarsonic acid (MMA) and dimethylarsine acid (DMA) (Akter et al., 2005). MMA and DMA are, in fact, considered only moderately toxic (Akter et al., 2005). Other As species, such as trimethylarsine oxide (TMAO) and tetramethylarsonium (TETRA) are also considered moderately toxic, while arsenobetaine (AsB), arsenocholine (AsC), and other arsenosugars (AsS) show no toxicity (Fattorini et al., 2006).

In aquatic environments, As can be incorporated into the food web, through different uptake routes: directly from solution across the entire body surface of the organisms, through specialized respiratory structures (eg, gills), or through the digestive epithelium with ingested food, water, sediments or suspended particles (Rahman et al., 2012).

Due to its toxicity, national and international authorities consider As within their regulations as a parameter for the protection of aquatic life. In Mexico, among the ecological criteria, the maximum permissible limit of As in water for the protection of aquatic life is set at 0.2 mg/L (DOF, 1989). For sediments, international regulations indicate a 17 mg/kg (CCME, 1999; EPA, 2002a) as a probable effect level (PEL) on freshwater aquatic organisms, while Mexican regulations need to be developed yet, for sediment quality purposes. For doing so, Mexico shall consider, as many other countries, biological parameters as they might provide better information than other descriptors for certain types of contamination (Cairns and Dickson, 1971; De la Lanza-Espino et al., 2011), such as phosphorus contamination in water, where chemical measurements may not accurately reflect a reduction in species diversity or how the growth and reproduction of other species may decline due to competitive exclusion (Holt and Miller, 2010). Furthermore, quantitative biological data is as easily accessible as physicochemical data.

In general, diverse feeding habits, very short life cycles, high reproductive rates and small sizes make microcrustaceans suitable as bioindicators. In this sense, the EPA (Environmental Protection Agency of the USA), developed a water quality standard using the cladocerans microcrustaceans *Daphnia pulex* and *Daphnia magna* as a bioindicators to determine acute toxicity in effluents and receiving waters to freshwater and marine organisms is the Method 2021.0 (EPA, 2002b), these species are highly sensitive to the presence of contaminants in water and therefore exhibits impacts on their level of reproduction, lifetime, and size of the individuals (Chen et al., 1999). In Mexico, the standard to evaluate acute toxicity in water analysis is the NMX-AA-087-SCFI-2010 (DOF, 2011), which, analogously to the Method 2021.0, uses *Daphnia magna* as a proxy. However, *Daphnia magna* is a species inhabiting cold and temperate climate being an exotic microcrustacean in Mexico, therefore it can not be considered representative for the monitoring of tropical aquatic ecosystems (Pérez-Legaspi et al., 2017). There is little research on tropical microcrustaceans, specifically cladocerans proposed to perform acute toxicity tests with some heavy metals (Do Hong et al., 2004; Freitas and Rocha, 2010; Hong and Li, 2007; Martínez-Jerónimo et al., 2008; Pérez-Legaspi et al., 2017; Rodgher et al., 2010), however, tropical microcrustaceans inhabiting contaminated freshwater with As have been poorly considered in these evaluations (Alvarado-Flores et al., 2019). Due to this, it is necessary to propose species of tropical microcrustaceans, widely distributed in Mexico that can be bioindicators of polluted environments, specifically with As.

A site that offers an excellent opportunity to study and understand As-related phenomena is Matehuala, San Luis Potosí, Mexico. In Matehuala, freshwater is contaminated with As due to current and historical metallurgical and mining activities (Martínez-Villegas et al., 2013; Pelallo-Martínez, 2006; Razo et al., 2004). Furthermore, As contamination has been reported to range from 4.78 mg/L to up to 158 mg/L (Martínez-Villegas et al., 2013), concentrations well above the recommended limits for the protection of aquatic life (0.2 mg/L) and the lethal concentration for zooplankton (3 mg/L). However, knowing this information raises the question of the existence of organisms in these conditions. This area gains importance and public interest since Matehuala contains recreation areas in which animal populations (including humans) are exposed to contact with contaminated water and sediment, and is a priority eco-region for the conservation and management of Mexico's aquatic biodiversity (Mercado-Salas and Suárez-Morales, 2012).

In this study, the species of microcrustaceans inhabiting freshwater were identified, as well as their ecological indicators. In addition, As concentrations in water and sediment were determined, as well as As speciation in the latter. A classification of the sampling points and species of microcrustaceans was made based on the concentration of As and alkalinity found in the water, in order to propose the bioindicator species. And, finally, to better understand any relationship between physicochemical parameters and ecological indicators, a correlation matrix was obtained and a principal components analysis was carried out.

Material And Methods

The study area corresponds to the municipality of Matehuala, San Luis Potosi, Mexico, formed by a set of closed basins, where rainfall and groundwater are the water sources. Soils are Xerosol type, with gypsum in the deep horizon and soil cemented with carbonate at a shallow depth (Gómez-Hernández et al., 2020).

Water and microcrustaceans samples were collected through two sampling campaigns (S1 and S2) at five different sites: Club de Tiro, Abrevadero, Laguna, Presa, and Canal. While the first three sites correspond to a complex with As contaminated water (Martínez-Villegas et al., 2013; Razo et al., 2004), the other two sampling points are As-free. Additionally, sediment samples were collected in an additional sampling campaign at Club de Tiro, Laguna y Presa. All samping sites were located within the urban area of Matehuala city (Fig. 1).

