

# Biodiversity of rocky intertidal benthic communities associated with copper mine tailing discharges in northern Chile

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## Abstract

Copper mine tailings have been discharged around the city of Chañaral, in northern Chile, for more than 60 years. This report summarizes a 17-month long monitoring study of species richness and biodiversity at five intertidal sites around the point of the tailing discharge. Total dissolved copper in sites close to the point of discharge varied between 8.72 µg/l and 34.15 µg/l, showing that there has not been a significant reduction since 1994. However, species richness has increased, suggesting a possible recovery of the system. While diversity of sessile organisms correlates negatively with dissolved copper, diversity of mobile invertebrates did not correlate with the metal concentration. To explain the observed results we discuss the role of algal turf interference on the distribution of mobile invertebrates at reference sites, a top-down effect caused by the absence of carnivores at impacted sites, and an avoidance strategy by some species to reduce their contact with contaminated seawater.

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## 1. Introduction

Different sources of anthropogenic inputs of copper to coastal zones have been reported (Lewis, 1995; Torres, 2003). Copper mining activities remain as one of the most significant worldwide sources of this metal into the environment due to the large volumes of released wastes, which usually affect relatively small areas of a given ecosystem, and because, in a number of cases, these wastes have a direct impact on coastal areas, far away from the mining operations. Copper mining wastes have caused severe and negative effects on the coasts of England (Bryan and Langston, 1992), Canada (Mardsen and DeWreede, 2000; Grout and Levings, 2001; Mardsen et al., 2003), Australia (Stauber et al., 2001) and Chile (Castilla, 1996; Correa et al., 1999;

Lancellotti and Stotz, 2004). On the western slopes of the Andes Mountains in South America, copper mining has historically been the most important economic activity. As a consequence, several areas of the northern Chilean coast have been affected by copper mine tailing discharges (Castilla and Nealler, 1978; Vásquez et al., 1999; Fariña and Castilla, 2001). One of these areas is Chañaral Bay, which, in 1938, began to receive wastes from the El Salvador copper mine (Castilla, 1983). Until 1975, more than  $150 \times 10^6$  metric tons of untreated tailings were continuously discharged, directly affecting more than 20 km of coastline (Castilla, 1983; Paskoff and Petiot, 1990). From 1976 to 1989 the discharge point was moved 10 km north of Chañaral Bay to Caleta Palito (26°16'S, 70°41'W), a rocky cove that received  $\approx 130 \times 10^6$  metric tons of tailings in 13 years. After the construction of an inland sedimentation dam in 1990, sediment-free wastewaters have been channeled from the dam to Caleta Palito at a flow rate of 200–250 l/s.

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Since 1978, studies on beach progradation and variations of the intertidal benthic communities around Chañaral and Caleta Palito have resulted in more than 14 reports assessing the potential effects of the mine tailings (Castilla and Nealler, 1978; Castilla, 1983; Paskoff and Petiot, 1990; Vermeer and Castilla, 1991; Correa et al., 1995; Castilla, 1996; Correa et al., 1996; Castilla and Correa, 1997; Riquelme et al., 1997; Correa et al., 1999; Correa et al., 2000; Lee et al., 2002; Ratkevicius et al., 2003; Lee and Correa, 2004). From these studies it is clear that the most important effects of the tailing discharges on the coastal zone around Chañaral are: the persistently high copper concentrations in coastal waters, beach progradation, and the virtual elimination of algae, several benthic herbivores and all benthic carnivores from the impacted area (Correa et al., 2000). While these studies have significantly improved our understanding of the effects of mine tailings on the biota, they nevertheless represent static pictures of the area, disconnected in time, where specific monitoring of temporal variation in biodiversity is absent. Furthermore, these studies have applied different methodological approaches that do not allow reliable comparisons. In addition, although copper has been reported as the main single pollutant in the Chañaral area (Correa et al., 2000), its current concentration in the surrounding coastal water is not known. This situation now becomes important, as local authorities claim to have achieved a reduction in mine tailing contamination, which could be leading to a possible recovery of the biodiversity in the coastal zone of Chañaral.

This study assesses the existence of (a) temporal variations in intertidal species richness and biodiversity in the rocky intertidal zone around the mine tailing discharges and (b) a possible ongoing process of biodiversity recovery in this coastal zone. The study includes comparisons between historical and new data and relates this information to the concentration of total dissolved copper in the surrounding coastal water.

## 2. Materials and methods

### 2.1. Study sites

Four sites covering 100 km of coastline around Chañaral were monitored at low tide (0.23–0.30 m) every two months, from February 2002 to June 2003 (Fig. 1). Among these, Guanillo (G) and Zenteno (Z) were considered reference sites due to their history of a lack of metal pollution. Palito (P) is located 200 m south from the current discharge point (Caleta Palito), and Achurra (A) is close to the old discharge site, at the northern extreme of Chañaral Bay. A fifth site, La Lancha (L), located 5 km north of Palito, was included

during the second year of monitoring. Local currents move northward in this part of the coast and, as a result, tailing sediments have accumulated in this site over time.

### 2.2. Seawater analysis

Since copper has been reported as the main single pollutant in the tailing discharge (Correa et al., 2000), we measured its total dissolved concentration in the water column around the studied sites. Two 500 ml seawater samples were collected from the shore with acid-washed polypropylene bottles, and kept at 4 °C during transportation to the laboratory. Seawater was first filtered through 0.45 µm cellulose acetate Millipore membrane filters, fixed with 0.5 ml concentrated nitric acid (pH < 2) and stored at 4 °C for analysis. Total dissolved copper concentration (TDC) as measured by anodic stripping voltammetry (ASV) using a Metrohm 757 VA processor following the methodology proposed by Metrohm (2000). Analytical grade reagents were used to prepare all relevant blanks and calibration curves. Analytical accuracy was tested against high purity reference material provided by the National Research Council of Canada (CRM-SW). In addition we recorded in situ variations in the surface temperature of coastal seawater during the study period using a submersible data logger (Optic Stow Away Temp®, ONSET Computer Corp., USA), which was deployed and fastened to shallow subtidal rocks at three of the study sites (G, P, Z). Dissolved oxygen and pH were also measured in situ at all of the sites using a WTW Multiline P4, Universal meter.

### 2.3. Biodiversity monitoring procedures

In order to determine intertidal biodiversity, two different approaches were applied. First, species richness was determined by one observer who walked slowly along the intertidal platform for 5 min recording all algal and invertebrate species encountered. This methodology was applied because it has been used during previous studies of these sites (Castilla and Correa, 1997; Correa et al., 1999). The second approach included the use of transects and quadrats. At each site three transects, at least 4 m apart, were defined perpendicular to the coast, from high to low along the intertidal gradient. The sampling unit, a 25 × 25 cm quadrat with a grid of 100 equally spaced intersection points, was positioned along the transects as many times as it fit. Algae and sessile invertebrates occurring beneath each intersection point, and all of the mobile invertebrates inside the quadrat, were identified to the lowest possible taxonomic level. Complete monitoring was not performed at Guanillo and La Lancha in June 2003 due to bad weather conditions.

