Ecotoxicology and Environmental Safety 145 (2017) 653–658

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Assessment of oxidative stress and bioaccumulation of the metals Cu, Fe, Zn, Pb, Cd in the polychaete *Perinereis gualpensis* from estuaries of central Chile

Hernán Gaete[a,*], Manuel Álvarez[a], Gabriela Lobos[b], Eulogio Soto[c], Carlos Jara-Gutiérrez[d]

[a] *Escuela de Ingeniería en Medioambiente, Facultad de Ingeniería, Centro de Investigación y Gestión de Recursos Naturales (CIGREN), Universidad de Valparaíso, General Cruz N° 222, Valparaíso, Chile*

[b] *Instituto de Química y Bioquímica, Facultad de Ciencias, Centro de Investigación y Gestión de Recursos Naturales (CIGREN), Universidad de Valparaíso, Av. Gran Bretaña, N° 1111, Playa Ancha, Valparaíso, Chile*

[c] *Escuela de Biología Marina, Facultad de Ciencias del Mar y Recursos Naturales, Universidad de Valparaíso, Av. Borgoño, N° 16.344, Montemar, Reñaca, Viña del Mar, Chile*

[d] *Laboratorio de Investigación-Estrés Oxidativo, Centro de Investigaciones Biomédicas (CIB), Facultad de Medicina, Universidad de Valparaíso, Hontaneda N° 2653, Valparaíso, Chile*

ARTICLE INFO

Keywords:
Biomarkers
Oxidative stress
Polychaete

ABSTRACT

The estuaries of the Aconcagua and Maipo Rivers of central Chile are receptors of residues that contain metals from anthropic activities including agriculture, mining and smelters, which have different levels in the two basins. This study postulates that the exposition to metals is different in the two estuaries and that their sediments contain bioavailable chemical agents that produce oxidative stress. The aim of the study was to evaluate the effect of estuarine sediments on the polychaete *Perinereis gualpensis* using oxidative stress biomarkers and to determine the metal concentrations in sediments and their accumulation in *P. gualpensis*. Sediments and organisms were collected in December 2015 and January 2016 in the estuaries. The Catapilco estuary was used as control, since its basin has little anthropic activity. The metal concentrations of Fe Cu, Pb, Zn and Cd were determined in tissues of the organisms and in sediments. The granulometry, conductivity, redox potential, pH and organic matter in sediments were determined, as well as catalase activity and lipid peroxidation. The results show that the concentrations of metals in sediments were higher in the estuary of the Aconcagua River: Cu: $48 \pm 2\ \mu g\ g^{-1}$; Fe: $154 \pm 19\ mg\ g^{-1}$, Pb: $20 \pm 3\ \mu g\ g^{-1}$ and Zn: $143 \pm 20\ \mu g\ g^{-1}$. In tissues, Pb and Fe were higher in the estuary of the Maipo River, while Cd was detected only in the Catapilco River mouth. Catalase activity was greater in the estuary of the Aconcagua River and lipid peroxidation in the estuary of the Catapilco River. Significant regressions were found between biomarkers of oxidative stress and metal concentrations in tissues of *P. gualpensis*. In conclusion, the sediments of the studied estuaries contain bioavailable chemical agents that provoke oxidative stress in *P. gualpensis*, which may be a risk for the benthic communities of these ecosystems. This species is proposed to monitor metals bioavailability and oxidative stress in estuarine sediments.

1. Introduction

Estuarine systems integrate marine, coastal and fluvial systems through water flow, sediment and dissolved substances, constituting a socio-ecological system. Although it is an ecosystem itself, it cannot act alone since it largely depends on other ecosystems. In addition to the processes and ecological functions, the cumulative effects of human activity should be included both upstream and downstream (Pallero et al., 2017)

Estuaries represent ecosystems of high productivity and are crucial in the life histories of many fish, invertebrates, birds, etc. (Monserrat et al., 2007; Díaz-Jaramillo et al., 2010). The sustainability of these water bodies is vital for coastal regions in ecological and economic terms. However, estuaries are potentially exposed to chemical contaminants transported by rivers from urban and industrial areas, because they are sediment deposition areas. Estuaries have become the principal reservoir for a large number of chemical substances introduced into the aquatic ecosystem by human activity (Thompson de Oliveira Lemos et al., 2014). Complex mixtures of contaminants can enter the aquatic environment via effluents or surface runoff, and thus aquatic organisms can be inevitably exposed (Blanchet- Letrouvé et al., 2013).

The deterioration of estuaries due to the presence of metals and organometals results mainly from anthropic activities. Due to their

* Corresponding author.
E-mail address: hernan.gaete@uv.cl (H. Gaete).

http://dx.doi.org/10.1016/j.ecoenv.2017.07.073
Received 18 April 2017; Received in revised form 26 July 2017; Accepted 31 July 2017
0147-6513/

tendency to accumulate in aquatic organisms, these contaminants compromise the water quality and the health of biotic estuarine communities (Tapia et al., 2009, 2012). Metals tend to bind covalently with macromolecules such as proteins and DNA. This binding causes oxidative stress (Srivastava et al., 2006; Fevzi et al., 2007). The transition metals, among other sources, change the metabolism of organisms by stimulating reactive oxygen species (ROS) production by the Fenton and Haber–Weiss reactions (Monserrat et al., 2007; Jara et al., 2014).

