American Journal of Environmental Protection, 2024, Vol. 12, No. 2, 21-30
Available online at http://pubs.sciepub.com/env/12/2/2
Published by Science and Education Publishing
DOI:10.12691/env-12-2-2

The Elemental Composition of Valves and Appendages in Seven Ostracod Strains from the Northeastern Yucatan Peninsula, Mexico: Baseline

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Received June 23, 2024; Revised July 25, 2024; Accepted August 01, 2024

Abstract The elemental composition of biological structures in ostracods cultured under standard conditions is a biological reference material and a baseline to study bioaccumulation due to exposure to inorganic compounds in laboratory experiments or samples collected from the environment in ostracods. Ostracods are used as paleoclimatic, and paleo-evolutionary study models, and are sentinels of climatic change and anthropogenic pollution. The main goal was to collect ostracods from the Yucatan peninsula and culture them in the laboratory under standard conditions to establish the elemental composition. Seven strains of ostracods of three species *Cypridopsis* cf. *vidua* (OF Müller), *Diaphanocypris meridiana* (Furtos), *Heterocypris* cf. *incongruens* (Ramdohr), were collected using a Wisconsin-type plankton net of 54 µm of mesh opening. We use EPA medium (NaHCO3, CaSO4•H2O, MgSO4•7H2O, and KCl) pH 7.5, and a hardness range of 80-100 CaCO3 mg L-1 to culture the ostracods, which were kept in a bioclimatic chamber with 25 + 2 oC and a photoperiod of 16:8 h light: darkness. Ostracods were fed commercial lettuce *Lactuca sativa*. A two-month acclimation period was enforced after which ostracod specimens were identified to genera and fixed with 5% formaldehyde. These samples were processed and photographed with the Scanning Electron Microscope with which the elemental analysis using X-ray diffraction was performed in valves and appendages. Our results indicate that most of the elemental Ca are in the valves (41% of total dry weight) when compared to appendages (4%). A total of twelve elements were found in valves and appendages: Al, Br, Ca, Cd, Cl, Cr, Cu, Mg, Na, O, and Si. Ostracods are excellent bioindicators of inorganic elements for the study of environmental contamination.

Keywords: *Bioaccumulation, Environmental Toxicology, Exploratory Strategies, Metal Toxicity, Zooplankton*

Cite This Article: Miguel Hernández-Pedraza, Jesús Alvarado-Flores, Marcelo Silva-Briano, Araceli Adabache-Ortiz, and Roberto Rico-Martínez, "The Elemental Composition of Valves and Appendages in Seven Ostracod Strains from the Northeastern Yucatan Peninsula, Mexico: Baseline." *American Journal of Environmental Protection*, vol. 12, no. 2 (2024): 21-30. doi: 10.12691/env-12-2-2.

1. Introduction

In Mexico at the Yucatan Peninsula, there is one of the most important freshwater aquifers in the region which is compared with Guatemala and Belize [1,2]. This is a karstic aquifer containing a vast diversity of aquatic ecosystems including freshwater, brackish, and estuarine [1,3,4]. There are also a lot of different types of reservoirs like cenotes (natural sinkholes), underground rivers, wetlands, mangroves, coastal lagoons, and caverns, among others. Saline intrusion and anthropogenic contamination (both inorganic and organic) are the main problems that the aquifers face in this region [5,6,7,8]. For instance, a total of 26 heavy metals have been detected in water, sediment, and biota of the Yucatan peninsula since 1987; Cd (16%) and Pb (14%) are predominant in the Yucatan peninsula [9].

The dispersion and magnitude of metal contamination in the aquifer are hard to estimate due to the different sources of contamination, the underground flows, and the karstic characteristics. However, the detection of metals in sentinel species is a useful tool that has been already used to study the patterns of metal dispersion in karstic aquatic systems of the Yucatan Peninsula. Alvarado-Flores *et al*. [10] reported the presence of Cd, Cu, Hg, and Pb in the external structures of cladocerans, copepods, diatoms, and rotifers from the state of Quintana Roo. However, no

reports of ostracods from the Yucatan peninsula have been reported so far, although ostracods are excellent bioindicators of metal accumulation and health of reservoirs at the Yucatan peninsula [11].

Ostracods are microcrustaceans of 0.5 to 3.0 mm in size. The body and soft structures are located inside an exoskeleton or carapace of two valves that secrete a chitinose-calcarean secretion, the valves are subjected by abductor muscles and dorsally articulated [12,13]. The ostracods are widely distributed and are common in aquatic ecosystems [14,15], but also in some terrestrial ecosystems or humid forests [14,16]. There are 8000 species worldwide and 2000 are freshwater which belongs to three superfamilies: Darwinuloidea, Cypridoidea y Cytheroidea [17]. The presence, absence, and relative abundance of ostracods are very well related to the physicochemical characteristics of the aquatic environments like conductivity, temperature, energy level, and levels of dissolved oxygen [18,19,20,21].

