Bull Environ Contam Toxicol (2007) 79:121–125
DOI 10.1007/s00128-007-9084-z

# Monitoring Heavy Metal Pollution in San Antonio Bay, Río Negro, Argentina

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Received: 20 June 2006 / Accepted: 15 March 2007 / Published online: 24 May 2007


Heavy metals naturally exist in low concentrations in water, as well as in sediments and in biota. Concerns abound when these elements are derived from anthropogenic sources because they become toxic when they reach threshold levels in chemical forms, and because they persist in the environment for a long time. Marine sediments form a reservoir for heavy metals because they are able to complex to other compounds due to their reactive properties. The accumulation of these pollutants in organisms is an indicator of their bioavailability in the environment, mainly for non-essential metals. Although the bioaccumulation factors between biota and sediments do not imply an accumulation-specific mechanism, they are indicative of the degree of exposure of the organisms that inhabit polluted sediments (Foster and Wright, 1988).

By 1994 some environmental impacts of a mine called Gonzalito began to be studied in the marine environment of San Antonio Bay (Fig. 1). The first report to detect the presence of a point source of pollution and the distribution of metals levels within the bay were carried out between 1994 and 1995 (Gil et al. 1997, 1999). The Gonzalito mine operated 107 km from San Antonio Oeste from its opening in the 1960s until its closure in the 1980s, and was involved in extraction of zinc, lead, silver and vanadium. In 1961 the company built an electrochemical plant within San Antonio Oeste city itself, for the production of lead and silver. The heavy-metal ores excavated from the mines were smelted in the plant. The waste produced in this process was piled in the open air in different locations around the city and on the edges of San Antonio Bay. We analyzed contaminant levels in sediments and in small mussels (*Brachydontes rodriguezi* d'Orb - Bivalvia, Mollusca) that cover soft and sandy sediments. The aim of the present study was to evaluate the heavy metal contamination levels in San Antonio Bay nine years after these concentrations were measured for the first time. In order to provide information to make recommendations for ecological or human health applications it is important to update those studies.

## Materials and Methods

San Antonio Bay, an ecologically productive and diverse system, is located in the northwest point of San Matías Gulf (40° 50' S - 65° 10' W), Río Negro, Argentina (Fig. 1). It has been included in the Western Hemisphere Shorebird Reserve Network because of the presence of more than 17 shorebirds species. In addition, it is important for regional economy since several exploited bivalves inhabit this reserve, such as blue mussel (*Mytilus edulis platensis*), oyster (*Ostrea puelchana*) and purple clam (*Amiantis purpurata*) (Yorio et al., 1998). Here, three profiles can be differentiated: surfaces covered with vegetation resistant to salinity, sandy beaches, and tidal surfaces covered with small mussels called ''mejillinares''. These mitillidae have been reported to be good biomarkers organisms because of their capacity to accumulate metals from the environment (food, water and detritus), their abundance in the study area, and their sessile life form (Escofet et al., 1977). They are also an important prey for Red Knots (*Calidris canutus rufa*)

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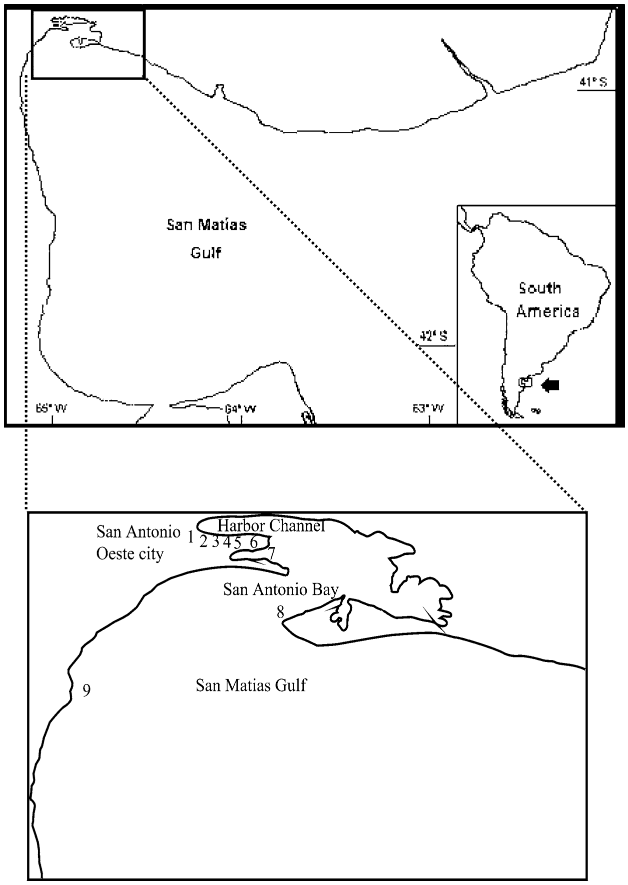


