Bull. Environ. Contam. Toxicol. (1994) 52:261–268

Environmental Contamination and Toxicology

Heavy Metals in Bivalve Mussels and Their Habitats from Different Sites along the Chilean Coast

I. De Gregori,[1] H. Pinochet,[1] D. Delgado,[1] N. Gras,[2] L. Muñoz[2]

[1]Chemistry Institute, Catholic University of Valparaíso, P.O. Box 4059, Valparaíso, Chile
[2]Neutron Activation Analysis, Nuclear Center "La Reina," Chilean Nuclear Energy Commission, P.O. Box 188-D, Santiago, Chile

Received: 3 April 1993/Accepted: 30 June 1993

It is well known that heavy metals have a great ecological significance due to their toxicity and accumulative behaviour playing a prominent role in marine ecosystems. They occur in all compartments in the marine environment with a tendency to accumulate in organism from different trophic levels of the marine webs (Amiard et.al.1987; Stoeppler 1992). Along this pathway, toxic trace metals become a potential hazard for man and mammals. Coastal and estuarine zones are more vulnerable to anthropogenic pollution with toxic metals and it must be kept in mind that these zones have high seafood production.

Chile is in a favourable position to develop fishing activities since it has approximately 4.500 km. of coastline bordering the South Pacific Ocean. The seawater is rich in nutrients and new aquaculture projets have been developed during the last year. However, these seafoods, like other marine organisms, are susceptible to being contamined by trace metals, produced especially by the mining and industrial processing of ores and metals (i.e. Cu, Mo). This activity is well known as an important source of heavy metals, due to the enormous quantities of waste products, some of which were formerly released directly to the marine environment. Actually there are many aspects demanding better and more detailed knowledge on the ocurrence, inventory, of the Chilean marine ecosystem. For these reasons we are developing a programme for monitoring some heavy metals in marine samples. The development of analytical quality control procedures and the analysis of toxic trace elements in fresh and canned mussels were recently published (De Gregori et.al. 1992). In this paper we present the results obtained for the Cadmium, Copper and Zinc contents in water, sediment and mollusc samples collected from different geographical areas located along the coast of Chile.

Correspondence to: H. Pinochet

MATERIALS AND METHODS.

In the period May - June 1992 bivalve molluscs, seawater and sediment samples were collected simultaneously from 8 different geographical sites located nearby the following cities (see Fig. 1):
1. Iquique (Cavancha beach) 2. Antofagasta (Huascar beach) 3. Chañaral (Pan de Azúcar Core) 4. La Serena (Lagunillas beach) 5. Valparaíso (Quintero bay) 6. Concepción (Arauco Gulf) 7. Valdivia (Corral bay) and 8. Puerto Montt (Chamiza beach at Ancud Gulf). The sites 1, 2 and 3 are located in the desert zone of Northern Chile; 4, 5 and 6 in the central zone, with moderate climate; 7 and 8 at the south, a rainy zone. The bivalve mussels chosen for study were Chorito Maico (common for all sampling sites), Almejas and Navajuelas chilenas (*Perumytilus purpuratus, Semelle solida* and *Tagelus dombeii* respectively). All sampling flasks, cells and materials for samples treatment and heavy metal determination were acid cleaned, rinsed with pure water and stored in polyethylene bags until used. The flasks for sample collection were then rinsed with sea water of the same sampling site. The water used was obtained from a Millipore Milli Q water purifying system which provided deionized water with particulary low levels of the trace metals under study. Reagents for treatments, digestions and analytical determination were of ultrapure quality (suprapur merck).