Location of the sampling points: 1) Club de Tiro, 2) Abrevadero, 3) Laguna, 4) Presa, and 5) Canal.

Ten water samples were collected in 60 mL polypropylene containers previously washed with 2% Extran® and 10% HNO_3 acid. Water samples were filtered through Whatman filter paper #40 (125 mm), acidified to pH < 2 with concentrated HNO_3, closed and stored at 4°C until analysis to determine As. For quality control and assurance, one laboratory blank, one field blank and one duplicate were collected at each sampling campaign.

Water temperature, pH, electrical conductivity, dissolved oxygen, and salinity were measured onsite using a multiparameter probe (HANNA Instruments Model 9829 Handheld MultiparameterWater Quality Meter). Additionally, alkalinity was determined by titration using an Automatic Titration Kit of the HACH brand model AL-DT.

As concentrations in water samples were determined by Inductive Coupling Plasma Optical Emission Spectroscopy (ICP-OES), using a Varian 730 ES spectrometer (EPA, 1994). Calibration with reference samples and blanks as well as replicate analyses for quality control were carried out to ensure the reliability of the analytical data. The calibration curve was in the range of 0.05-20 mg/L, while the detection limit was 0.001 mg/L.

Additionally, 100 L of freshwater were filtered through a 50 µm mesh to capture zooplankton; once in a collecting glass, zooplankton organisms were fixed with concentrated ethyl alcohol (90%) until analysis. All zooplancton specimens were sorted and taxonomically examined using specialized literature (Elias-Gutierrez et al., 2008; Korovchinsky, 1992). Adult abundance (organisms/L) was estimated by total counting on an Eclipse E-400 compound microscope for each species. The diversity was calculated using the Shannon Index (Shannon, 1948): $H' = -\sum pi \ln(pi)$, where pi is the relative abundance of the ith species.

Sediment samples were collected at Club de Tiro, Laguna, and Presa in aluminum foil bags using a shovel, previous cleanup of rocks and vegetation. All sediment samples were kept at 4°C until As analysis. In the laboratory, the sediment samples were dried in an oven at 60°C for 24 h. Then, 0.25 g of homogenized samples were predigested in 4 ml of 65% HNO_3 overnight at room temperature in triplicates. Subsequently, the samples were gently shaken and digested in the microwave using the BASIC OPEN program with a ramp of 5 min to 55°C and 10 min of digestion. Finally, each digest was diluted to 50 ml in 1% HNO_3 and centrifuged for 5 min at 2500 rpm. Total As concentrations in the supernatants were determined by inductively coupled plasma mass spectrometry (ICP-MS Agilent 7700 Series) (Hossain et al., 2012; Mestrot et al., 2011). Additionally, As speciation was determined in dry sediment samples, crushed in an Agate mortar. For doing so, 0.25 g of homogenized samples were predigested in 4.8 ml of 1% HNO_3, in order to recover the most possible amount of As in the sample. Subsequently, microwave digestion was carried out using the BASIC OPEN program with a ramp of 5 min to 55°C and maintaining this temperature for 10 min. After, the supernatants were centrifuged for 5 minutes at 2500 rpm and As speciation in sediments was determined using an ICP-MS Agilent 7700 Series coupled to an high performance liquid chromatography (HPLC Agilent 1260 Infinity) with an anion exchange PRP X-100 HPLC column (Hossain et al., 2012; Mestrot et al., 2011; Viacava et al., 2020) to determine inorganic As (As(III) and As(V)) and arsenicals (TMAsO, DMAs(V) or MMAs(V)).

The correlation between physicochemical (water temperature, electrical conductivity, dissolved oxygen, pH, salinity, alkalinity, As) and ecological (richness, Shannon index, and abundance) variables was determined in order to establish any relationship among the hydrogeochemistry and the attributes of the organisms. PCA was carried out using OriginPro, Version 2016 (OriginLab Corporation, Northampton, MA, USA).

Results

Table 1 shows water temperature, pH, electrical conductivity, dissolved oxygen, alkalinity and salinity. Water temperature ranged from 16.9 to 23.7°C, pH was between 6.7 and 8.3, electrical conductivity ranged from 2364 to 3282 μs/cm, dissolved oxygen ranged from 0.6 to 3.7 mg/L, alkalinity was between 11.2 and 296 mg$CaCO_3$/L, and salinity ranged between 0.7 and 1.7‰. Water pH values are the results of the buffer capacity of limestone (Razo et al., 2004), while electrical conductivity values are the result of calcite and gypsum dissolution in the aquifer (Gómez-Hernández et al., 2020). Small variations on water temperature and dissolved oxygen were observed, however they are typical of groundwater at tropical altitudes (Caspers et al., 1981), suggesting typical environmental conditions. A high transparency, as well as a low amount of algae or aquatic vegetation and low biomass were observed in the studied water bodies, which allowed their classification as "oligotrophic systems" according to Roldán and Ramírez, (2008).

Table 1
Water temperature (T), pH, electric conductivity (EC), dissolved oxygen (DO), alkalinity (Alk), and salinity (Sal) measured *in situ* at two sampling campaigns (S1 and S2) at the different sampling sites. S.D.= Standard deviation.

