Heavy metal concentrations in sediment, benthic invertebrates and fish in three salt marsh areas subjected to different pollution loads in the Tagus Estuary (Portugal)

Susana França [a], Catarina Vinagre [a], Isabel Caçador [a,c], Henrique N. Cabral [a,b,*]

[a] *Instituto de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[b] *Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[c] *Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*

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Heavy metals occur in the environment both as a result of natural processes and as pollutants from human activities (Garcia-Montelongo et al., 1994). Contamination of the marine environment by metals has risen in recent years due to the global population increase and industrial development (Arellano et al., 1999).

The ocean burden of heavy metals and different kinds of pollution is now a serious environmental concern and public interest redarding this issue has been increasing (Cohen et al., 2001). Research efforts have focused primarily on salt marshes, estuaries and coastal environments since these highly productive and sensitive areas are often directly and most seriously affected and exposed to this problem because of their proximity to sources of pollution (Arellano et al., 1999; Cohen et al., 2001). Nevertheless, in recent years effluent discharges have been reduced considerably and the main concern has therefore shifted from the impact of direct loading to possible effects of contaminants in the sediment caused by these discharges (Berge and Brevik, 1996).

Metals tend to accumulate in sediments from where they may be released, moving up through the food chain (Nabawi et al., 1987). Little is known about the bioavailability of sediment associated contaminants to marine organisms (Berge and Brevik, 1996). However it is becoming increasingly important to understand metal accumulation within food webs, because once these heavy metals reach man, they may produce chronic and acute ailments (Nabawi et al., 1987). Regarding these aspects, various estuarine and coastal species have been studied with respect to their differing abilities to concentrate certain metals, and the effects that metal exposure can produce on them (Howard and Brown, 1983).

The present study was conducted in the Tagus estuary, one of the largest estuaries on the Atlantic coast of Europe. Salt marsh habitats cover a large area of this estuary and have great ecological value for this ecosystem, namely in terms of nutrient regeneration, primary production, habitats for fish and birds and as shoreline stabilizers (Caçador and Vale, 2001).

In contrast to many cases in Europe where pollutants from industrial regions are discharged into rivers and brought to the estuaries via fluvial action, in the Tagus most pollutants are discharged directly into the estuary (Caçador et al., 1996). Previous studies have shown that Tagus salt marshes incorporate large quantities of anthropogenic metals into sediments (Vale, 1986). Furthermore, the Tagus estuary acts as a nursery area for several fish species (Costa, 1982; Cabral, 1998) that are caught for human consumption, such as sole, maigre, seabass and mullets, among others. The main nursery grounds are located near saltmarsh areas where benthic invertebrates, the main fish prey, are particularly abundant.

The main aim of this study was to evaluate heavy metal contamination through the food web and to compare metal levels in tissues of several organisms and in sediments. Three salt marsh areas, subjected to different metal loads, were chosen and then concentrations of Cd, Cu, Pb and Zn were measured in species that were abundant in the estuary, and which belong to different trophic levels in the estuarine food web.

The Tagus estuary (38°40′N; 9°15′W) is one of the largest estuaries on the Atlantic coast of Europe, with a length of 50 km and an area of 325 km^2, of which about 40% is intertidal area. The mean depth is <10 m and the greatest depths, ca. 40 m, are found near the mouth of the estuary. The mean river flow is 400 $m^3 s^{-1}$, being highly variable both seasonally and interannually. Salinity varies from 0‰, 50 km upstream

* Corresponding author. Address: Instituto de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal. Tel.: +351 21 750 00 00; fax: +351 21 750 00 09.
E-mail address: hcabral@fc.ul.pt (H.N. Cabral).