Protocols for trace metal analyses of total tissue of a mollusc samples have been previously described (De Gregori et.al. 1992, 1993). Water samples were filtered through precleaned 0.45 μm pore size membrane (each filter was rinsed with 1M HCl and then about of 1 lt of filtered sample was discarded). The water sample was acidified and stored deep frozen at-20°C until analysis was carried out. For metal analysis preconcentration with Chelex-100 was done by a batch method. Chelex 100 resin (200-400 mesh, Na form, Bio Rad) was purified by pouring 100 mL of resin into a polypropylene column, washing five times with 250 mL of 2,5 M nitric acid in order to eliminate trace metals from the resin and removing the excess of acid by rinsing with 150 mL of water. Finally the resin was transformed into the ammonium form by adding three 50 mL aliquots of 2 M ammonia. The column was rinsed in order to remove the excess of ammonia. The acidified sea water sample (500 g) was weighed in a precleaned Schot-Duran glass bottle of 500 mL and the pH was adjusted to 5.0 - 5.5 with 1 M ammonia. Chelex-100 (6-8 mL) and 10 mL of 1M ammonium acetate buffer (pH 5.8) were added to the sample and shaked for 64 hr. The resin was collected in a polypropylene column (i.d. 7mm) and washed with 30 mL of 1M ammonium acetate buffer in order to elute the alkaline and alkaline earth metal ions (Boniforti and Queirazza 1984). Trace metals were eluted with 10 mL of 2.5 M nitric acid (0.3 - 0.5 mL min^{-1}) and collected in a 10 mL cleaned glass flask.

The trace elements (Cd, Cu and Zn) were determined by graphite furnace atomic absorption spectrophotometry (GFAAS) using a GBC 950 PB atomic absorption spectrophotometer with a deuterium background corrector with an autosampler

(PAL 3000) and quantified by the standard addition method. For Cd analyses a L'vov platform was used (L'vov 1991) and $(NH_4)H_2\,PO_4/Mg(NO_3)_2$ was added as modifier (Bozsai et.al. 1990).

Sediment samples were stored at - 20°C, thawed in a microwave oven and dried for at least 48 h at 50°C. The dry sample was homogeneized and then sieved through nylon sieves supported in a home made demountable plastic plate and shaked with a Tyler Rotap. The fraction smaller than 200 μm was taken for analysis. Dry sediments (1.5-2.0 g) were digested with a mixture of 9 mL of HNO_3 c.sp. + 3 mL HCl c.sp. using an Uniseal Teflon high pressure decomposition system. The Teflon vessel was covered with a teflon plate and allowed to stand overnight at room temperature and then the closed Uniseal system was heated at 150°C for 3 h. The cooled sample was quantitatively filtered with precleaned filter paper (HCl 1M) and then made to 50 mL with water. Cd was analyzed by GFAAS with L'vov platform, Cu and Zn by flame AAS. Blank samples were run simultaneously with samples and the autozero of the spectrometer was made with the respectively blank.

Zn determination of mollusc samples was carried out by instrumental neutron activation analysis; Cd and Cu with radiochemical separations. For Zn, 0.5 g samples were prepared in pellet form. Standard solutions were prepared from high-purity reagents onto Whatman N° 41 filter paper, then dried under an infrared lamp and later pressed into pellets. Samples and standards were irradiated for 24 h at a thermal neutron flux of 1-2 x 10^{13} n cm^{-2} sec^{-1} using the neutron facilities of La Reina Nuclear Center. After a suitable cooling time, the measurements were carried out using a Canberra high purity Ge detector coupled to a S 100 computer system. Calculations were made using the 1115 KeV gamma-line of ^{65}Zn. (Muñoz et.al. 1992)

For Cd analysis 0.5 g of samples or standards were irradiated in similar conditions. After 3 days cooling time, samples were inmediately digested in a mixture of sulphuric acid and perhydrol, and passed through an analytical grade anion exchange resin Bio-Rad AG 2x8, 100-200 mesh chloride form resin column which retained cadmium. The resin was removed and counted 24 h after on a high resolution gamma spectrometry system using the 336 KeV gamma-line of ^{115}Cd (^{115}In).

For the determination of Cu, the samples and standards were irradiated for 5 min at the same neutron facility. After a cooling time of 1 h the samples were digested with the same reagent mixture and passed through an analytical grade chelating resin Chelex-100, 100-200 mesh sodium form column, in order to retain the element of interest. The resin was then removed and the radionuclide ^{64}Cu was measured immediately using the 511 KeV gamma-line. The yield of these radiochemical separations were controlled using certified reference materials (CRM) (Gras et.al 1992).

Results for metal determinations in sediment and mollusc samples are reported on dry weight basis (d.w.).

RESULTS AND DISCUSSION.

Monitoring trace metals in environmental samples requires strict quality control of the analysis. Proper application of quality assurance requires the analysis of CRM that match as closely as possible the matrix type and the elemental level of the real samples. The accuracy of the analytical methodologies were assessed using the BCSS-1 Coastal marine sediment, the Estuarine marine sediment SRM 1646 and the Oyster tissue NBS 1566. With the aim to apply analytical quality control for water analysis in identically experimental conditions of real samples and to avoid the use of 500 mL of a water CRM in triplicate, a recovery study was controled in each set of analyses.