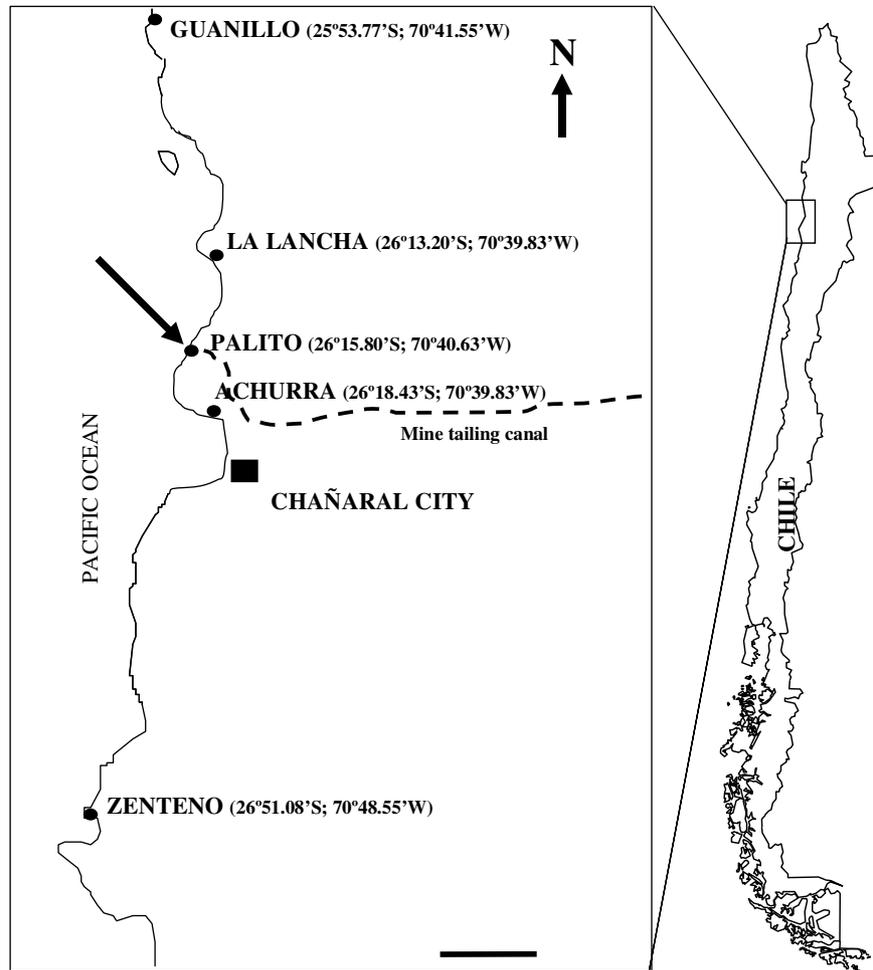


Fig. 1. Chañaral area and study sites. The discharge point of the copper mine tailing is indicated (arrow).

#### 2.4. Data analysis

Significant differences in TDC between sites were determined considering all seawater samples taken during the study period in a one-way (between subjects) ANOVA. A post hoc Tukey's multiple comparison test was applied to determine which sites were significantly different from the others (Zar, 1999). Prior to this statistical analysis, we checked assumptions of a normal distribution of the data and homogeneity of variance by application of the Kolmogorov–Smirnov test and Levene's test, respectively. Standard procedures of data transformation (Zar, 1999) were included when required.

The Jaccard's coefficient of similarity (Jaccard, 1901; Sneath, 1957) was applied to species richness data in order to assess similarity in species composition between sites (Multi-variate Statistical Package 3.01, Kovach Computing Services, UK). Similarity between sites was first calculated considering the presence of species for the entire period of study. Subsequently, similarity was determined using only the species observed at each site

during summer and winter, separately for each year (i.e. February 2002, July 2002, January 2003, June 2003). Patterns of similarity in species composition were contrasted using cluster diagrams of the Jaccard's coefficient calculated for each combination of sites during each date. Clusters were constructed using unweighted average linkage methods.

The density of mobile invertebrates was quantified by counting all individuals inside the entire quadrat, whereas the cover of sessile organisms (algae and sessile invertebrates) was obtained as percentage cover by summing all of the intersection points on the quadrat grid where they were present (Castilla, 1988). Density and percentage cover were determined in relation to the total area and grid of intersection points, respectively, generated by the sum of all quadrats along each transect.

Utilizing this data of density and percentage cover, we calculated the diversity of sessile organisms and mobile invertebrates for each site and date using the Shannon–Wiener diversity index. In order to assess the level of occupation of primary space in each site, the percentage of bare rock was also obtained from percentage cover

data and plotted against time. Differences in primary space occupation and biodiversity within each site over time were determined by one-way repeated measures (within subjects) ANOVA. Sites were then compared on four different monitoring dates (February 2002, July 2002, January 2003 and June 2003) by a one-way (between subjects) ANOVA. The post hoc Tukey's multiple comparison test was applied to determine which sites were significantly different from the others (Zar, 1999). Prior to the first statistical analysis, we checked data for the assumption of homogeneity of covariance by the application of the Mauchly's test of sphericity (Kinneer and Gray, 2000). When this assumption was not fulfilled, the more conservative Greenhouse-Geisser test was applied (Kinneer and Gray, 2000). Assumptions of a normal distribution and homogeneity of variance were also checked prior to the second analysis, as mentioned above. In order to test the degree to which TDC and intertidal biodiversity were related, we calculated the Pearson's correlation ( $r$ ) (Zar, 1999). For this, we utilized the TDC data obtained during the entire study period, and the corresponding diversity data (all transects) for sessile organisms and mobile invertebrates.

Finally, since algae were the main users of primary space, the relationship between their spatial dominance and temporal incidence was analyzed in order to identify the most representative species. Mean percentage cover considering all transects and dates were plotted against the frequency of presence of each species during the entire study period.

### 3. Results

#### 3.1. Seawater parameters and total dissolved copper

Dissolved oxygen ranged between 8.52 and 9.05 mg/l and pH varied between 7.98 and 8.13 between all sites, but remained almost constant within each site during the entire study period. Temperature, on the other hand, showed the expected seasonal variation at the three continuously monitored sites (G, P, Z), with values as low as 12°C in winter and up to 18°C in summer.

Our results of TDC showed that concentration of this metal continues to be significantly higher ( $p < 0.001$ ) at those sites near to the current tailing discharge (P and L) than in the non-impacted sites (G and Z) (ANOVA,  $F_{4,37} = 23.49$ ). The post hoc Tukey's multiple comparison test however, demonstrated no significant differences between any of these sites and Achurra (A), where TDC values fall between those measured at the other sites. Thus, the sequence of mean TDC concentrations measured in this study was La Lancha > Palito > Achurra > Zenteno > Guanillo (Table 1). It is also clear that values of TDC experienced the widest fluctuations in those sites nearer to the discharge point

Table 1  
Mean, minimum and maximum values of total dissolved copper concentration (TDC) considering the entire period of study

Site	Mean (µg/l)	Minimum (µg/l)	Maximum (µg/l)	Number of samples
Guanillo	3.13	1.62	4.55	8
La Lancha <sup>a</sup>	20.86	9.85	34.15	8
Palito	17.04	8.72	25.64	10
Achurra	7.18	6.33	9.83	6
Zenteno	5.58	4.01	7.52	6

Sites are listed from north to south.

