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UNITED NATIONS ENVIRONMENT PROGRAMME

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

PROCEEDINGS OF THE FAO/UNEP/IOC WORKSHOP ON THE BIOLOGICAL EFFECTS OF POLLUTANTS ON MARINE ORGANISMS

(Malta, 10-14 September 1991)

Organised jointly with the Euro-Mediterranean Centre
on Marine Contamination Hazards (Council of Europe)

Edited by G.P. Gabrielides

MAP Technical Reports Series No. 69

In cooperation with

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This volume is the sixty-ninth issue of the Mediterranean Action Plan Technical Reports Series.

This series contains selected reports resulting from the various activities performed within the framework of the components of the Mediterranean Action Plan: Pollution Monitoring and Research Programme (MED POL), Blue Plan, Priority Actions Programme, Specially Protected Areas and Regional Marine Pollution Emergency Response Centre for the Mediterranean.

Ce volume constitue le soixante-neuvième numéro de la série des Rapports techniques du Plan d'action pour la Méditerranée.

Cette série comprend certains rapports élaborés au cours de diverses activités menées dans le cadre des composantes du Plan d'action pour la Méditerranée: Programme de surveillance continue et de recherche en matière de pollution (MED POL), Plan Bleu, Programme d'actions prioritaires, Aires spécialement protégées et Centre régional méditerranéen pour l'intervention d'urgence contre la pollution marine accidentelle.

PREFACE

The present volume of the MAP Technical Reports Series contains the proceedings of the Workshop on the Biological Effects of Pollutants on Marine Organisms which was convened by FAO, UNEP and IOC and organised jointly with the Euro-Mediterranean Centre on Marine Contamination Hazards (Council of Europe) in the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean (MED POL - Phase II) which constitutes the scientific and technical component of the Mediterranean Action Plan. The Workshop was hosted by the Foundation for International Studies, Valletta, Malta, from 10-14 September 1991.

Two key-note papers and 19 other papers were presented at the Workshop. As decided, the papers were reviewed by other participants of the Workshop namely, Dr. John Widdows, Plymouth Marine Laboratory, U.K., Dr. Ken Renton, Dalhousie University, Halifax, N.S., Canada, Dr. David Abel, University of Sunderland, U.K. and Mr. Richard Lloyd, Chelmsford, U.K. The authors normally responded to the referee's comments; in case of disagreement, the referee's comments as well as the author's reply appear at the end of the paper. In two cases, the referees had strong objections to the papers and as a result they do not appear in the present publication. All other papers appear in full (in alphabetical order of the senior author's name) as Annex IV to the report while the discussions which took place and the recommendations appear in the main body of the report. The views expressed in the papers are those of the authors and do not necessarily represent the views of either FAO, UNEP or IOC.

The discussions concentrated primarily on the applicability of various biological effects techniques, in field studies, on a routine basis, and on their significance and interpretation vis-à-vis marine pollution risk assessment. In order to make the most rapid progress towards routine applications, the choice of techniques (test species, experimental and monitoring procedures) should be based on a few selected approaches which are well established and documented. A major research activity should therefore be to develop and apply these techniques within the Mediterranean, bearing in mind both the oceanographic and the biotic peculiarities of the area as well as laboratory organisation and availability of resources.

The Workshop recommended, among other things, the establishment of a Working Group to formulate a pilot biomonitoring exercise for implementation by selected Mediterranean institutions in their respective areas. Such a Working Group was set up and will have its first meeting in Malta, in November 1992. Training was also recommended and the FAO/UNEP/IOC Training Workshop on the Techniques for Monitoring Biological Effects of Pollutants in Marine Organisms was organised and took place in Nice, France from 14-25 September 1992.

Final editing and compilation of this volume was done by Mr. G.P. Gabrielides, FAO Senior Fishery Officer (Marine Pollution) at the Co-ordinating Unit for the Mediterranean Action Plan, while Ms Vanta Papapanagiotou was responsible for the typing.

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THE FATE OF LEAD IN A BENTHIC BIVALVE (Venus verrucosa)

by

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A B S T R A C T

The bio-kinetics of lead in a benthic clam, Venus verrucosa were investigated. Lead contents in various body organs of bivalves freshly collected from contaminated coastal sites, and then after 1 month and 2 months depuration, were investigated by anode stripping voltammetry. Histochemical and secondary-ion emission spectroscopy techniques were employed to localize lead at the cellular and subcellular levels.