The heavy metals Cd, Cu and Zn concentrations of different samples collected at 8 sampling sites along Chilean coast are listed in Table 1. Almejas samples were not caught in sites 2 and 3, even though in the past this zone was well known as producer of this species. Navajuela is a mollusc that is found only in natural banks in estuarine zones of southern Chile.

According to these data, in all kind of samples, Cd is the metal found in lowest concentration followed by Cu and Zn. This behaviour was expected and correlated inversely with their toxicity. The Cd levels in water were similar and ranged between 4 to 21 ppt. The Cu concentrations show a wider variation than Cd; the levels ranged between 0.27 to 2.7 ppb with an outlier value at Chañaral (3.7 ppb). Zn levels were similar showing a factor not more than two between the lowest and the highest value.

Figures 2 and 3 show the metal concentrations in sediments and Chorito maico mussels respectively. In sediments, the Cd concentrations were similar with the only exception being the value obtained at Iquique. In general, the copper levels decrease from north to south with values between 40 to 10 μg/g with two clearly polluted areas, Chañaral and Quintero. At these stations the mussels studied also present the highest values for Cu. This behaviour can be explained by the fact that Quintero station is located in proximity of one of the biggest copper smelters and electrorefinery plants of Chile, receiving airborne particulate matter containing great concentrations of heavy metals. In the Chañaral zone in turn, the sewage and sweepings of a copper mine located in the Andes mountains were released, producing in consequence that the Chañaral harbour became blocked.

Another anomalous behaviour can be emphasized from the sediments results: Chañaral is the only sampling station where the Zn/Cu concentration ratio was lower than 1 (0.5).

Table 1. Trace metal of marine samples colected along the Chilean coast.

SITES	METAL	WATER (ppb)	SEDIMENT (ppm)	CHORITO (ppm)	ALMEJA (ppm)	NAVAJUELA (ppm)
Iquique	Cd	0.16 ± 0.06	0.78 ± 0.12	15.0 ± 2.2	1.3 ± 0.5	
	Cu	0.27 ± 0.02	42.2 ± 3.3	13.4 ± 1.3	7.8 ± 0.6	
	Zn	9.7 ± 0.6	88.7 ± 2.8	122 ± 15	208 ± 4	
Antofagasta	Cd	0.15 ±0.03	0.18 ± 0.01	4.4 ± 0.9		
	Cu	1.6 ±0.3	22.4 ± 0.6	120 ± 15		
	Zn	8.7 ± 2.7	25.6 ± 3.4	106 ± 6		
Chañaral	Cd	0.05 ± 0.01	0.06 ± 0.01	4.7 ± 0.5		
	Cu	3.7 ± 0.8	102 ± 5	134 ± 10		
	Zn	7.7 ± 3.2	50 ± 2	107 ± 6		
Coquimbo	Cd	0.10 ± 0.02	0.07 ± 0.01			
	Cu	0.5 ± 0.2	15.1 ± 2.2			
	Zn	13.6 ± 2.4	19.5 ± 1.0			
Quintero	Cd	0.08 ± 0.02	0.09 ± 0.01	4.0 ± 0.2	1.1 ± 0.2	
	Cu	1.2 ± 0.3	54 ± 2	137 ± 10	31 ± 6	
	Zn	11.1 ± 1.4	83 ± 5	134 ± 2	66 ± 2	
Concepción	Cd	0.21 ± 0.07	0.12 ± 0.02	6.1 ± 0.3	4.9 ± 0.5	
	Cu	1.6 ± 0.2	10.7 ± 1.5	13.4 ± 0.4	3.6 ± 0.5	
	Zn	6.7 ± 1.1	31.6 ± 3.2	133 ± 14	79 ± 2	
Valdivia	Cd	0.05 ± 0.01	0.22 ± 0.03	4.2 ± 0.1	0.85 ±0.15	5.4 ± 1.0
	Cu	1.8 ± 0.2	14.3 ± 2.2	7.7 ± 1.5	7.6 ± 1.0	2.4 ± 0.2
	Zn	8.4 ± 2.0	81 ± 4	63 ± 1	50 ± 7	71 ± 2
P. Montt	Cd	0.12 ± 0.07	0.09 ± 0.01	2.2 ± 0.1	2.2 ± 0.2	6.6 ±0.5
	Cu	2.1 ± 0.9	9.8 ± 1.4	7.4 ± 0.1	9.7 ± 0.6	6.6 ±1.5
	Zn	10.1 ± 2.8	48 ± 3	233 ± 30	94 ± 1	139 ±2

In the light of our observations Zn levels in sediments, do not reflect a clear geographical trends.

