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Spatial and Temporal Comparison of Copper Bioaccumulation in the Mussel *Aulacomya ater* (Molina) from Jorgillo Location (23°45'S; 79°27'W) and Dichato Location (36°32'S; 71°56'W), Chile

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Since Chile is recognized as an "oceanic country", it appears quite pertinent to understand and know the biological and physicochemical processes of the coastal environment, whether natural or anthropic origin, with emphasis in areas of defined productive processes, such as those in the northern Chile, where one of the principal industries is the copper mining.

Copper is an essential element at minimum concentration, playing a role in the catalyzis of many enzimatic systems, required for the healthy growing of the organisms (Cheung *et al.* 1999), but in higher concentrations is toxic, and persist in the environment (Sobral & Widdows 1997; Cohen *et al.* 2000), being transformed and becoming toxic (same than other heavy metals), not only through mortality but also through physiological alterations, such as respiration rate (Nicholson 1999), and damage at a membrane level (Riveros *et al.* 2002).

The response of animals to metals present in the aquatic environment and/or in their food, greatly depend on the capacity of the species to regulate the levels of bioaccumulation (regulation mechanisms) in their tissues. The marine invertebrates (*e.g.* bivalves), can bioaccumulate metals within certain limits, and may be utilized in monitoring programs for quantification of trace metal levels in water, sediments, and marine organisms (Rainbow 1995; Franco *et al.* 2002; Paulson *et al.* 2003), for the following: i) establish the spatial-temporal trends of the element concentrations; ii) establish the origin of sources pollution through concentrations gradient, and iii) compare the concentrations in situ against current standards for regulation purposes.

Given the importance of characterize the bioaccumulator capacity and efficiency from marine species of the chilean coasts (*eg*., bivalves), in front of different physicochemical conditions of the marine environment, in this study are compared the copper concentrations in the soft tissues (gill, muscle, gonad, and digestive gland) of the bivalve mytilid *Aulacomya ater*, obtained in different periods of the year from two coastal locations and its relation with the copper concentrations in water and phytoplankton.

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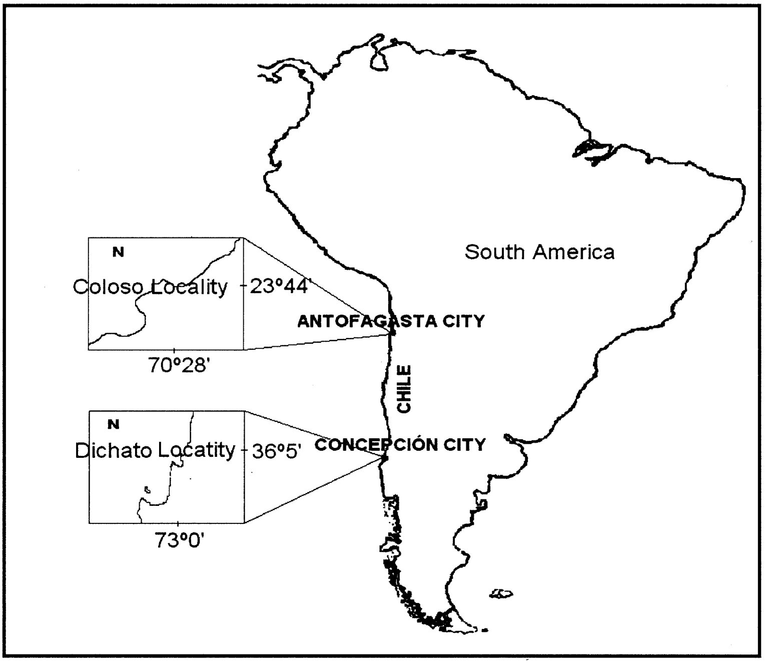

Figure 1. Locations of the two sampling sites in the chilean coasts

MATERIALS AND METHODS

Every other two months from November 1998 through November 1999, were collected individuals of *Aulacomya ater* (N=30, tamaño 5-6 cm) from two locations of the chilean coast: Jorgillo site in the northern Chile and Dichato site in South-Central Chile (Figura 1). In the laboratory each individual was dissected obtaining four well differentiated tissues, namely: gill, muscle, gonad, and digestive gland, in order to determine the copper concentrations separately in each one of them.

In parallel were collected samples of seawater and phytoplankton. The water was collected through a previously treated Van Dorn bottle, at depths near the sectors from where the analyzed individuals had been collected. As for obtaining the phytoplankton samples, a WP-2 type net was made with a mesh aperture of < 50 µm and not longer than 80 cm far from the net bucket, which facilitate a better handling for obtaining the samples. The net was fixed to an outboard motor boat utilizing a cord 5 m long and dragged along the area of study until to obtain the amount of the sample required for the analyzes.