Site	**Sampling**	**T (°C)**	**pH**	**EC (µs/cm)**	**DO (mg/L)**	**Alk ($mgCaCO_3$/L)**	**Sal (‰)**
Club de Tiro	S1	22.5	6.7	2762.0	1.3	219.0	1.4
	S2	21.5	6.7	3209.0	0.6	296.0	1.7
	Mean	22.0	6.7	2985.5	0.9	257.5	1.5
	S.D.	0.7	0.0	316.0	0.5	54.4	0.2
Abrevadero	S1	21.1	7.0	2364.0	3.4	121.0	0.7
	S2	20.5	7.5	2376.0	0.7	193.0	1.3
	Mean	20.8	7.2	2370.0	2.0	157.0	1.0
	S.D.	0.4	0.3	8.4	1.9	50.9	0.4
Laguna	S1	20.9	7.5	2423.0	3.6	90.0	1.2
	S2	20.4	8.3	2591.0	0.9	11.2	1.3
	Mean	20.6	7.9	2507.0	2.3	50.6	1.2
	S.D.	0.3	0.6	118.8	1.8	55.7	0.1
Presa	S1	23.7	7.3	2892.0	2.6	103.0	1.6
	S2	18.7	7.7	3282.0	0.9	142.0	1.7
	Mean	21.2	7.5	3087.0	1.7	122.5	1.6
	S.D.	3.5	0.3	275.7	1.2	27.6	0.1
Canal	S1	18.8	7.4	2528.0	3.7	127.0	1.2
	S2	16.9	7.8	2888.0	1.3	169.5	1.5
	Mean	17.8	7.6	2708.0	2.5	148.2	1.3
	S.D.	1.3	0.3	254.5	1.7	30.1	0.2

Figure 2 shows As concentrations in water samples, which ranged between < 0.01 mg/L and 53.23 mg/L. At Club de Tiro, As concentrations were 38.98 mg/L and 53.23 mg/L, during S1 and S2, respectively. In Abrevadero, As concentrations were 1.62 mg/L and 5.91 mg/L, during S1 and S2, respectively. In Laguna, As concentrations were 1.9 mg/L and 5.3 mg/L, during S1 and S2, respectively. Canal and Presa showed the lowest concentrations of As in water with values between < 0.01 mg/L and 0.2 mg/L, respectively. More importantly, 6 out of the 10 water samples were above the maximum permissible levels of As in water for irrigation, recreation and protection of aquatic life (CCME, 2001; DOF, 1989). All contaminated water samples were collected from Club de Tiro, Abrevadero and Laguna. Furthermore, some of these samples were also above the As lethal concentration reported for zooplankton (3 mg/L) (Chen et al., 1999), highlighting a likely risk for microcrustacean life in the impacted water bodies.

As concentrations in water simples, in squares the data of sampling 1 and in triangles the data of sampling 2, additionally the maximum permissible As concentration in natural waters (0.2 mg/L) in Mexico (DOF, 1989) as well as the As lethal concentration for zooplankton (3 mg/L) are presented in dotted lines (Chen et al., 1999).

Figure 3 shows total As concentration in sediment samples for contaminated and non-contaminated sampling sites. Total As concentrations in sediment were 2472.84 ± 611.48 mg/kg at Club de Tiro, 553.48 ± 9.11 mg/kg at Laguna, and 10.37 ± 5.39 mg/kg at Presa, which largely exceed the international guideline values for As in sediments for quality purposes (5.9 mg/kg) (CCME, 1999) as well as the probable effect level of As for freshwater aquatic organisms (17 mg/kg) (CCME, 1999; EPA, 2002a). So far, up to date, no regulations are available in Mexico for sediment quality. Additionally, a high mobility of As, from the sediments, was found in this study, which accounted for 47.20% of the total As at Club de Tiro, 52.19% of the total As at Laguna, and 100% at Presa as determined by the recovery of As using 1% HNO_3. Considering that As recoveries in acid digestions using concentrated acids (> 65%) are between 85 and 99.9% (Davidowski and Sarojam, 2012), As mobility at Club de Tiro and Laguna using mild acid might have been limited by the experimental conditions (solid to solution ratio and reaction time), highlighting the possibility of being higher than the values obtained in this study and the risk of the samples. We also found that the mobile As was actually As(V). No methylated As (III) species were found in sediments. This, likely due to the minerals that were suggested to control the mobility of As in the study area, which accounted for calcium arsenates (Martínez-Villegas et al., 2013). Similarly, in this study, the oxidation state of As was + 5.

Total As concentration in sediment samples, recovery percentages of As in sediment digestions using 1% HNO_3 and the maximum permissible concentration of As for the protection of healthy aquatic systems (17 mg/kg) is presented in red dotted line (CCME, 1999).

Table 2 shows the ecological indicators for each sampling site and time. Abundance ranged from 0 to 25.17 org/L, richness was between 0 and 4, and Shannon index ranged from 0 to 1.24, showing that the site with the greatest species richness was Laguna. For Canal, we found absence of zooplankton likely due to the water flow that may complicate the survival of the organism in the stream of the channel. Typically, Shannon index values are between 2 and 3, values less than 2 are considered low in diversity and greater than 3 are high in species diversity (Margalef, 1972). Therefore, the sampling sites studied here exhibited low diversity, with Club de Tiro exhibiting the lowest diversity, with Shannon indexes of zero at two sampling campaigns.

Table 2
Ecological indicators obtained from microcrustaceans samples.