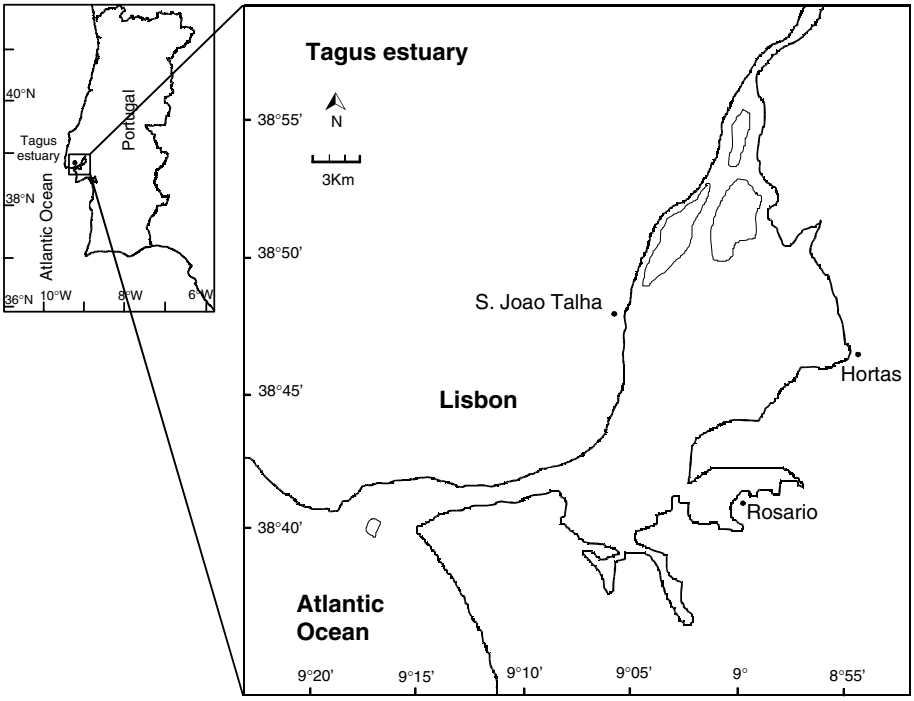

Fig. 1. Location of sampling sites in the Tagus estuary.

from the mouth, to nearly 37‰ at the mouth of the estuary. Three saltmarsh areas were sampled: Rosário, Hortas and S. João Talha (Fig. 1). *Spartina maritima* (Poales: Poaceae), *Sarcocornia fruticosa* (Caryophyllales: Chenopodiaceae) *Sarcocornia perenis* (Caryophyllales: Chenopodiaceae) and *Halimione portulacoides* (Caryophyllales: Chenopodiaceae) are the main species of the Tagus estuary salt marshes.

In surveys carried out from January until March 2003, sediment samples were collected in the three saltmarsh areas, using a 12 cm diameter core, down to a depth of 20 cm. Ten sediment samples were collected at each site. Sediment subsamples were removed and immediately frozen for determination of heavy metals concentrations.

Sediments were transported to the laboratory and then sieved through a 0.5 mm nylon mesh to collect specimens of worms (*Nereis diversicolor*) and burrowing bivalves (*Scrobicularia plana*). Several beam trawls were performed near the three salt marsh areas, in order to collect specimens of brown shrimp (*Crangon crangon*), shore crab (*Carcinus maenas*), grey mullet (*Liza ramada*), sole (*Solea senegalensis*) and sand goby (*Pomatochistus minutus*).

The organisms were subsequently grouped into three composite samples of ca. 20 individuals.

Muscle tissue samples of about 5–10 g were obtained from all species for heavy metal determinations, except for *C. maenas* for which the gills were separated and for *S. plana* for which the whole soft parts of the individuals were used.

For heavy metal determinations, sediment samples were dried to constant weight at 80 °C and 1 g was extracted with 10 ml of HNO_3/HCl (3:1 v/v) twice at 130 °C (Otte, 1991). The procedure was then repeated and the two extracts added together. The metal concentration in the solutions was determined by atomic absorption spectrometry (Perkin–Elmer 4000). Standard additions and sludge reference materials were used (EC standard CRM 145 and 146). The biological samples were dried to constant weight at 60 °C and the percentage of water in the tissues determined. Dry tissue (2 g) was subsequently digested in a mixture of nitric and perchloric acid (suprapure quality; 9:1) as described by Julshmamn et al. (1982). The metal concentrations were determined by atomic spectrometry on a Perkin–Elmer 4000 spectrometer. Standard curves were used for the determination of Cu and Zn, whereas standard addition procedures were employed for the calculation of Cd and Pb.

The differences in the heavy metal contamination of the sediment of the three sampling sites and in the different groups of organisms were evaluated by a two-way ANOVA. The data were log transformed for homogeneity of variance. Tukey tests were performed whenever the null hypothesis was rejected and a significance level of 0.05 was considered. All test procedures were performed in SPSS.

In order to assess the accumulation of metals by the organisms from the sediment, enrichment factors were calculated using the ratio between the metal concentration in the organism's tissues and the metal concentration in the sediment.