In Chorito maico mussel the highest value for Cd was found in samples from Iquique (15 μg/g), the northest station, and the lowest value at Puerto Montt (2.2 μg/g), the southest sampling station. Iquique is the same site where the Cd concentration in sediments was also the highest. This station is located at a big city of 150.000 people where different kind of marine and coastal activities such

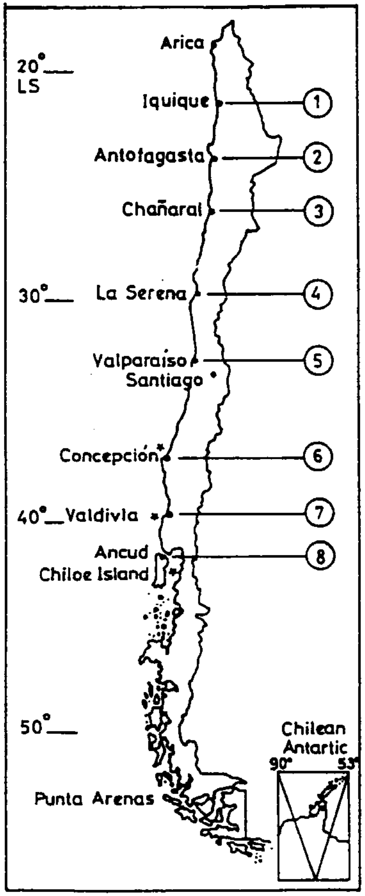

Figure 1. Map of Chile showing the sampling areas.

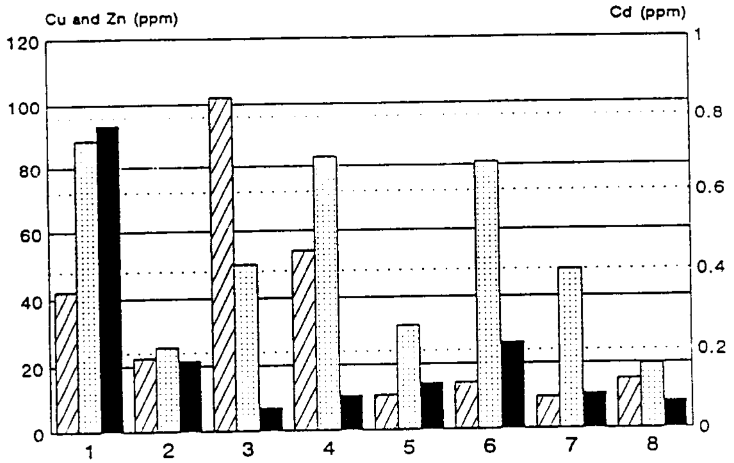

Figure 2. Metal concentrations in sediments.

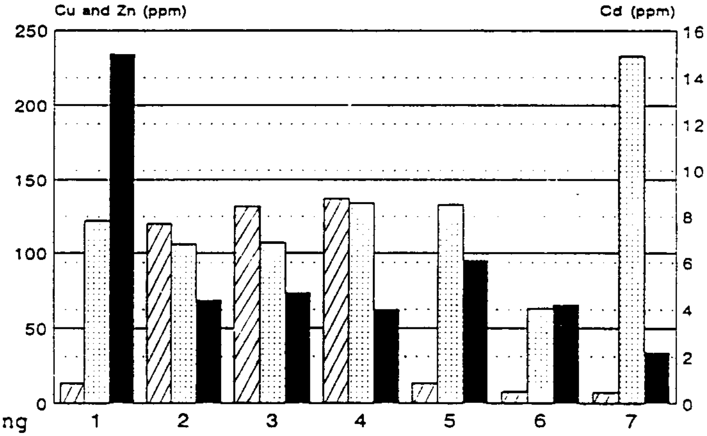

Figure 3. Metal concentrations in Chorito maico.