Most of the lead body burden was found to be sequestrated in the kidneys, presumably after entry through ingestion of lead contaminated particles and passage through the digestive gland. Some loss of lead to the gills was evident during the 2 month depuration period. Renal spherocrystals were found to be the most important subcellular depositary sites for this contaminant. A likely transport mechanism of lead from one body organ to another is suggested.

1. INTRODUCTION

A rapid increase in the number of operating automobiles over the past decades has greatly accelerated the deposition of lead in the marine environment (Zaroogian et al., 1979). This is more so in a small and densely populated island like Malta which has one of the highest densities of car traffic in Europe. Semi-enclosed inshore coastal sites near densely urbanized areas may be expected to receive a significant amount of lead contamination. These include, the local harbours which are also exposed to industrial activities.

The benthic bivalve, Venus verrucosa is an important component of benthic communities in such harbour areas, because of its relative abundance. Moreover, it is often consumed by man and has a significant commercial value.

Bivalves have often been used as bioindicator species to monitor levels of contamination in the marine environment. However, while much is known about the fate, bioaccumulation, and depuration of contaminants in Mytilus spp, very little information is available with respect to Venus spp. Various biological responses of this species to a range of contaminants, have been investigated in our laboratory (Axiak and George, 1987a,b; Axiak and Galea, 1988; Axiak et al., 1988). In the present study, the fate and mobilization of accumulated lead within the various body compartments of this clam were investigated.

2. MATERIALS AND METHODS

Specimens of Venus verrucosa (of size range: 45-55mm) were collected by SCUBA divers from Marsamxett and Grand Harbour between July 1990 and February 1991. They were immediately transported to the laboratory in clean polythene plastic bags, within a maximum of 1 hour of being collected.

2.1 Investigations on freshly collected specimens

A subsample (9 individuals) of freshly collected specimens were dissected and their main organs (namely: digestive gland, kidney, mantle folds, muscular foot, gills and siphons) were fixed in 70% alcohol saturated with hydrogen sulphide for 41 hours. They were then dehydrated, cleared in chloroform and embedded in paraffin wax. Sections were cut at 6 µm with a rotary microtome, deparaffinized and brought down to water. Histochemical staining for lead was carried out according to Sumi et al. (1983). 2-3 mg of the chelate Bromopyridylazo-diethylaminophenol (Br-PADAP) were dissolved in 1 ml dimethylsulphoxide and a drop of 0.5MNaOH. To the solvent 15 mls of 0.1M thiourea and 15 mls of 0.1M potassium cyanide (masking agents for iron, copper and zinc) were added. The different sections were stained in this solution for twenty minutes and afterwards washed in deionized water. They were allowed to dry completely in air at room temperature and mounted in DPX.

Another subsample of three bivalves were similarly dissected and fixed in 70% gluteraldehyde for 30 minutes. Tissues were washed in three changes of 3.5% cacodylate buffer and stored in same buffer until further analysis using secondary-ion emission spectroscopy. Such analysis were carried out by Prof. C. Chassard-Bouchaud at the Laboratoire de Biophysique (Université Paris, France). A Cameca SMI-300 fitted with an electrostatic deflector, with ionic oxygen as primary ions, was employed. The images of the distribution of the secondary ions (lead) were obtained directly with appropriate optics (Chassard-Bouchaud, 1991).

A further subsample of 12 individuals was used for lead analysis using anode stripping voltammetry. These specimens were stored at 8°C overnight. Shells were then washed with distilled water to remove any source of metal contamination. Bivalves were then dissected after the internal soft parts were rinsed with deionized water and allowed to drain on clean tissue paper. Organs were pooled according to type, frozen at -20°C overnight and then homogenized. Digestion was carried out in concentrated nitric acid for 15 hours at room temperature and then for two 6-hour digestion cycles at 115°C. The clear digests were diluted to a known volume with deionized water and then analyzed for lead content using a Metrohm 646 VA anode stripping voltameter.

2.2 Investigations during depuration phase

Freshly collected bivalves were introduced to clean sea water in a recirculating seawater system, within a maximum of 1 hour of being collected from the field. Animals were placed in glass aquaria measuring 60 by 39 by 40 cm and each holding 55 l of water. Seawater was circulated through these aquaria at a rate of 1.5 to 2 l min^{-1}. The bottoms of these aquaria were covered with clean sediments to a depth of 5 cm, and in which the clams could easily burrow. Water temperatures were kept at 19 to 21°C and salinities at 36 to 38 ppt throughout the depuration investigation period which lasted for two months. During this period, bivalves were fed on 'Liquifry Marine', supplied by Liquifry Co. Ltd., Dorking, U.K. (Batch Code: 0308).