Organisms respond to chemical agents through the antioxidant systems located in subcellular organelles, providing protection. They have been traditionally called non-enzymatic defenses and enzymatic defenses. The former do not interact directly with free radicals generated from O_2 (O^{-2}) or the product of dismutation, while the latter have to do with the repair or removal of damaged biological structures (Pal Yu, 1994; Monserrat et al., 2007). The enzymes known as Phase I (solubilization) and Phase II (conjugation) of xenobiotic metabolism are essential in the process of detoxification, involving on one hand ROS and on the other hand free radical-metabolizing enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferases). These enzymes are good biomarkers for determining biological effects of exposure to pollutants (Díaz-Jaramillo et al., 2013).

Metals tend to accumulate in aquatic ecosystem sediments, depending on factors such as grain size, organic matter and redox potential (Gray and Elliott, 2009). Therefore, organisms inhabiting these sediments (benthic assemblages) may be exposed to metals and exhibit toxic effects (Campana et al., 2012; Fossi et al., 2012). These organisms are key to the food web of estuarine ecosystems. They include polychaetes, which are commonly used in eco-toxicological bioassays (Díaz and Reish, 2009) as indicators of bioaccumulation of chemical agents, the health status of subtidal sediments and contamination by the analysis of community variables (Cañete et al., 2000; Giangrande et al., 2005; Dean, 2008).

The effect of contamination on organisms that live in the sediments in Chile has been determined through changes in the structure of communities such as diversity, species richness, and abundance (Bertrán et al., 2001a). These ecological methods are time-consuming and expensive. Chemical analytical methods may detect and determine concentrations of the chemical agents, but these do not provide direct information on potentially adverse effects. Therefore, there is currently growing interest in using molecular biomarkers to determine the oxidative damage produced by metals in organisms which inhabit aquatic environments, as a useful tool to provide early warning (Carvalho-Neta, Abreu-Silva, 2010; Jara et al., 2014)

The nereidid polychaete *Perinereis gualpesis* is very abundant in estuaries of southern Chile, with densities greater than 2000 ind/m^2 (Jaramillo et al., 2001). Preliminary studies have shown that this species is an efficient biomonitor for heavy metals in these environments (Bertrán et al., 2001b; Díaz-Jaramillo et al., 2017). The sub-organism response level in this species has been proposed as a biomarker and it could be a potential tool to determine the state of environments experiencing numerous types of anthropogenic stressors.

The estuaries of the Aconcagua and Maipo Rivers of central Chile are considered category one priority sites for biodiversity conservation in the Valparaíso Region (CONAMA, 2005). However, important anthropogenic activities such as mining, industry and agriculture take place in their basins. These activities generate wastes containing metals such as copper, zinc, iron, manganese, arsenic and lead, among others, which can reach these aquatic ecosystems and accumulate in the estuarine sediments. Since the basins of these rivers have different levels of human activity, it is likely that there are different levels of exposure in the sediments of their estuaries. There is currently no information about the levels of exposition of benthic organisms in these estuaries. This information is fundamental to determine the environmental risk due to metals in these estuary zones. This study postulated that the estuarine sediments of these rivers contain concentrations of bioavailable chemical agents that cause oxidative damage and antioxidant response in the macrozoobenthos organisms. Thus the aim of this study was to evaluate the effect of estuarine sediments on the polychaete *Perinereis gualpensis* using oxidative stress biomarkers and to determine the metal concentrations in sediments and the bio-accumulation in *P. gualpensis*.

2. Materials and methods

2.1. Study area

The Maipo River basin covers an area of 15,304 km^2. The basin covers almost 100% of the Metropolitan Region and a minimal area of the Regions of Valparaiso (San Antonio and Valparaiso Provinces) and Libertador Bernardo O'Higgins (Cachapoal Province). The total population in the basin is 4668,473. The average annual flow is 99 m^3/s. The Aconcagua River basin covers an area of 7200 km^2 with a total population of 1000,000 people. It is located in the southern end of the area of the transverse or semi-arid valleys in the Valparaiso Region. The average annual flow is 32 m^3/s. An Andean mining company that belongs to the state company Codelco, one of the largest copper producers in the world, is located in the upper part of the basin, and the Chágres smelter, an important copper smelter, is located in the Catemu zone. This study also used the Catapilco River estuary as a control zone; it has a small basin and activity is mainly agriculture and tourism (Fig. 1).