<sup>a</sup> Values correspond to analysis of samples obtained between January and June 2003.

(Table 1). Since the Levene's tests applied to the data showed some violation of homogeneity of variance, ANOVA was performed following log-transformation of the data.

#### 3.2. Species richness and similarity

Considering all sites and the entire study period, we identified a total of 84 entities (Table 2). From these, 63 were identified to the species level, 12 to the genus level and 8 were classified at higher taxonomic levels. A non-calcareous crustose alga did not fit in any taxonomic group and, thus, remained as "green crust". All identified entities were treated as different taxa or putative species, which allowed biodiversity analysis and comparisons between sites.

From the 84 entities identified, 37 were algae, 15 were sessile invertebrates and 32 were mobile invertebrates (Table 2). It should be noted that 19 algae and 11 invertebrate species were present exclusively at the reference sites (G and Z), and only 3 algae and 2 invertebrate species were never recorded at these sites. The phaeophycean Order *Scytosiphon* deserves special attention, as it later became clear that our initial conclusion that only *S. lomentaria* was present in the area had to be re-considered. For the purposes of this study, and based on conclusive but still unpublished information we assigned two different entities, with the status of species, to the genus *Scytosiphon* (Table 2). The persistently higher levels of copper recorded at the impacted sites were associated with a clear decrease in the total number of species (Fig. 2A). Considering the entire study period, the number of species recorded in Guanillo and Zenteno was 67 and 75, respectively, contrasting with the 47 and 42 recorded in Achurra and Palito, respectively. Overall, less than half of the species recorded at the control sites were found at their impacted counterparts at the end of the study period. It is worth mentioning that the magnitude of the difference was not clear at the beginning of the study, particularly for Achurra (Fig. 2A). Species richness at the fifth site, La Lancha, was initially lower than at the two other impacted sites, with a total of 32

Table 2

List of species (or entities classified at the lowest possible taxonomic level) observed at the studied sites considering the entire period of study

Species	G	L	P	A	Z
<b>Algae</b>					
<b>Chlorophyta</b>					
<i>Ulva compressa</i>	X	X	X	X	X
<i>Ulva linza</i>					X
<i>Rama novaezelandiae</i>					X
<i>Codium dimorphum</i>	X				X
<i>Codium fragile</i>					X
<i>Ulva</i> sp.	X	X	X	X	X
<i>Chaetomorpha</i> sp.					X
<i>Cladophoropsis</i> sp.	X				X
<b>Rhodophyta</b>					
<i>Porphyra columbina</i>	X		X		X
<i>Polysiphonia paniculata</i>	X		X	X	X
<i>Corallina officinalis</i>	X			X	X
<i>Gelidium chilense</i>	X				X
<i>Hildenbrandia lecanellieri</i>	X	X	X	X	X
<i>Centroceras clavulatum</i>					X
<i>Chondrus canaliculatus</i>	X				X
<i>Bangia atropurpurea</i>		X	X		X
<i>Ahnfeltiopsis durvillaei</i>	X				X
<i>Ahnfeltiopsis furcellata</i>	X			X	X
<i>Grateloupia doryphora</i>	X				X
<i>Corallina</i> sp.	X				X
<i>Lithothamnium</i> sp.	X	X	X	X	X
<i>Mesophyllum</i> sp.	X				X
<i>Gelidium</i> sp.	X				X
<i>Rhodoglossum</i> sp.	X				X
<i>Ceramium</i> sp.		X			
<b>Phaeophyta</b>					
<i>Ralfsia confusa</i>	X				X
<i>Glossophora kunthii</i>	X		X	X	X
<i>Scytosiphon</i> sp1		X	X	X	
<i>Scytosiphon</i> sp2		X	X	X	
<i>Hinckesia mitchelliae</i>	X		X	X	
<i>Halopteris hordacea</i>	X		X	X	X
<i>Lessonia nigrescens</i>	X				X
<i>Petalonia fascia</i>	X				X
<i>Colpomenia sinuosa</i>	X			X	X
<i>Ectocarpus</i> sp.	X	X	X	X	X
<b>Others</b>					
Cyanophyceae	X				X
“Green crust”	X	X	X	X	X
<b>Invertebrates</b>					
<b>Sessiles</b>					
<i>Jehlius cirratus</i>	X	X	X	X	X
<i>Notochthamalus scabrosus</i>	X		X	X	X
<i>Balanus laevis</i>	X	X		X	X
<i>Balanus flosculus</i>	X			X	
<i>Austromegabalanus psittacus</i>		X	X	X	
<i>Semimytilus algosus</i>	X	X	X	X	X
<i>Perumytilus purpuratus</i>		X	X	X	X
<i>Phymactis clematis</i>	X	X	X	X	X
<i>Anthothoe chilensis</i>	X		X		X
<i>Phymanthea pluvia</i>	X	X	X	X	X
<i>Bunodactes</i> sp.	X	X	X	X	X
Porifera	X		X	X	X
Ectoprocta	X			X	X
Spirorbidae	X			X	X
Phoronidae	X			X	

(continued on next page)

Table 2 (continued)

Species	G	L	P	A	Z
<b>Mobiles</b>					
<i>Nodilittorina peruviana</i>	X	X	X	X	X
<i>Nodilittorina araucana</i>	X	X	X	X	X
<i>Scurria scurra</i>	X				X
<i>Scurria zebrina</i>	X	X	X	X	X
<i>Scurria ceciliana</i>	X	X	X	X	X
<i>Scurria araucana</i>	X	X	X	X	
<i>Scurria bohemita</i>			X	X	X
<i>Scurria parasitica</i>	X	X	X	X	X
<i>Scurria viridula</i>	X	X	X	X	X
<i>Siphonaria lessoni</i>	X	X	X	X	X
<i>Trimusculus peruvianus</i>	X				X
<i>Fissurella crassa</i>	X	X	X	X	X
<i>Fissurella limbata</i>	X	X	X	X	X
<i>Fissurella maxima</i>		X		X	X
<i>Concholepas concholepas</i>	X	X	X	X	X
<i>Nucella crassilabrum</i>			X		
<i>Leptograpsus variegatus</i>	X	X	X	X	X
<i>Taliepus dentatus</i>	X				X
<i>Loxechinus albus</i>	X				X
<i>Tetrapygyus niger</i>	X				X
<i>Enoplochiton niger</i>	X				X
<i>Acanthopleura echinata</i>	X			X	X
<i>Heliaster helianthus</i>	X				X
<i>Meyenaster gelatinosus</i>					X
<i>Tegula atra</i>	X				X
<i>Stichaster striatus</i>	X				X
<i>Chiton granosus</i>			X		X
<i>Acanthocyclus gayi</i>	X			X	X
<i>Betaeus</i> sp.	X		X	X	X
Amphipoda	X	X	X	X	X
Acarina			X	X	X
Polychaeta	X				X

species recorded during the three sampling dates (Fig. 2A). When algal species richness was analyzed separately, similar but more pronounced differences between reference and impacted sites became apparent (Fig. 2B). Although species richness of invertebrates was also generally higher at non-impacted sites (Fig. 2C), differences with those sites nearer to the tailing discharge were small, reaching similar values at different times during the study period.

