# Is δ-aminolevulinic acid dehydratase activity in bivalves from south-west Iberian Peninsula a good biomarker of lead exposure?

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## ARTICLE INFO

## ABSTRACT

The coast of Huelva is considered as a heavily contaminated area where the Tinto and Odiel rivers discharge after running through a metalliferous mining area in the Iberian Pyrite Belt and end in common estuary called Ría of Huelva. Lead is a highly toxic and widely distributed element in the aquatic environment; therefore there is a great interest in assessing the impact of this contaminant on aquatic organisms. To study the bioavailability and sources of lead, the bivalve species *Chamelea gallina* was collected from six sampling sites along the coast of Huelva. Besides the effect of this metal on the enzyme activity, δ-aminolevulinic acid dehydratase (ALA-D) was determined in the whole tissue. Results show the inverse relationship between the lead concentration and the activity of ALA-D measured in whole tissues ($r^2 = 0.7$). Individuals collected from the Ría of Huelva estuary had the highest levels of total lead concentrations and the lowest activity of ALA-D. Lead isotope analyses demonstrated the different sources of lead contamination and also confirmed the influence of Ría of Huelva on the metal input to the marine environment.

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Lead is a highly toxic contaminant due to its ability to accumulate in living organisms and inhibit the activities of essential enzymes. The main sources are mining and industrial activities, sewage waste and the remains of leaded gasoline used in the past. The study area, the coast of Huelva, is situated on the Iberian Pyrite Belt, which is one of the most famous sulfide mining regions in the world. The coast receives considerable fluvial contaminant input from Ría of Huelva formed from the Odiel and Tinto Rivers (Machado et al., 2005). Pb contamination of the study area is related to sulfide minerals outflow which, besides wastes from the industrial complex located at the junction of the two rivers, contribute to considerable lead input to the marine environment (Martins et al., 2007). The epibenthic suspension feeder, *Chamelea gallina* is abundant along the whole Atlantic coast of southern Spain and highly appreciated for human consumption (Usero et al., 2005). The activity of δ-aminolevulinic acid dehydratase (ALA-D) is considered as a specific and useful biomarker of lead pollution (Aisemberg et al., 2005). This enzyme catalyzes the condensation of two molecules of δ-aminolevulinic acid to porphobilinogen (PBG), which is an important step in the heme biosynthesis. In the presence of this pollutant the activity of ALA-D is inhibited. To investigate the influence of lead contamination and its adverse biological effect, bivalves were collected along the coast of Huelva and total lead concentration and activity of ALA-D were determined in the whole tissues. This survey was carried out as a part of a multidisciplinary study (INTERREG IIIA, UTPIA) to investigate the distribution and sources of lead. In the framework of this project lead isotopes (204Pb, 206Pb, 207Pb, 208Pb) were determined to identify the origins of lead contaminant.

Fig. 1 shows a map indicating the six sampling sites. At each sampling site approximately 20 individuals were collected. Three of them were frozen individually in liquid nitrogen immediately after dissection, and the rest (about seventeen organisms) were placed in plastic containers filled with seawater and transferred to the laboratory. Samples for analysis of ALA-D inhibition were stored at $-80\,°C$, while samples for lead determination were placed in clean aerated seawater for 48 h to depurate the digestive tract. After dissection pooled samples were freeze-dried and prepared for total Pb and lead isotope analyses. In our laboratory total lead concentration was determined in duplicate by ICP-OES (Optima 2000 DV, Perkin Elmer), bicinchoninic acid assay (Smith et al., 1985) was used for the measurement of total protein concentration and the method described by Nakagawa et al. (1995) was followed for determination of the inhibition of ALA-D. The identification of distinctive isotope compositions of lead was carried out by GEO-MEDIC Laboratory using multi collector ICP-MS.