In the southern region of Mexico bordering Belize and Guatemala, several species of ostracods of the 23 genera have been reported, *Cypria, Heterocrypris, Monoculus, Alicenula, Bradleystrandesia, Chlamydotheca, Cypretta, Cypria, Cyprideis, Cypridopsis, Cyprinotus, Cypris, Cytheridella, Darwinula, Eucrypris, Heterocypris, Keysercypria, Neocypridopsis, Paracythereis, Peryssocytheridea, Pseudocandona, Strandesia, and Vestalenula*, living in these environmental conditions: minimum temperature of 19.1°C and maximum of 32°C, minimum pH of 6.9 and maximum of 9.3, with an average dissolved oxygen of 4.49 mg/L, and an average conductivity of 1858 µS/cm [22]. It is important to mention that the genus *Cypridopsis*, *Cypretta and Diaphanocypris* is a highly distributed species complex in Yucatan, with genetic and morphological variations distinguished in two important regions, the north and south. Their wide distribution and adaptability make them a representative genus for studies of ecotoxicology [23], ecology and climate change [22,24].

Ostracod valves and appendages represent an interface of the environmental mineralogical changes that vary accordingly with environmental contamination, because calcium carbonate exoskeleton can be fossilized conserving information about the environment they inhabited [25]. Therefore, ostracods are excellent bioindicators of climatic change and environmental deposition of inorganic substances. This is the reason that we need to study the elemental composition of ostracods cultured in standard laboratory conditions, which would contribute significantly to creating a reference baseline for studies of bioaccumulation of metals in zooplanktonic ostracods.

2. Material and Methods

Collection of ostracods

Ostracods were collected using a Wisconsin-type plankton net of 54 µm of the mesh size. Between three and six pulls of the net were performed. A 500 mL jar was obtained and divided into equal 250 mL parts. The first part was preserved in 5% formaldehyde to identify ostracods. The second jar was taken to the ecotoxicology laboratory at the Unit of Water Sciences to isolate, identify and culture the ostracods. We used the dichotomic keys of Nagler *et al*. [26] to identify ostracods under an inverted microscope Nikon Eclipse (Tokyo, Japan), model TS 100, and a stereoscopic microscope Nikon SMZ745, model C-LEDS. The collection sites were the following: Punta Laguna (20.649855 N, -87.640872 W), Cenote Rancho Viejo (21.202770 N, -86.840863 W), Humedal La Esperanza (20.974068 N, -87.420709 W), Cenote Azul-Ha (20.835528 N, -87.323713 W), Cenote Corchalito (21.025016 N, -87.203505 W), Cenote Leona Vicario (20.961734 N, -87.273946 W), and Tecnológico de Valladolid (20.722959 N, -88.196682 W).

Isolation and culture of ostracods

To develop the ostracod cultures, the organisms collected were placed in 10 mL of EPA medium consisting of 96 mg/L of $NaHCO_3$, 60 mg L^{-1} of $CaSO_4 \cdot 2H_2O$, 60 mg L^{-1} of $SO_4 \cdot 7H_2O$, and 4 mg L^{-1} of KCl [27], adjusted at a pH of 7.5; with a hardness of 80-100 mg L^{-1} of $CaCO_3$. The maintenance of the organisms was achieved by using the protocol of Hernández-Pedraza *et al.*, [28]. The cultures were maintained for one year under controlled conditions in a bioclimatic chamber at 25 $\pm$ 2°C and a photoperiod of 16:8 light: darkness and fed with commercial lettuce (*Lactuca sativa*). We observed the growth of ostracods especially of the species *Cypridopsis vidua* of the RV-2 strain, to determine the maximum number of organisms produced at 25 days under controlled conditions: 5 organisms in 2 ml with a 1x1 cm piece of lettuce, using 24-well plate (Corning).

Analysis of Elementary Composition using Scanning Electron Microscopy/ X-Ray Dispersive Spectroscopy

The ostracods after a two-month acclimation period were used for the analysis of scanning electron microscopy/X ray dispersive spectroscopy (SEM/EDS-X). Twenty to twenty-five specimens (15-30 d of age) were fixed in 5% formaldehyde for at least 24 h. Then, they were washed three times with deionized water and dehydrated with gradual concentrations of alcohol (60, 70, 80, 90, 96, and 99.9 %) for 30 minutes in each alcohol solution. After dehydration, the samples were placed in Samdri®-PVT-3D equipment to submit them to a critical point and eliminate alcohol through the application of liquid CO_2. Posteriorly, the specimens were placed in an aluminum cylinder with graphite for scanning and coated with a gold coat to take some scanning electron photographs, and from those photographs, the EDS-X allows for the elemental analysis.

The spectral signal of each element was detected by using the software INCA suite 3.04. For the analysis an acceleration voltage of 20,00 kV; time = 50 s; elevation = 35; the number of iterations = 4-6. The histogram contains an “x” axis (energy units: kilo electronvolts), and a “y” axis (intensity), with a penetration range of 1 µm (Alvarado-Flores *et al*., 2019). This technique allows for both qualitative and quantitative analysis according to Sigee *et al*. [29], Newbury and Ritchie [30], and Alvarado-Flores *et al*. [31].

Ostracod map

The map was made with the data of geographic coordinates of the collection sites, layers of vector

information of the Mexican territory, the Valladolid hydrogeological reserve and the Cancun-Tulum corridor obtained from the INEGI databases were used, the map was prepared with software version QGIS 3.30.1-´sHertogenbosch.

