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Copper, Zinc and Lead Bioaccumulation in Marine Snail, *Strombus gigas*, from Guacanayabo Gulf, Cuba

O. Díaz Rizo · S. Olivares Reumont · J. Viguri Fuente · O. Díaz Arado · N. López Pino · K. D'Alessandro Rodríguez · J. O. Arado López · A. Gelen Rudnikas · G. Arencibia Carballo

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Abstract Levels of copper, zinc and lead were determined in sediments and edible muscle of marine snail *Strombus gigas* collected from Guacanayabo Gulf, Cuba. The concentration range of each metal in marine snail muscle on mg kg^{-1} wet weight varied as follows: Cu = 6.4–32.6, Zn = 20.4–31.1 and Pb = 0.2–2.3; and in corresponding sediments (on mg kg^{-1} dry weight) as: Cu = 157–186, Zn = 56–94 and Pb = 20–37. The average biota-sediment accumulation factors (BSAFs) obtained for studied metals are less than unity in all cases, indicating that only a little fraction of metal content in the sediments is bioavailable, independently of their possible enrichments in the sediments. The concentrations of copper and lead in some of the marine snails are above typical public health recommended limits.

Keywords Heavy metals · *Strombus gigas* · Sediments · Guacanayabo gulf · Cuba

Guacanayabo gulf is the largest gulf of Cuba. Hundreds of rivers and streams have its outlets in Guacanayabo waters, including Cauto River – the largest Cuban river. A deep range between 2 and 13 m, altogether with an average temperature around the 25°C and an annual average oxygen dissolved concentration above 5, create an historical habitat for species of high relevance in the Fishing Industry. Recently, an average enrichment for Cu (5,1), Pb (11,7) and Zn (1,3) in Guacanayabo gulf surface sediments in the last 20 years and a Zn and Cu levels in oyster (*Crassostrea rhizophorae*) above public health recommended limits were determined (Díaz Rizo et al. 2010). The aim of the present study was to investigate the bioaccumulation of those heavy metals in common in Guacanayabo mollusc: marine snail (*Strombus gigas*), popular called "Cobo", taking into account its use as food by local population.

O. Díaz Rizo (✉) · S. Olivares Reumont · O. Díaz Arado · N. López Pino · K. D'Alessandro Rodríguez · J. O. Arado López · A. Gelen Rudnikas
Instituto Superior de Tecnologías y Ciencias Aplicadas, Salvador Allende Ave. and Luaces, POB 6163, 10600 La Habana, Cuba
e-mail: odrizo@instec.cu

J. Viguri Fuente
Universidad de Cantabria, Ave. Los Castros s/n, 39005 Santander, España

G. Arencibia Carballo
Centro de Investigaciones Pesqueras, 5th Ave and 246 st, 17100 Playa, La Habana, Cuba

Materials and Methods

The surface sediments were collected using a Van Veen snapper in 5 points of the gulf (Fig. 1) during the same journey. Stations were selected to be near of the outlets of the main rivers and the drainage and sewage systems of Manzanillo city. All samples were dried at 50°C. Large rock debris, mollusc skeletons and organic debris were removed before sieving. The fraction smaller than 1 mm was ground to a fine powder (<63 μm) in an agate mortar. The pulverized samples were newly dried at 60°C until obtaining a constant weight.

Strombus gigas were collected in the same sediment collection day. At each stations 3 young specimens of nearly equal full length (7 ± 1 cm) in order to minimize the effect of body size, were stored at −20°C in metal-free plastic bags kept in an insulated ice box until arrival to the laboratory, where the samples were thawed and dissected to separate the edible muscle. Right after all were cleaned

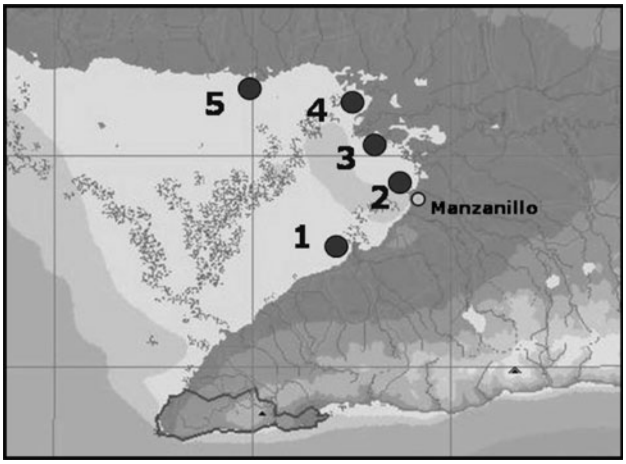

Fig. 1 Location of studied stations in Guacanayabo gulf

with distilled water, weighed, lyophilized and homogenized in an agate mortar.

