QUANTITATIVE AND QUALITATIVE STUDIES ON BIOACCUMULATION OF LEAD (Pb) IN COCKLE *ANADARA INFLATA* REEVE

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ABSTRACT

The aim of this study was to determine the effects of exposure time and lead concentration on the accumulation of lead in cockles (*Anadara inflata* Reeve). Concentrations of lead (Pb) in tissues of cockle were measured and histological examination of the tissues was carried out. Histological observations revealed the occurrence of lead binding at the end of the experiment, but did not show the effects of concentration and exposure time.

INTRODUCTION

Heavy metals, even though some are essential in trace amounts, are toxic to marine organisms at relatively low concentrations (Roberts, 1976). Lead is one of these widely distributed metals, which are very toxic, causing serious nervous disorders and death if concentrations get high. As with mercury, organic lead compounds are persistent and concentrated in the tissues of organisms. The principal source of lead pollution in the marine environment appears to be the exhaust of vehicles run with leaded fuel (Clark 1986). Lead eventually reaches the water with precipitation and air-borne particles. In addition, lead has found its way into all sort of products, such as paints and ceramics and sooner or later some of it will reach the ocean.

Absorbtion of heavy metals from solutions is generally by passive diffusion across gradients created by adsorbtion onto the surface, and by binding of constituents to body fluids (Clark 1986). One important pathway is when metals are present in food passing food-collecting structures such as the bivalve gills. According to Brooks and Rumsby (1975) there might be three pathways whereby heavy metals in trace concentrations are concentrated by marine organisms. 1) Ingestion of particulate material suspended in sea water. 2) Ingestion of elements via their preconcentration in food material and complexing of metals by co-ordinate linkages with appropriate organic molecules. 3) Incorporation of ions into physiologically important systems and uptake by exchange, for example, via mucous sheets of the bivalves. Furthermore, uptake of heavy metals will be affected by the physiological condition of the animal, by temperature and salinity, and by the physico-chemical form of the metal (Roberts 1976).

The water containing food (and lead) enters the pallial cavity through the inhalant siphon before passing through the gill ostia into the suprabranchial chamber. Food particles are bound into mucous strings on the gill lamellae and carried to the labial palp via ciliated grooves on the lamellar margins (Bayne *et al.* 1979). From the palps, food is directed into the mouth, passes through oesophagus and enters the stomach and directly towards the digestive tubule duct opening or towards the intestine. Intracel-

lular and extracellular digestion occurs in the digestive gland, which also stores nutrient reserves and regulates their transfer to other tissues.

The cockle (*Anadara inflata* Reeve) is widely distributed and is a suitable species for monitoring of heavy metal pollution in Indonesia. The species is good for biomonitoring because it is sedentary, it is common, and it concentrates heavy metals. The binding sites of heavy metals can be monitored by histological examination (Giam *et al.* 1988). However, little has been done so far in relation to bioaccumulation of heavy metals and their presence in organs of cockles. The aim of this study was to fill some of these gaps in our knowledge.

MATERIALS AND METHODS

A total of 480 individuals of *A. inflata* Reeve (2.5-3.0 cm shell length) were collected from Jepara waters. Cockles were measured and acclimatised for 10 days in a flow-through water system using 9 aquaria (50 litre capacity). Salinity was 32-33 ‰ and temperature 28-29.5 °C. Cockles were fed *Chlorella* sp. added daily.

For the bioaccumulation experiment aquaria were stocked with 48 individuals/aquarium. Three levels of lead as Pb $(NO_3)_2$ were maintained: 0.03 ppm (natural Pb concentration at Jepara waters), 0.05 ppm (limit of Pb concentration permitted in Indonesian waters by Ministry of Living Environment Regulation No. 02/Men-KLH/I/1998), and 0.1 ppm (belongs to Group II of industrial waste (very polluted) under Regulation of Ministry of Living Environment No. 03/Men-KLH/I/1998).

For analyses of lead concentrations, 6 cockles were sampled on day 3, 7, 14, 21, 28, and 35. The samples, along with samples of cockles before acclimatisation (D_{-10}) and beginning of experiment (D_0) were analysed using an Atomic Absorption Spectrophotometer (APHA, 1992). For qualitative studies, 2 cockles were analysed histologically at the beginning, the middle, and the end of the experiment (D_{35}) using the 'chromate' method of Pearse (1972). The binding site of lead in the organ was shown by the colour (yellow).

RESULTS

The levels of lead in the soft tissues of cockles are shown in Table 1 and Figure 1. The analysis of variance showed that the level of Pb in the medium (exposure level), the exposure time significantly influenced the

Table 1. Average levels of Pb (ppm ± SD) in soft tissue of *Anadara inflata* Reeve.