3. Results

Seven strains of ostracods belonging to three different species of the family Cyprididae were identified and cultured from the samples collected. The strain RV-1 collected from Rancho Viejo corresponds to *Diaphanocypris meridiana* (Furtos). The strains AH, CO, LE, RV-2, and TV, belong to the species *Cypridopsis cf vidua* (OF Müller). The strain PL belongs to *Heterocypris* cf *incongruens* (Ramdohr). Figure 1 includes Scanning Electron Microscopy (SEM) photographs of the valves and appendages of the three species and seven strains collected. Figure 1a corresponds to *Diaphanocypris meridiana* (strain RV1), and *Cypridopsis cf vidua* strains CO, LE, and TV. Figure 1b includes *Heterocypris* cf *incongruens* strain PL, and *Cypridopsis cf vidua* (strains AH and RV-2). *Cypridopsis* cf *vidua* strain RV-2 produces an average of 76.57 individuals after 25 days with a standard deviation of 17.47 individuals (n=14). This represents enough individuals to obtain a sample for X-ray analysis and to establish a culture for toxicity tests, in the other strains we do not analyze the total individual produced at 25 days. However, using the same culture conditions, we obtained sufficient organisms to culture and maintain the strains as a rutinary culture ostracods lab. In the Figure 2, we showed the geographical distribution of ostracods from the northeast of Yucatan peninsula.

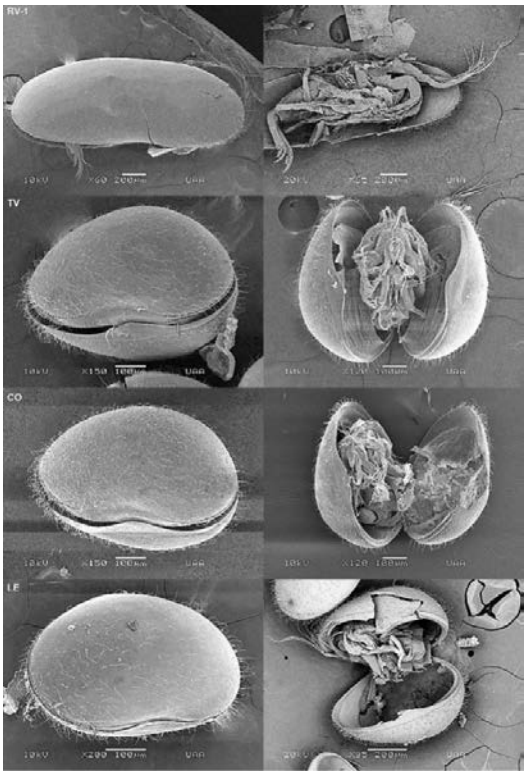

Figure 1a. SEM Photographs of *Diaphanocypris meridiana* (strain RV1), and *Cypridopsis cf vidua* strains CO, LE, and TV

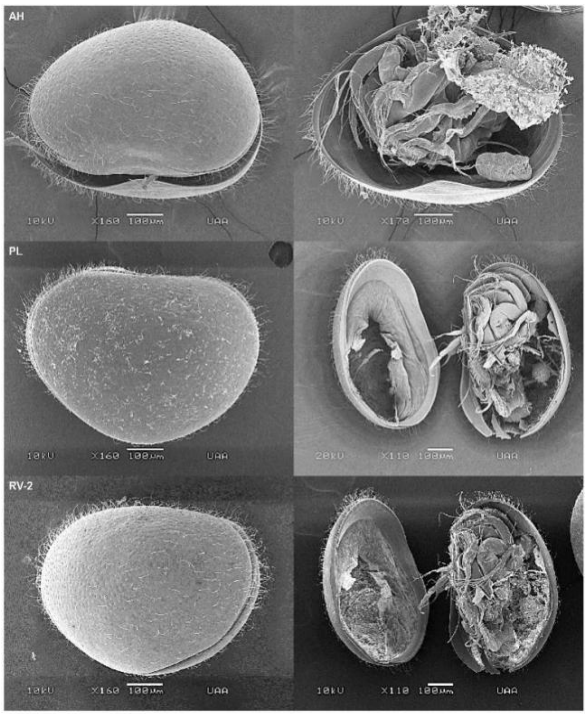

Figure 1b. *Heterocypris* cf *incongruens* strain PL, and *Cypridopsis cf vidua* (strains AH and RV-2)

A total of 38 photographs were analyzed to study elemental composition. Six each of the strains RV-1, and TV4, 4 from the carapace, and 2 from the appendages in both cases. Five each of the strains CO, LE, and RV2, 4 from the carapace, and 1 from the appendages in all three cases. Five of the strains AH and PL, 3 from the carapace, and 2 from the appendages in both cases.

A total of twelve elements were detected as components of valves and appendages (Table 1). This data represents a first attempt to establish a baseline for the elemental composition analysis of three ostracod species of the Yucatan Peninsula. In the appendages, we detected: Al, Ca, Cd, Cl, Cr, Cu, P, and S. In the valves, we detected: Al, Br, Ca, Cd, Cr, Cu, Mg, Na, and Si.

In Figure 3, the macroelements C and O were eliminated from the analysis, and the potential technical contaminants Au, Ir, Hf, Nb, and Pt were also eliminated. Figure 3 shows the presence or absence of the rest of the twelve elements present in the seven strains of ostracods cultured under laboratory conditions. Figure 4 was elaborated from the mean weight values in percentages of each element in each structure and presented as bar graphs. The percentages of calcium composition (based on the dry weight) of calcium in the valves of the seven strains cultured in this work are shown in Figure 5.

Table 2 shows the percentages of C, Ca, and O in valves and appendages of ostracods collected from the Yucatan Peninsula in this work. Ca is the most abundant element in the elemental composition of the ostracod valves (Figure 5). The percentage of the dry weight of Ca in valves varies between 30-40% of the total composition (Table 2). In appendages, Ca represents less than 10% (Table 2).

