# Heavy metal concentrations in different tissues of some marine organisms from the Mediterranean (Castellon, Spain)

F. HERNANDEZ, J. MEDINA, J. ANSUATEGUI and M. CONESA

Environmental Laboratory, University College of Castellon, University of Valencia, P.O.Box 224, Castellon (Spain)

Samples of *Sardina pilchardus, Mullus barbatus, Mullus surmuletus, Mytilus galloprovincialis, Carcinus mediterraneus and Thunnus thynnus* were collected in three points of the Castellón coast (Vinaroz, Castellón, Burriana) during the months of April, July & October, 1986, and July & October,1987. These were analysed for total Hg, Cd, Pb and Cr.

Samples identification and preparation were carried out according to the method recommended by the FAO Fisheries Technical Paper nº 158 (Bernhard,1978).

Different tissues were analysed in order to know the degree of accumulation of heavy metals: the whole body for *M. galloprovincialis* and *C. mediterraneus*; and muscle, digestive, liver, gills, kidney and gonads for *S. pilchardus, M. barbatus, M. surmuletus* and *T. thynnus*.

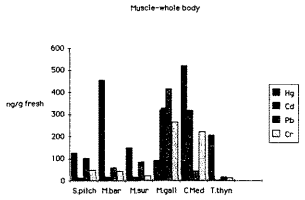
The high number samples makes the digestion procedure in teflon reactors under pressure very tedious. However, the use of open flasks allows one to work comfortably with a large number of samples. For this reason, a digestion procedure with $HNO_3$ conc. in erlenmeyer flasks covered with a glass was applied. This procedure was as follows: 0.05-0.9 g of lyophilised tissue were introduced into a 100-ml erlenmeyer flask and 10-ml $HNO_3$ conc. (65%) were added. Samples were digested on a hot plate at a temperature of 70-80 ºC during approximately 24 h. After cooling solutions were quantitatively transferred to a 25-ml beaker and diluted with water to the mark.

Due to the risk of losses of metals by applying this procedure of digestion (specially for Hg and Cd), we have carefully obtained the recoveries for five replicates of two standards of Hg, Cd, Pb and Cr, and also the accuracy of the procedure (ten replicates) using a sample of *M. galloprovincialis* for intercalibration (IAEA, MA-M-2/TM). The results indicated in the Table show that no losses of metals occurred during the digestion of samples.

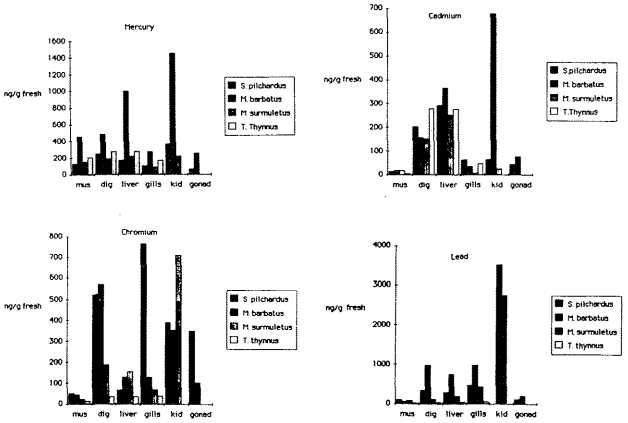
Analyses of total Hg were carried by the Cold Vapour Tehnique AAS; Cd and Cr were determined by graphite furnace AAS with deuterium background and by standard adittions method; Pb was analysed by graphite furnace in the presence of 0.5% $(NH_4)_2HPO_4$ as matrix modifier.

| | Hg | | Cd | | Pb | | Cr | |
|---|---|---|---|---|---|---|---|---|
| standard (µg/ml) | 0.2 | 0.5 | 0.1 | 0.2 | 1.0 | 2.0 | 0.5 | 1.0 |
| recovery (%) | 108 | 108 | 107 | 103 | 102 | 104 | 115 | 103 |
| std. deviation (%) | 20 | 14 | 7.2 | 4.5 | 6.2 | 3.7 | 13 | 4.1 |
| Accuracy | 1.00 ± 0.13 | | 1.83 ± 0.24 | | 1.68 ± 0.26 | | 1.36 ± 0.35 | |
| Reference Values (µg/g dry weight) | 0.93 ± 0.16<br>0.95(0.85-1.06) | | 1.50 ± 0.12<br>1.32(1.16-1.54) | | 1.79 ± 0.54<br>1.92(1.53-2.5) | | 1.64 ± 0.36<br>1.25(0.95-1.62) | |