The heavy metal concentrations in sediments were estimated by X-Ray Fluorescence Analysis (XRF) using the Certified Reference Materials (CRM) IAEA-SL-1 "Lake Sediment" (Dybczynski and Suschny 1974), IAEA-Soil-5 (Dybczynski et al. 2007), IAEA Soil-7 (Pszonicki 1984), BCR-2 "Basalt Columbia River" (Wilson 1997) and BCSS-1 "Marine sediment" from the Canadian National Research Council as standards. All samples and CRM were mixed with cellulose (analytical quality) in proportion 4:1 and pressed at 15 tons into the pellets of 25 mm diameter and 4–5 mm height. Pellets were measured using Canberra Si(Li) detector (150 eV energy resolution at 5,9 keV, Be window thickness = 12.0 μm) coupled to a MCA. A ^{238}Pu (1.1 GBq) excitation source with ring geometry was used. All spectra were processed with WinAxil code (Winaxil 2005). Detection Limits were determined according Padilla et al. (2007) (in concentration units) as $L_D = 3\sigma/\text{mt}$, where m is the sensibility in counts.seg^{-1} per concentration unit, σ is the standard deviation of the area of the background windows (peak window at 1.17 times the FWHM) and t is the measuring time (6 h).

The accuracy was evaluated using the SR criterion, proposed by McFarrell et al. (Quevauviller and Marrier 1995):

$$\mathrm{SR} = \frac{|C_X - C_W| + 2\sigma}{C_W}\,100\%$$

where C_X – experimental value, C_W – certified value and σ is the standard deviation of C_X. On the basis of this criterion the similarity between the certified value and the analytical data obtained by proposed methods is divided into three categories: SR $\leq$ 25% = excellent; 25 < SR $\leq$ 50% = acceptable, SR > 50% = unacceptable. The analysis of five replica of the CRM IAEA-356 "Polluted Marine Sediment" (IAEA 1994) is presented in Table 1. All heavy metals (Cu, Zn and Pb) determined by ES-method are "excellent" (SR $\leq$ 25%) and the obtained results shows a very good correlation ($R = 0.9999$) between certified and measured values.

Table 1 XRF analysis of CRM IAEA-356 (mean ± SD, n = 5, mg kg^{-1}), SR values and Detection Limits

Metal	Certified value	Measured value	SR (%)	L_D (mg kg^{-1})
Cu	365	360 ± 29	17	16
Zn	977	958 ± 45	11	5
Pb	347	362 ± 22	22	4

Lyophilized marine snail samples were transported to University of Cantabria, Spain, for chemical analyses. Methods for extracting heavy metals from tissue samples were derived from the US. Environmental Protection Agency (EPA) standard method 3052 (EPA 1996). About 0.5 g dried sample was transferred into HP 500 vessel for digestion with 9 mL of ultra pure nitric acid. The vessels were then inserted into a microwave (CEM MARS 5X) and heated at a temperature profile reaching 180°C in approximately less than 5.5 min and remaining at 180°C for 9.5 min at 600 W. After cooling, the vessel contents are filtered, centrifuged, diluted to volume, and then transferred into plastic containers and kept under refrigeration at about 4°C previous to analysis. All reagents were analytical grade or Suprapur quality (Merck, Germany). Milli-Q water (Millipore, Bedford, MA, USA) was used in all experiments. Cleaning of plastics and glassware was carried out by soaking in 14% (v/v) HNO_3 for 24 h and then rinsing with water.

Quantification of metals in the extract was performed using a combination of inductively coupled plasma emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS). All digested samples were analyzed for Cu and Zn by ICP–AES (Perkin Elmer plasma 400) due to the high contents in biologic samples according to the US EPA Method 6020A (EPA 2007a). ICP-AES was calibrated by standard addition methods and freshly prepared metal salt (Merck multielement standard) solutions. Practical minimum detection limits against replicated procedural blanks were 0.053 mg L^{-1} for Cu and 0.132 mg L^{-1} for Zn. The Pb analysis was performed by ICP–MS (Agilent Technologies 7500ce model) according to the US EPA Method 6010C (EPA 2007b); analytical multielement standard solutions for ICP-MS were prepared by diluting a mixed standard solution. Minimum detection limit of Pb was 0.041 μg L^{-1}. Concentrations reported are the mean and standard deviation values of three replicate measurements (corrected for dilution when was necessary). Analytical quality was assured by using a certified reference sample of NIST CRM 1566b oyster tissue. The percentage of recovery for all metals ranged between 85% and 110% of certified values.

In order to estimate the rate at which metal appears in the selected specie and associates sediment, the biota-sediment accumulations factors (BSAFs) were calculated for selected metals in the studied marine snails according to the formula (Szefer et al. 1999):

$$\mathrm{BSAF} = C_X/C_S$$

where C_X and C_S are the mean concentrations of metal in the organism and the corresponding sediment, respectively.

Results and Discussion

The average Cu, Zn and Pb concentrations determined by ED-XRF in Guacanayabo Gulf coast sediments are given in Table 2. Pb and Zn distributions shows maximum in stations near of Manzanillo city (1 and 2), and Cu concentration is practically uniform in all studied stations.