2.2. Biomonitor

P. gualpensis (Annelida, Polychaeta, Nereididae) is a polychaete endemic to Chile, inhabiting estuaries with mixo-oligohaline and euhaline saline surface water. It is found in intertidal and subtidal environments and estuaries with a higher proportion of silt and clay. It has a distribution from the southern province of Hualpén (Fig. 1)

3. Sampling

Sampling was performed in December 2015 and January 2016. The sampling points were: Aconcagua River estuary, 32°55′3.66″ S, 71°30′28.26″ W; Maipo River estuary, 33°37′8.57″S, 71°37′45.40″W and Catapilco River estuary, 35°37′53.74″S, 71°25′44.89″W. The organisms were separated and collected from the sediments manually using a 500 µ sieve. Four replicates were taken at each site. The collected sediments were transported to the laboratory in plastic containers and frozen at −20 °C. The organisms separated were transported to the laboratory, washed and then frozen at −80 °C. In the laboratory the identification of *P. gualpensis* specimens was made according to Sampertegui et al. (2013). The arrangement of paragnaths in all areas of the jaws as well as the length of tentacular cirri were used.

3.1. Characterization of sediments (chemical analysis)

Total organic matter (TOM) in sediments was determined by weight loss after ignition at 475–500 °C for 4 h. Redox potential was measured *in situ* using a Mettler Toledo electrode. Sediment grain sizes were determined according to Folk (1980) and Blott and Pye (2001).

3.2. Determination of metals in tissues and sediments

The tissues were dried in an oven at 60 °C for 4 days or until constant mass. Then samples were pulverized, homogenized and stored in plastic bottles in a desiccator until analysis. Samples of tissue were digested by first adding 10 ml HNO_3 and then 5 ml of H_2O_2 to 0.6 g of dry tissue. Samples were then heated at 180 °C for 2 h with reflux. After cooling, 1 ml $HClO_4$ was added and the mixture was boiled in an open system at 180 °C until 2 ml. A transparent digest was obtained. It was dissolved in 25 ml deionized water. O.25 g of sediments were dried and

