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Presence of Cd-binding proteins in freshwater Crayfish Procambarus clarkii and Brine Shrimp Artemia

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Metallothioneins, a metal binding proteins, have a high affinity for various toxic metals, particulary cadmium and mercury. Metal binding proteins have been observed in mammals [1] and a variety of marine invertebrates [2], however, there is very little information available on metal binding proteins in freshwater invertebrates, and particulary in frehwater crustaceans [3]. The presence of such proteins has been variosly suggested as indicating involvement in uptake, storage, transport and elimination of toxic metals [4] and in the routine metabolism of metals [2]. Cadmium binding proteins observed in invertebrates have similar characteristics to mammalian metallothionein: low molecular weight, stable to acid and heat treatment, inducible by metal exposure, low ultraviolet absorption at 280 nm and high absorption at 254 nm, a characteristic absorbance dissapeared on acidification and reappeared with neutralization [1]. This report describes results on the characterization of Cd-BPs obtained from very euryhaline brine shrimp *Artemia* exposed to cadmium. For metodological purposes these results have been compared with those of the freshwater crayfish *Procambarus clarkii* . Induction of Cd-BP was achieved by water exposure at a concentration of 0.1 ppm and 3.0 ppm for *P. clarkii* and *Artemia* , respectively. In accordance with followed method by Engel and Brouwer [4] two midgut glands of *P. clarkii* or 0.5 gr of *Artemia* body were minced and homogenized in Tris-HCl (0.06 M) and NaCl (0.01 M) at pH=8.6 with 0.1 mM PMSF to prevent protease activity and 1 mM DTT to maintain reducing conditions. The homogenate was centrifuged at 30000 g for 45 min at 4°C. The supernatant was heat treated and 60°C for 10 min and centrifuged again at 30000 g for 45 min at 4°C. A portion of the 2 mL supernatant was then applied to a column of Sephadex G-75 (2.6 x 60 cm) and eluted with the same buffer (pH=8.6). Absorbances of the fractions collected were measure at 254 and 280 nm. Spectral changes on acidification and neutralization proteins fractions were determined. Cadmium concentrations was determined by flame (*P. clarkii*) in a Perkin-Elmer model 5000 atomic absorption spectrofotometer a deuterium background corrector. In *Artemia* samples cadmium was determined by graphite furnace with Zeeman background corrector.

Figure 1A is a Sephadex G-75 elution profile derived from midgut gland tissue pooled from two crayfish. Low levels of cadmium occur in the void volume. One cadmium peak is cleary resolved. Cadmium was accumulated in the low molecular weight range of 18000-20000. This fraction had high ultraviolet absorption at 254 nm and a higher 254/280 ratio. Figure 1B show a Sephadex G-75 elution profile derived from *Artemia* sample. In this case a important amount of cadmium was present in the void volume. Two peaks were resolved at the low molecular weight. The first one corresponded to 18000-20000 range and the second one to 8000-11000 range.In both *P. clarkii* and *Artemia* Cd-BP fractions were scanned on a U.V. absorbance spectrophotometer and found a maximal absorbance at about 254 nm with minimal absorbance at 280 nm. This maximal absorbance disappeared on acidification and reappeared with neutralization, indicating the presence of a mercaptide-metal bound (peculiar Cd-BP) [1].

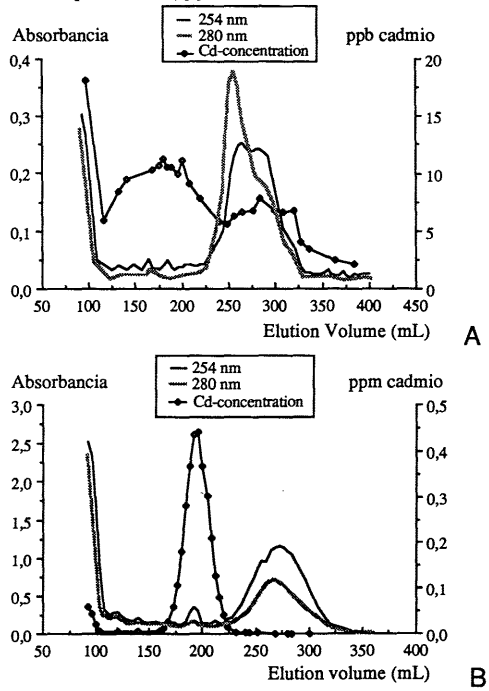

Figure 1. A) Sephadex G-75 elution profile for *Artemia* sample. B) Sephadex G-75 elution profile for midgut gland (*P. clarkii*). In both absorbance at 254 and 280 in arbitrary units, flow rate=50 mL/hr.

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Estimation of nutrients (N and P) along the Romanian Black Sea Coast

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Along the Romanian Black Sea coast (246 Km) several landbased sources are acting. Their importance is different depending on the volume and chemical composition.

The organic and mineral load determined some physico-chemical and biological modifications in the marine environment from influenced areas. In the last decades significant changes especially massive phytoplankton blooms,ocurred in biological cycle. They were induced by the enriching of sea water with nutrients. Owing to this situations it became important to evaluate the N and P input in the marine shallow waters, to identify the sources and to establish each contribution.

The quality of marine waters along the Romanian coast is mainly under the influence both of Danube river and waste water discharges. Because of flow and chemical composition variations; periodical estimations should be performed in order to quantify the source contributions and to establish adequate measures for preventing the environment degradation (DRAGASANU,VASILESCU and STOINA,1960; RUDESCU NICULESCU and CHIVU, 1965; PECHEANU, MIHNEA, SERBANESCU and CUINGIOGLU, 1977).

The paper analyses 1983-1986 data for Danube influence and 1987 for the southern sources. Concerning the Danube water the mean values for $N-NO_2$, $N-NO_3$, $N-NH_4$ during 1983-1986 and $P-PO_4$ during 1985-1986 were calculated. They were presented in the table.

Nutrients were determined by spectrophotometry. 1983 UNESCO method was utilised for $P-PO_4$. $N-NO_2$, $N-NO_3$ and $N-NH_4$ were measured by Murphy & Riley and Koroleff methods.

From the table it can be concluded the importance of the Danube in enriching marine waters along the Romanian coast with nutrients (N and P). Its share consists in more than 99% for N ($NO_2 + NO_3 + NH_4$) and 86.65% as $P-PO_4$ from the total quantities introduced in Romanian Black Sea waters/year.

However because of the distance as well as dilution and dispersion phenomena in the southern part of the coast the Danube contribution is diminished.The local sources acting in this well developed from industrial and tourist's point of view area are determining in biological processes especially in the shallow waters (till 20 m isobate). The 5378 t/year $P-PO_4$,although represent only 13.26% from the total input, are in such a quantity that phosphorus is no more a limitative factor for phytoplankton blooms in this zone.

Parametere	$N-NO_2$	$N-NO_3$	$N-NH_4$	$N-(NO_2+NO_3+NH_4)$	$P-PO_4$
A. Average input of the Danube water tons/year	6 848	189 181	33 050	229 080	34 924
B. Average input of southern sources tons/year	21	236	727	1002	5 378
Total input tons/year	6 869	189 417	33 777	230 082	40 302
B/A % ratio	99.69	99.87	97.84	99.56	86.65

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