The copper concentrations in seawater ($\mu g*L^{-1}$), phytoplankton ($\mu g*g^{-1}$), and tissues ($\mu g*g^{-1}$ wet weight), were measured through atomic absorption spectrophotometry according to UNEP/FAO/IOC/ IAEA (1984). The analyzes were realized with the support of an atom trap system, to enhance the residence time of gaseous atoms and with standards addition (Bader, 1980). The addition curve of the standard calibration was carried out analyzing DORM-1.

The copper concentrations of the three matrices (seawater, phytoplankton, and tissues), were normalized utilizing natural Logarithm (Ln), and then a variance analysis (ANOVA) was realized in order to evaluate the existence of differences between locations, and between months. Besides, a Pearson correlation was done between the copper concentration in sea water v/s phytoplankton, seawater v/s tissues, and phytoplankton v/s tissues.

RESULTS AND DISCUSSION

No significant differences were detected in the copper concentrations in seawater, between locations ($p>0.01$), excepting with the time (months) for each one of the sites ($p<0.01$) (Table 1).

Table 1. ANOVA of two ways of the transformed results (Ln) of the copper concentrations in seawater and phytoplankton, and tissues of the two studied locations, namely, Dichato site, and Jorgillo site between November 1998 and November 1999.

		DF	MS Effect	F	p-level
Between Locations	Seawater	1	0.19	1.55	0.210
	Phytoplankton	1	118.69	185.49	0.001
	Tissue	1	22.62	87.39	0.000
Inside of Location	Dichato Seawater	6	0.29	10.69	0.001
	Dichato Phytoplankton	6	17.32	335.84	0.001
	Dichato Tissue	3	0.501	16.336	0.000
	Dichato Month	6	3.072	100.205	0.000
	Jorgillo Seawater	6	0.41	19.88	0.001
	Jorgillo Phytoplankton	6	5.41	231.46	0.001
	Jorgillo Tissue	3	1.947	14.549	0.000
	Jorgillo Month	6	0.964	7.205	0.000

In the Dichato site the four analyzed tissues (gill, muscle, gonad, and digestive gland) presented significant differences between the months and between the tissues ($p<0.01$).(Table 1), being the months November 1998 and November 1999, which in general showed the higher copper concentrations.

The relation between the months presenting the maximum and minimum concentrations go from 3.8 times in the digestive gland to 6 times in the gonad. In all the cases the higher ratios were found between the spring month (November) and summer months (January-March), which form a group significantly different than the others ($p<0.01$) (Fig. 2).

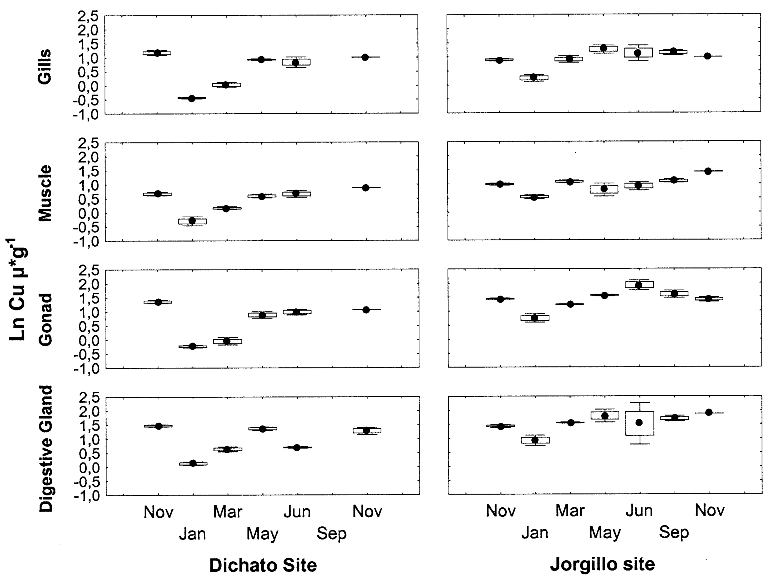

Figure 2. Total copper concentration (wet weight) in soft tissues of *Aulacomya ater* for the two sites studied from November 1998 through November 1999.

The Jorgillo and Dichato sites presented important differences in the copper concentrations between different months and analyzed tissues ($p<0,01$) (Table 1). The major concentrations in the tissues were recorded between July and November for the gonad and the digestive gland, and in November for muscle, with values between 3.0 and 4.3 times higher than the determined minimum concentrations, which corresponded to January (summer), being this month significantly different than the others ($p<0.01$). The exception was the gill, since it did not render evidence of significant differences ($p>0.01$), except of January which presented the lowest value of copper concentrations of all the samplings (Table 1).

Differences were also detected in the copper concentrations in the tissues of *Aulaconya ater*, between sites ($p<0.01$) (Table 1), being in general the concentration values in organisms of the Jorgillo site, higher than those determined in the Dichato (Fig. 2). However, a high correlation exist in the copper concentration for each one of the tissues studied, between both sites ($p<0.01$) (gill: 0.71; muscle: 0.66, and gonad: 0.53), except of copper in the digestive gland with a "r" of 0.24 ($p>0.01$).