Total lead concentrations were highest in samples collected near the estuarine mouth (1.58 μg g$^{-1}$ d.w. for stations 1), while organisms found further from the river outflows accumulated less lead
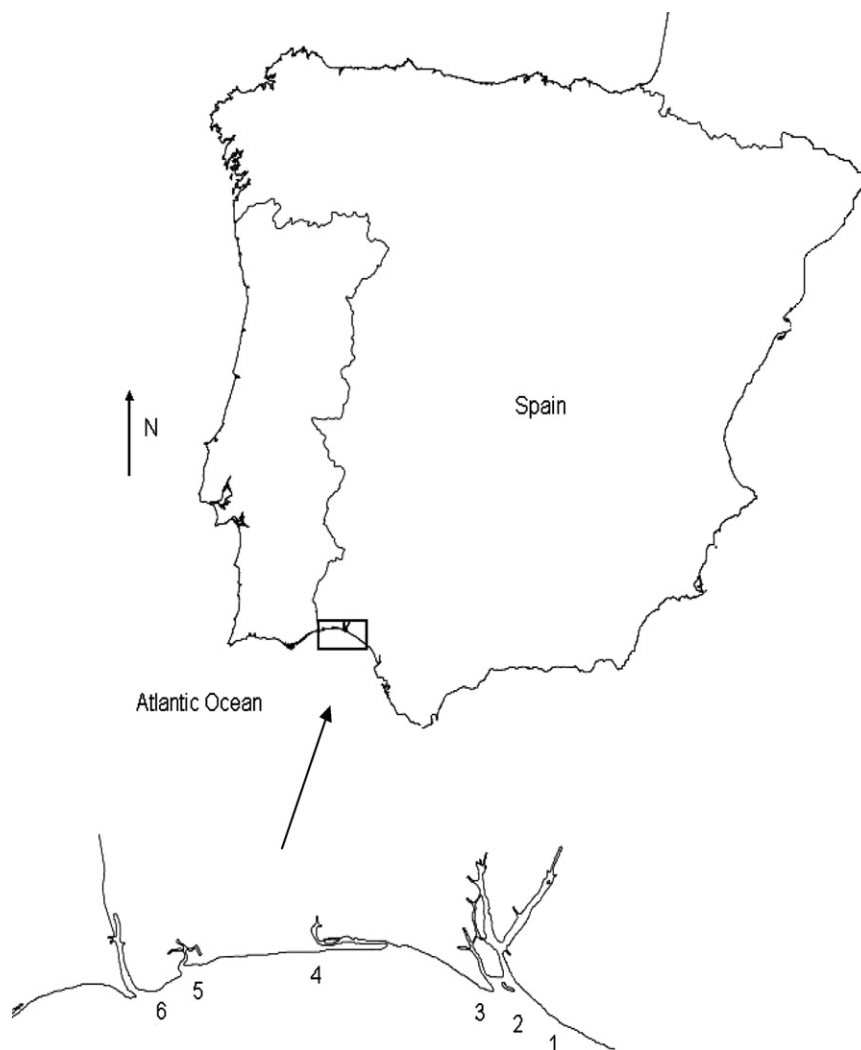
**Fig. 1.** Map of the studied area in south-west Spain showing the locations of six sampling sites selected along the coast of Huelva.

(0.60–0.84 $\mu$g g$^{-1}$ d.w.). Although *C. gallina* is commonly found not only the Atlantic coast, but also in the Mediterranean Sea, limited surveys were conducted on the bioaccumulation of lead. Castro et al. (1999) recorded much lower lead concentrations in *C. gallina* (0.07–0.30 $\mu$g g$^{-1}$ d.w.) collected from fishing areas located in the coast of Almería (SE Spain), suggesting that coast of Huelva is a heavily lead-contaminated area. It is well established that higher concentration of Pb causes higher inhibition of ALA-D (Hodson et al., 1984), which is in accordance with our findings, as the lowest activities of ALA-D were found in tissues collected from the estuarine mouth (Fig. 2). For several reasons it is difficult to compare our results to other reports; most studies were performed in the blood or particular tissues of vertebrates and not in the whole tissues; moreover the experiment-related variables (acclimation period, temperature) differed as well. ALA-D inhibition was determined by Campana et al. (2003) in the blood, liver and kidneys of *Halobatrachus didactylus*, with considerably higher levels obtained than those of the present study (7.54; 37.7; 75.4 ngPBG/min/mg protein). The strong inverse relationship ($r^2 = 0.7$) between ALA-D and Pb concentrations found in this survey also confirms that ALA-D inhibition increased with the increase of Pb concentrations. The activity of this enzyme is sensitive to a variety of metals due to its sulfhydryl groups; therefore to propose the activity of ALA-D as a specific biomarker for lead contamination, further investigation is required. Alves Costa et al. (2007) found that the activity of ALA-D in blood of the fish *Hoplias*
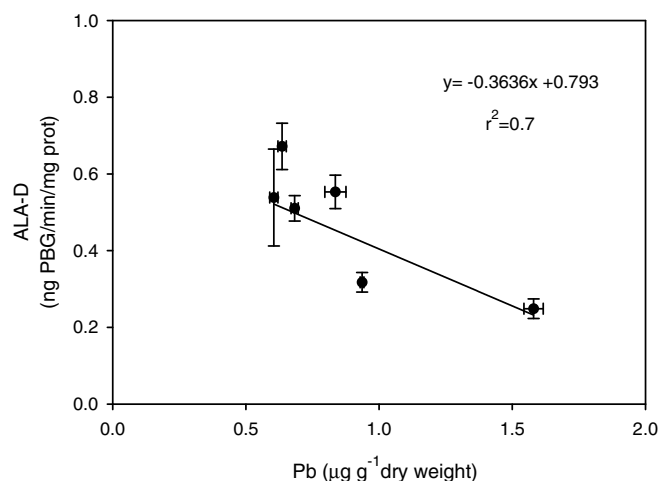


**Fig. 2.** Relationship between the activity of ALA-D (ng PBG min$^{-1}$ mg prot$^{-1}$) and Pb concentration ($\mu$g g$^{-1}$ d.w.) measured in the whole tissues of *Chamelea gallina*. Vertical error bars represent the standard deviation about the mean of three determinations, while horizontal error bars represent the standard deviation about the mean of two determinations.

*malabaricus* was affected not only by lead but by methylmercury as well.

The isotope studies indicated that the anthropogenic lead input is related to mining and industrial activities. On the other hand the strong increase in 206Pb/204Pb ratio with decreasing lead contamination also confirms that Ría of Huelva contributes to the lead input into the marine ecosystem on a large scale (Martins et al., 2007).

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