The amount of Ca is different in the zooplankton collected in the field and that of species reared in the laboratory as shown in Table 3.

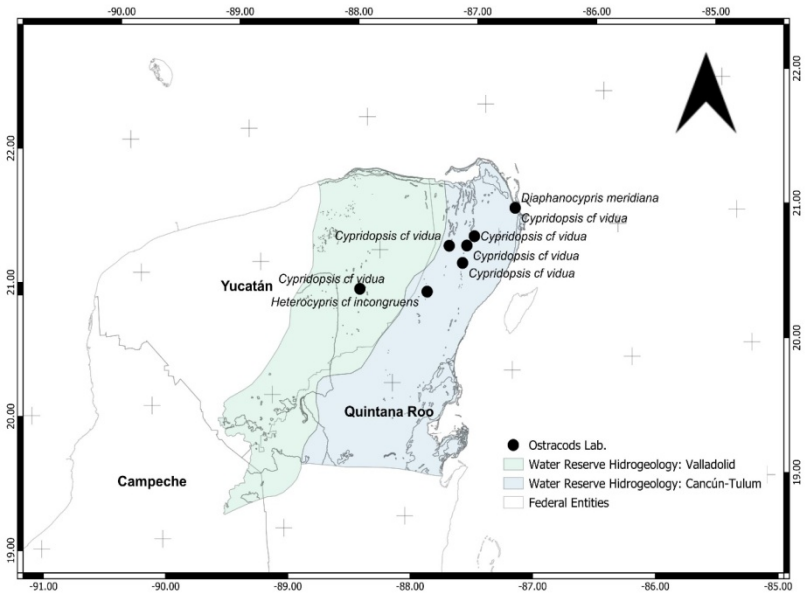

Figure 2. Geographical location of the three species of ostracods grown in the laboratory from the seven localities located in the northeast of the Yucatan peninsula, note that the largest number of sites is within the groundwater hydrogeological reserve of the Cancun-Tulum corridor

Table 1. Elemental composition (%) of valves and appendages of the ostracods from the Yucatan Peninsula. *Diaphanocypris meridiana* strain RV-1, and *Cypridopsis cf vidua* strains CO, LE, AH, RV-2 and TV. *Heterocypris* cf *incongruens* strain PL

Strain	Structure	C	O	Br	Na	S	Mg	Ca	Cu	Cd	P	Al	Si	Ir	Cr	Cd	Pt	Nb	Cl	Hf	Total
RV-1	Valve	11	32					54				1	2								100
RV-1	Valve	65	32					2				1									100
RV-1	Appendage	62	37												0	1					100
RV-1	Valve	47	34				2	17		0											100
RV-1	Valve	24	41					35				0									100
RV-1	Appendage	55	37					7				0									100
TV	Valve	36	23					16				0						25			100
TV	Appendage	59	35					5				1							0		100
TV	Valve	9	16					73				1									100
TV	Appendage	61	36					2												0	100
TV	Valve	20	47				0	29				5									100
TV	Valve	21	28					51													100
CO	Valve	17	52		0		1	30	0		0	0									100
CO	Appendage	59	32					4	3			3									100
CO	Valve	15	46				1	39													100
CO	Valve	9	13	3				68	7												100
CO	Valve	64	31					2	1			2									100
LE	Valve	18	46				1	35													100
LE	Valve	27	29					36				8									100
LE	Valve	20	34					45				1			0						100
LE	Valve	16	26					57				0	0	0							100
LE	Appendage	60	35					4				1									100
AH	Appendage	57	39					3													100
AH	Valve	32	41				0	26										1			100
AH	Valve	12	20				1	66				1									100
AH	Appendage	68	28			0		2			1	0									100
AH	Valve	53	18					6									9	14			100
PL	Appendage	55	31					10	2			1								2	100
PL	Valve	12	31				1	55	1			1	0								100
PL	Valve	17	47				1	34	1												100
PL	Appendage	60	29					2	0			0						9			100
PL	Valve	12	31				1	55	1			1	0								100
RV-2	Valve	36	30				1	31	2						0						100
RV-2	Valve	48	30					18	5												100
RV-2	Valve	60	35					4	1			0									100
RV-2	Appendage	55	36					7	1			1									100
RV-2	Valve	11	31				1	54	2			1									100

Table 2. Descriptive statistics of Ca, C and O present in the appendages and shell of ostracods from the Yucatan peninsula, the values are expressed in % weight. App = Appendages, and Val = Valves

	App-Ca	*Val-Ca*	*App-C*	*App-O*	*Val-C*	*Val-O*
Mean	4.293	41.900	59.201	34.192	27.425	32.430
Typical error	0.665	3.585	1.149	1.070	3.522	1.952
Median	3.610	37.280	59.450	34.960	20.075	30.990
Mode	#N/A	#N/A	#N/A	#N/A	12.230	30.670
Standard Deviation	2.489	16.815	3.812	3.549	17.961	9.953
Variance	6.195	282.759	14.533	12.594	322.605	99.059
Kurtosis	-0.207	-0.951	1.712	-0.935	-0.316	-0.385
Asymetry coefficient	0.865	0.160	0.943	-0.404	0.990	0.112
Range	8.070	57.340	13.270	11.090	55.750	38.510
Minimum	1.520	15.730	54.600	28.350	9.130	13.450
Maximum	9.590	73.070	67.870	39.440	64.880	51.960
Sum	60.100	921.790	651.210	376.110	713.060	843.180
N	14	22	11	11	26	26
Confidence level (95.0%)	1.437	7.456	2.561	2.384	7.255	4.020