In the figure are shown the mean concentrations of each metal found in muscle (*S. pilchardus, M. barbatus, M. surmuletus, T. Tynnus*) and in whole body (*M.galloprovincialis, C.mediterraneus*). As can be seen, moluscs and crustaceans are generally the most contaminated organisms, whereas fishes present a minor metallic content, except for Hg (especially in *M. barbatus* and *T. Thynnus*)




In next figures the degree of accumulation of each metal in different tissues is shown.
A one-way analysis of variance (ANOVA) indicate significative differences of metal levels in the different tissues analysed. The order of accumulation for Hg and Cd were as follows: muscle < digestive < liver < kidney; the content of these two metals in gills and gonads is not high, and similar to that of muscle. A different order is observed for Cr, the order being: muscle < liver < digestive < kidney, with high levels in gills and gonads in *S. pilchardus*. For Pb, similar concentrations are found in digestive, liver and gills, and lower that those found in kidney; gills and kidney seem to be the tissues where a major accumulation of Pb is produced. For all the organisms analysed, the muscle is the tissue which clearly presents a minor content of heavy metals

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# Study of toxicity and bioaccumulation of Mercury, Cadmium, Chromium and Lead in the Crayfish Procambarus clarkii

J. MEDINA*, J. DIAZ-MAYANS**, A. PASTOR*, F. HERNANDEZ*, J. DEL RAMO** and A. TORREBLANCA**

* Environmental Laboratory, University College of Castellon, P.O.Box 224, Castellon (Spain)
** Animal Fisiology Laboratory, Faculty of Biological Sciences, Burjassot, Valencia (Spain)

In the present study, adult intermolt speciments of the crayfish *Procambarus clarkii* were collected in Lake Albufera (Valencia, Spain) and taken immediately to the laboratory where they were maintained in 300-l aquaria and for 15 days, at 20ºC with a daily diet of pork liver.

Groups of 10 crayfish were kept in tap water at several metal concentrations, each group in a 15-l experimental aquarium. Ten more crayfish used as a control were kept in 15-l tap water, without adding any metal. Only crayfish weighing between 15 and 20 g were used. The degree of toxicity of Hg, Cd and Cr on crayfish at various temperatures has been studied. All tests have been conducted under static conditions. The LC50 96 h values were calculated using the method of Litchfiel and Wilcoxon (1949). The results show the Hg is the most toxic of metals tested, while Cr presented very low toxicity (0.5 g Cr(VI)/l caused the death of only 40% of the population)

It has been proved that the toxic effects of Hg and Cd increased with increasing temperature. The effect of temperature on the Hg toxicity was more marked than in the cadmium toxicity. The responses of crayfish to Hg and Cd was further investigated with respect to different exposure times. In general, the increase in percent mortality was related to both time and metal concentration, with the highest mortality occurring after 48 h of metal exposure. However, in the case of Hg, the highest mortality occurred between 24 and 72 h for 24 ºC, and between 24 and 48 h for 28ºC.

Table 1 shows the 96-h LC50 values (mg/l) and the 95% confidence limits for Hg and Cd at 20, 24 and 28ºC with *Procambarus clarkii* Each 96-h LC50 value represents the mean of three replicates.

In conclusion, the *Procambarus clarkii* from Albufera Lake present a high resistence to heavy metals pollution. The importance of metallothioneins in the detoxification events of heavy metals is already known. These kinds of mechanisms are probably related to the resistence and accumulation ability of heavy metals in this crayfish.

| Temperature (ºC) | mercury | cadmium |
|---|---|---|
| 20 | 0.79 (0.58 - 1.08) | 58.5 (41.8 - 81.9) |
| 24 | 0.35 (0.21 - 0.56) | 34.8 (28.1 - 43.2) |
| 28 | 0.14 (0.08 - 0.23) | 18.4 (10.7 - 31.6) |

For experiments on metal accumulation, crayfishes from Albufera Lake were divided in groups of 10 animals each. These were kept in 15-l experimental aquaria containing increasing concentrations of Hg, Cd, Cr, and Pb. Ten more crayfish served as control and were kept in 15-l of clear water. After 96 h of metal exposure at 20ºC, the animals were transferred to clean water, free of any contamination, and kept there for an additional 5 h. Gills, midgut gland, antennal glands and muscle of each crayfish were disected, lyophilised and homogenised. Sample digestion was carried out with $HNO_3$. The content of heavy metal on each tissue was determined by flameless AAS, by using the standard additions method for Cr and Cd, or in the presence of $(NH_4)_2HPO_4$ as matrix modifier for Pb. Analyses of Hg were carried out by AAS Could Vapour Technique, by using NaBH4 as reductor agent and argon as purging gas.