Table 3 presents metal concentrations found on the edible muscle of the marine snail *S. gigas* in all studied stations. The results have shown significant differences between stations in the accumulated concentrations of each of the trace metals in the marine snail. Given the fact that accumulated concentrations are integrated records of the bioavailability of each metal to the marine snail, it follows, therefore, that the bioavailability of the metals varies geographically in the Guacanayabo Gulf. The different metals show different patterns of bioavailability. The highest accumulation of Pb comes from snails collected nearly to Manzanillo City (St. 2) while Zn and Cu have maximums of concentration at St. 4 and St. 1, respectively.

The reason for the observed geographical variation in the bioavailability of the metals is caused most probably by the input of these metals in dissolved and/or particulate form through anthropogenic activity in the Guacanayabo Gulf. Such activities include dredging, agricultural use of metal-containing fertilizers and pesticides, the emission of untreated sewage and also metal contaminated effluents from medical use and industrial units. For example, high Pb content in marine snails from St. 2 might be associated with poor sewage treatment by the battery recycling plant located in Manzanillo city.

The average metal concentrations in organisms and sediments from the studied stations were used for calculating BSAFs and are shown in Fig. 2. BSAF factor refers to the bioavailability of given metals presents in the sediment. The average BASFs obtained for Cu, Pb and Zn were less than unity. That fact is an indicator that a little fraction of these metal contents present in Guacanayabo gulf coast sediments is bioavailable, regardless their metal enrichments. But, in comparison with oysters (*C. rhizophorae*) (Díaz Rizo et al. 2010), the marine snail accumulate Pb with a rate higher than the latter in an order of magnitude.

Cobo from the Guacanayabo Gulf is used as a local food source. Public health limits for the concentrations of toxic metals are usually quoted in terms of fresh (wet) weight. Cuban regulations (NC 2006) specify a maximum allowable limit for lead is 0.2 mg kg^{-1}. Taking that information into account, snails located around stations 1, 2 and 4 exceed Pb limit. Limits for the rest of the elements have not been specified yet. On the other hand, the limits recommended by the Ministry of Agriculture Fisheries and Food (MAFF) for England and Wales are 20 mg kg^{-1} wet wt for

Table 2 Average concentrations (mean ± SD, n = 4, mg kg^{-1}) for heavy metals determined in Guacanayabo Gulf coast sediments

Metal	St. 1	St. 2	St. 3	St. 4	St. 5
Cu	169 ± 14	158 ± 13	169 ± 14	157 ± 13	186 ± 15
Zn	90 ± 6	94 ± 6	92 ± 6	56 ± 4	60 ± 5
Pb	37 ± 4	35 ± 3	21 ± 3	20 ± 3	24 ± 3

Table 3 Heavy metal average concentrations (mean ± 1 SD, n = 3, mg kg^{-1} wet wt.) determined in *Strombus gigas* from Guacanayabo Gulf

Metal	St. 1	St. 2	St. 3	St. 4	St. 5
Cu	32,6 ± 0,5	24,0 ± 0,6	16,1 ± 0,7	6,4 ± 0,3	7,1 ± 0,3
Zn	21,5 ± 0,9	20,4 ± 0,4	23,1 ± 0,6	31,1 ± 0,8	23,3 ± 0,5
Pb	0,5 ± 0,1	2,3 ± 0,4	0,2 ± 0,1	1,1 ± 0,3	0,2 ± 0,1

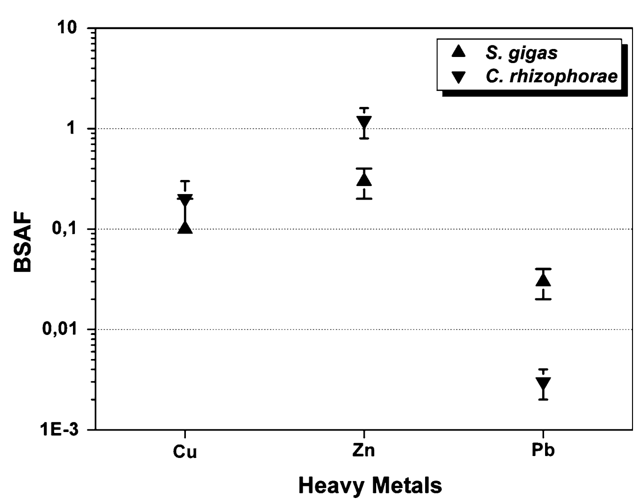

Fig. 2 Biota-sediment accumulation factor values (main and range) in *Strombus gigas* and comparison with those for oysters

copper, 50 mg kg^{-1} wet wt for zinc (MAFF 1981), and 1 mg kg^{-1} wet wt for lead (MAFF 1982). Then, the most contaminated marine snails from Guacanayabo Gulf, especially at stations 1–2 surroundings, may carry a health risk due to their Pb and Cu concentrations.

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