After 1 month and 2 months of depuration in clean seawater, samples were collected from the aquaria and analyzed histochemically (9 individuals), and for tissue lead content (12 individuals) using anode stripping voltammetry as indicated above.

2.3 Sediment analysis

Sediment samples were collected from Marsamxett at the same site from which animals were collected. Superficial sediments (top 5 - 8 cm) were collected by SCUBA divers from a depth of 7.2 m, stored in prewashed plastic containers and immediately transported to the laboratory. Here they were dried at 100°C, sieved to a fine fraction (<2mm in particle diameter) and digested in concentrated nitric acid at room temperature for 15 hours, and then for a further period of 6 hours at 115°C. The digest was filtered and then diluted to 20 ml with deionized water before being analyzed by Anode stripping voltammetry. Sediments contained in depuration aquaria were similarly treated for subsequent analysis of lead content.

3. RESULTS

The superficial sediments from Marsamxett Harbour were found to carry a lead content of 118 $\mu g\ g^{-1}$ dry weight, while the sediments in the depuration aquaria had 12 $\mu g\ g^{-1}$ dry weight of lead.

The lead concentrations in the various body parts of freshly collected bivalves and then after 1 month and 2 months depuration are presented in Table 1. Also shown are the percentage distribution of lead within the various body compartments and the percentage of fresh weight of the given body part to the whole soft body weight of the animal. Freshly collected bivalves had a total lead body burden of 1.45 $\mu g\ g^{-1}$ of fresh weight. Over 83% of this contaminant was located in the kidney, though this organ constituted only 7.4% of the total soft body weight of the animal. No lead was detected in gills, siphons and muscular foot of this group of bivalves, while the remaining body burden of lead was localized in the digestive gland (16%) and the mantle and mantle folds (0.5%).

After one month depuration in clean seawater, 86% of the body burden of lead was still localized within the kidneys, with the remaining being found in the muscular foot. No lead was found in the gills, siphons, mantle or digestive glands.

The overall lead body burden did not decrease even after two months of depuration in clean seawater. In fact during this period, the relative distribution of lead within the kidneys was actually higher than in freshly collected bivalves. Therefore, by this time over 93% of the lead body burden was located within the kidneys, with almost 6% being located in the digestive glands and only 0.9% being found in the gills.

The above findings were confirmed by the histochemical localization of lead at the cellular level. Positive control tissue sections which, were previously immersed in a saturated solution of lead nitrate for 24 hours and then treated histochemically as described above, exhibited a magenta to purple depositions of Br-PADAP within various parts of the tissues. This was considered as a positive reaction towards lead.

Table 1

Lead concentrations and % distribution of lead load in the various body parts of Venus verrucosa.

Body Part		Freshly Collected	1 month Depuration	2 months Depuration
Gills	Pb Conc.(µg g^{-1})	-	-	0.173
	% Distrib. of Pb	0	0	0.86
	% of Body Weight	9.5 + 1.3	9.8 + 2.0	8.9 + 2.6
Siphons	Pb Conc.(µg g^{-1})	-	-	-
	% Distrib. of Pb	0	0	0
	% of Body Weight	6.6 + 3.2	6.1 + 0.7	5.4 + 1.7
Kidney	Pb Conc.(µg g^{-1})	15.89	9.22	26.8
	% Distrib. of Pb	83.5	86.1	93.2
	% of Body Weight	7.4 + 2.1	7.3 + 1.6	6.2 + 2.7
Mantle Margin	Pb Conc.(µg g^{-1})	0.03	-	-
	% Distrib. of Pb	0.5	0	0
	% of Body Weight	26.6 + 3.9	26.1 + 3.7	27.2 + 3.1
Muscular Foot	Pb Conc.(µg g^{-1})	-	0.41	-
	% Distrib. of Pb	0	13.9	0
	% of Body Weight	34.6 + 4.2	35.4 + 5.2	32.9 + 5.2
Digestive Gland	Pb Conc.(µg g^{-1})	1.96	-	0.551
	%Distrib. of Pb	16.0	0	5.963
	% of Body Weight	14.8 + 5.2	14.8 + 5.0	19.3 + 3.9

N.B. Lead concentrations in the various body parts are shown in µg g^{-1} of fresh soft body weight.
% Distribution of Lead in the various body parts is calculated as the percentage ratio between the lead burden of the organ to the total lead body burden.
% of Body Weight shows the percentage fraction of the weight of the organ to the weight of the total fresh soft body.