Site [As mg/L]	Sampling	Abundance (org/L)	Richness	Shannon Index
Club de Tiro 38.98–53.23	S1	0.32	1	0
	S2	25.17	1	0
Abrevadero 1.62–5.91	S1	1.45	3	0.49
	S2	0.25	1	0
Laguna 1.9–5.3	S1	3.91	4	0.67
	S2	2.49	4	0.89
Presa 0.01–0.2	S1	11.54	4	1.24
	S2	7.88	2	1.12
Canal < 0.01	S1	0.21	2	0.19
	S2	0.00	0	ND

Table 3 shows the cladoceran (Crustacea; Branchiopoda: Ctenopoda, Anomopoda) and copepod (Crustacea: Copepoda, Cyclopoida) species inhabiting the surveyed systems in both sampling periods.

In non-contaminated sampling points (0.0-0.2 mg/L), 5 species of microcrustacean were found (*Simocephalus punctatus, Alona glabra, Eucyclops leptacanthus*, *Macrocyclops albidus* and *Pleuroxus (Picripleuroxus) quasidenticulatus*). In the moderately contaminated sampling points (1.61–5.91 mg/L), 4 species were found (*Latonopsis australis, Pleuroxus (Picripleuroxus) quasidenticulatus, Eucyclops chihuahuensis*, *Acanthocyclops americanus Paracyclops chiltoni*), while in the most contaminated sampling point (up to 53.23 mg/L) only one species was found (*Pacacyclops chiltoni*).

Additionally, two species were found to cohabite As free and contaminated water, namely *Pleuroxus (Picripleuroxus) quasidenticulatus* and *Macrocyclops albidus.* One species was found cohabiting As moderately contaminated and highly contaminated water, while 4 species were found only in non contaminated water. Large species like *Simocephalus punctatus* (Cladocera: Anomopoda) were the most abundant in absence of As (6.06 org/L), whereas smaller microcrustacean, like the copepod *Paracyclops chiltoni* (Copepoda: Cyclopoida), inhabiting the most As contaminated water, showed an abundance up to 25.17 org/L, which was, in turn, the highest abundances found among all sampling points.

Table 3
Microcrustacean species inhabiting non-contaminated and contaminated freshwater in Matehuala, San Luis Potosi, Mexico.

Level of As (mg/L)	**Sampling point**	**Specie**	**Distribution***	**Highlights**
0.00–0.02	Presa	*Simocephalus punctatus* (Orlova-Bienkowskaja, 1998)	Canada, U.S.A., and North of Mexico (Nearctic species).	Intermediate alkalinity ranged from 100–170 $mgCaCO_3/L$; zooplankton of longer body size with abundances ranged from 10–100 org/L.
	Presa	*Alona glabra* (Sars, 1901)	South America and some localities in Central Mexico.	
	Presa	*Eucyclops leptacanthus* (Kiefer, 1956)	South and Central America, and North of Mexico.	
	Presa and Canal	*Pleuroxus (Picripleuroxus) quasidenticulatus* (Smirnov, 1996)$^{-}$	Cosmopolitan, recorded in Europe, Asia, North, Central and South America.	
	Canal	*Macrocyclops albidus albidus* (Jurine, 1820)$^{+}$	Cosmopolitan, in North, Central and Southeastern of Mexico.	
1.61–5.91	Laguna	*Latonopsis australis* (Sars, 1888)	Presumably cosmopolitan, probably a species complex.	Low alkalinity from 11 to 190 $mgCaCO_3/L$; zooplankton of medium body size, with abundance ranged from 0 to < 10 org/L.
	Laguna	*Macrocyclops albidus albidus* (Jurine, 1820)$^{+}$	Cosmopolitan, in North, Central and Southeastern of Mexico.	
	Laguna	*Acanthocyclops americanus* (Marsh, 1893)	Considered as a cosmopolitan species.	
	Abrevadero and Laguna	*Pleuroxus (Picripleuroxus) quasidenticulatus* (Smirnov, 1996)$^{-}$	Cosmopolitan, recorded in Europe, Asia, North, Central and South America.	

* Distribution and body size data found in the Barcode of Life Data System v4 database (BOLD, 2021).

−,+ Species found in two sites and different concentrations of As.

Level of As (mg/L)	Sampling point	Specie	Distribution*	Highlights
	Abrevadero and Laguna	*Eucyclops chihuahuensis* (Suárez-Morales and Walsh, 2009)	Possibly endemic to a few localities in the North of Mexico.	
5.91–53.23	Abrevadero and Club de Tiro	*Paracyclops chiltoni* (Thomson, 1883)	Cosmopolitan, widely distributed in North and South America.	High alkalinity from 220 to 300 $mgCaCO_3$/L; zooplankton of short body size, with abundance ranged from 1 to 60 org/L.
* Distribution and body size data found in the Barcode of Life Data System v4 database (BOLD, 2021).				
–,+ Species found in two sites and different concentrations of As.				

Figure 4 shows the PCA. PC1 (38.31%) combined electrical conductivity, salinity, alkalinity, As and abundance, while the PC2 (28.39%) combined pH, richness and Shannon index. These two main components explained 66.70% of the total variability of the data. Table 3 shows the parameter coefficients to each principal component.