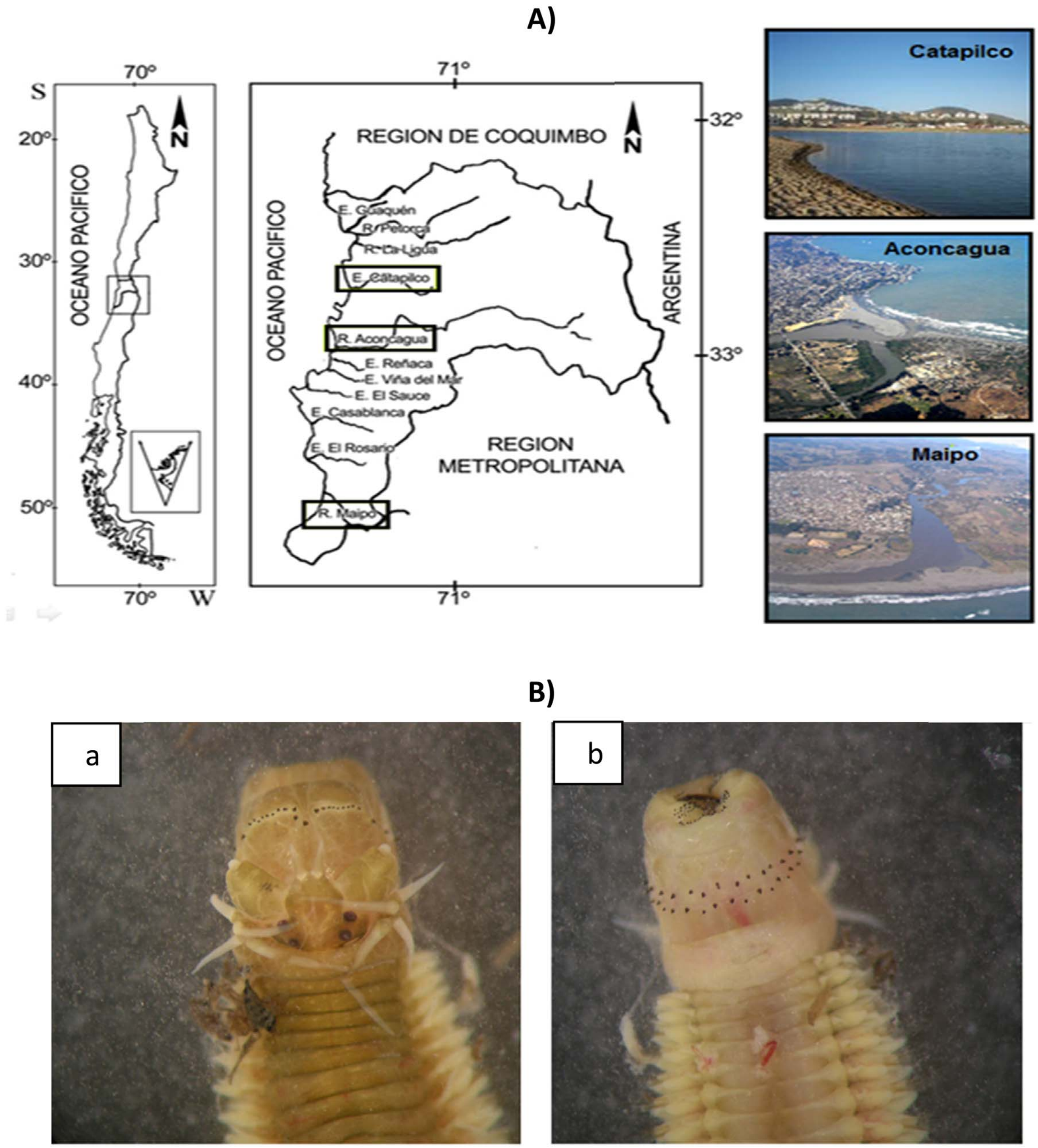

Fig. 1. A): location of the mouths of Catapilco, Aconcagua and Maipo Rivers; B) *P. gualpensis* in ventral view (a) and dorsal view (b).

sieved with a 0.5 mm frame, followed by a pre-digestion by adding 2 ml of HNO_3, H_2O_2 and 2 ml of HCl. Finally digestion was carried out in an Ethos Easy microwave. The concentrations of metals were determined by Inductive Coupling Plasma optical emission spectrometry (ICP-OES, Optima 2000 DV, Perkin-Elmer). The Supplementary Table 1 shows the results of the quality control of metal determinations with reference material.

3.3. Determination of catalase

Catalase was determined spectrophotometrically as described in Aebi (1984), by detecting the loss of absorbance at 240 nm of a solution of H_2O_2 0.3 M when 100 H_2O_2 of sample and 2.9 ml PBS pH 7.8 (Na_2HPO_4 and NaH_2PO_4 50 mM) were added. Values were expressed as units of enzyme/mg protein, using the total protein determined with the Lowry method.

3.4. Determination of TBARS: malondialdehyde (MDA)

This analysis was performed as described by Esterbauer et al. (1982). The pellet from centrifuged muscle macerate was treated with 10% trichloroacetic acid (final concentration) and centrifuged for 15 min at 3000 RPM to eliminate proteins. Then 1 ml of the supernatant was mixed with 0.33% (w/v) thiobarbituric acid, which reacts with the aldehydes in the sample. Samples were boiled for 30 min and their absorbance spectrum (400–600 nm) was recorded in a UV–visible spectrophotometer, to determine the concentration by extrapolation from a malondialdehyde calibration curve.

3.5. Data analysis

Comparison of antioxidant activity, oxidative damage and metal concentrations in tissues between sampling sites was performed after tests of normality and homoscedasticity (Kolmogorov–Smirnov and

Table 1
Physical and chemical parameters and granulometry of sediments in the estuaries.

Parameters	Estuaries		
	Aconcagua	Maipo	Catapilco
TDS ppm	200	110	6800
pH	7.8	8.2	8.7
Conductivity dS/m	40	120	1200
Temperature °C	18	23	21
Redox Mv	301	330	375
M.O, %	1.9	2.0	2.1
Shells %	0	0	3.8
Very coarse sand %	0	0	23.3
Coarse sand %	0	0	61.8
Medium sand %	4.7	21.9	4.7
Fine sand %	1.7	23.1	4.2
Very fine sand %	74.7	5.9	2.2
Coarse lime %	18.9	49.1	0

TDS: Total dissolved solids; O.M: Organic matter.

Cochran tests respectively), the Kruskal–Wallis test was applied for non-Normal samples using a significance level of 5%. Spearman correlation was performed to determine the relationships between variables. All tests were performed using SPSS program.

4. Results and discussion

4.1. Physical and chemical parameters of sediments in the estuaries

The pH of sediments tended to alkalinity, while there were similar percentages of organic matter among estuaries (Table 1). These results suggest that although the basins have different levels of human impact, they have the same self-purification or assimilative capacity. The pH and organic matter were similar to the values reported by Díaz-Jaramillo et al. (2013) in the Lenga and Raqui estuaries. However, the organic matter was lower in our study compared to the Tornagaleones and Valdivia Rivers, which have values between 1.55% and 10.13%. These differences could be due to the characteristics of the basins of the Tornagaleones and Valdivia Rivers, which are wetlands in zones of humid temperate forests that would provide greater amounts of organic matter. The low redox potential in the Aconcagua estuary indicates that chemical agents would have low tendency to form metallic oxides, favoring the solubility of the metal salts in the pore water of the sediments. The higher electrical conductivity in the Catapilco estuary may be due to formation of a coastal lagoon at the time of this study, which could have increased the concentration of salts, similar to that reported by Gaete et al. (2014).

The grain size was different between the estuaries. In Catapilco it was composed of coarse sand, while in the Aconcagua River there was silt and fine sand, and fine sand in the Maipo River (Table 1). These differences may be due to the different geological characteristics of the basins, erosive processes, soil uses, drainage areas and physical processes such as flows and tides, among others. In the Aconcagua estuary the sediment characteristics were consistent with those reported by Martínez and Cortez (2007). These authors indicated a strong influence of currents (tidal and fluvial) and erosive processes. The predominance of finer particles of the estuaries of the Aconcagua and Maipo Rivers at the time of the study suggests that sedimentation processes were favored.