Considering the entire period of study, we were able to distinguish two main groups with less than 45% similarity from the analysis of species composition (Fig. 3A). The first group (cluster 1 in Fig. 3A) was comprised of the two non-impacted sites and the second group (cluster 2 in Fig. 3A) included the three impacted sites. Within the latter group, La Lancha was slightly different from Palito and Achurra (cluster 3 in Fig. 3A). When species composition was analyzed separately for summer and winter, the level of similarity between groups was reduced (Fig. 3B–E). However, similarity between sites remained relatively constant through time ( $\approx 25\%$ ), and thus, the two distinct groups (i.e. impacted and non-impacted sites) remained clearly identifiable.

### 3.3. Bare rock and diversity of algae and sessile invertebrates

Mean percentage of bare rock varied during the study period (Fig. 4). However, repeated measures ANOVA performed independently on data from each site indicated no significant differences ( $p > 0.05$ ) between monitoring dates for any site. Comparisons of percentage of bare rock between sites were significantly different ( $p < 0.05$ ) for all monitoring dates analyzed (Table 3). A post hoc Tukey's multiple comparison test, however, demonstrated no significant differences between reference sites (G and Z), but significant differences between these two reference sites and Palito for all monitoring dates, with the exception of February 2002, when Zenteno did not differ significantly from Palito (Fig. 4). In February 2002, Achurra also showed no significant differences with the control sites; however, it did differ from control sites on all monitoring dates performed after February 2002. During the last two analyzed dates, the percentage of bare rock at Achurra showed no significant differences with Palito. The fifth site, La Lancha, showed no significant differences with any other site during the dates on which it was analyzed.

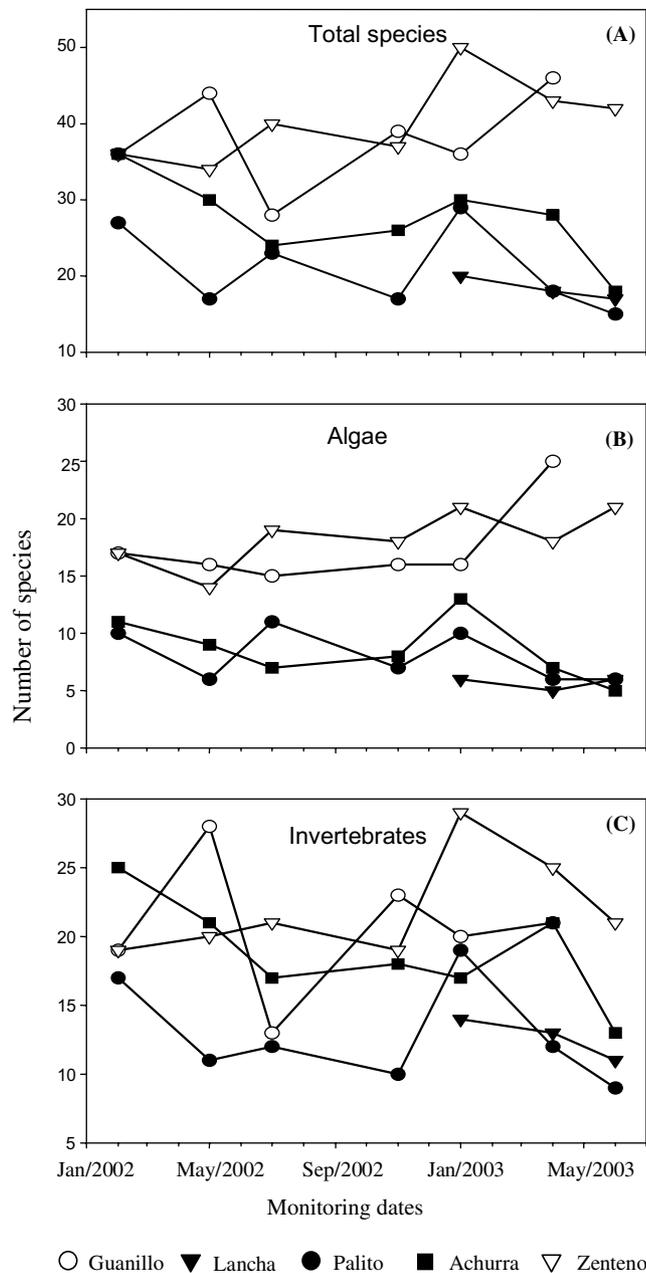


Fig. 2. Temporal variation in species richness. (A) Total number of species; (B) Species of algae; (C) Species of invertebrates.

Although the mean diversity of sessile organisms, including algae and sessile invertebrates, ranged from 0.79 to 0.97 at reference sites and from 0.47 to 0.75 at Palito (Fig. 5), variations in time were not significant at any site ( $p > 0.05$ ). Comparisons between sites showed significant differences only in February 2002 and July 2002 (Table 3). In February 2002, the post hoc Tukey's multiple comparison test indicated significant differences between Palito and all other sites (Fig. 5). In July 2002, significant differences were observed between Palito and reference sites, but not between

Palito and Achurra. In January 2003, due to a drop in diversity at Zenteno coupled with an increase in diversity at Palito, no differences were observed. After this monitoring date, some evidence of differentiation was observed, but statistical analyses did not detect significant differences (Table 3). The high and significant ( $p < 0.001$ ) negative correlation between TDC and diversity of sessile organisms is clearly shown in Fig. 6.

Spatial dominance and incidence of algal species showed important differences between sites (Fig. 7). Guanillo and Zenteno had more than 11 algal species recorded at least 8 times during the study period, and the red alga *Hildenbrandia lecanellieri* displayed the highest incidence at both sites. In Guanillo, *Ulva* sp. and *Lithothamnium* sp. were reported the same number of times. In terms of dominance, *Ahnfeltiopsis furcellata* and *Ulva* sp. had the highest mean percentage cover in Guanillo and Zenteno, respectively. The red alga *Centroceras clavulatum* was also important at the two reference sites, although it had a low incidence (recorded less than 6 times). As indicated above, the percentage of bare rock was high in Palito and, as a consequence, the incidence and dominance of algal species was low (Fig. 7). Only four species appeared more than eight times during the study which, ranked from high to low incidence, were: *Scytosiphon* sp2, *Ulva compressa*, *Porphyra columbina* and *Ulva* sp. When present, *U. compressa* also had the highest algal dominance. Algal incidence in Achurra was similar to that in non-impacted sites; however, dominance as mean percentage cover was lower. In addition, in terms of incidence, *U. compressa* was also important at this site.