Figure 4 and Table 4 suggest that PC1 was a factor that combined abiotic and biotic aspects related to mineral solubility and abundance of zooplankton, which was supported by a positive correlation between the level of electrical conductivity and salinity, as well as salinity and abundance and alkalinity and As (Table 5). Salinity is considered one of the most important environmental factors that shapes the biodiversity and abundance of zooplankton (Ojaveer et al., 2010; Perumal et al., 2009; Yuan et al., 2020). It has been reported that the groups of zooplankton most affected by salinity are rotifers and cladocerans, showing a decrease in their abundances and diversities (Ojaveer et al., 2010; Yuan et al., 2020). On the other hand, copepods show little effect on abundance with increasing salinity, and even positive correlations have been reported between salinity and abundance of copepods in freshwater (Perumal et al., 2009), as in the case of this work. In this study, electrical conductivity showed a relationship with salinity as a result of calcite and gypsum dissolution. In the study area, water evaporation and mineral dissolution explain the positive correlation between the concentration of As and alkalinity as well (Table 5).

PC2 was a biotic factor related to pH, which was supported by a positive correlation between pH and richness. It has been reported that, although zooplankton organisms show a high degree of tolerance to changes in pH, negative effects in abundance and richness have been related to moving away from neutrality (Echaniz et al., 2012).

PCA biplot showing PC1 and PC2 as well as the contribution variables of each component. The physicochemical and ecological variables are presented in blue and the sampling sites in red.

Table 4
Extracted eigenvectors.

	Coefficients of PC1	Coefficients of PC2
Abundance	**0.36559**	0.26023
Shannon	0.21621	**0.36870**
Richness	-0.07763	**0.46492**
T	0.05217	-0.21034
pH	-0.20972	**0.47342**
EC	**0.44508**	0.17796
DO	-0.34911	-0.13026
Alk	**0.38742**	-0.31489
Sal	**0.41569**	0.21547
As	**0.35213**	-0.34292

Table 5
Pearson's correlation matrix between ecological and physicochemical parameters, showing values > 0.5 in red and <-0.5 in blue.

	Abundance								
Shannon	0.5326	**Shannon**							
Richness	0.2623	0.5277	**Richness**						
T	-0.1504	0.0847	0.1367	**T**					
pH	-0.0706	0.1243	0.5687	-0.5386	**pH**				
EC	0.8075	0.4491	0.0299	-0.0823	-0.1309	**EC**			
DO	-0.4005	-0.2664	-0.0354	0.1027	-0.1218	-0.5328	**DO**		
Alk	0.3017	0.1005	-0.6239	0.0647	-0.7276	0.4316	-0.4161	**Alk**	
Sal	0.5963	0.5067	0.0745	-0.0208	0.0398	0.8536	-0.6225	0.3780	**Sal**
As	0.1966	-0.1773	-0.3765	0.3923	-0.6856	0.3941	-0.4567	0.7549	0.3429

In this survey, the cosmopolitan *P. chiltoni* probed to be a copepod highly tolerant to extremely high concentrations of As. The presence of *P. chiltoni* has been recorded in systems with different trophic states and in the littoral or planktonic habitats in worldwide freshwater systems (Lansac-Tôha et al., 2002). In this study, *P. chiltoni* was the only species inhabiting a system with more than 50 mg/L of As with Shannon Indexes of zero. That is, *P. chiltoni* seems to be resistant to severe stress and thrive in extreme conditions, providing additional evidence as an extremophile crustacean as previously reported in Mendoza-Chávez et al., (2021).

Based on the results from this study, 4 species inhabiting the Canal and Presa (*Simocephalus punctatus, Alona glabra, Eucyclops leptacanthus* and *Macrocyclops albidus*) may exhibit potential as likely bioindicators vulnerable to As contamination and alkalinity. Species of the genus *Simocephalus*, *S. mixtus* and *S. serrulatus* have been reported

(Martínez-Jerónimo et al., 2008; Nogueira et al., 2008) to show great sensitivity in toxicological tests and bioassays, even with better sensitivities than the commonly used tested *Daphnia*. Therefore, we suggest *Simocephalus puntactus* as a likely bioindicador for As contamination in freshwater, genus that meet the characteristics of bioindicators, such as short life cycles, diverse feeding habits, small sizes and high reproductive rates (Chakri et al., 2014; Murugan and Sivaramakrishnann, 1973; Sharma and Pant, 1982). Especially because, unlike *Daphnia*, the species recommended for toxicity tests in the country's regulations, this species is distributed in Mexico (Elias-Gutierrez et al., 2008; Young et al., 2012).

On the other hand, species inhabiting water contaminated with As, such as *Latonopsis australis, Pleuroxus (Picripleuroxus) quasidenticulatus, Eucyclops chihuahuensis*, *Acanthocyclops americanus* and *Paracyclops chiltoni*, could be studied in the future to better understand As methylation in freshwater organisms, as well as their possible adaptations to survive.

Conclusions

As contamination in freshwater and sediment from Matehuala account for up to 53.23 mg/L and 2472.84 mg/kg, respectively. Such values are orders of magnitude higher than the Mexican guidelines for the protection of aquatic life (0.2 mg/L and 17 mg/kg). Yet, microcrustaceans, as the extremophile *P. chiltoni*, were found in this contaminated environment, while *Simocephalus punctatus, Alona glabra, Eucyclops leptacanthus* and *Macrocyclops albidus* inhabiting As free freshwater in the study area could served as bioindicators of As contamination conditions, especifically, the species *S. punctatus*, whose genus has shown good results in ecotoxicology. Microcrustacean species reported in this study offer opportunities to better understand the incorporation of As in the trophic chain and likely morphologic/genotypic As adaptations.