4.2. Metal concentrations in sediments and tissues

The concentrations of metals in tissues and sediments varied significantly between the estuaries. In tissues, the concentrations of Cu and Zn were higher in the estuary of the Aconcagua River; Fe and Pb were higher in the Maipo River, and Cd was detected only in Catapilco. The higher

Table 2
Metal concentrations (mean ± standard deviation; n = 4) in tissues of *P. gualpensis* and sediments. Different letters represent significant differences (p < 0.05) among estuaries.

Parameters	Unit	Aconcagua	Maipo	Catapilco
Tissue				
Cu	$\mu g\ g^{-1}$	112.4 ± 12^{a}	29 ± 0.6^{b}	13.6 ± 0.8^{c}
Fe	$mg\ g^{-1}$	51 ± 7^{a}	163 ± 3^{b}	7 ± 1^{c}
Zn	$\mu g\ g^{-1}$	109 ± 12^{a}	94 ± 5^{b}	45 ± 3^{c}
Pb	$\mu g\ g^{-1}$	1.3 ± 0.2^{a}	2.2 ± 0.2^{b}	0.3 ± 0.1^{b}
Cd	$\mu g\ g^{-1}$	< LD	< LD	0.88 ± 0.03
Sediment				
Cu	$\mu g\ g^{-1}$	48 ± 2^{a}	23.2 ± 0.4^{b}	11 ± 1^{c}
Fe	$mg\ g^{-1}$	154 ± 19^{a}	62 ± 8^{b}	16 ± 1^{c}
Zn	$\mu g\ g^{-1}$	143 ± 20^{a}	55 ± 5^{a}	17 ± 1^{b}
Pb	$\mu g\ g^{-1}$	20 ± 3^{a}	0.08 ± 0.01^{b}	0.56 ± 0.05^{b}
Cd	$\mu g\ g^{-1}$	< LD	< LD	< LD

< LD: below detection limit.

concentration of Cu in tissues from the Aconcagua estuary may be due to the pH, which was lower than in the other estuaries, increasing its bioavailability. On the other hand, the lower concentration of Fe in the tissues in the estuary of the Aconcagua River could be due to its tendency to form less soluble oxides which makes difficult its ingestion and bioaccumulation. Concentrations had the following decreasing order: Aconcagua estuary: Fe > Cu > Zn > Pb > Cd; Maipo estuary: Fe > Zn > Cu > Pb > Cd and Catapilco estuary: Fe > Zn > Cu > Cd > Pb (Table 2).

The metal concentrations in sediments were higher in the estuary of the Aconcagua River; Cd was not detected in the three estuaries. Metals in sediments showed the following descending order in the three estuaries: Fe > Zn > Cu > Pb > Cd (Table 2). The higher concentrations of metals in tissues and sediments in the estuary of the Aconcagua River could be related to mining activity that is carried out in the basin by Codelco Andina mining company, one of the most important in the world, as well as by the copper smelter of Chágres, located in Catemu. These activities generate wastes containing heavy metals that are directly or indirectly discharged to the Aconcagua River (Gaete et al., 2007). Studies of metals in estuarine sediments in Chile are scarce. Bertrán et al. (2001a) reported concentrations of copper and lead in the Biobio River similar to those of this study. UACH (2016) reported similar values of copper concentrations in the central axis of the Cruces River in 2014 and 2015, ($11.5-63.8\ mg\ g^{-1}$) except for the Aconcagua River that were higher in our study. This was also true for lead ($8.8-12.8\ mg\ g^{-1}$), zinc ($34.8-127.0\ mg\ g^{-1}$) and iron (19.820–64.434 $mg\ g^{-1}$), except for the latter compared to sediments of the Maipo River that were higher in our study. The differences in the concentrations of the metals in sediments among estuaries may be related to the geological characteristics of basins, river flows and industrial activities carried out in the basins such as mining. Pizarro et al. (2010) reported that there are nine large mining companies in the Aconcagua river basin, while in the Maipo River there are three.

4.3. Bioaccumulation factor (BCF)

Copper showed the highest bioaccumulation factor (BCF) in the Aconcagua estuary, Fe and Pb had maximum values in the Maipo estuary and Zn in Catapilco (Table 3). The differences in the BCF can be explained by the different affinities of tissues for metals. Cu has low

Table 3
Bioaccumulation factors (BAFs) in *Perinereis gualpensis*.

