DIFFERENCES IN METALLIC CONTENT BETWEEN MARINE VERTEBRATES AND INVERTEBRATES LIVING IN OCEANIC ISLANDS

Enrique Lozano-Bilbao*, José María Espinosa**,
Gonzalo Lozano*, Arturo Hardisson***,
Carmen Rubio***, Dailos González Weller•,
Soraya Paz***, Ángel J. Gutiérrez***

Abstract

The metallic content in each class of organism varies in different ways, depending on metabolism, habitat behavior, and where it is found in the trophic network. In this study, 845 specimens of different types of marine invertebrate and vertebrate organisms of the Canary Islands have been analyzed, of them the content of 20 metals and trace elements has been analyzed (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sr, V and Zn) in mg / kg. In the PCoA analyzes it is clearly seen how the invertebrate and vertebrate organisms are separated according to their metallic content, there being significant differences between these two groups in each of the trace elements and metals. Invertebrate species having the highest concentration in all metals and trace elements, may have a higher concentration of metals than vertebrates because they have a very fast growth, and with it a high metabolic rate that causes higher concentrations of the elements to bioaccumulate.

Keywords: vertebrate, invertebrate, trace elements, metal.

DIFERENCIAS EN EL CONTENIDO EN METALES DE LOS VERTEBRADOS E INVERTEBRADOS MARINOS QUE VIVEN EN ISLAS OCEÁNICAS

Resumen

El contenido metálico en cada clase de organismo varía de diferentes formas; según el metabolismo, el hábitat y el lugar de la red trófica en que se encuentre. En este estudio se han analizado 845 ejemplares de diferentes tipos de organismos vertebrados e invertebrados marinos de Canarias, de ellos se ha analizado el contenido de 20 metales y elementos traza (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sr, V y Zn) en mg/kg. En los análisis de PCoA se observa claramente cómo los organismos invertebrados y vertebrados se separan según su contenido metálico, existiendo diferencias significativas entre estos dos grupos en cada uno de los elementos traza y metales estudiados. Las especies de invertebrados tienen la mayor concentración en todos los metales y oligoelementos, pueden tener una mayor concentración de metales que los vertebrados debido a que tienen un crecimiento muy rápido, y con ello una alta tasa metabólica que hace que se bioacumulen concentraciones más altas de los elementos.

Palabras clave: vertebrados, invertebrados, elementos traza, metal.

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INTRODUCTION

The Animalia kingdom comprises two large groups, vertebrates and invertebrates, which are very varied internally. Vertebrates represent a small group in the animal kingdom, although due to their location on the trophic scale, they play a key role for life on Earth (Fischer *et al.*, 2012).

Although we find cartilage tissue in different organisms of the animal kingdom, bone, dentin and enamel are exclusive tissues of vertebrates (Grillner, 2018; Hedges, 1998; Kumar *et al.*, 2017; Roach *et al.*, 2005). The main constituent mineral of hard tissues is hydroxyapatite, a form of calcium phosphate (Kawasaki and Weiss, 2003; Kikuchi *et al.*, 2004; Pezzotti *et al.*, 2016). The shells and other hard tissues of invertebrates are made of a different substance, carbonate.

Invertebrates represent many animals on Earth, and they do not possess a notochord or dorsal cord, nor a vertebral column, nor an articulated internal skeleton. In this set 95% of the known living species are found, between 1.7 and 1.8 million species (Brittain and Eikeland, 1988; Kurtz and Franz, 2003; Ratcliffe *et al.*, 1985). These differences cause them to have different metabolic rates, being higher in invertebrates that have a much shorter life expectancy and that have large amounts of nutrients for their growth and development, having fewer detoxification mechanisms, therefore accumulating toxins and other xenobiotics in the body (Brockington and Clarke, 2001; Heikens *et al.*, 2001; Mason *et al.*, 2000; Livingstone, 1991).

Trace elements and metals are incorporated into the marine trophic network in various ways, such as the anthropic of discharges from the coast to the ocean, runoff in plantation areas, contamination by factories, etc. Naturally, we find the effects of upwelling that ascends nutrients and elements from the lower layers of the ocean, sandstorms from deserts that enrich the ocean, etc. (Afandi *et al.*, 2018; Lozano-Bilbao *et al.*, 2019b, 2020d; Ruilian *et al.*, 2008; Qing *et al.*, 2015).

