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Metal Concentrations in Age-Groups of the Clam, *Megapitaria squalida*, from a Coastal Lagoon in Mexico: A Human Health Risk Assessment

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Abstract

The present study shows the human health risk of Cd, Cu, Hg and Zn by consumption of clams *Megapitaria squalida* from Northwest Mexico, collected in 2013. The mean concentration for each metal in the soft tissue was: Zn > Cu > Cd > Hg; and mean values of 68.89 ± 37.59—30.36 ± 27.19, 8.77 ± 1.35—6.80 ± 0.36, 4.47 ± 0.21—3.18 ± 0.63 and 0.99 ± 0.81—0.52 ± 0.16 µg/g, respectively. Clam age was significantly negatively correlated ($p < 0.05$) with soft tissue Zn concentrations. For all metals there is a low level of human health risk associated with the consumption of *M. squalida*, but it is necessary to determine the specific characteristics of the human population of the study site.

Keywords Metals · Clams · Age · Risk assessment

Some metals are considered essential to living organisms since they form part of proteins and enzymes, however, most of them are toxic, even at low concentrations (Esposito et al. 2018). Metal contamination in coastal aquatic systems is mainly present in estuaries and around densely populated human settlements due to anthropogenic inputs of Cd, Cr, Cu, Hg, Pb and Zn, which are introduced to the environment at a higher proportion in comparison to natural sources (Ke and Wang 2018). Sentinel organisms have been used in order to evaluate marine and estuarine systems. Molluscs, especially those which are filter feeders, act as biomonitors for assessing the magnitude of environmental pollution and, therefore, are appropriate indicators for determining the presence and levels of metals in natural habitats (Guo and Feng 2018).

For these reasons, some species of mussels and oysters have been successfully used as biomonitors for metals in temperate and tropical waters since 1980. Because such organisms are often used for human consumption, potential human health risk can be assessed by determining metal concentrations in the edible soft tissue. *Megapitaria squalida* is a bivalve mollusc which is distributed from Baja California to Peru; this organism settles in sediments and is compatible with commercial, artisanal and recreational fishing (Aragón-Noriega 2016). *M. squalida* shows a seasonal reproductive cycle, with a long reproductive activity period from January to August, and an inactivity period from September to December. Additionally, spawning occurs when animals reach a total length of 92 mm (total length) and 2 years of age (Arellano-Martínez et al. 2006).

The metal content in bivalves could be affected by environmental (seasonal changes, pH, salinity, temperature) and physiological (age, sex, size, reproductive cycle, growth rate, nutritional status) factors (Alavian et al. 2017). In the

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present study, the concentrations of Cd, Cu, Hg and Zn were evaluated at different ages, in the soft tissue of the clams *Megapitaria squalida* obtained from the Navachiste lagoon system (which receives metals from urban discharge and agricultural run-off) in Sinaloa, Mexico (Southeast Gulf of California) to establish the potential risk to human health from its consumption.

Materials and Methods

Organisms were collected from a clam bank in Navachiste lagoon, Sinaloa, Mexico during March, April, June and October of 2013 (Fig. 1). These organisms were collected manually through free diving and stored in plastic containers. All field and laboratory materials used in this study were acid washed (Moody and Lindstrom 1977). To determine the age, the valves of each organism were used to observe the external hyaline bands with a stereoscope (Metcalfe-Smith et al. 1996; Leyva-Velázquez 2015). Afterwards, the edible soft tissue portions of the organisms were lyophilized (− 49 °C and 133×10^{-3} mBar) and were submitted to acid digestion with HNO_3 (trace metal grade) for 4 h at 120 °C in Teflon SAVILLEX containers. After digestion, the samples were analyzed for Cd, Cu and Zn through atomic absorption flame spectrophotometry (Ruelas-Inzunza et al. 2009a), and Hg was determined through cold vapor atomic absorption spectrophotometry (Ruelas-Inzunza et al. 2009b).

For accuracy of the technique, samples were analyzed in triplicate and blanks were used; certified fish protein reference material DORM-3 (National Research Council.

Canada) was analyzed with a recovery of between 90.27 and 104.34%, with a detection limit of 0.01 µg/g. Since data were not normal (Kolmogorov–Smirnov and Bartlett'stests), the existence of significant differences among the mean concentration of Cd, Cu, Hg and Zn, calculated for different ages, was determined using non parametric ANOVA (Kruskall–Wallis) and Dunn's multiple comparison tests. Spearman correlations were used to determine the relationship among different metals and different age of clams. All analyses were performed under a confidence level of $\alpha = 0.05$ (Zar 1999).