### 3.4. Diversity of mobile invertebrates

Although variation of mobile invertebrate diversity was not significant ( $p > 0.05$ ) within monitoring dates at any of the sites, a different situation was observed when comparisons were performed between sites (Fig. 8). Mean diversity index obtained from Guanillo was lower than that obtained from Achurra and Palito during the entire study period. Achurra had the highest mean index throughout the study period, with the exception of April 2003, when the indices obtained from Zenteno and La Lancha were higher. When a one-way ANOVA was used to test differences between sites, significant differences were found only in July 2002 (Table 3), when the diversity of mobile invertebrates in Achurra was similar to that of Palito and Zenteno, but not to that estimated for Guanillo (post hoc Tukey's multiple comparison test). In contrast to the diversity of sessile organisms, no correlation between TDC in coastal seawater and diversity of mobile invertebrates was observed (Fig. 9).

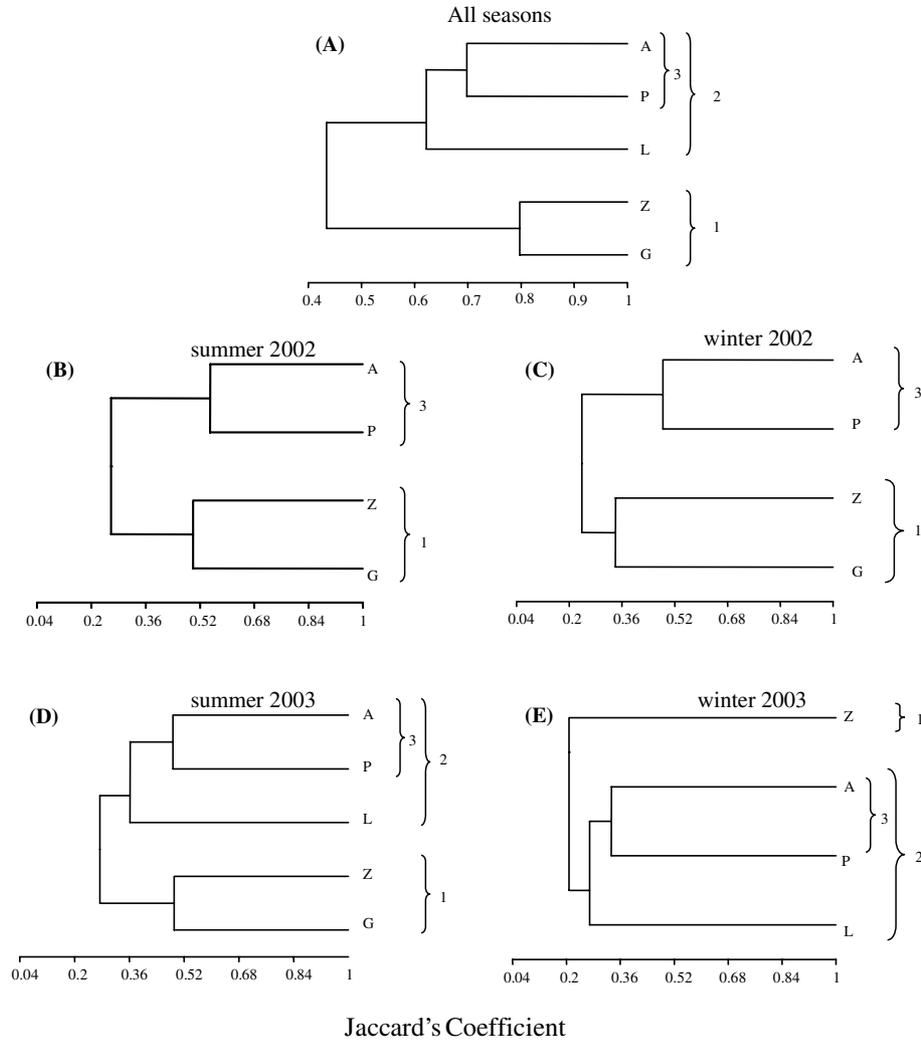


Fig. 3. Cluster analysis of the similarity in site species composition. (A) Data considering the entire study period; (B) Data from February 2002; (C) Data from July 2002; (D) Data from January 2003; (E) Data from June 2003. G: Guanillo, L: La Lancha, P: Palito, A: Achurra and Z: Zenteno.

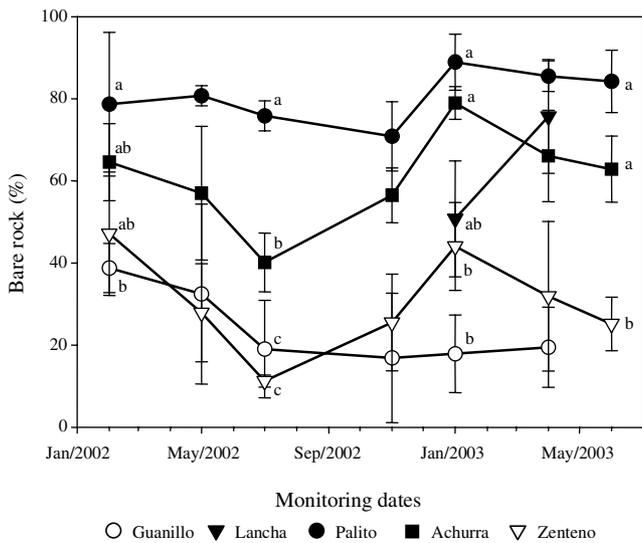


Fig. 4. Percentage of bare rock. Values represent the mean  $\pm$  SD from three replicates. Letters indicate significant differences between sites.

#### 4. Discussion

The study presented here summarizes data obtained after 17 months of regular monitoring at five rocky intertidal sites around the point of discharge of copper mine tailings in northern Chile. Results show high and variable TDC concentrations in the coastal seawater from Palito and La Lancha, the closest sites to the current discharge of the waste waters from the copper mine El Salvador. Our results agree with previous information reported by Correa et al. (1996) and Castilla and Correa (1997) who attributed this variability to the potential contribution of other small-scale operations scattered downstream from the mine which could illegally discharge additional copper-containing wastes into the canal before it reached the sea. This certainly is not the situation today, as all small-scale operations have been dismantled. However, regardless of the eventual occurrence of copper-containing wastes added to the

Table 3

Summary of one-way (between subjects) ANOVA performed on: (I) percentage of bare rock, (II) diversity of sessile organisms (Shannon index,  $H'$ ), and (III) diversity of mobile invertebrates (Shannon index,  $H'$ ) between sites on different monitoring dates

Site	Source	df	MS	F	P
<i>February 2002</i>					
I	Site	3	789.88	5.49	0.03
	Error	7			
II	Site	3	$8.63 \times 10^{-2}$	10.97	0.005
	Error	7			
III	Site	3	$8.58 \times 10^{-2}$	2.38	0.16
	Error	7			
<i>July 2002</i>					
I	Site	3	1940.86	33.82	0.001
	Error	7			
II	Site	3	$8.41 \times 10^{-2}$	6.27	0.02
	Error	7			
III	Site	3	$8.99 \times 10^{-2}$	6.32	0.02
	Error	7			
<i>January 2003</i>					
I	Site	4	2377.49	13.87	0.001
	Error	9			
II	Site	4	$2.92 \times 10^{-2}$	3.02	0.09
	Error	8			
III	Site	4	$9.57 \times 10^{-2}$	3.99	0.05
	Error	8			
<i>June 2003</i>					
I	Site	2	2279.17	41.91	0.001
	Error	5			
II	Site	2	$7.17 \times 10^{-2}$	5.40	0.06
	Error	5			
III	Site	2	$1.89 \times 10^{-3}$	0.04	0.96
	Error	5			