**Fig. 1** San Antonio Bay Study Area Station 1 (Pile: open air deposit), stations 2 to 6 (Harbor Channel: 2-Near Pile; 3-Alpesca; 4– West San Antonio Harbor; 5-Beach; 6-Punta Verde), stations 7 and 8 (sites external to the Harbor Channel: 7-Punta Delgado; 8- East San Antonio Harbor), station 9 (site out of the bay)

(Gonzalez et al., 1996), snail (*Trophon geversianus*), and sea anemones.

In November 2003 samples of intertidal sediments were collected with plastic spatulas. Previous work (Gil et al., 1997, 1999) has shown that local currents would transport heavy metals from the mine site unevenly throughout the bay. As a result, we divided the study area into three sampling regions: the open-air deposit (station 1, thePile), the zone under direct influence of the mine run-off (stations 2 to 6, the harbor channel'') and the zone external to the harbor channel (stations 7 and 8). A station out of the bay was also sampled (station 9), where a mollusk hatchery is located (Fig. 1).

The sediments were stored in a freezer at –20°C until they could be analyzed. In the laboratory samples were dried at 60°C until a constant weight was reached, and sifted. The granulometric fraction lower than 62 μm was removed and analytically treated in order to determinate its organic matter content (lost by calcination, 450°C for 4 hours) and heavy metal concentration (cold-extraction with 0.5 N HCl (Merck, p.a.) (Agemian and Chau, 1976).

Zinc (Zn), copper (Cu) and lead (Pb) analyses were carried out using an Atomic Absorption Spectrophotometer (AAS) air-acetylene flame, while cadmium (Cd) levels were measured using a graphite furnace. All extractions were carried out in duplicate and blanks were processed as the samples. Results are reported on a dry weight. Detection limits were 0.4 $\mu g.g^{-1}$ for Zn, 0.6 $\mu g.g^{-1}$ for Cu, 1.8 $\mu g.g^{-1}$ for Pb, and 0.2 $\mu g.g^{-1}$ for Cd. The following variation coefficients were obtained from seven replicates (the respective averaged concentrations in brackets): Zn 2.9% (9,980), Cu 3.0% (6,452), Pb 10.9% (4,128), Cd 6.3% (35.7). Because no certified sediment exists for weakly-bound metal concentrations, recoveries for this analytical procedure was performed by the standard addition method, obtaining: 96.6% for Zn, 104.7% for Cu, 102.9% for Pb and 98% for Cd.

A sample of 500 small mussels, *Brachydontes rodriguezi* (shell length 23.5 ± 3.6 mm) was taken from the intertidal area at stations 4, 5, 6, 8 and 9 (Fig. 1) during the same sampled sediments season. Individual condition index (the wet flesh weight to wet shell weight ratio) and bioaccumulation factor (the tissue metal concentration to sediment metal concentration ratio) were calculated. Zn, Cu, Pb and Cd concentrations were analyzed by digesting 2–10 g wet tissue with concentrate $HNO_3$ (Merk, p.a.), evaporating the sample at 140°C and muffle calcinations until white ashes. All extractions were carried out in triplicate and blanks were processed as the samples. Results are reported on a dry weight. Detection limits were 1.10 $\mu g.g^{-1}$ for Zn, 0.39 $\mu g.g^{-1}$ for Cu, 0.28 $\mu g.g^{-1}$ for Pb and 0.08 $\mu g.g^{-1}$ for Cd. Overall analytical precision, expressed as the variation coefficients for the three replicates, were less 10% for all metals. Recoveries after analysis of reference material (National Institutes of Standards and Technology -standard reference material 1566a - Oyster tissue) (table 1) were 1.1% for Zn, 0.39% for Cu%, 0.28% for Pb and 0.08% for Cd.