Day		Lead Level (ppm)	Average ± SD
-10		-	3.682 ± 0.084
0		-	3.297 ± 0.071
		0.003 (B1)	3.254 ± 0.143
3	(A1)	0.005 (B2)	4.073 ± 0.295
		0.1 (B3)	4.986 ± 0.344
		0.003 (B1)	3.213 ± 0.158
7	(A2)	0.005 (B2)	4.855 ± 0.243
		0.1 (B3)	5.668 ± 0.243
		0.003 (B1)	3.273 ± 0.122
14	(A3)	0.005 (B2)	5.943 ± 0.328
		0.1 (B3)	7.165 ± 0.333
		0.003 (B1)	3.344 ± 0.133
21	(A4)	0.005 (B2)	6.598 ± 0.148
		0.1 (B3)	10.378 ± 0.551
		0.003 (B1)	3.377 ± 0.145
28	(A5)	0.005 (B2)	8.205 ± 0.469
		0.1 (B3)	11.828 ± 0.476
		0.003 (B1)	3.453 ± 0.103
35	(A6)	0.005 (B2)	9.025 ± 0.210
		0.1 (B3)	13.155 ± 0.302

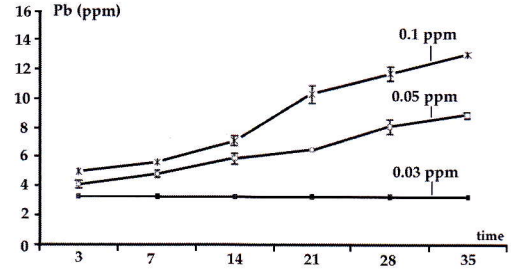

Fig 1. The average lead level in the soft tissues of *A. inflata* Reeve measured over time (days).

level of Pb accumulated by cockles. Duncan's New Multiple-Range test was applied to distinguish the effect between treatments. Only the treatments A6B3, A5B3, and A4B3 (Table 1) were significantly different.

Regression analysis showed that there was a relation between exposure time (X) and level of bioaccumulation in the soft tissue of cockles (Y) (Table 2). The exposure level of Pb influenced the bioaccumulation and depended on the exposure time (Table 3).

Table 2. *Anadara inflata.* Regression equations of bioaccumulation (y) as a function of exposure time (x)

Lead level (ppm)	Regression equation	r
0.003	$y = 3.194 + 0.935\ 10^{-3}\ x$	0.569
0.005	$y = 3.679 + 0.154\ x$	0.986
0.1	$y = 3.953 + 0.273\ x$	0.985

Table 3. *Anadara inflata.* Regression equations of bioaccumulation (y) as a function of exposure level of Pb (x).

time (days)	Regression equation	r
3	$y = 3.195 + 17.801\ x$	0.953
7	$y = 3.292 + 25.220\ x$	0.963
14	$y = 3.423 + 39.954\ x$	0.964
21	$y = 3.071 + 72.687\ x$	0.995
28	$y = 3.368 + 86.965\ x$	0.991
35	$y = 3.153 + 99.832\ x$	0.994

Staining of sections showed that lead was concentrated in gills, digestive tract and connective tissues (Figure 2). The colour intensity did not differ visibly as a function of exposure level of lead at exposure time.

DISCUSSION

After 10 days acclimatisation the lead level in the tissue of cockles had only slightly decreased (0.365 ppm). This was due to the natural Pb level in the water, which was constant at 0.003 ppm. The exposure level of Pb and exposure time highly influences the bioaccumulation in the tissue of cockles in accordance with other studies, *e.g.* Ritz *et al.* (1972); Bryan (1984); Petrocelli (1984). Also, Schultz-Baldes 1974 *in* Roberts (1976) found that uptake of lead into the tissues of *Mytilus edulis* was a linear function of lead concentration in the medium.

Our histological studies showed that lead was concentrated in the digestive tract, connective tissues and gill lamellae. This was in accordance with Pearse (1972).

By the staining we had expected to be able to see an effect of time and concentrations as found by Loomis (1978). However, the yellow colour intensity in various organs of cockles could not be differentiated. The yellow colour was formed by the reaction of potassium chromate in the acetic acid solution (Vogel 1979) as follows:

$$Pb^{2+} + CrO_4^{2-} \longrightarrow PbCrO_4 \downarrow_{(yellow)}$$

Water (food and lead) contacted the gills of the cockle during feeding. Gills of marine invertebrates are permeable and act as an effective pathway for uptake of lead (Mance 1987). Through feeding, the food was brought to the digestive tract in which uptake of lead took place (Connel & Miller 1983; Giam & Lee 1987). The connective tissues, which support and supply nutrients to other tissues, were also stained yellow.

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