Table 3. Analysis of the composition of Ca (weight %) in zooplankton using x-ray microanalysis. *Diaphanocypris meridiana* strain RV-1, and *Cypridopsis cf vidua* strains CO, LE, AH, RV-2 and TV. *Heterocypris* cf *incongruens* strain PL

Specimens	Ca (Weight %)	Origin	References
Ostracods Valves	28.75	Environmental	Alvarado-Flores Jesús, 2017; datos no publicados
Ostracods RV-1	35.39 ± 18.50	Laboratory	This study
Ostracods TV	42.25 ± 25.28	Laboratory	This study
Ostracods CO	45.39 ± 19.81	Laboratory	This study
Ostracods LE	43.27 ± 10.36	Laboratory	This study
Ostracods AH	32.68 ± 30.18	Laboratory	This study
Ostracods PL	44.13 ± 14.86	Laboratory	This study
Ostracods RV-2	34.25 ± 18.37	Laboratory	This study
Rotifers	1.40 ± 0.96	Environmental	Alvarado-Flores *et al*., 2017
Cladoceran	1.64 ± 1.08	Environmental	Alvarado-Flores *et al*., 2017
Copepods	1.42 ± 0.42	Environmental	Alvarado-Flores *et al*., 2017
Diatoms	16.67 ± 6.48	Environmental	Alvarado-Flores *et al*., 2017
Dinoflagellates	5.95 ± 1.05	Environmental	Alvarado-Flores *et al*., 2017
Lecane bulla	0.26 ± 0.11	Laboratory	Alvarado-Flores *et al*., 2017
Lecane quadridentata	0.52 ± 0.09	Laboratory	Alvarado-Flores *et al*., 2017
Brachionus cf *ibericus*	0.65 ± 0.80	Laboratory	Alvarado-Flores *et al*., 2017
Brachionus calyciflorus	0.77 ± 0.46	Laboratory	Alvarado-Flores *et al*., 2019

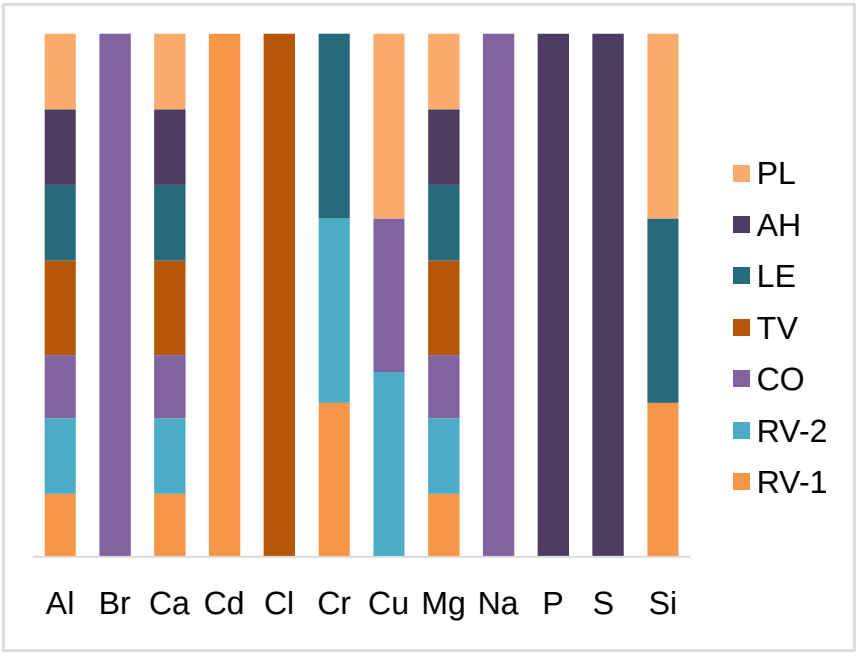

Figure 3. Presence and absence of the elemental composition of seven ostracod strains. *Diaphanocypris meridiana* strain RV-1, and *Cypridopsis cf vidua* strains CO, LE, AH, RV-2 and TV. *Heterocypris* cf *incongruens* strain PL