Statistical analyses were conducted using parametric tests when the data indicated a normal distribution. Non-parametric analyses were conducted on condition index

**Table 1** Analysis of reference material (NIST - standard reference material 1566a -Oyster tissue)

| Element | Certified values ($\mu g.g^{-1}$) | Present study ($n$ = 3) ($\mu g.g^{-1}$) |
|---|---|---|
| Zn | 830.00 ± 57.00 | 818.00 ± 10.00 |
| Cu | 66.00 ± 4.00 | 61.00 ± 1.90 |
| Pb | 0.30 ± 0.01 | < 2.00 |
| Cd | 4.00 ± 0.30 | 4.00 ± 0.10 |

ratio data. Comparisons between stations were performed according to metal concentrations in sediments and mussels using analysis of variance (ANOVA), followed by Turkey's HSD test (Sokal and Rohlf, 1979), when data normality and equal variance were proved. Mussels condition index between stations were compared by the Kruskal-Wallis (KW) test followed by post hoc $t$ comparisons.

## Results and Discussion

All sediment samples were mainly sandy, with low fine content (between 0.1% at station 9 and 10% at station 1). Organic matter varied between 2 and 13%, respectively.

**Table 2** Concentration of heavy metals (dry weight) in sediments along the San Antonio Bay coast. (nd: no detectable)

| Station | Zn (μg.g$^{-1}$) | Cu (μg.g$^{-1}$) | Pb (μg.g$^{-1}$) | Cd (μg.g$^{-1}$) |
|---|---|---|---|---|
| 1 | 9,980 ± 298 | 6,452 ± 194 | 4,198 ± 450 | 37.5 ± 22.0 |
| 2 | 44.6 ± 4.0 | 5.0 ± 0.3 | 25.0 ± 1.5 | nd |
| 3 | 32.8 ± 5.0 | 3.0 ± 0.0 | 28.0 ± 6.0 | nd |
| 4 | 44.0 ± 16.0 | 7.9 ± 0.1 | 37.8 ± 1.6 | nd |
| 5 | 41.2 ± 0.2 | 3.0 ± 0.0 | 28.0 ± 0.2 | nd |
| 6 | 37.0 ± 23.0 | 3.0 ± 0.3 | 16.2 ± 1.7 | nd |
| 7 | 4.4 ± 0.2 | 0.7 ± 0.0 | 6.2 ± 1.7 | nd |
| 8 | <1 | 3.0 ± 0.0 | 8.0 ± 1.6 | nd |
| 9 | <1 | 3.0 ± 0.0 | 13.0 ± 0.2 | nd |

Sediment metal concentrations are shown in Table 2. The highest Zn, Cu, Pb and Cd levels were recorded at the Pile itself (station 1), with significantly higher levels than in all the remaining sites (Turkey's test; $p < 0.05$). The highest of Zn, Cu and Pb in the marine sediments were found within the Harbor Channel (stations 2 to 6) which, according to the standards proposed by Albaigés (1989), may be considered as moderately polluted with Zn and Cu and polluted with Pb. The lowest levels of Zn, Cu and Pb of all the studied samples were measured in the site off the bay (station 9), while Cd was not detected in any of the sites.

No significant correlation was observed between metal and organic matter contents ($p > 0.05$), but correlation coefficients among Zn, Cu, and Pb concentrations were found positive in all cases: Zn-Cu: 0.61, Zn-Pb: 0.85, Cu-Pb: 0.78 ($p < 0.05$). This suggests that these three metals likely shared a common origin, which in this case would be associated with old mine residuals (Gil et al., 1999). Thus, Bonuccelli et al. (2004) reported that these elements leach from the Pile to the Harbor Channel through an acid drainage; these acids, resulting in fluid transport by the rain, can dissolve heavy metals. Bioaccumulation of metals can occur when they are released from the sediments via exposure to dissolved and particulate phases present in the water column, as well as through trophic transfer (Burton, 1992).