LOCATIONS

1. Iquique
2. Antofagasta
3. Chañaral
4. Quintero
5. Concepción
6. Valdivia
7. Puerto Montt
8. Coquimbo

as transport, fisheries, recreation and industry are carried out. In addition effluents originating from fish and canning industries and municipal wastes find their way into its waters.

A good correlation was found between Cd concentrations of Chorito maico and the Cd concentration in sediments (r=0.951). This preliminary results could indicate that Cadmium in sediments can be evaluated using *Perumytilus purpuratus* (Chorito maico) as biomonitor. This behaviour needs a more exhaustive study.

The Cu concentrations in Chorito maico are close to a mean value of 10 μg/g with the exception of the values obtained for Antofagasta, Chañaral and Quintero where the values were 12 fold higher (120; 134 and 137 μg/g respectively). The behaviour of Quintero and Chañaral was already described and at Antofagasta the high value for Cu in this mussel, can be explained by the proximity of this sampling station to a harbour for shipping copper concentrates.

The Zn concentrations for Chorito maico range between 63 and 134 ppm, not taken into account the value for Puerto Montt station, where the value found was 21 fold bigger (233 μg/g). For this behaviour we do not have explanation but this continental area has some polymetallic ores. The results obtained for Navajuela samples allow us to corroborate that this mollusc presents high cadmium concentration levels compared to those found in Almejas, another bivalve mussel of the same habitat. This behaviour is independent of the geographical zone from where they were caught and was observed by us in previous studies and also by Ostacpczuk (1990).

In general it is important to remark that heavy metals levels found in water, sediment and mollusc samples are comparable to those reported for the same matrices from the coastal areas of the Southeast Pacific ocean (Hungspreugs 1988), except the high values found specially for Cu in sediments and in Chorito maico mussel from the zones of Chile with recognized industrial heavy metal pollution.

Acknowledgments. We thank the Fondo Nacional de Desarrollo Científico y Tecnológico de Chile (FONDECYT) for their financial support of this work (Project 92-715). We are also grateful to the Dirección General de Investigación, Universidad Católica de Valparaíso and the Comisión Chilena de Energía Nuclear.

REFERENCES

Amiard J C, Amiard-Triquet C, Berthet B, Metayer C (1987) Comparative study of the patterns of bioaccumulation of essential (Cu, Zn/and non-essential (Cd,Pb) trace metals in various estuarine and coastal organisms. J Exp Mar Biol Ecol 106: 73-89.

Boniforti R, Queirazza G (1984) Intercomparison of five methods for the determination of trace metals in sea water. Anal Chim Acta

162: 33-46.

Bozsai G, Schlemmer G, Grobenski Z (1990) Determination of Arsenic, Cadmium, Lead and Selenium in highly mineralized waters by Graphite-Furnace atomic absorption spectrometry. Talanta 37: 545-553.

De Gregori I, Delgado D, Pinochet H, Gras N, Thieck M, Muñoz L, Bruhn C, Navarrete G (1992) Toxic trace elements in Chilean seafoods: development of analytical quality control procedures. Sci Total Environ 111: 201-218.

De Gregori I, Delgado D, Pinochet H, Gras N, Thieck M, Muñoz L (1993) Lead, Copper and Mercury levels in fresh and canned bivalves mussels "*Tagelus dombeii*" (Navajuela) and "*Semelle sólida*" (Almeja) from the Chilean coast. Sci Total Environ (In press).

Hunpreugs M (1988) Heavy metals and other non-oil pollutants in Southeast Asia. Ambio 17: 178-182.

Gras N, Muñoz L, Cassorla V, Castillo P (1992) Preliminary homogeneity study of an house-reference material using Neutron Activation Analysis and X-ray Fluorescence. Fresenius J Anal Chem 334:250-253.

L'vov BV (1991) A personal view of the evolution of graphite Furnace Atomic Absorption spectrometry. Anal Chem 63: 924A-931A.

Muñoz L, Gras N, Guzmán C, Thieck M, Vanska L (1992) Development of an automatic system for Neutron Activation Analysis. J Radioanal Chem 167:97-101.

Ostapczuck P, Froning M (1990) Heavy metal determination in canned mussels from different countries. Fresenius J Anal Chem 337: 104.

Stoeppler M (1992) Hazardous metals in the environmental, techniques and instrumentation. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.