Sections taken from freshly collected bivalves, exhibited a highly marked light red precipitate in the renal concretions. These concretions or spherocrystals, were amorphous and had pleiomorphic radial structures (especially evident in sections stained with heamatoxylin and eosin). They were located within the renal glandular cells forming the walls of the kidney tubules.

A weak Br-PADAP precipitate was detected in the ciliated epithelium of the mantle folds; the connective tissues between the pallial muscles and outer epithelia, and the digestive cells of the tubules of the digestive glands of freshly collected specimens.

Tissue sections which were analyzed with secondary-ion emission spectroscopy, exhibited lead in the renal spherocrystals. The digestive gland also showed distinct localization of lead within the digestive cells and the interstitial regions.

After one month and two months depuration in clean seawater, only the renal spherocrystals continued to exhibit a positive reaction to Br-PADAP.

4. DISCUSSION AND CONCLUSIONS

The level of lead in the sediments (118 $\mu g\ g^{-1}$) collected from the same site as that of the bivalves, was found to be comparable or higher than those of other Mediterranean inshore sediments exposed to urban and industrial pollution (eg. Thermaikos Gulf; 71.2 $\mu g\ g^{-1}$ dry weight; Voutsinou-Taliadouri, 1981; Gulf of Venice: 45 $\mu g\ g^{-1}$ dry weight, Angela et al., 1981). Marsamxett Harbour is in fact a semi-enclosed body of water which is surrounded on all its sides by heavily urbanized areas. Though a number of yacht marinas as well as a small ship repairing yard are located here, it may not be considered as a heavily industrialized area. Therefore it may be suggested that the main source of lead contamination in this area is car traffic.

The total lead body burden of freshly collected specimens from this area was found to be 1.45 $\mu g\ g^{-1}$ fresh weight. Mytilus galloprovincialis collected from other similarly urbanized areas such as the Golf of Genoa and Rijeka Bay (UNEP/FAO, 1986) showed maximum lead body burdens of approximately 2 $\mu g\ g^{-1}$ fresh weight.

Uptake of lead by bivalves can either occur through adsorption of ions at membrane-water interphases, by absorption or passive diffusion across semi-permeable membranes into body fluids or by ingestion of the metal with food. No significant levels of lead were located within the external body parts (eg. siphons, gills, and muscular foot) of freshly collected Venus verrucosa. However, 16% of the total body burden was located within the digestive glands. This suggests that the major uptake mechanism is through ingestion of lead contaminated particles by this filter feeder. Bryan (1973) observed that on exposure to lead, Mytilus edulis showed a lower concentration of lead in the gills as compared to the mantle margin, with much higher levels in the kidneys and digestive gland.

The present study has shown that once inside the body, lead is almost completely sequestrated by the kidney of Venus verrucosa. Histochemically, this lead was found to be mostly associated with renal spherocrystals. These renal refractory concretions are only found in invertebrates and are concerned with synthesis of an inorganic matrix in which inorganic matter may be precipitated as phosphates, carbonates or oxalates (Martoja and Truchet, 1983). Accumulation of soluble metal-binding proteins within the lysosomal system of the bivalve kidney has also been postulated (Simkiss and Mason, 1984). Both the renal lysosomes as well as the renal spherocrystals may act as the major depositary sites of this contaminant in Venus verrucosa, thus limiting the injury to the other body parts.

The present study have shown that during depuration, most of the lead originally found in the digestive gland of this species was transported to the kidney. Moreover, at the end of 2 months depuration, traces of lead were found

to increase in the gills. This suggests that these sites may also be sequestrating lead from other body organs. The transport of lead from one organ to another may be occurring via some metallo-protein plasma ligand. No amoebocytes loaded with lead were ever detected histochemically throughout the depuration period.

The total lead body burden of the present species did not decrease over a two-month depuration period. Similarly, Mauri and Orlando (1982) have reported that renal spherocrystals of the clam Mercenaria mercenaria are only eliminated after prolonged depuration in clean seawater. This long residence time of lead within the body of Venus verrucosa suggests the possibility of biomagnification to higher trophic levels. Moreover, it may constitute a health hazard through its consumption by man.

5. ACKNOWLEDGEMENTS

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