Declarations

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Yadira J. Mendoza-Chavez: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. Jose L. Uc-Castillo: Formal analysis & Investigation. Adrian Cervantes-Martínez: Methodology, Investigation, Formal analysis, Writing - review & editing. Martha A. Gutierrez-Aguirre: Methodology, Formal analysis, Writing - review & editing. Nadia Martínez-Villegas: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Data Availability

All data generated or analysed during this study are included in this published article.

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Figures

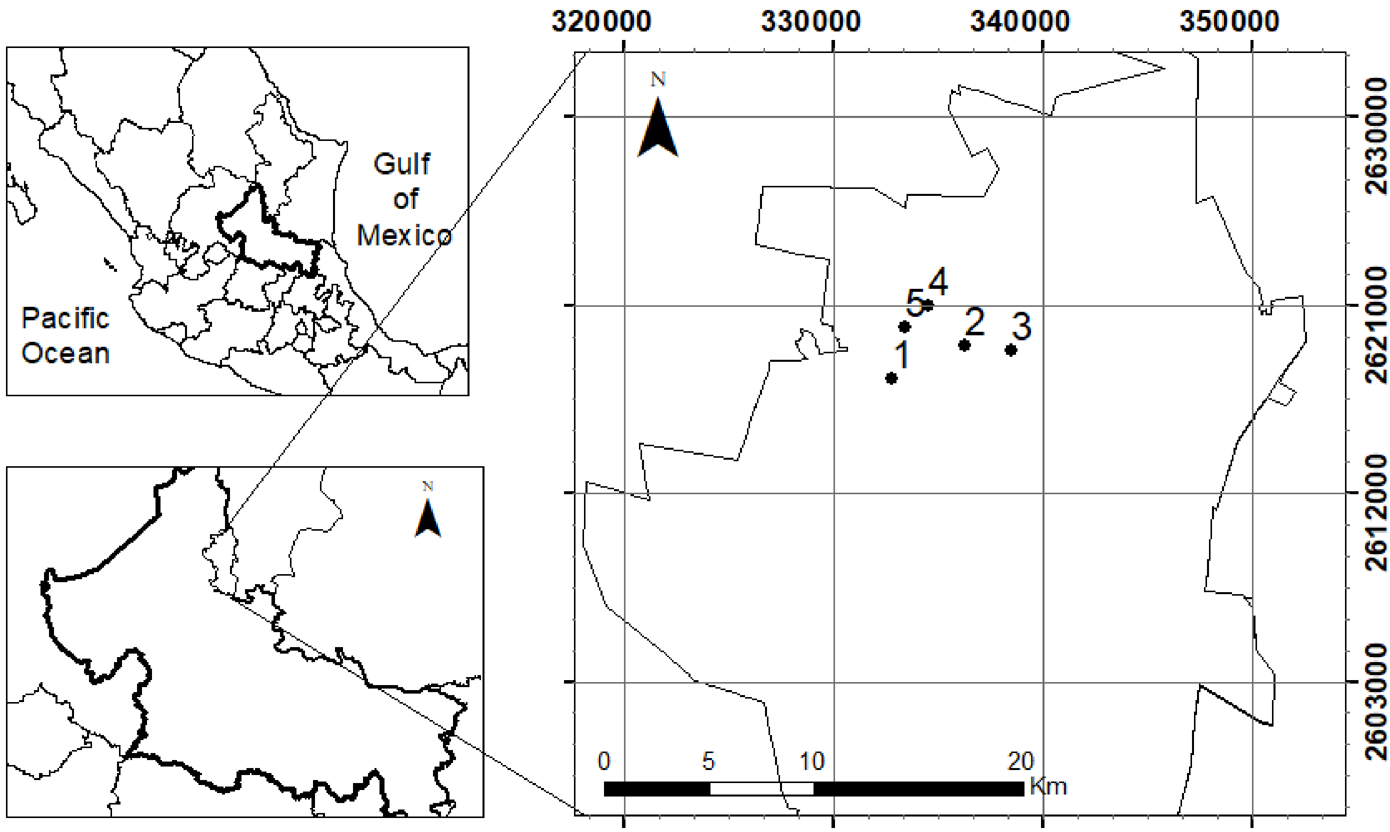

Figure 1

Location of the sampling points: 1) Club de Tiro, 2) Abrevadero, 3) Laguna, 4) Presa, and 5) Canal.