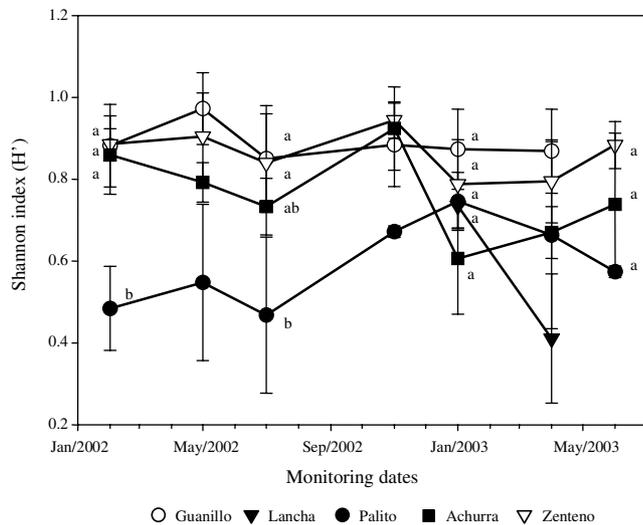


Fig. 5. Diversity of sessile organisms (algae and sessile invertebrates). Values represent the mean  $\pm$  SD from three replicates. Letters indicate significant differences between sites.

canal before reaching the sea, it is clear that the levels of total dissolved copper in the waste water before the

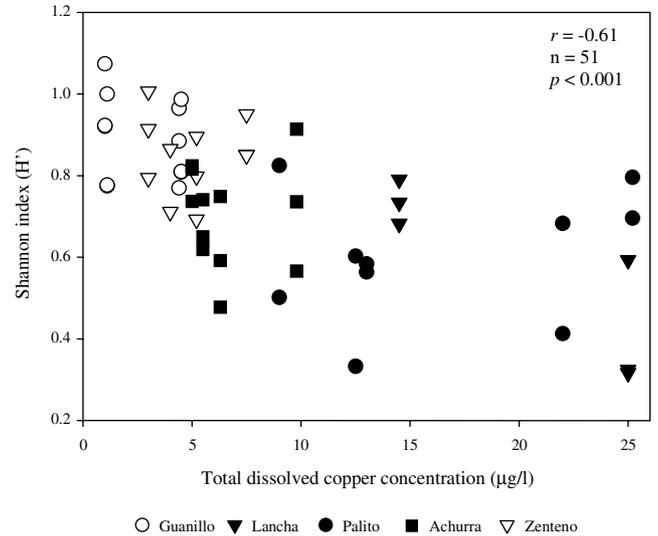


Fig. 6. Correlation between total dissolved copper (TDC) in coastal seawater and diversity of sessile organisms (algae and sessile invertebrates). All monitoring dates and diversity data obtained were included in the analysis, with the exception of the highest value of TDC measured in seawater during the study period ( $34.15 \mu\text{g/l}$ , see Table 1), since diversity was not determined when this value was obtained.

discharge point have decreased significantly in recent years, from  $2416.2 \mu\text{g/l}$  in 1995 (Correa et al., 1996) to  $31.4\text{--}71.7 \mu\text{g/l}$  in 2003 (this study, data not shown). Thus, even though the canal remains as a persistent source of copper to the coastal environment, its impact seems to be reduced in comparison to other sources, all of which are related to copper mining activities. Today, re-suspension of old coastal sediment deposits is likely the most important source of copper to the water column. This is supported by two recent studies of intertidal and shallow subtidal sediments present on beaches in the area under the influence of the mining waste products. The first study (Lee et al., 2002) demonstrated that, while labile copper in the water was between  $10$  and  $50 \mu\text{g/l}$  depending on the site, the effective copper concentration in the sediment was always greater than  $100 \mu\text{g/l}$  and, in some cases, greater than  $1000 \mu\text{g/l}$ . The second study (Ramírez et al., in press) confirmed that copper is the main metal in the sediments of the beaches affected by the mining wastes, and demonstrated that an important fraction is found in a labile form, easily released from the sediment to the water column—and available to the biota. This information regarding the availability of copper in the environment, together with factors that could modulate the levels of copper in the water (e.g. strong wave action and high currents that affect the study area, particularly during winter storms, and atmospheric dry deposition of fine particles from the copper mine tailing beaches), may be the cause of the temporal variability observed in TDC concentrations.

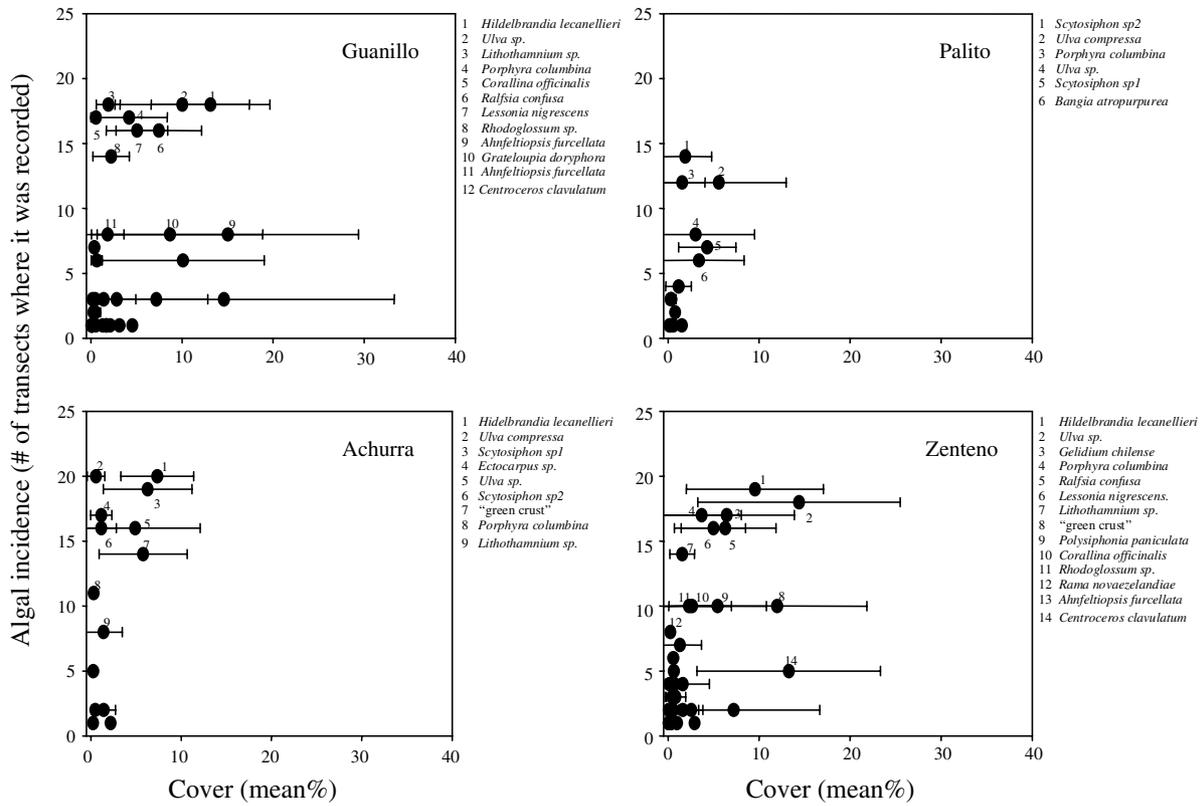


Fig. 7. Representation of the relative importance of algal species. Numbers have been assigned to species that were recorded more than eight times and to species with an important contribution to cover.