Estuaries	Cu	Fe	Pb	Zn
Aconcagua	2.30	0.32	0.06	0.75
Maipo	1.20	2.60	26.7	1.60
Catapilco	1.30	0.44	0.51	2.57

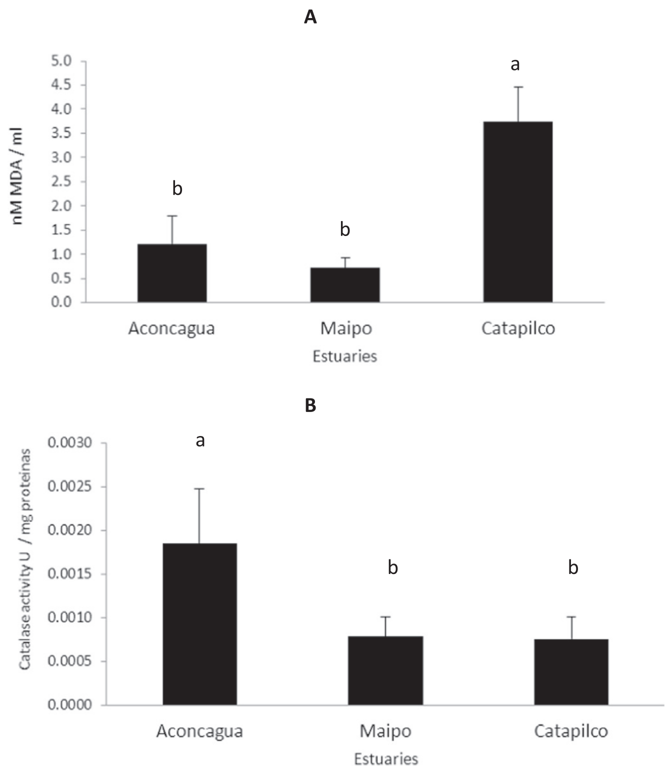

Fig. 2. A) Lipid peroxidation (LPO). **B)** Catalase activity in *P. gualpensis*. Different letters represent significant differences ($p < 0.05$) among estuaries.

bioaccumulation and can be easily excreted. It is known that essential elements such as copper are more regulated than non-essential ones such as Pb, which has the highest BCF (ATSDR, 1990, Moreno, 2003). The concentrations of copper, iron and lead in tissues of *P. gualpensis* reported in our study are higher than those reported for the Valdivia River estuary (Velásquez, 2005) in *Mytilus chilensis*. Guiñez et al. (2015) reported copper and zinc concentrations in *Emerita analoga* lower than those obtained in this, which suggests that *P.gualpensis* has greater capacity for bioaccumulation. The higher concentration of Pb in tissues of *P. gualpensis* than in the sediments in the Maipo River estuary, can be due to the redox potential that was the lowest among estuaries. This could promote the formation of more soluble and bioaccumulative chemical species. On the other hand, it could be that the Pb be more difficult to excrete by its capacity of adsorption to the organic matter, in this respect Moreno (2003) reports factors of concentrations of 500 in insects, which is greater than found in our study.

4.4. Biomarkers

Lipoperoxidation was higher in the Catapilco estuary, and catalase activity had its maximum value in the Aconcagua estuary (Fig. 2). Although the lowest concentrations of metals except for Cd were in the Catapilco estuary, the organisms there showed the highest oxidative damage, which could be due to other chemical agents that were not measured in this study such as pesticides. It also might be explained by Cd, which was detected only in this estuary. Sun and Zhou (2008) reported a relationship between concentrations of cadmium and lipid peroxidation in the polychaete *Nereis diversicolor*. Another factor could also be the salinity. Freitas et al. (2015) reported that salinity provoked oxidative stress in the polychaete *Diopatra neapolitana*. In our study, the Catapilco estuary showed the highest conductivity due to increased salinity. Zhao et al. (2013) found that salinity increase may elevate the potential ecological risk of Cd and Mn, while Cu and Pb seem to be more conservative. The higher salinity can generate chemical species of metals with greater mobility (Zhao et al., 2013) and therefore pass the biological barriers and interact with the components of the cellular membranes, generating alterations in their permeability and provoking lipoperoxidation. The higher catalase activity in the Aconcagua estuary could be explained by the higher concentration of copper bio-accumulated, which causes oxidative stress and antioxidant response (Geracitano et al., 2004). The minor oxidative damage in the other estuaries could be related to Zn, which can induce the production of metallothioneins (Marín et al., 2016). The thiol groups in these proteins can immobilize metals, decreasing their bioavailability and toxic effects. Nevertheless, significant correlation between metal concentrations and catalase activity and also lipoperoxidation were observed (Supplementary Table 2). This suggests that these metals are bioavailable and cause the variation both in catalase activity and MDA levels. Our results indicate that the mechanisms of detoxification due to the generation of reactive oxygen species (ROS) provoked by metals, generate the antioxidant response of catalase. Cataldo et al. (2011) report similar result in earthworms in sites contaminated by metals. On the other hand, although an antioxidant response of catalase was observed, the results show damage to the cell membranes of *P. gualpensis* at increasing the bioaccumulated metals, which explains the increase of MDA that is a product of the lipoperoxidation. According to the above, the significant relationship between these variables and the concentrations of metals in tissues suggests that these biomarkers in *P. gualpensis* are sensitive to bioaccumulated metals.

