METALLOTHIONEIN AND TRACE METAL MEASUREMENT IN MARINE BIOTA

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Introduction

Metallothionein is a low molecular weight protein found in both vertebrates and invertebrates. It has a function in intracellular availability of zinc and copper, although roles for detoxification of some metals have been put forward (Kagi and Schaffer 1988). It is known to bind to non-essential metals such as mercury silver and cadmium, these metals induce the synthesis of the metallothionein protein and it is therefore been suggested as a useful biomarker for environmental metal contamination (George and Olsen 1994). The aim is to assess the use of the electrochemical method of differential pulse polarography (Olafson and Olsson 1991) to measure metallothionein in a variety of marine species and tissues, develop the methods at CEFAS Burnham laboratory to be used as a biomarker of metal contamination in the marine environment.

Metallothionein will be measured in the livers of *Limanda limanda* in five sites off the coast of England, One a reference site of known low metal contamination the other four having varying degrees of trace metal contamination. In all the samples, the total metal content of the tissue and that in the extracted cytosol will be measured. The metallothionein will be normalised to protein content.

Sampling

Samples were collected during the summer of 1999 using the RV Cirolana. Samples of liver were taken from individual animals and placed immediately in liquid nitrogen until their return to the laboratory, where they are stored at -80°C until analysis. For each site a number of livers were bulked on board ship and stored using the same procedure.

Method

The method used is that recently applied to the BEQUALM intercalibration analysis on metallothionein. It is based upon the method of Olafson and Olsson 1991.

The cytosol is extracted from the tissues in Triz buffer below 4°C; dithiothreitol is used to prevent protein oxidation. The supernatant is separated by centrifugation. This fraction is used for the analysis of the metallothionein, protein and the metals Cu and Zn. A portion of the liver was taken and digested for total metals.

The Metallothionein is measured after heat denaturation at 95°C by differential pulse polarography using a hexaminecobalt chloride electrolyte with an EG &G polarography unit with a mercury dropping electrode. Quantification is by reference to rabbit type I and II metallothionein, Figure 1 and 2. Protein was analysed by the Lowry method on the extracted cytosol. Metals analysis was by nitric acid digestion of the cytosol and liver, followed by determination by inductively coupled plasma spectrometry. (ICP-MS), Figure 3 and 4.

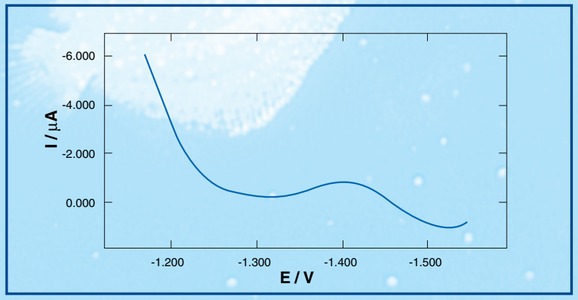

Figure 1. Typical Metallothionein Peak from DPP

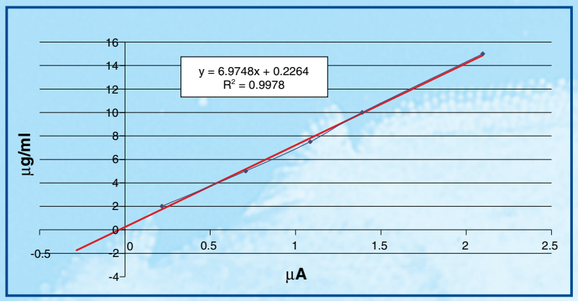

Figure 2. MT Calibration

Figure 3. Polarography equipment

Figure 4. ICP-MS

Results and Discussion

The bulked livers at each of the five sites were analysed in addition to eight individual livers at each site. Each sample was analysed for its protein content and metallothionein results were expressed as in terms of protein. Samples were analysed for Cadmium, Copper and Zinc in the cytosol fraction. The results are given in table 1 and 2. There does not appear to be a good correlation between any of the metals measured and metallothionein in the bulk samples, however there is a strong regression coefficient between copper and MT, but not for cadmium and zinc between sites from the individual means. Figures 5-8.

The results for the individual samples of liver for metallothionein show a great deal of variability; this is mainly attributable to the species chosen and the timing of the sampling. The species was initially chose as representing fish which can be found right around the coasts of England and Wales, The timing was due to availability of ship time. Unfortunately Limanda limanda do move from area to area and so will be exposed to different areas of contamination.