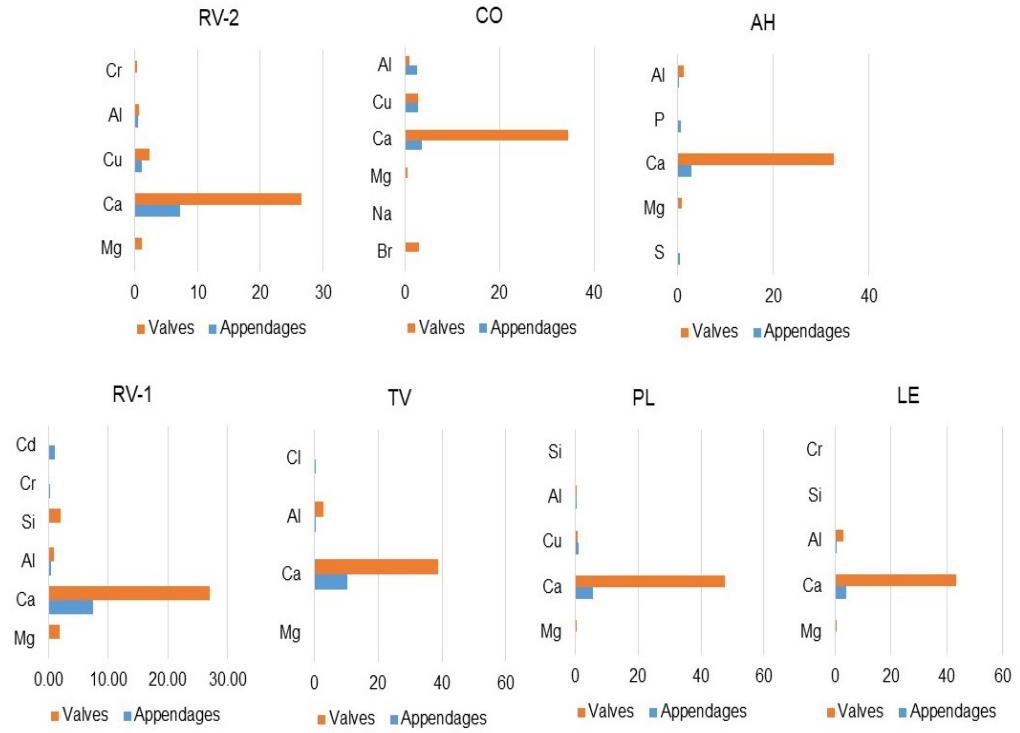

Figure 4. Elemental composition (weight %) of valves and appendages of seven ostracod strains. *Diaphanocypris meridiana* strain RV-1, and *Cypridopsis cf vidua* strains CO, LE, AH, RV-2 and TV. *Heterocypris* cf *incongruens* strain PL

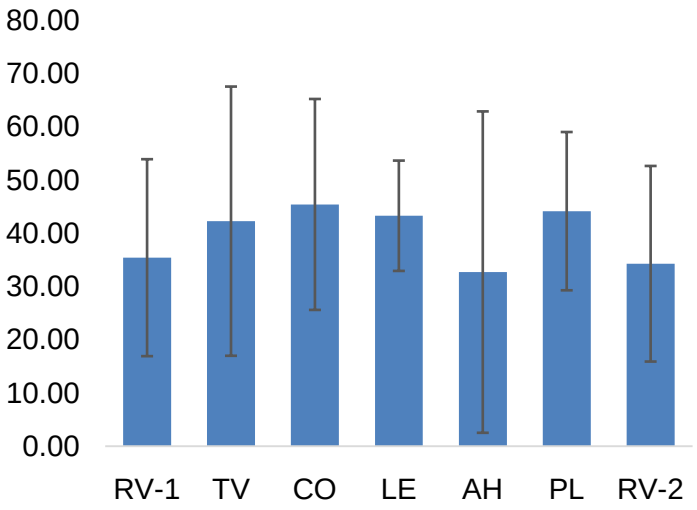

Figure 5. Mean and standard deviation of the weight (%) of Ca in the carapace of the seven ostracod strains from the Yucatan Peninsula. *Diaphanocypris meridiana* strain RV-1, and *Cypridopsis cf vidua* strains CO, LE, AH, RV-2 and TV. *Heterocypris* cf *incongruens* strain PL

In general, the valves have the higher amount (wt%) of Ca and Al than the appendages as shown in Figure 4, where a comparative bar graph of valves of the amount in wt% of detected elements was used.

Ca is the most important element in the elemental composition of ostracod valves and in the column graph of Figure 5. The amount (weight %) of Ca in valves is between 30-40% of the total composition, with based on the analysis of the elemental composition by X-rays, while in appendices the values are below 10% (weight), according to the descriptive statistics shown in Table 2.

A total of twelve elements were detected in all samples of valves and appendages assessed in this contribution. Only Br, Mg, Na, and Si were detected in all valve samples. Only Cl, P, and S were detected in all appendage samples. The metals Cd, Cr, and Cu were detected in both appendages and valves. Silicon (Si) was only detected in three strains of ostracods LE, PL, and RV-1. In the strains, RV-1 and RV-2 more elements were detected. In the strains of TV only four elements were detected (Al, C, Ca, and O). In all other strains, 5-6 elements were detected. The relation between Ca and C in ostracods averages 1.73, with a minimum of 1.148 in RV-1 strain, and a maximum of 2.37 observed in the CO strain (Table 4). Among species, the relation is *Heterocypris* > *Cypridopsis* > *Diaphanocypris*. Curiously the Mg/Ca relationship is inverse: *Diaphanocypris* > *Cypridopsis* > *Heterocypris* (Table 4). In addition, we performed the Mg/C relation

with the following result: *Heterocypris* > *Cypridopsis* > *Diaphanocypris*. Finally, the Al/C relationship was: *Cypridopsis* > *Diaphanocypris* > *Heterocypris*.