In conclusion, in the sediments of the estuarie of Aconcagua river found the higher metals concentrations, while in tissues of *P.gualpensis* the Fe and Pb concentrations were higher in the estuarie of Maipo river. The sediments of estuarie of Aconcagua river contain bioavailable chemical agents that provoke oxidative stress in *P. gualpensis*, which may be a risk for the benthic communities of these ecosystem. The concentrations of metals in tissues were related to oxidative stress biomarkers measured in *P. gualpensis*. This species is proposed to monitor metals bioavailability and oxidative stress in estuarine sediments

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was financed by the Centro de Investigación y Gestión de Recursos Naturales (CIGREN) of Universidad de Valparaíso (DIUV/2003).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.07.073.

References

Aebi, H., 1984. Catalase in vitro. Method Enzymol. 105, 121–126.
ATSDR, 1990. Toxicological Profile for Copper. US Departament of Health and Human Services. Public Health Service, Atlanta, GA. ⟨www.astdr.ced.gov⟩.
Bertrán, C., Arenas, J., Parra, O., 2001a. Macrofauna del curso inferior y estuario del río Biobío (Chile): cambios asociados a variabilidad estacional del caudal hídrico. Rev. Chil. Hist. Nat. 74, 331–340.
Bertrán, C., et al., 2001b. *Perinereis gualpensis* Jeldes (Annelide, Polychaeta) como biomonitor de metales pesados en la desembocadura del rio Biobio (Chile). Inform. Tecnológica 12 (4), 59–62.
Blanchet- Letrouvé, et al., 2013. Vg mRNA induction in an endangerred fish species (Anguilla anguilla) from the Loire estuary (France). Ecotoxicol. Environ. Saf. 97, 103–113.
Blott, S.J., Pye, K., 2001. Gradistat: a grain size distribution and statistics package the analysis of unconsolidated sediments. Earth Surf. Process Landf. 26 (11), 1237–1248.
Campana, O., et al., 2012. Sub-lethal effects of copper to benthic Invertebrates explained by sediment properties and dietary exposure. Environ. Sc. Technol. 46, 6835–6842.
Carvalho-Neta, R., Abreu-Silva, A., 2010. Sciades herzbergii oxidative stress biomarkers: an in situ study of an estuarine ecosystem (São Marcos' Bay, Maranhão, Brazil). Braz.