Table 1. Bulked samples

Site ref	MT µgMT/mg prot	Cd µg	Cu mg	Zn mg
stn 41	3.44	31.9	0.77	2.26
stn 59	5.79	4.20	0.59	1.83
stn 145	4.51	6.00	0.42	1.21
stn 156	12.70	4.00	0.51	1.53
stn 159	0.76	24.9	0.15	0.86

Table 2. Means of individuals

Site ref	MT µgMT/mg prot	Cd µg	Cu mg	Zn mg
stn 41	2.32	13.30	0.29	2.50
stn 59	4.11	20.50	0.49	1.91
stn 145	8.17	20.90	1.24	3.20
stn 156	6.88	10.70	0.86	2.35
stn 159	5.02	13.90	0.61	2.27

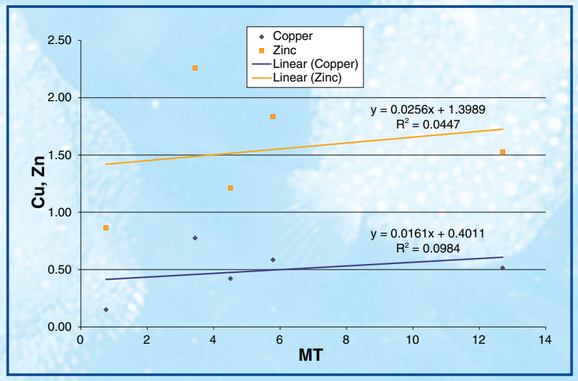

Figure 5. Bulk samples Mt vs Cu and Zn

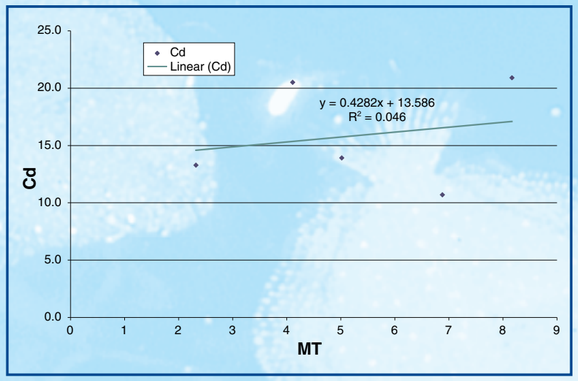

Figure 6. Individual Means MT vs Cd

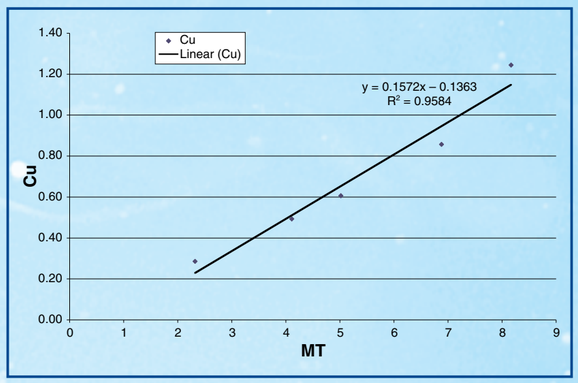

Figure 7. Individual Means Mt vs Cu

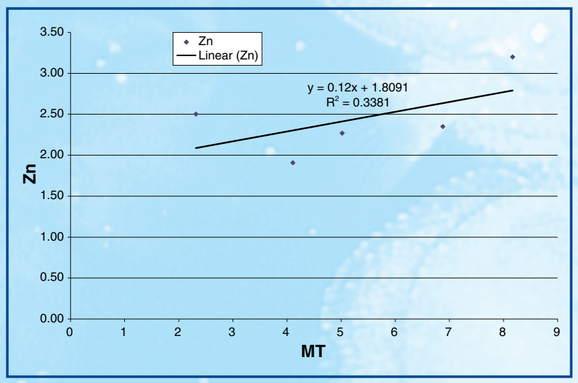

Figure 8. Individual Means MT vs Zn

Conclusion

It is possible that metallothionein measurement can be used to evaluate sites of certain metal contamination and show that the metals are having a direct influence on the biota of the area. Future work will need to include a number of other metals such as Hg and Ag and other species such as invertebrates. Care will need to be taken to exclude factors, which also influence the production of metallothionein.

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