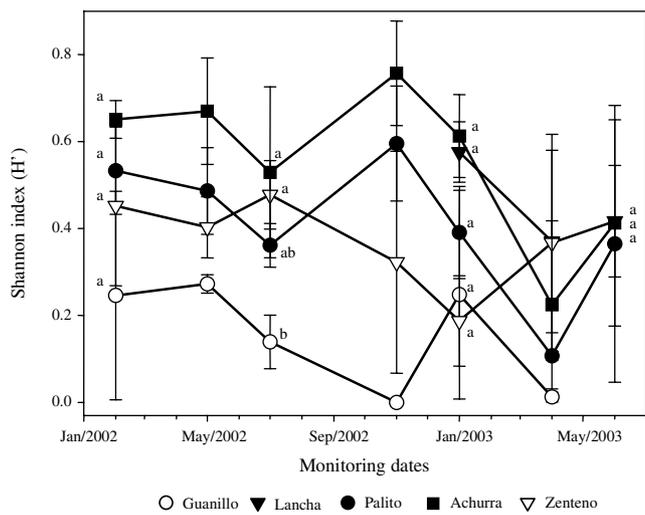


Fig. 8. Diversity of mobile invertebrates. Values represent the mean  $\pm$  SD from three replicates. Letters indicate significant differences between sites.

When historical data are considered (Table 4), it can be seen that some of the TDC values obtained from Palito during this study were the lowest since this site was first studied in 1994. The mean TDC concentration, however, remains within the historical range and within the range reported for other sites affected by copper

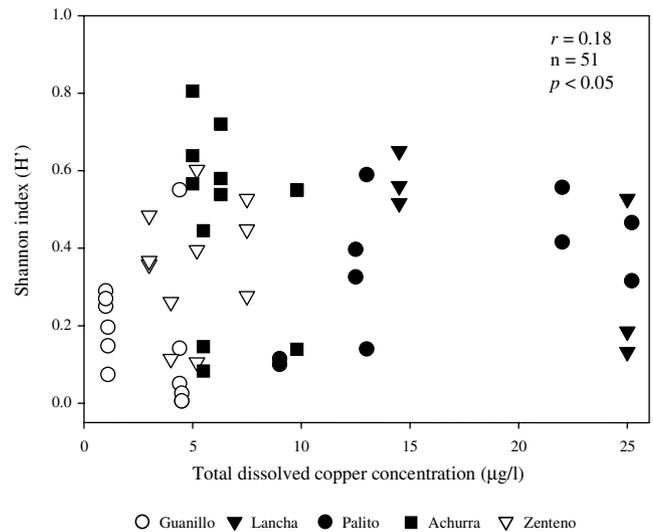


Fig. 9. Correlation between total dissolved copper (TDC) in coastal seawater and diversity of mobile invertebrates. All monitoring dates and diversity data obtained were included in the analysis, with the exception of the highest value of TDC measured in seawater during the study period (34.15 µg/l, see Table 1), since diversity was not determined when this value was obtained.

mine tailing discharges in northern Chile (Fariña and Castilla, 2001). It has to be emphasized that the values reported here for Palito are measurements made in

Table 4  
Historical data of total dissolved copper (TDC) concentrations at Palito

Year	Mean (µg/l)	Min. (µg/l)	Max. (µg/l)	Reference
1994	–	26.8	31.8	Castilla (1996) <sup>a</sup>
1994	29.3	25.7	32.9	Correa et al. (1995) <sup>a</sup>
1995	14.13	–	–	Correa et al. (1996)
1995	–	10.0	40.7	Castilla and Correa (1997) <sup>a</sup>
1996	–	10.2	13.9	Castilla and Correa (1997) <sup>a</sup>
1999	30.0	29.3	30.7	Correa et al. (1999)
2000	14.1	–	–	Correa et al. (2000) <sup>a</sup>
2002	–	10	20	Ratkevicius et al. (2003)
2003	17.04	8.72	25.64	This study

<sup>a</sup> Fifty meters south of the discharge point at Caleta Palito.

water samples from 200 m south of the current dumping site. Directly at the point of discharge point, however, values as high as 54 µg/l and 88 µg/l were reported in 1986 and 1995, respectively (CIMM, 1996). Although values of TDC measured at La Lancha were lower than those previously reported by Correa et al. (1999), they were higher than those from Palito. According to this, La Lancha continues to be the coastal site around Chañaral with the highest level of TDC, and one of the most copper-polluted coastal sites in the world (see review by Lewis, 1995). As indicated in previous reports (CIMM, 1996; Correa et al., 1999), the high TDC values at this site could reflect the influence of northward coastal currents on tailing dispersal and the aforementioned re-suspension of old tailing sediments by wave action. From the information provided by our study we conclude that there has not been a significant reduction in the level of copper in the coastal marine environment affected by the mine tailing discharges in Palito, in comparison with the levels reported in 1994.

Despite this first conclusion, and also considering historical data, the number of intertidal species in Palito and La Lancha has increased considerably during this period (Table 5). The increase was observed in algal as well as invertebrate species, leading to a total species richness 2.5 and 3.6 times higher than the values observed in June 1996 in Palito and La Lancha, respectively (Castilla and Correa, 1997). Although the present study coincides with the reports included in Table 5, regarding the pattern of species richness decline towards most impacted sites (Palito and La Lancha) and an increase in the number of species with time, conclusions about a possible biological recovery of the area must be made with caution. This caveat is given since comparisons with previous studies at the reference sites also show a current increase of species richness. This observation suggests that the increase in species richness around Chañaral could be the result of a more general, large-scale phenomenon that is not necessarily related to a reduction in the toxicity of the coastal water around the mining discharges. Moreover, possible variability

Table 5  
Historical data on species richness in Palito and La Lancha

Year	Algae	Invertebrates	Total	Reference
<i>Palito</i>				
1976–1994 <sup>a</sup>	5	1	6	Castilla (1996) <sup>b</sup>
1996	6	11	17	Castilla and Correa (1997) <sup>b</sup>
1995–1999	6	15	21	Correa et al. (1999)
2003	14	28	42	This study
<i>La Lancha</i>				
1976–1994 <sup>a</sup>	5	1	6	Castilla (1996) <sup>b</sup>
1996	4	5	9	Castilla and Correa (1997) <sup>b</sup>
1995–1999	5	6	11	Correa et al. (1999)
2003	10	22	32	This study

<sup>a</sup> Presence assessed by at least five consecutive or non-consecutive observations.