J. Oceanogr. 4 (58), 11–17 (N° special).
Cañete, I., Leighton, G., Soto, E., 2000. Proposición de un índice de vigilancia ambiental basado en la variabilidad temporal de la abundancia de dos especies de poliquetos bentónicos de bahía Quintero, Chile. Rev. De. Biol. Mar. Y. Oceanogr. 35 (2), 185–194.
Cataldo, J., et al., 2011. Use of molecular biomarkers in *Eisenia foetida* to assess copper toxicity in agricultural soils affected by mining activities. J. Soil Sci. Plant Nutr. 11, 57–70.
CONAMA, 2005. Estrategia y Plan de Acción Para la Conservación de la Diversidad Biológica. Ministerio del Medio Ambiente de Chile, Región de Valparaíso, pp. 160.
Dean, H.K., 2008. The use of polychaetes (Annelida) as indicator species of marine pollution: a review. Rev. De. Biol. Trop. 56, 11–38.
Díaz-Jaramillo, M., et al., 2010. Biomonitoring of antioxidant and oxidative stress responses in *Perinereis gualpensis* (Polychaeta: nereididae) in Chilean estuarine regions under different anthropogenic pressure. Ecotoxicol. Environ. Saf. 73, 515–523.
Díaz-Jaramillo, M., et al., 2013. Biochemical and behavioral responses in the estuarine polychaete *Perinereis gualpensis* (Nereididae) after in situ exposure to polluted sediments. Ecotoxicol. Environ. Saf. 89, 182–188.
Díaz-Jaramillo, et al., 2017. Sublethal effects in *Perinereis gualpensis* (Polychaeta: nereididae) exposed to mercury-pyrene sediment mixture observed in a multipolluted estuary. Ecotoxicology 26, 792–801.
Díaz, V., Reish, D., 2009. Polychaete in environmental studies. Annelids Mod. Biol. 203–227.
Esterbauer, K., et al., 1982. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-$Fe2+$ in rat liver microsomes. Biochem. J. 208 (1), 129–140.
Fevzi, Y., et al., 2007. Heavy metal levels in two fishes species *Leuciscus cephalus* and *Lepomis gibbosus*. (l) Food Chem. 100, 830–835.
Folk, R.L., 1980. Petrology of Sedimentary Rocks. Hemphill Publishing Company, Austin, pp. 181.
Fossi, O., et al., 2012. Intersite variations of a battery of biomarkers at different levels of biological organisation in the estuarine endobenthic worm Nereis diversicolor (Polychaeta, Nereididae). Aquat. Toxicol. 114–115, 96–103.
Freitas, R., et al., 2015. The effects of salinity changes on the Polychaete Diopatra neapolitana: impacts on regenerative capacity and biochemical markers. Aquat. Toxicol. 163, 167–176.
Gaete, H., et al., 2007. Metales pesados y toxicidad de aguas del Río Aconcagua en Chile. Quím. Nova 30 (4), 885–891.
Gaete, H., et al., 2014. Evaluación de la genotoxicidad de las aguas costeras de Chile central sobre los peces *Mugil cephalus* y *Odontesthes brevianalis*. Hidrobiológica 24 (3), 271–279.
Geracitano, L.A., et al., 2004. Oxidative responses in two populations of *Laonereis acuta* (Polychaeta, Nereididae) after acute and chronic exposure to copper. Mar. Environ. Res. 58, 1–17.
Giangrande, A., Margherita, L., Musco, L., 2005. Polychaetes as environmental indicators revisited. Mar. Pollut. Bull. 50, 1153–1162.
Gray, J., Elliott, M., 2009. Ecology of Marine Sediments. From Science to Management. Oxford, New York, USA, pp. 225.
Guiñez, M., Valdés, J., Castillo, A., 2015. Contenido de metales en sedimentos y en *Emerita análoga* (Stimpson, 1857), en bahía Mejillones del Sur, Chile. Lat. Am. J. Aquat. Res. 43 (1), 94–106.
Jara, C., et al., 2014. Oxidative stress in the mollusk *Echinolittorina peruviana* (Gasteropoda: littorinidae, Lamarck, 1822) and trace metals in coastal sectors with mining activity. Ecotoxicology. http://dx.doi.org/10.1007/s10646-014-1253-3.
Jaramillo, E., Contreras, H., Quijón, P., 2001. Variabilidad estacional e interanual en las abundancias poblacionales de la macroinfauna intermareal del estuario del rı´o Queule, centro-sur de Chile. Rev. Chil. Hist. Nat. 74 (2), 455–468.
Marín, L., et al., 2016. Metalotioneínas en bivalvos marinos. Lat. Am. J. Aquat. Res. 44 (2), 202–215 (2016).
Martínez, C., Cortez, C., 2007. Características hidrográficas y sedimentológicas en el estuario del río Aconcagua, Chile Central. Rev. De. Geogr. Norte Gd. 37, 63–74.
Moreno, D., 2003. Toxicologia Ambiental: evaluación de riesgo para la salud humana.Ed. McGraw Hill, España, pp. 370.
Monserrat, J.M., et al., 2007. Pollution biomarkers in estuarine animals: critical review andnew perspectives. Comp. Biochem. Phys. C 146, 221–234.
Pal, Yu, B., 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 73 (1), 139–162.
Pallero, C., Barragán, J.M., García, E., 2017. Management of transboundary estuaries in Latin America and the Caribbean. (http://dx.doi.org/) Mar. Policy 76, 63–70. http://dx.doi.org/10.1016/j.marpol.2016.11.014.
Pizarro, P., et al., 2010. Heavy metals in northern Chilean rivers: spatial variation and temporal trends. J. Hazard. Mater. 181, 747–754.
Sampertegui, S., et al., 2013. Morphological and molecular characterization of *Perinereis gualpensis* (Polychaeta: Nereididae) and its phylogenetic relationships with other species of the genus off the Chilean coast, Southeast Pacific. Cah. De. Biol. Mar. 54, 27–40.
Srivastava, S.S., et al., 2006. Copper-induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla certicillata* (L. F.) Royle. Aquat. Toxicol. 80, 405–415.
Sun, F., Zhou, Q., 2008. Oxidative stress biomarkers of the polychaete *Nereis diversicolor* exposed to cadmium and petroleum hydrocarbons. Ecotox. Environ. Safe 70, 106–114.
Tapia, J., et al., 2009. Study of the copper, chromium and lead content in *Mugil cephalus* and *Eleginops maclovinus* obtained in the mouths of the Maule and Mataquito rivers (Maule Region, Chile). J. Chil. Chem. Soc. 54 (1), 36–39.
Tapia, J., et al., 2012. Heavy metals in the liver and muscle of *Micropogonias manni* fish from Budi Lake, Araucania region, Chile: potential risk for humans. Environ. Monit. Assess. 184, 3141–3151.
Thompson de Oliveira Lemos, R., Martins de Carvalho, P., Zanardi-Lamardo, E., 2014. Petroleum hydrocarbons in water from a Brazilian tropical estuary facing industrial and port development. Mar. Pollut. Bull. 82, 183–188.
UACH, 2016. Programa de Monitoreo Ambiental Actualizado del Humedal del Río Cruces y sus Ríos Tributarios: Informe Anual Correspondiente al Período Abril 2015. Universidad Austral de Chile, Valdivia, Chile, pp. 676.
Velásquez, D., 2005. Determinación de metales pesados en biota (*Mytilus chilensis*) y sedimentos de la bahía de corral, Provincia de Valdivia, X Region. Tesis para obtener el título de químico farmacéutico. Universidad Austral de Chile, Valdivia, Chile.
Zhao, S., et al., 2013. Salinity increases the mobility of Cd, Cu, Mn, and Pb in the sediments of Yangtze Estuary: relative role of sediments' properties and metal speciation. Chemosphere 91, 977–984.