Table 4. Relationship between the elemental composition of Ca, Mg and Al with carbon and the Mg/Ca ratio using the detected % weight values of valves and appendages of the seven ostracod strains. *Diaphanocypris meridiana* strain RV-1, and *Cypridopsis cf vidua* strains CO, LE, AH, RV-2 and TV. *Heterocypris* cf *incongruens* strain PL

Strain	Ca/C	Mg/C	Al/C	Mg/Ca
RV-1	1.148	0.007	0.026	0.112
TV	2.048	0.003	0.063	0.013
CO	2.370	0.014	0.015	0.017
LE	1.826	0.008	0.069	0.019
AH	1.299	0.026	0.021	0.020
PL	2.216	0.027	0.025	0.015
RV-2	1.230	0.028	0.017	0.025

4. Discussion

As baseline of elemental composition in ostracods from Yucatan peninsula, 12 constituent elements of valves and appendages of ostracods culture in laboratory conditions were detected as a material references for ecotoxicology studies on the dispersion and bioaccumulation of metals, metalloids, and metallic nanoparticles such as TiO_2 and ZnO_2 (considering emerging pollutants both) in aquatic environments of the karstic zone of the Yucatan peninsula. The elements that the ostracods accumulate in their valves are all those elements that are dissolved in the water, for example, in culture organisms they adsorb and assimilate the elements that are added in the reconstituted media or liquid media, instead in the medium environment are all those elements that are dissolved or available in the water, and also, they absorb all the elements that are in their diet, either in the laboratory or that available in their ecological niche. When we compare the diverse of elements that are detected in ostracod cultures in the laboratory with zooplankton species from environmental samples, according to the study carried out by Alvarado-Flores *et al*. [10], we observe differences, there are more elements in collected organisms than in cultivated ones, for example, in the presence and detection of Cd, Cu, Zn, Hg, and Pb in zooplankton from environmental samples: in rotifers a total of 13 elements were detected (Al, Na, Mg, Si, P, S, Cl, K, Ca, Cu, Zn, Fe, and Pb), in cladocerans 10 elements were detected (Al, Mg, Si, S, Cl, Ca, Cu, Br, Cd, and Hg), in copepods 11 elements were detected (Al, F, Mg, Si, P, S, Cl, Ca, Cu, Br, and Cd).

Hg appears in cladocerans of environmental samples, while in cultures it has never been detected, and the metals Cd and Pb were detected in rotifers, cladocerans, ostracods, and copepods by X-ray diffraction and by quantification by atomic absorption in whole digested organisms (with HNO_3) collected in the environment by Pérez-Yañez [9] in a region of Quintana Roo, named as sinkhole route of Puerto Morelos. In our cultured ostracods, we detected by X-ray diffraction the metals Cd, Cu, and Cr, which were not added to the liquid media where the reference ostracods cultured. We attribute its presence to the diet to which the cultures were established since commercial lettuce was used and it was randomly acquired from commercial centers, therefore, we suggest that for future studies it is recommended to use microalgae grown in the laboratory to avoid contamination by cross-contamination and the presence of these metals in reference or control materials.

In general, the constituent elements of cultured ostracods and even environmental samples are found in the valves and their appendages immersed in ostracod chitin: a specialized tissue to fix intracellularly and extracellularly the elements dissolved in water and thereby maintain homeostasis organisms by letting cations and anions in and out, and/or fixing them in their valves. This mechanism of absorption and adsorption of elements is carried out by the internal epidermal cells of the ostracod, through the endocuticle towards the exocuticle and epicuticle, which fix the dissolved elements in the form of crystals in the valves through a dynamic process that serves to the formation and molting of its shell in ostracods [12,32]. The main scaffolding and structural support of the valves and appendages are chitin and Ca. When observing the amount of Ca expressed as a weight percentage in different groups of zooplankton (see Table 3), the ostracods together with the diatoms are the groups that have the greatest amount of Ca, followed by copepods, cladocerans, and rotifers.

In the epicuticle, high levels of P are quantified, and in the endocuticle, high levels of Ca and Mg, for example, in total in the ostracod *Skogsbergia lerneri*, up to 6 elements have been quantified (Na, Mg, P, S, Si, and Ca) in the valves. And the weight in % of Ca oscillates between 20-40% of the total composition of the shell in various stages of *S. lerneri*: Instar1 stage, early-middle, late-middle, and adult according to what was done by [32]. And the element S, in this species of ostracod, its weight in % of the composition of the shell is very high because it is a marine ostracod.

Our reference organisms, the three ostracod species analyzed, show an average Ca value of 41% in valves and 4% in appendages. While the high amount in % by weight of C and O is different in valves and appendages, that is, there is a higher % by weight of C and O in appendages than in valves, according to Table 2. This is because In the valves there is a greater calcification than in the appendages. The high concentrations of C in % of weight in appendages suggest that they are more rigid or structurally strong since the appendages are used to move, reproduce, and feed, while the valves are a hydrodynamic and flexible tissue in which communication is prioritized. intra and extracellular with water and other cellular communication molecules, where biomineralization is favored as it is a tissue that is constantly in replacement or ecdysis as the ostracod grows.

The differences in elementary detection between valves and ostracod appendages are not random, it is a structure and function relationship, for example, in the valves it was observed that only Mg, Br, Na, and Si can be found. While the elements Cl, S, and P can only be detected in the appendages. The S and P are structural elements together with the large amount of C wt% in the appendages, while the Mg, Ca, Na and Si elements in the valves are elements that contribute to the homeostatic dynamics of the ostracod. All these microelements were added to the ostracod culture media: NaHCO3,

CaSO4·H2O, MgSO4·7H2O, and KCl.