All organisms have evolved and developed detoxification techniques for compounds that are harmful to your body, either the accumulation of these elements in the body such as in fat or in organs such as the hepatopancreas in molluscs and arthropods or the liver in most vertebrates. There are mechanisms of excretion of harmful substances that depend on the chelating substances that organisms can create (Bustamante *et al.*, 2008; Lozano-Bilbao *et al.*, 2020b, 2019a; Raimundo *et al.*, 2005; Rainbow and Luoma, 2011; Saénz de Rodrigáñez *et al.*, 2005). The main

* Departamento de Biología Animal y Edafología y Geología, Unidad Departamental de Ciencias Marinas, Universidad de La Laguna, 38206 La Laguna, Santa Cruz de Tenerife, Spain.

** Fundación del Sector Público Estatal Observatorio Ambiental Granadilla (Unidad Técnica), 38001, Santa Cruz de Tenerife, Spain.

*** Departamento de Obstetricia y Ginecología, Pediatría, Medicina Preventiva y Salud Pública, Toxicología, Medicina Legal y Forense y Parasitología. Área de Toxicología, Universidad de La Laguna, 38200 La Laguna, Santa Cruz de Tenerife, Spain.

• Servicio Público Canario de Salud, Laboratorio Central, Santa Cruz de Tenerife, Spain. Corresponding author: lozaenr@gmail.com.

objective of this research is to verify the possible group (invertebrate and vertebrate) by metallic content and trace elements of species.

MATERIAL AND METHODS

A total of 845 samples of 20 different species were collected from Canary Islands (Spain (15°30'0''W; 28°0'0''N)): *Scomber colias* Gmelin, 1789, *Trachurus picturatus* (Bowdich, 1825), *Sardina pilchardus* (Walbaum, 1792), *Serranus cabrilla* (Linnaeus, 1758), *Mullus surmuletus* (Linnaeus, 1758), *Diplodus sargus* (Linnaeus, 1758), *Sarpa salpa* (Linnaeus, 1758), *Chelon labrosus* (Risso, 1827), *Sparisoma cretense* (Linnaeus, 1758), *Anemonia sulcata* (Pennant, 1777), *Sepia officinalis* Linnae, 1758, *Octopus vulgaris* Cuvier, 1797, *Loligo vulgaris* Lamarck, 1798, *Patella aspera* Röding, 1798, *Patella candei crenata* D'Orbigny, 1840, *Palaemon elegans* Rathke, 1837, *Plesionika narval* (Fabricius, 1787), *Physeter macrocephalus* Linnae Stenella frontallis (Cuvier, 1829) and *Tursiops truncatus* (Montagu, 1821). Part of this data was used in (Lozano-Bilbao *et al.*, 2020a). In the present study, live verbs were not manipulated, the samples were taken from stranded animals (cetaceans) and fish markets (fish).

Treatment of the samples

The sample consisted of a portion of muscle between 10-15 g. The samples were dried in an oven at a temperature of 70°C for 24 hours. Subsequently, they were incinerated in a muffle-furnace for 48 hours at 450°C ± 25°C, until obtaining white ashes.

Obtained the white ashes, they were filtered with a 1.5% HNO_3 solution until 25 mL of total volume for the subsequent determination of the metallic content (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sr, V y Zn) by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Lozano-Bilbao *et al.*, 2018b).

Statistical analysis

In order to study existence of differences in the content and relative composition of metals and trace metals among the analyzed samples, a statistical analysis was performed, using a distance-based permutational multivariate analysis of variance (PERMANOVA) with Euclidean distances (Anderson and Braak, 2003).

A one-way design was used with the fixed factor of "Organism type" with two levels of variation:

Vertebrate = *Scomber colias*, *Trachurus picturatus*, *Sardina pilchardus*, *Physeter macrocephalus*, *Stenella frontallis*, *Tursius truncatus*, *Serranus cabrilla*, *Mullus surmuletus*, *Diplodus sargus*, *Sarpa salpa*, *Chelon labrosus* and *Sparisoma cretense*.