Metal concentrations in mussel tissue revealed the same pattern as that observed in sediments: the highs near the mine site and the lows in the site outside the bay.

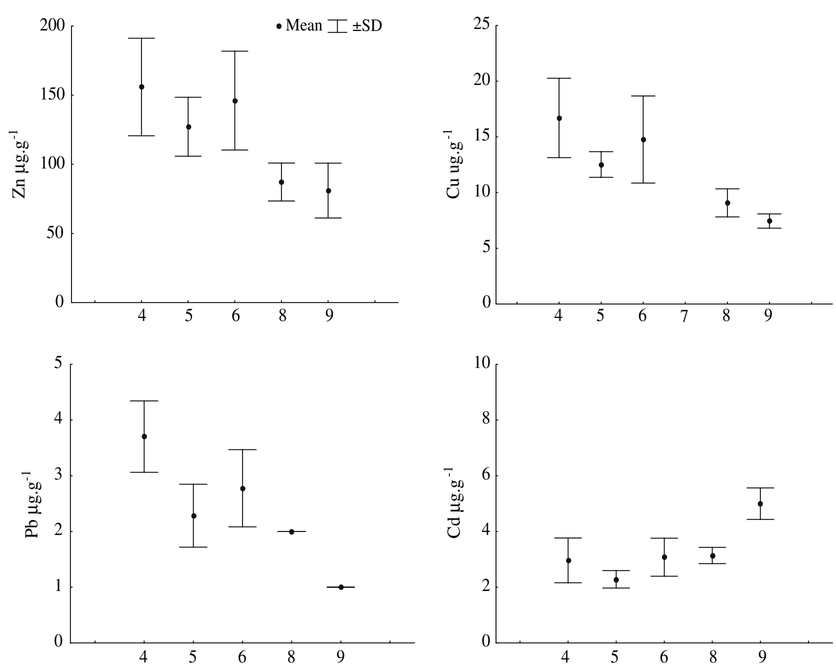


**Fig. 2** Concentration of Zn, Cu, Pb and Cd (dry weight) in *Brachydontes rodriguezi* tissue along San Antonio Bay

**Table 3** Bioaccumulation factors (tissue metal concentration to sediment metal concentration ratio)

| Station | Zn | Cu | Pb | Cd |
|---|---|---|---|---|
| 4 | 3.52 | 2.11 | 0.10 | 14.81 |
| 5 | 3.08 | 4.17 | 0.08 | 11.42 |
| 6 | 3.95 | 4.92 | 0.17 | 12.31 |
| 8 | 87.22 | 3.03 | 0.25 | 15.69 |
| 9 | 81.00 | 2.48 | 0.16 | 25.00 |

**Table 4** Post hoc comparisons between stations for condition index. Kruskal-Wallis $p < 0.05$

| Stations | ε i,j | CV |
|---|---|---|
| 4 - 8 | 121.0094937 | 27.95032886 * |
| 4 - 5 | 109.9428571 | 28.89136671 * |
| 4 - 9 | 94.14010989 | 26.95105105 * |
| 8 - 9 | 26.86938378 | 27.87844775 |
| 8 - 5 | 11.06663653 | 29.75835625 |
| 5 - 9 | 15.80274725 | 28.82183263 |

* significant difference

Concentrations of Zn and Cu were higher in mussels collected in the harbor channel than those collected outwards (Fig. 2). Notably, Pb concentrations were significantly higher in organisms from station 4 than in mussels from outside sites (Turkey's test, $p < 0.05$). In this station (the most inner site at Harbor Channel where organisms were found), the values measured were near or slightly higher than those considered to represent high concentrations according to the international mussel watch project (1995) (Zn: 210 $\mu g.g^{-1}$, Cu: 13 $\mu g.g^{-1}$, Pb 4 $\mu g.g^{-1}$).