Whereas in the Jorgillo site the concentration fluctuated between 24.5±0.28 μg^*g^{-1} (November), and 136.2±10.18 μg^*g^{-1} (March); in the same manner were no correlations between the copper concentrations in the tissues, and the copper concentrations in the water and phytoplankton ($p>0.01$) (Table 2), even when the studied species are phytoplanktivorous (Cancino & Becerra 1978).

Regarding the copper concentrations in phytoplankton, significant differences were found between locations ($p<0.01$), and between months ($p<0.01$), existing a high correlation for this parameter between locations ($r=0.65$ $p=0.0065$) (Table 2); thus, in Dichato site the concentration of total copper in phytoplankton fluctuated between 3.58±2.17 $\mu g*g^{-1}$ (January) and 55.4±7.07 $\mu g*g^{-1}$ (March).

Table 2. Correlation Analysis between copper concentrations in seawater and phytoplankton, and copper concentration in tissues for the organisms.

			N	r	p
Dichato site	Gills	Seawater	19	0.269	0.333
		Phytoplankton	21	-0.193	0.492
	Muscle	Seawater	19	**0.580**	**0.023**
		Phytoplankton	21	-0.295	0.287
	Gonad	Seawater	19	0.085	0.764
		Phytoplankton	21	-0.205	0.464
	Digestive Gland	Seawater	19	0.130	0.643
		Phytoplankton	21	0.003	0.991
Jorgillo site	Gills	Seawater	21	0.082	0.717
		Phytoplankton	21	-0.226	0.312
	Muscle	Seawater	21	0.279	0.209
		Phytoplankton	21	-0.190	0.398
	Gonad	Seawater	21	**-0.473**	**0.026**
		Phytoplankton	21	-0.202	0.368
	Digestive Gland	Seawater	21	-0.350	0.110
		Phytoplankton	21	-0.159	0.479

The seasonal variations of the copper concentrations found in the tissues of the species under study both in the Jorgillo and Dichato locations, have been largely documented for other species of the northern hemisphere (Phillips 1976a; Cossa *et al.* 1980; Cain & Luoma 1990), reporting seasonal variations in function of the growth rate, reproductive cycles, salinity and temperature (Phillips 1976b; Swaileh 1996; Serafim & Bebianno 2001). In this respect, *A. ater* presents reproductive periods well defined with marked spawning during summertime (Cancino & Becerra 1978).

Parallel to this study were evaluated the reproductive development through histological slices in females of the two locations, establishing that the reproductive process occur synchronously in both locations with a month displacement, probably due to the different temperature values between both locations (*i.e.* Jorgillo 13.8-24.2°C Arcos 1998; Dichato 10-19°C Ahumada *et al.* 1979).

The temperature is a factor that affects the metabolism of the poikilothermics, being observed a decrease in the gonadal productivity related with a decrease in the metabolism (Brey 1995), having implications in the low concentrations of the bioaccumulated copper, for the organisms of both locations in the summer months, and not only in the gonad, but also in the four analyzed tissues, which reveal a pattern of seasonality in the copper bioaccumulation of the mytilid (Swaileh 1996; Serafim & Berianno 2001) .

On the contrary of the above, Phillips (1980) described that the lost of corporal mass during the spawning months would cause an increase of the copper concentration in certain tissues. However, the decrease of copper in tissues of *A. ater* during the summer period have been also described for the oyster *Crassostrea virginica* (Lauenstein *et al.* 1990), and *Mytilus edulis*, for which is indicated a relation between the weight decrease and the copper concentration (Boalch *et al.* 1981). This process occurs during the spring and summer periods, coinciding with the species spawning (Kreeger *et al.* 1995). Besides, the variation of metal concentrations in organisms in relation with the seasons of the year is largely documented.

As if taken into account some characteristics described for mytilids utilized as bioindicators in the seas of the northern hemisphere, such as: i) large spatial contribution (Amiard *et al.* 1986; Nolan & Dahlgaard 1991); ii) acclimation and resistence under significantly different environmental conditions (Rainbow *et al.* 1999; Paulson *et al.* 2003), and iii) efficiency in pollutant bioaccumulation (*e.g.*, metals), through ingestion together with their food (Radenac 1997), it could be selected the *Aulacomya ater* as the mytilid bioindicator for the southern hemisphere, since it complies with the above characteristics (*e.g.* easy manipulation and feasibility) to be utilized for both juveniles and adults.

The results obtained show the following conclusions:

1) The copper concentrations in the tissues of *Aulacomya ater* in the two locations present seasonal variations, being the concentrations higher in the Jorgillo site than those found in Dichato site.

2) The comparison of the two locations showed a high correlation between the copper concentrations in the distinct tissues, which would be confirming a similarity of the organisms behaviour concerning the metal bioaccumulation. This condition is generally indicated for the mytilids as one of the most important characteristics at the time of being selected as bioindicators in monitoring programs (Phillips 1976a; Radenac *et al.* 1997).

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