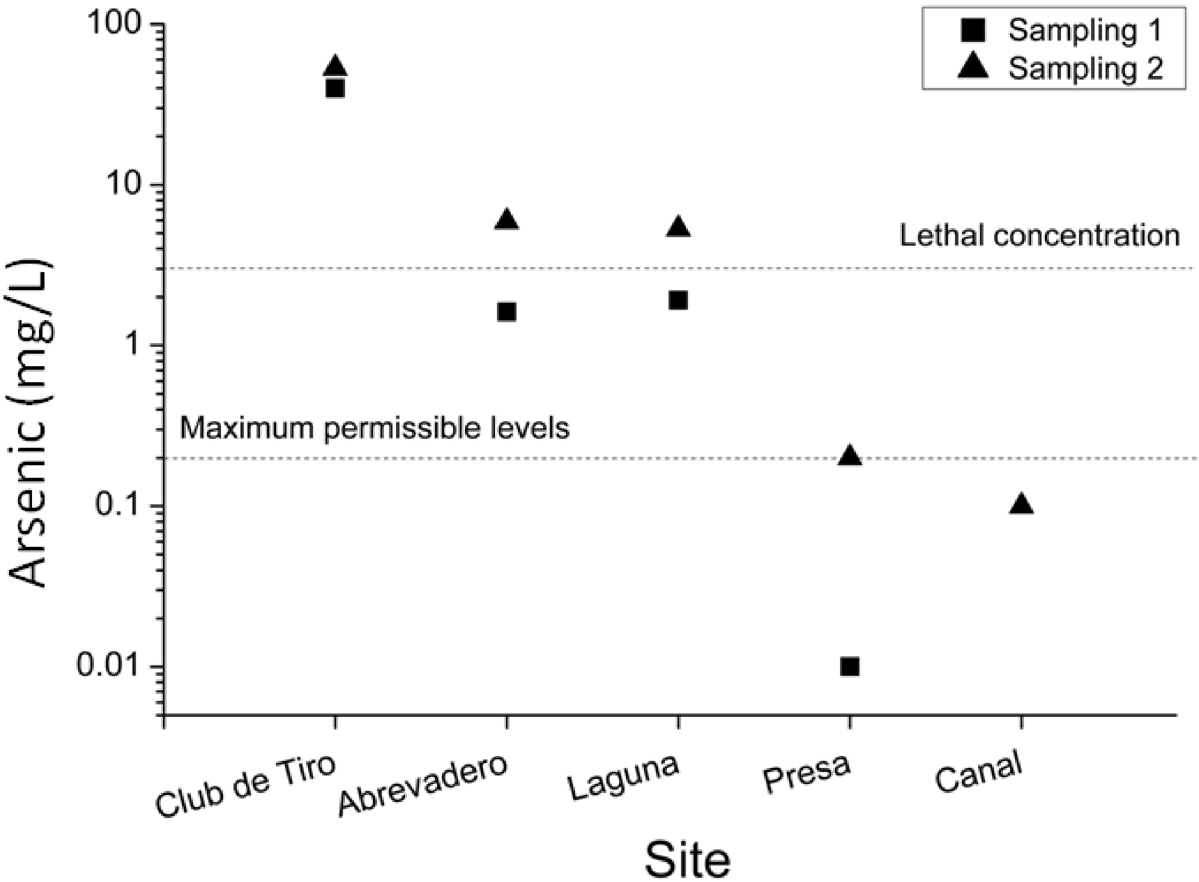

Figure 2

As concentrations in water simples, in squares the data of sampling 1 and in triangles the data of sampling 2, additionally the maximum permissible As concentration in natural waters (0.2 mg/L) in Mexico (DOF, 1989) as well as the As lethal concentration for zooplankton (3 mg/L) are presented in dotted lines (Chen et al., 1999).