<sup>b</sup> Fifty meters south of the discharge point at Caleta Palito.

due to different monitoring efforts and observer experience in data collection must be considered when interpreting results from different studies. Historical differences in species richness could also be attributed to the time of the year when monitoring was carried out. The present study showed that similarity between sites varied in time and, when data from a particular month was considered, it was lower than when data of the entire study period was included in the analysis. Therefore, sound estimations of species richness recovery based on comparisons with reference sites will likely depend on the time of the year when the study is performed and the length of time which the study considers.

In order to reduce the potential variability resulting from the application of different visual efforts and different observer experience during sampling, the present study applied the additional, objective methodology of predefined transects and quadrats to assess biodiversity. By utilizing this methodology species richness determined by visual monitoring varied during the year, but the biodiversity of sessile organisms and primary space occupation (i.e. the opposite of the percentage of bare rock) did not change significantly at any site. Since the biodiversity index applied here considers both species richness and evenness (Krebs, 1972), any temporal increase in the number of species observed at these sites is counterbalanced by the dominance of a few species. In contrast, any reduction in the number of species is counterbalanced by an enhanced species evenness.

In relation to the differences between sites, the lower mean diversity of sessile organisms observed at impacted sites using transects and quadrants is consistent with the information obtained from the visual monitoring. In this context, the fact that significant differences were found only during the first year of monitoring supports the possibility of an ongoing process of biodiversity recovery at these sites. This is also in agreement with the lack of significant differences in diversity of mobile

invertebrates between reference and impacted sites during most of the study period. However, lower mean diversity values at reference sites are not consistent with the general trends obtained here and elsewhere (Castilla, 1996; Castilla and Correa, 1997; Correa et al., 1999). As indicated above, both species richness and species evenness are considered in the biodiversity index applied in our study. Thus, if we accept that the number of species determined by transects and by visual monitoring is truly representative of the situation in each site, species evenness then becomes responsible for these unexpected results. This is the result of dominance by only a few of the numerous species recorded at the reference sites, combined with a high species evenness at the impacted sites, despite the presence of fewer species. This pattern may be real, or could result from biased data recording that is intrinsically associated with the applied methodology. High algal cover at the reference sites, where a thick algal turf is formed, may limit the primary space available for mobile invertebrates, interfering with their homogenous distribution on intertidal platforms. In this case, although most mobile invertebrates could be recorded during both the visual monitoring and counted inside the quadrats, only those individuals able to move within or above the algal turf were registered. In contrast, at the Palito study site algal cover is low and, thus, the fewer species present are homogeneously distributed in space, and easily recorded.

Two alternative hypotheses could help to understand the higher diversity of mobile invertebrates at the impacted sites. First, high diversity levels of mobile invertebrates in Palito could reflect a toxicity avoidance strategy. Animals that recruit to this site may tend to move toward the upper levels of the intertidal zone in order to reduce their contact with copper-enriched seawater. This would result in easier recording of mobile invertebrates by both visual and quadrat monitoring in Palito, as compared to the reference sites, where animals remain at lower intertidal and shallow sub-tidal levels. This avoidance strategy could reduce the possible detrimental effects of TDC on mobile invertebrates and, thus, community parameters such as species abundance and richness should be independent of the level of copper enrichment in the surrounding coastal seawater. This hypothesis was supported by the correlation analysis performed using the data obtained in this study, where no relationship was found between these two variables. On the other hand, sessile organisms like algae and sessile invertebrates are not able to avoid the possible effects of copper in the water and, thus, a negative relationship such as the one revealed by the correlation analysis, should exist between the diversity of sessile organisms and TDC in seawater. Second, the absence of some keystone species at the impacted sites may be considered as a clear-cut difference with the control sites, and although standard statistical comparisons may not

detect these differences, their effects can nevertheless be profound. For instance, the absence of high-level carnivores in Palito and La Lancha, such as the starfishes *Heliaster helianthus* and *Meyenaster gelatinosus*, could reduce predation pressure at these sites and, thus, foster the development of larger populations of a more herbivore species. Thus, although quantitative estimators suggest that diversity seems higher at these sites, a qualitative analysis of the intertidal assemblage of organisms reveals a simplified community structure. In undisturbed intertidal sites, on the other hand, it is expected that these carnivore species directly regulate the abundance of herbivores (i.e. Dayton et al., 1977; Tokeshi et al., 1989; Espoz and Castilla, 2000) and indirectly regulates algal abundance through a top-down effect (Hunter and Price, 1992; Correa et al., 2000). In terms of species richness, the absence of the kelp *Lessonia nigrescens* may also have a strong effect. Indeed, *L. nigrescens* is considered a bioengineer (sensu Jones et al., 1994) due to the rich diversity of organisms living within its holdfast (Vásquez and Santelices, 1984) and to the effect of the frond canopy on the removal of mobile invertebrates, allowing the recruitment of understory algal species (Santelices and Ojeda, 1984). For example, Vásquez and Santelices (1984) found up to 49 species of macro-invertebrates living in a total of 79 sampled holdfasts, with 7–15 species per holdfast. Thus, although species diversity associated to *L. nigrescens* holdfasts was not estimated in the control sites, the absence of this kelp at impacted sites is likely to be accompanied by the absence of a significant number of invertebrate and algal species.

Finally, characterization of the diversity of mobile invertebrates by means of species richness and evenness (Shannon–Wiener) has not been reported previously in this area nor other areas impacted by copper mine tailing discharges in northern Chile. Therefore, this study represents the starting point for future assessments of biodiversity at these impacted areas. However, it is important to highlight the need for testing the ecological and ecotoxicological hypotheses mentioned above prior to reaching final conclusions regarding past and current effects of the copper mine wastes on the biota, as well as on potentially ongoing recovery processes.

## 5. Conclusions

This study indicates that there has not been a significant reduction of the total dissolved copper concentration (TDC) at the coastal zone around Chañaral since 1994, in spite of the drastic reduction of the concentrations of this metal in the water discharges. High TDC values were observed in Palito and especially in La Lancha, which remains as the most impacted site. However, levels of TDC at the contaminated sites are highly

variable in time, probably due the re-dissolution of the metal from old tailing sediments deposited in the area which are re-suspended by coastal currents and wave action and due to atmospheric dry deposition from the tailing beaches. Despite the lack of a recent reduction in TDC, our study showed an increased species richness which, in turn, suggests the occurrence of a recovery process at the impacted area. However, this result must be considered with reservation since species richness varies with time at both the impacted and reference sites and, thus, the results of comparisons between these sites, and with historical data, are influenced by the time of the year when the studies were performed. On the other hand, biodiversity indices did not vary with time, suggesting a counterbalance between these two community parameters (i.e. richness and evenness) at all sites. Our results also showed a lower mean diversity of mobile invertebrates in the reference sites, which could represent a true pattern or may be a biased result associated with the practical limitations of the applied methodology. If this methodology is to be used in the future for biodiversity assessments at these or other impacted sites, this situation needs to be clarified through additional testing of complementary ecological and ecotoxicological hypotheses.

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