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TABLE 1. MEAN METAL AND TRACE ELEMENT CONCENTRATIONS (MG/KG), STANDARD DEVIATION AND STATISTICAL PARAMETER OF THE PAIR TEST (PERMANOVA) BETWEEN INVERTEBRATE AND VERTEBRATE							
	Vertebrate			Invertebrate			Vertebrate vs. Invertebrate
Al	4.060	±	3.342	5.828	±	5.943	0.001*
B	0.189	±	0.188	0.871	±	1.040	0.001*
Ba	0.208	±	0.191	0.417	±	0.534	0.001*
Ca	696.853	±	935.076	797.231	±	1006.970	0.001*
Cd	0.040	±	0.112	0.531	±	0.819	0.001*
Co	0.006	±	0.006	0.022	±	0.027	0.001*
Cr	0.157	±	0.274	0.207	±	0.258	0.001*
Cu	0.891	±	0.613	1.877	±	2.073	0.001*
Fe	13.352	±	25.180	27.332	±	37.495	0.001*
K	2074.230	±	759.03	935.876	±	790.995	0.001*
Li	0.494	±	0.458	0.587	±	0.665	0.001*
Mg	281.871	±	84.911	387.106	±	450.859	0.001*
Mn	0.255	±	0.407	0.494	±	0.573	0.001*
Mo	0.013	±	0.023	0.068	±	0.078	0.001*
Na	671.279	±	285.698	1333.420	±	1062.840	0.001*
Ni	0.171	±	0.434	0.366	±	0.755	0.001*
Pb	0.059	±	0.074	0.316	±	0.637	0.001*
Sr	1.455	±	1.834	1.870	±	1.716	0.001*
V	0.108	±	0.547	0.179	±	0.216	0.001*
Zn	6.970	±	5.888	5.438	±	4.126	0.001*

* p<0.01

Invertebrate = *Loligo vulgaris*, *Anemonia sulcata*, *Sepia officinalis*, *Octopus vulgaris*, *Patella aspera*, *Patella candei crenata*, *Palaemon elegans* and *Plesionika narval*.

Relative dissimilarities among the groups were studied using a principal coordinate analysis (PCoA) where metals that best explained data variability were represented as vectors.

In all analyzes, 9999 permutations of exchangeable units and a posteriori pairwise comparisons were used to verify the differences between the levels of the significant factors (p-value <0.01) (Anderson, 2004). The statistical packages PRIMER 7 & PERMANOVA + v.1.0.1 were used for the statistical analyzes.

RESULTS

Table 1 shows the metal average concentration (mg/kg) by vertebrate and invertebrate species. K (2074 ± 759 mg/kg) level in vertebrates is higher than K (936 ± 791 mg/kg) in invertebrates. Levels of the other metals and trace elements stand out in invertebrates.

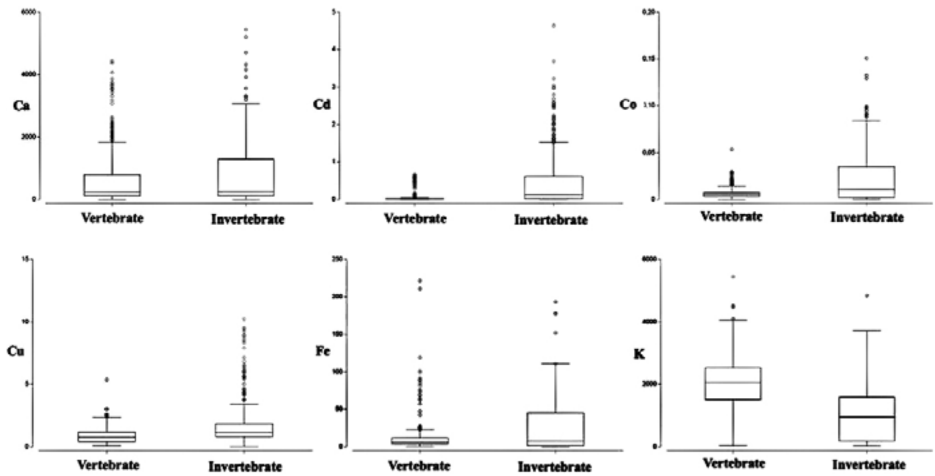

Figure 1. Boxplot graphs showing mean values for the metal contents, and minimum, maximum mg/kg (w.w.), for each kind of organism.

According to the type of organism (vertebrates or invertebrates), PERMANOVA revealed significant differences in the content of trace elements and metals between vertebrates and invertebrates ($F = 254.63$; $p = 0.001$). The PERMANOVA results better explained the variability of the data for all metals and trace elements, since all presented significant differences, with invertebrates showing the highest concentration in all metals and trace elements except for K (fig. 1). These differences are clearly seen in the PCoA (fig. 2), in this graph it is observed how the vertebrate and invertebrate samples are clearly separated due to the content of metals and trace elements that they contain in the muscle.