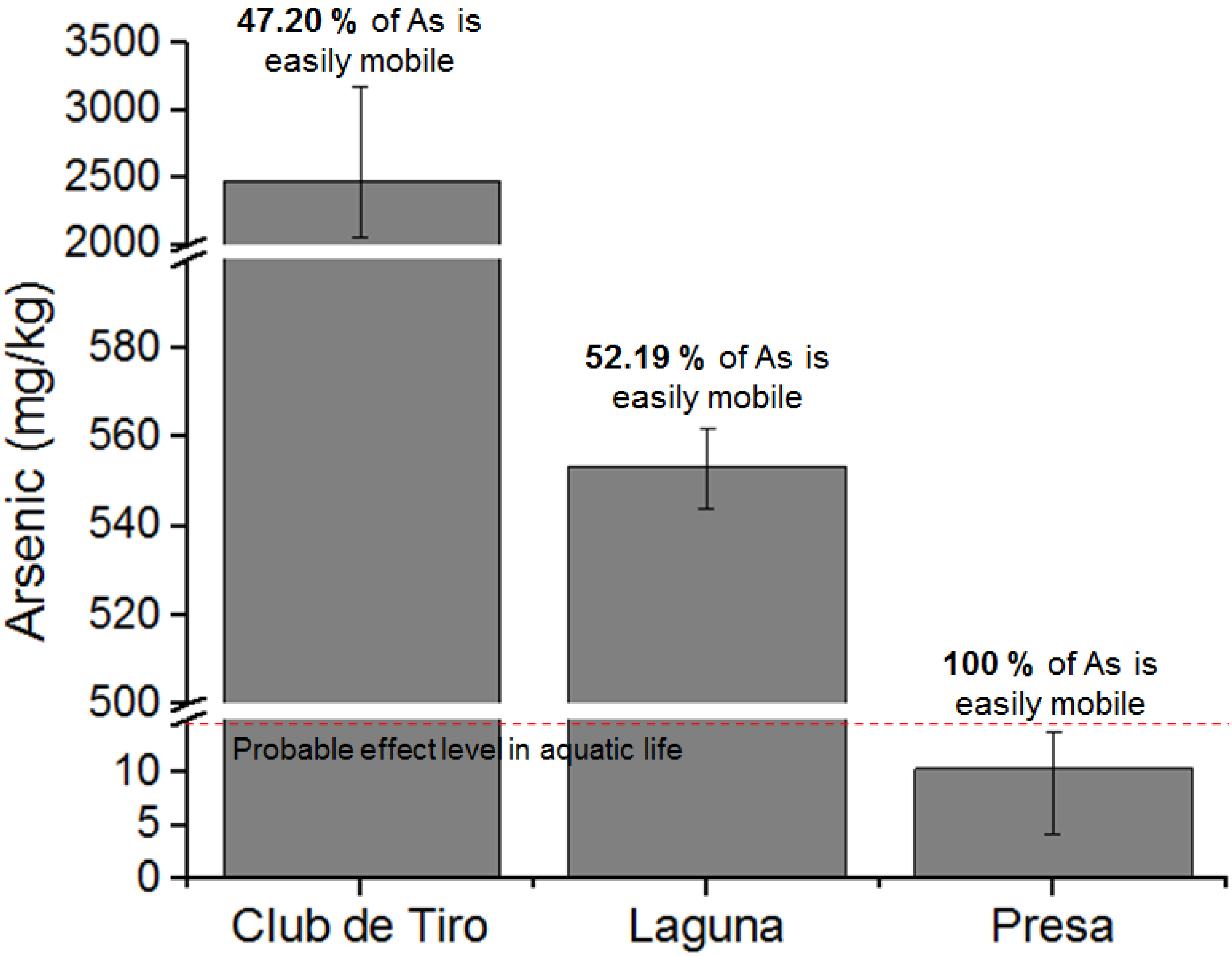

Figure 3

Total As concentration in sediment samples, recovery percentages of As in sediment digestions using 1% HNO_3 and the maximum permissible concentration of As for the protection of healthy aquatic systems (17 mg/kg) is presented in red dotted line (CCME, 1999).

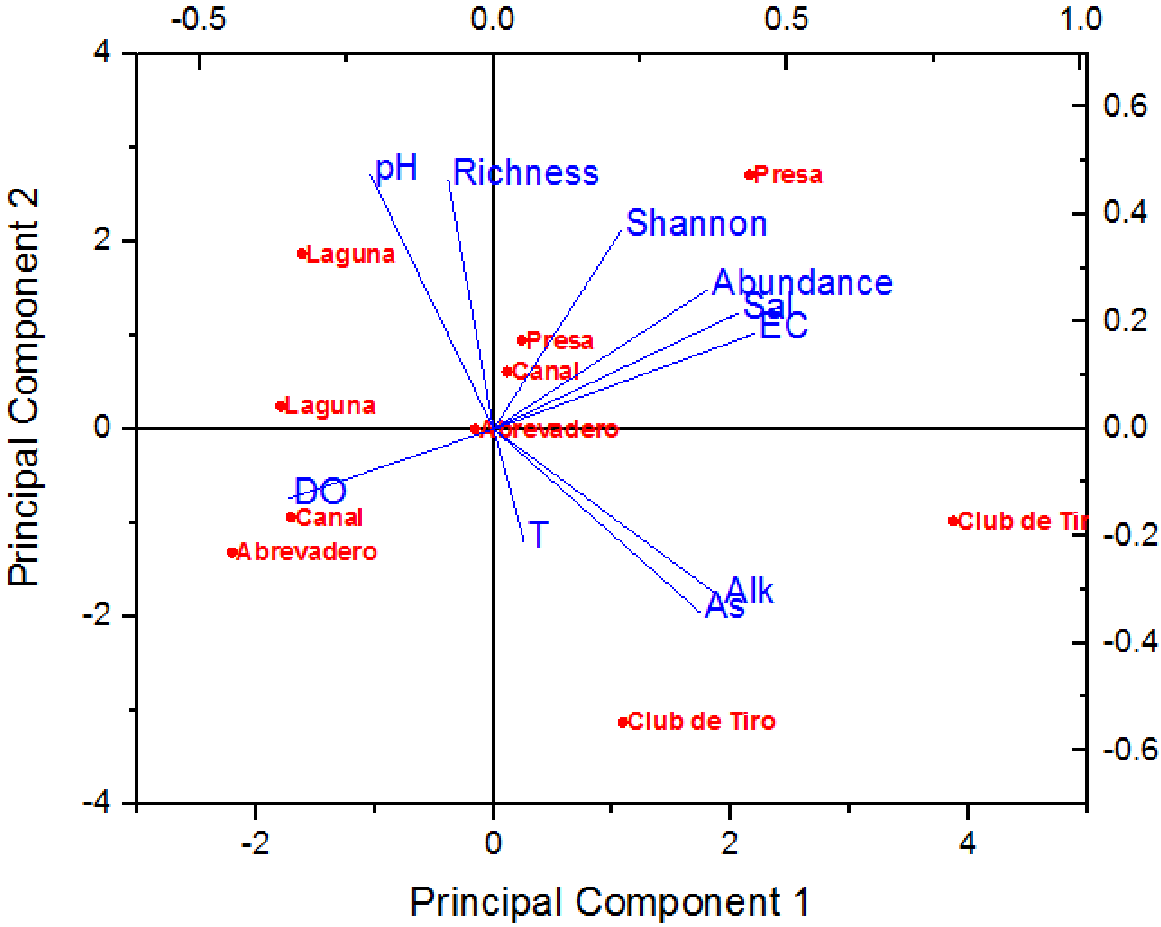

Figure 4

PCA biplot showing PC1 and PC2 as well as the contribution variables of each component. The physicochemical and ecological variables are presented in blue and the sampling sites in red.