The elements detected in the ostracod valves are accumulated due to a biomineralization process at a sub-micrometric scale, that is, the ostracod valves are composed of crystalline granules embedded in an organic matrix of chitin according to Branson *et al*. [33] in the marine ostracod of the genus *Krithe*. The incorporation of Ca into the ostracod valves according to Gussone and Greifelt [34] in marine ostracods shows that there is a great variation in the elemental deposition of Ca, Mg, and Sr in the ostracods of the *Buntonia* sp., *Cytherella* sp., *Henryhowella* sp., *Krithe* sp., *Poseidonamicus* sp., and *Ruggieria* sp. Our relationships between Ca/C, Mg/C, Al/C, and Mg/Ca (Table 3) in three different species of freshwater ostracods, cultured in the laboratory, show differences in the relationship between elements, despite being cultured under the same conditions. These differences are due to: a) they belong to different genera, b) the karstic region where they were collected, and c) their evolutionary origin. Therefore, it is important to analyze different species of ostracods, to understand the biomineralization processes of elements available in aquatic systems, analyzing the elemental composition of ostracod valves. With this, we can understand the hydrogeochemical adaptations of ostracods and assertively design a hydrogeochemical indicator in ostracods potentially associated with climate change. In addition, the biomineralization of toxic pollutants such as dangerous metals present in the air, water, and sediment could be detected and related to specific climatic changes.

The ostracods in the Yucatan peninsula, according to the study carried out by Macario-González *et al*. [22] indicate the existence of species complexes, they note that in the northern part of the peninsula, there is a large morphotype, while in In the southern zone of the peninsula, there is the small morphotype in the genera *Cypretta*, *Alicenula* and *Cypridopsis*, this last genus, with a high probability of being a species complex in the Yucatan peninsula, due to its great adaptability and presence in karstic ecosystems, makes it makes an excellent bioindicator of accumulation of inorganic polluting elements in water. We have as reference material 5 strains labeled as *Cypridopsis* cf *vidua* strains CO, LE and TV, AH and RV-2.

The *C. vidua* species complex is an excellent candidate for studies on the bioaccumulation of metals present in the environment that end up being incorporated into the water cycle, which will be deposited inside the shell. The karst system of the Yucatan peninsula has a large number of calcium carbonates, an essential element for zooplankton, especially for ostracods [35,36,37]. However, the diet of ostracods affects the formation of the valves, if this is rich in calcium there is a secondary thickening and formation of numerous protuberances [38]. As previously mentioned, ostracod valves are formed by biomineralized Ca and Mg in a layer of chitin during its last ecdysis from minerals dissolved in water.

How long does the calcification process take in ostracods? In the subclass Podocopida, the genus *Leptocythere* begins its calcification just after ecdysis (molting), but the degree of calcification remains the same as in the juvenile until approximately 35 h after ecdysis. The marginal fold of the adult specimen then begins to calcify from its distal part around 40 h after ecdysis, and short, simple pore channels can still be seen on the free margin. The marginal pore channels become more branched and narrower as calcification progresses beyond 100 hours post ecdysis (Yamada and Keyser, 2009). Calcium carbonate ($CaCO_3$) precipitates early in this precipitation/evaporation process and is virtually insoluble except at low salinities [39]. The point at which $CaCO_3$ precipitation begins to occur is called the branch point of calcite. Beyond this branch point, the precipitation of $CaCO_3$ causes the concentration of certain trace metals. In conclusion, the biomineralization process in ostracods is fast, which makes them excellent bioindicators to study adsorption processes, bioaccumulation of metals in contaminated environments, and laboratory experiments. In addition, their relatively short life cycles (more than 30 days) and their permanence in the environment and high distribution throughout all the aquatic ecosystems of the peninsula, the ostracods are a database of the past and present of the presence and absence of inorganic pollution in water and the environment.

For decades, studies in ostracods have focused on analyzing the chemistry of ostracod trace elements and their environmental importance, where he established that the absorption of certain elements such as Mg, Ca, and Sr in ostracod valves depends on the temperature and salinity of the water [40]. The absorption of the elements differs considerably from the species, for the majority of the epifaunal species the content of elements is related to the stationary cycles and correlated with the increases in temperature and/or with the variations of the Mg/Ca and Sr/ ratios. Ca of the water and the infaunal species are predominantly related to the interstitial chemistry of the sediments [41]. Ostracods are undoubtedly excellent environmental indicators [42] and in laboratory experiments for the quantification, bioaccumulation, and assimilation of hazardous elements in the environment, and the recent use of nanoparticles or metallic nanomaterials for multiple uses in industry, medicine, and technology. The elemental composition of ostracods from the Yucatan peninsula must be extended in environmental samples, in laboratory and experimental controls to decipher the biomineralization dynamics of priority elements such as TiO and ZnO nanoparticles, and heavy metals such as Cd, Pb, Cu, Cr, Zn, Li, and Al, because they have been detected in the karstic aquatic ecosystems of the Yucatan peninsula, the main source of drinking water for the southern region of the country. And the ostracods are a sentinel organism of the environmental pollution footprint of the new era, the Anthropocene.

ACKNOWLEDGMENT

Special thanks to Eduardo Cejudo for sharing the samples from the collection sites, and to Tecnológico de Valladolid and his Environmental Engineering students for giving us the sample where the TV strain was collected and identified. We thank Sergio Cohuo for advising us on the identification of two species of ostracods. To the CONAHCYT chairs project # 2944, the first author thanks CONAHCYT for the scholarship awarded # 815661. We thank the students of the Universidad Politécnica de

Quintana Roo and the Universidad del Caribe for helping to preserve laboratory cultures during their stays at CICY.

Statement of Competing Interests

The authors have no competing interests.

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