Furthermore, the hazard quotient (HQ) equation HQ = E/RfD (Newman and Unger 2002), was used to determine the risk to human health, where E is the level of exposure and RfD is the reference dose for each metal (Cu:10; Cd:1.0; Hg:0.5 and Zn:300, all in µg/kg/day; US Environmental Protection Agency (EPA) 2000; US Food and Drug Administration (FDA) 2006). The level of exposure was calculated as E = C*I/W, where C is the concentration of each metal in µg/g (wet weight), I is the estimated daily ingestion of the clam in the Mexican population (0.19 kg/person/year = 0.52 g/person/day; National Aquaculture and Fishing Commission (CONAPESCA) 2017) and W is the average weight of an adult consumer (70.7 kg, National Wearing Industry Agency (CANAIVE) 2012).

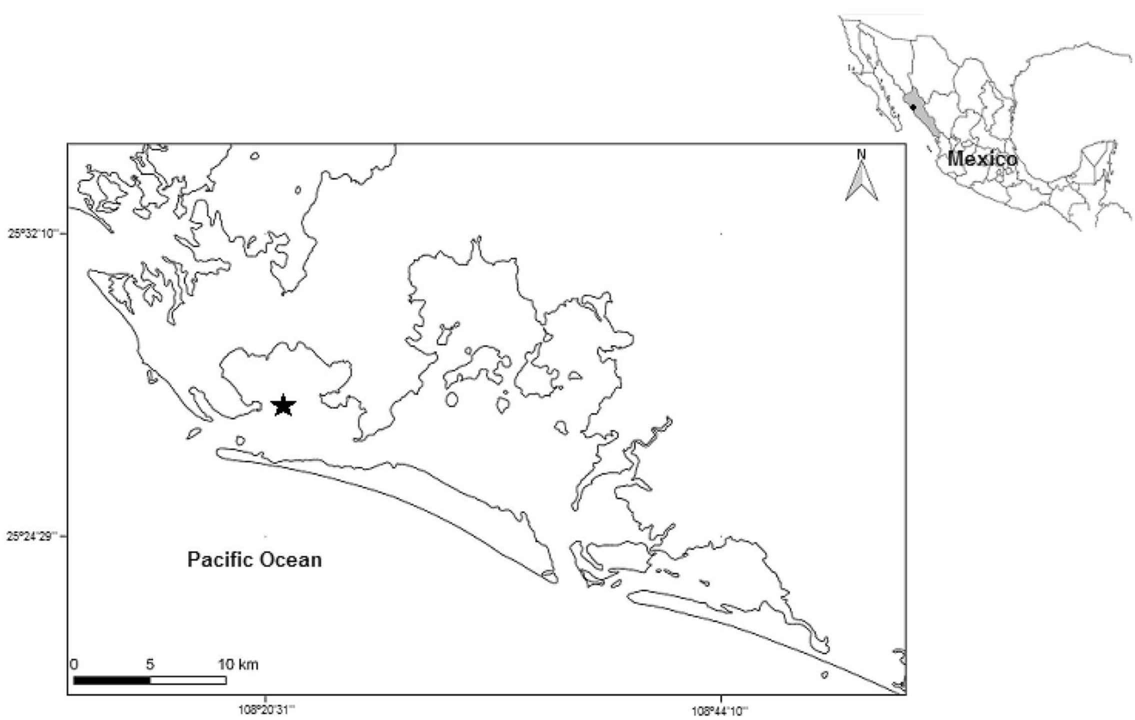

Fig. 1 *Megapitaria squalida* sampling site (filled star) located in Navachiste Lagoon, Sinaloa, Mexico

Results and Discussion

The mean concentration for each metal in the soft tissue was: Zn > Cu > Cd > Hg; and the mean intervals were 68.89 ± 37.59—30.36 ± 27.19, 8.77 ± 1.35—6.80 ± 0.36, 4.47 ± 0.21—3.18 ± 0.63 and 0.99 ± 0.81—0.52 ± 0.16 µg/g, respectively (Table 1). As expected, essential metals are more abundant than non-essential metals, which are due to the fact that copper and zinc play a fundamental role in the metabolic processes of all organisms, participating in different enzyme activities and regulating mechanisms such as hemocyanin protein synthesis (Harris 1991).

The metal content variability observed in the clams of the present study could be due to the bioavailability of the metals, which is in turn affected by (a) the mobilization of metals in interstitial waters and its chemical species (these organisms are benthonic); (b) the biogeochemistry of the sediment; (c) competition between metals to enter the organism and (d) the effects of bioperturbation (Baqueiro-Cárdenas et al. 2007).