Bioaccumulation factors of metals are shown in table 3. Values for Pb were one to two orders of magnitude lower than those for the other elements, which would probably indicate a low bioavailability of Pb retained in the sediments. These observations are in agreement with the results reported by Boisson et al. (1998), who suggested that mussels might be considered as good bioindicators of Pb contamination accumulated from the dissolved rather than from the particulate source.

Mussels with high concentrations of Zn, Cu and Pb showed worse body condition indices than those with low concentrations of these metals ($r = 0.84$; $r = 0.90$; $r = 0.95$, respectively). Values were significantly lower (Table 4) in organisms from station 4 than those from other stations (Fig. 3). These results suggest that pollution by heavy metals is one of the factors influencing the health status of *Brachydontes rodriguezi*, although other causes could be involved.

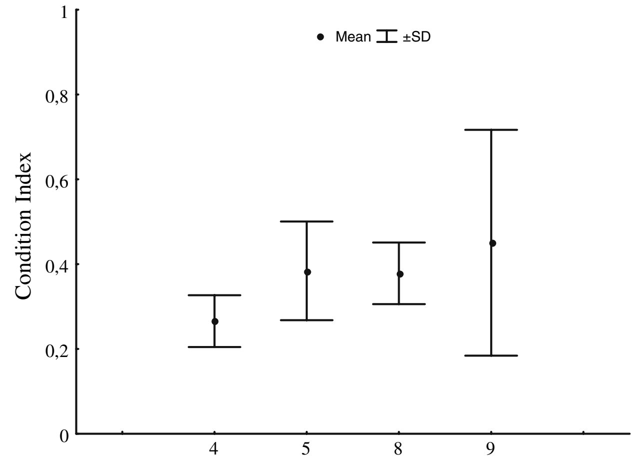


**Fig. 3** Condition index (wet flesh weight to shell weight ratio) in *Brachydontes rodriguez* at four stations from San Antonio Bay

Cadmium was recorded in all tissue samples within the range of 2.28 – 5.00 $\mu g.g^{-1}$, although was not detected in sediments. This pattern has been reported in other localities of Patagonia (Gil et al., 1997) and in the rest of the world (Berrow, 1991). It can be explained because of soluble forms prevalence in seawater (Balls, 1985) being incorporated by phytoplankton and then by mollusks. Interestingly the observed distribution of this metal differed from the others, showing significantly higher value (5.00 $\mu g.g^{-1}$) at the external site (station 9) compared to the others (Turkey's test; $p < 0.05$). It approaches that of a high background level according to the international mussel watch project (1995) and, despite it not implying direct damage to organisms or humans, it deserves attention considering the presence of the mollusks hatchery. Cd levels measured in this study are consistent with concentrations recorded in other bivalve species of other localities of the Patagonian coast (1.12–6.74 $\mu g.g^{-1}$) (Gil et al., 1997) and are low enough to suggest a natural source. Goldberg et al. (1984) for example, attributed high Cd concentrations in mussels of the west coast of the United States to the supply resulting from water upwelling enrichment. Events of upwelling have been reported in several opportunities along the Patagonian coast, including San Matías Gulf (Acha et al., 2004; Glorioso, 2000; Guerrero and Piola, 1997; Piola and Rivas, 1997) and therefore they would be investigated.

Future research should be directed at the fate of these metals at higher trophic levels. Because *Brachydontes rodriguezi* is almost the only prey taken by, a threatened species (Gonzalez et al., 1996), contaminant levels in *Calidris canutus rufa* should be measured to assess the fate of these contaminants through trophic transfer. In terms of human health, our analyses should be expanded to include those bivalves that are harvested for human consumption. Although future studies will be pursued, enough is known already to require immediate action. Overall, our study demonstrates that waste piles from the abandoned mine are still leaching various metals to the environment more than two decades after the mine's closure, and consequently that waste piles have not been treated appropriately. Actions should be taken to reduce the levels of leaching as soon as possible, while researchers continue to map the environmental legacy of this long and intense source of contamination.

**Acknowledgements** This study was supported by project ARG/02/G31 carried out by Fundación Patagonia Natural and by the Instituto de Biología Marina y Pesquera Almirante Storni. We are also indebted to Rob Williams for his help translating the manuscript in to English.

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