Next tables show the metal levels (µg/g dry weight) in some tissues of the crayfish after 96 h of metal exposure at several concentrations:

| µgHg(II)/l | gills | midgut gland | ant glands | muscle | TOTAL |
|---|---|---|---|---|---|
| 0 | 0.93 ± 0.51 | 0.08 ± 0.06 | ---- | 0.02 ± 0.01 | 1,03 |
| 50 | 69.8 ± 24.1 | 1.09 ± 0.61 | 40.1 ± 9.2 | 1.29 ± 0.10 | 112 |
| 100 | 83.7 ± 18.8 | 2.60 ± 1.40 | 122 ± 153 | 0.80 ± 0.08 | 209 |
| 250 | 249 ± 66 | 13.6 ± 5.8 | 697 ± 194 | 3.59 ± 0.52 | 963 |
| µg Cd(II)/l | | | | | |
| 0 | 1.24 ± 0.40 | 0.50 ± 0.13 | 3.08 ± 0.82 | 0.02 ± 0.01 | 4,84 |
| 3.2 | 1.58 ± 0.42 | 0.41 ± 0.15 | 2.75 ± 2.19 | 0.03 ± 0.01 | 4,77 |
| 10 | 3.98 ± 1.00 | 0.49 ± 0.21 | 1.33 ± 0.51 | 0.10 ± 0.04 | 5,90 |
| 32 | 12.8 ± 5.2 | 0.72 ± 0.46 | 1.94 ± 0.95 | 0.60 ± 0.28 | 16,1 |
| 100 | 37.3 ± 10.5 | 2.41 ± 1.74 | 5.17 ± 3.87 | 0.98 ± 0.43 | 45,9 |
| mg Cr(VI)/l | | | | | |
| 0 | 13.1 ± 1.6 | 1.00 ± 0.40 | 38.2 ± 5.0 | 0.41 ± 0.22 | 52,7 |
| 10 | 67.2 ± 17.0 | 20.3 ± 3.5 | 37.5 ± 9.2 | 1.80 ± 0.41 | 127 |
| 37 | 89.4 ± 13.3 | 55.9 ± 25.0 | 147 ± 42 | 3.93 ± 1.20 | 296 |
| 136 | 230 ± 69 | 189 ± 99 | 286 ± 88 | 7.32 ± 1.51 | 712 |
| 500 | 541 ± 125 | 462 ± 102 | 1170 ± 202 | 32.1 ± 3.4 | 2250 |
| mg Pb(II)/l | (*) | (*) | (*) | (*) | (*) |
| 0 | 0.22 ± 0.11 | 0.007 ± 0.003 | 0.11 ± 0.04 | 0.02 ± 0.01 | 0,36 |
| 10 | 3.11 ± 1.96 | 0.24 ± 0.13 | 3.25 ± 1.42 | 0.03 ± 0.02 | 6,63 |
| 50 | 30.4 ± 21.3 | 0.38 ± 0.35 | 2.97 ± 2.66 | 0.11 ± 0.08 | 33,9 |
| 100 | 35.2 ± 16.5 | 0.52 ± 0.50 | 3.54 ± 3.43 | 0.24 ± 0.23 | 39,5 |

(*) Concentrations expressed in mg/g dry weight

It can be concluded that: 1)After sublethal heavy metal exposure, *Procambarus clarkii* accumulate important amounts of Hg, Cd, Cr and Pb. 2)The heavy metal distribution among several tissues of the crayfish is function of the heavy metal concentration used. Commonly, the gills and antennal glands present a high content, whereas the muscle is the organ which accumulates lowest amounts of metals. 3)This crayfish presents both high resistence and high capacity for heavy metal accumulation. Since these animals are consumed directly by man a potential human health hazard exists.

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