In the present study no significant relation ($p > 0.05$) was observed between Cd, Cu and Hg content with the age of the clams, only Zn content had a significant ($p < 0.05$) relation (Table 2). Metcalfe-Smith et al. (1996) observed that As, Cd, Mn, Zn, Hg and Fe were higher in older mussels (*Elliptio complanata* and *Lampsilis radiata radiata*), while Cu content was higher in younger mussels. These authors point out that relationships between size and metal content in organisms were more significant at polluted sites than at clean ones, due to a failure in the metal regulation mechanisms of the organisms that inhabit pollution sites, and metal body content increases with size and age.

This Zn-age relationship could indicate that younger organisms have higher metal uptake rates and require more Zn because this metal is required for metabolic processes; besides, younger organisms have a higher metal uptake rates. In this context, Riisgård and Hansen (1990), in an experimental study with the bivalve *Mytilus edulis*, concluded that the accumulation rate was dependent on the size: smaller organisms had a higher inorganic mercury incorporation rate than larger organisms. Metcalfe-Smith et al. (1996) pointed out that bivalves with slower growth rates had higher metal content. Khristoforova et al. (2002) reported that with the growth of the clams *Tridacna crocea*, the content of Fe, Cu, Zn and Cr in their soft tissues decreased. Wang et al. (2013) observed that older *Tegillarca granosa* bivalve specimens (3-years old) accumulated significantly less cadmium and copper than the younger organisms (1-year old). Alavian et al. (2017) commented that age had a negative effect on content of Ni and Pb in the oyster *Saccostrea cucullata*. These results indicate that there is a metal-bivalve species specific relationship. However, the Zn decreases in older organism observed in the present study could be by a combination of growth dilution (Ke and Wang 2018) and the effect of the reproductive cycle, because this metal is required for this physiological process (Páez-Osuna et al. 1995). Besides, most of the *M. squalida* clams used in the present study (115 of 173) were at reproductive age, ≥ 2 years-old (Table 1) (Arellano-Martínez et al. 2006).

Table 3 presents metal concentrations in clams from different coastal zones around the world. Metal content differences are due to differences in geographic locations, environments and anthropogenically affected ecosystems, as well as biological differences among species (Esposito et al. 2018). However, metal contents in the soft tissues of the present study, are within the range of values listed in that table. Although, Hg contents in the present study are higher than those reported previously in Mexico.

Table 2 Correlation coefficients (ρ) of Cd, Cu, Hg and Zn in relation to the age of the clam *Megapitaria squalida*

	Cd	Cu	Hg	Zn
Age	0.245 p = 0.053	0.055 p = 0.669	0.016 p = 0.900	−0.347 p = 0.05

n = 24 for each metal

Table 1 Mean concentration and standard deviation (µg/g, dry weight) for Cd, Cu, Hg and Zn in the different age groups for the clam *M. squalida*

Age in years (n)	Size (mm)	Cd	Cu	Hg	Zn
0 (21)	0–57.5	3.18 ± 0.63[a]	7.14 ± 1.41[a]	0.87 ± 0.74[a]	67.41 ± 22.317[ab]
1 (37)	47.5–67.5	3.45 ± 0.63[a]	8.53 ± 1.94[a]	0.52 ± 0.21[a]	66.64 ± 12.29[a]
2 (50)	47.5–77.5	3.82 ± 1.09[a]	8.09 ± 1.60[a]	0.99 ± 0.81[a]	68.89 ± 37.59[ab]
3 (32)	67.5–77.5	3.68 ± 0.50[a]	8.77 ± 1.35[a]	0.87 ± 0.70[a]	36.54 ± 29.84[ab]
4 (21)	67.5–77.5	3.63 ± 0.82[a]	7.95 ± 1.09[a]	0.52 ± 0.16[a]	30.36 ± 27.19[b]
5 (12)	77.5–91.5	4.47 ± 0.21[a]	6.80 ± 0.36[a]	0.68 ± 0.06[a]	61.44 ± 2.85[ab]

n = number of clams

Same letters indicate a lack of significant differences among data in the same column (One-way non-parametric ANOVA, α = 0.05)

Table 3 Metal concentration (µg/g, dry weight) in the soft tissue of different clam species from different sites around the world