DISCUSSION

The growing of marine vertebrate species, generally, is slowly than invertebrate species. This is due to the large resources that are required for the formation of the bones and organs (Golling *et al.*, 2002; Naiche *et al.*, 2005; Schier, 2003). Vertebrate organisms have more detoxification mechanisms than invertebrates, and like them, they have the liver, which is a storage organ for many toxins that regulates most of the chemical levels in the blood and excretes bile, which helps to break down fats and prepares them for later digestion and absorption. The liver acts processing the blood and separates in its components, balances them, and creates nutrients for the body to use. It also metabolizes substances present in the blood to make them easier for the body to use (Angulo, 2002; Brasch, 1980; Eastwood and Couture, 2002; Tal *et al.*, 2017).

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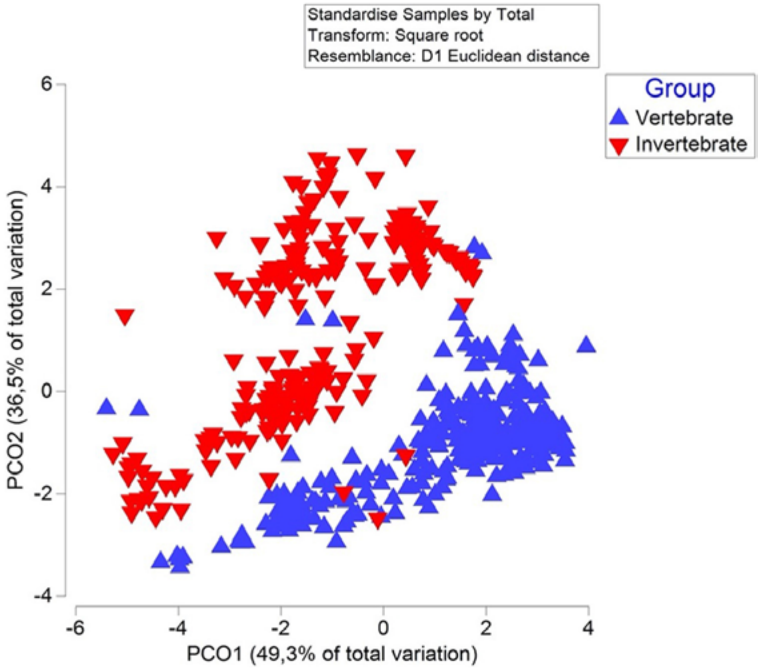

Figure 2. Principal coordinate analysis (PCoA) showing the first two axes (85.8% of variability), based on Euclidean distances of square-root-transformed data and standardise of the metal and trace element content in the groups of vertebrate and invertebrate.

In general, the growing process of the marine invertebrate species is faster than vertebrate species and have a younger age period, presenting an accelerated growth in the first stages of life, this first phase being only an increase in mass with a very high metabolic rate and high nutrient requirements, while having few detoxification mechanisms so that high concentrations of toxins or heavy metals are not harmful to your health (Brockington and Clarke, 2001; Dallinger, 1994; Livingstone, 1991). Subsequent development involves physiological and structural changes in that mass (Bergquist *et al.*, 2000; Brittain and Eikeland, 1988; Gallardo *et al.*, 1997; Sutton *et al.*, 2001). Therefore, invertebrates will bioaccumulate most of the metals and trace elements in the early stages of life, and it should be noted that different organisms, when growing very quickly, can reach large sizes and change the diet during growth as they can. this is the case of cephalopods (Gales *et al.*, 1993; Lacoue-Labarthe *et al.*, 2011; Piatkowski *et al.*, 2002; Storelli *et al.*, 2006). Mollusks and arthropods have developed the hepatopancreas, which acts as a warehouse for all these toxins and heavy metals, even so it is not enough and it has been observed in many studies that invertebrates such as anemones, cephalopods and crustaceans have high concentrations of metals and trace elements (Adami *et al.*, 2002; Dallinger and Prosi, 1988; Fischer and Dietrich, 2000; Iijima *et al.*, 1998), which in the case of cephalopods have very high Cd concentrations in muscle. (Bustamante *et al.*, 2002; Carvalho *et al.*, 2005; Lozano-Bilbao *et al.*, 2020a; Storelli *et al.*, 2006, 2005). For all this, they may have higher concentrations of metals and trace elements than vertebrate species. Invertebrate marine species are the most widely used as bioindicators of marine pollution; each species can be useful for more than one biomarker and by accumulating trace metals and elements more easily, it is easier to know the state of the ecosystem thanks to them (Dolenec *et al.*, 2011; Li *et al.*, 2019; Lionetto *et al.*, 2003; Lozano-Bilbao *et al.*, 2020c, 2018a).

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