Site	Species	Metal			
		Cd	Cu	Hg	Zn
Sardinia lagoons, Italy[a]	*Ruditapes decussatus*	0.18*	8.8*	0.10*	60*
Laizhou Bay, China[b]	*Mactra veneriformis*	0.88*	4.44*	0.02*	39.6*
Homa Lagoon, Aegean Sea[c]	*Tapes decussatus*	0.10–0.33	4.8–9.6	0.07–0.13	52.9–82.2
Black Sea coast[d]	*Chamelea gallina*	0.68–4.12*	6.4–28.7*	NA	36–121*
Venice Lagoon, Italy[e]	*Ruditapes philippinarum*	0.4–0.88*	7–11*	0.2–0.8*	70–120*
Tagus estuary, Portugal[f]	*Ruditapes philippinarum*	0.24–0.40*	NA	0.08–0.52*	56.8–120*
SE Gulf of California, Mexico[g]	*Megapitaria squalida*	NA	NA	0.28	NA
Coatzacoalcos estuary, Mexico[h]	*Polymesoda caroliniana*	1.01	9.39	NA	112.7
Coatzacoalcos estuary, Mexico[h]	*Corbicula fluminea*	0.45	9.0	NA	65.9
Coatzacoalcos estuary, Mexico[i]	*Polymesoda caroliniana*	NA	NA	0.14	NA
Coatzacoalcos estuary, Mexico[i]	*Corbicula fluminea*	NA	NA	0.09	NA
SE Gulf of California, Mexico[j]	*Megapitaria squalida*	1.5–11.1	5.4–18.7	NA	47.2–64.6
Navachiste, Sinaloa, Mexico[k]	*M. squalida*	3.70	7.83	0.74	55.2

NA not analyzed

*Converted to dry weight considering 80% of water content

[a]Esposito et al. (2018)

[b]Liu et al. (2017)

[c]Bilgin and Uluturhan-Suzer (2017)

[d]Gedik and Ozturk (2018)

[e]Sfriso et al. (2018)

[f]Chiesa et al. (2017)

[g]Romo-Piñera et al. (2018)

[h]Ruelas-Inzunza et al. (2009a)

[i]Ruelas-Inzunza et al. (2009b)

[j]Méndez et al. (2006)

[k]This study

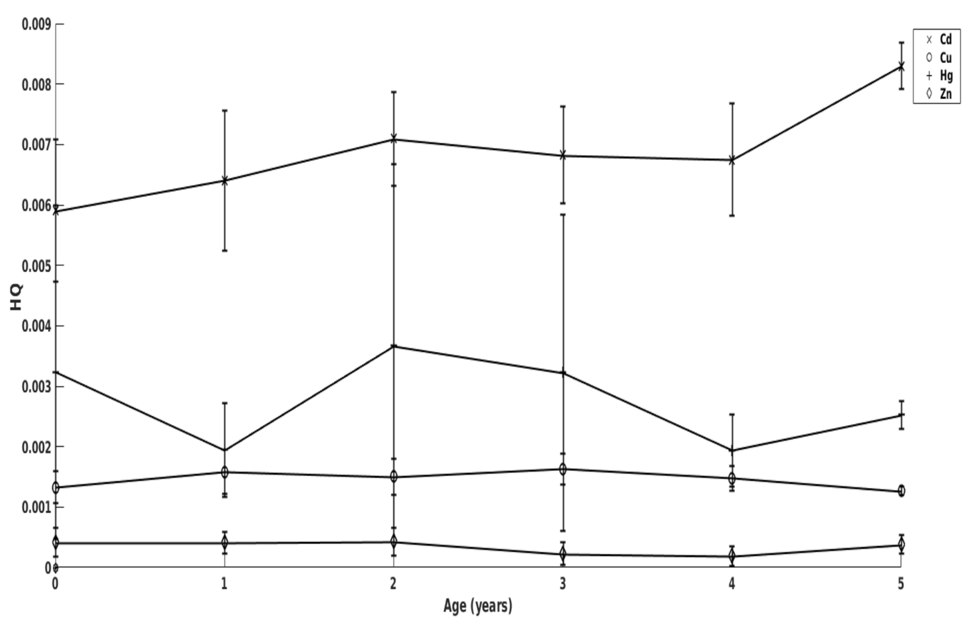

Fig. 2 Hazard Quotient (HQ) mean values (± SD) for Cd, Cu, Hg, and Zn in *Megapitaria squalida* for each age class from Navachiste Lagoon, Sinaloa, Mexico

Figure 2 shows the HQ values calculated in the present study. For all metals there is a low level of risk associated with the human consumption of *M. squalida.* It is important to mention that this value was calculated for the general Mexican human population, which has a mean clam consumption of 0.19 kg/person/year, which is too low. In this context, we would recommend that these values should be re-evaluated, because the regional consumption (coastal communities) may be higher due to the higher frequency of marine food consumption (Delgado-Alvarez et al. 2015).

According to the HQ values, the consumption of *M. squalida* does not represent a risk for the human population around the study area. However, it is necessary to determine specific characteristics about the human population around the study site such as gender, weight, age and the *M. squalida* consumption frequency. Likewise, in future studies, biotic factors should be included such as phenotypic differences, sex, and the reproduction stage of *M. squalida*, as well as the chemical properties of each metal and their relation to abiotic factors such as salinity, temperature, pH and interactions with other metals, in summary all of the multifactorial variables which can alter metal bioaccumulation rates in the soft tissue of organisms should be considered.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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