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MERCURY, CADMIUM AND LEAD LEVELS IN MARINE ORGANISMS *(MYTILUS GALLOPROVINCIALIS LMK.)* COLLECTED ALONG THE ITALIAN COASTS

R. GIORDANO *(a)*, P. ARATA *(b)*, S. RINALDI *(a)*, L. CIARALLI *(a)*, M. GIANI *(b)*, M. RUBBIANI *(a)* and S. COSTANTINI *(a)*

(a) Laboratorio di Tossicologia Applicata, Istituto Superiore di Sanità, Rome, Italy
(b) Istituto Centrale per la Ricerca Scientifica e Tecnologica Applicata alla Pesca Marittima, Rome, Italy

Summary. – The growing importance assumed in the last ten years by contamination of the marine environment caused by heavy metals has evidenced the necessity to constantly deepen the knowledge of pollutant concentration that can reach man through the food chain. Particularly interesting is the determination of potentially toxic elements in marine organisms which through accumulation and concentration processes can provide a direct correlation with the ecosystem's degree of contamination. The present study takes into account the determination of mercury, cadmium and lead in samples of Mytilus galloprovincialis Lmk. *collected in 41 sites along the Italian coast, between August 1986-April 1987. Analyses were performed using both the electrothermal (cadmium and lead) and the cold vapour (mercury) atomic absorption spectrometry techniques. Results showed that, in general, the degree of contamination is low, with a few exceptions where the concentration values were greater than the mean values.*

Riassunto (Livelli di mercurio, cadmio e piombo in organismi marini (*Mytilus galloprovincialis Lmk.*) prelevati lungo le coste italiane). - *L'importanza crescente che negli ultimi dieci anni ha assunto il fenomeno della contaminazione dell'ambiente marino causata da metalli pesanti ha evidenziato la necessità di approfondire costantemente la conoscenza delle concentrazioni di questi inquinanti che, attraverso la catena alimentare, possono giungere all'uomo. Particolarmente interessante risulta il dosaggio degli elementi potenzialmente tossici in organismi marini che, attraverso fenomeni di accumulo e concentrazione dei metalli, possono fornire una diretta correlazione con la contaminazione dell'ecosistema. Il presente studio ha riguardato la determinazione di mercurio, cadmio e piombo in campioni di* Mytilus galloprovincialis Lmk. *prelevati in 41 siti lungo le coste italiane dall'agosto 1986 all'aprile 1987. Le determinazioni sono state eseguite mediante spettrofotometria di assorbimento atomico con fornace di grafite (cadmio e piombo) ed a sviluppo di vapori freddi (mercurio). I risultati ottenuti hanno mostrato che, in generale, il grado di contaminazione è basso, ad eccezione di alcuni siti che hanno presentato valori superiori alla media.*

Introduction

The role of heavy metals as pollutants is nowadays widely recognized [1-4]. The possibility that the coastal marine environment may be contaminated by persistent chemicals has drawn the attention to the importance of measuring the concentration of pollution in the ecomarine system [5].

Geological weathering and erosion of the earth's crust release and transport heavy metals into the marine environment, mainly through rivers and surface runoff. Other natural sources include deep sea volcanism and the atmosphere. Certain elements, most notably mercury, cadmium and lead have been reported [6, 7] in anomalous high concentrations in the marine environment apparently related to naturally occuring deposits. However, anthropogenic activities such as mining, industrial processing of ores and metals, agriculture, as well as the use of metal components, have caused increased inputs of heavy metals into oceans. Other sources, including combustion of fossil fuels, smelting and the use of lead gasoline release heavy metals into the atmosphere, that are subsequently transported to the sea. In the light of these considerations, the importance of knowing the heavy metal concentrations in the marine environment, their sources, dispersion, dissipation and their possible interrelations with growth, reproduction and food chain dynamics of biota utilized by man is obvious.

To assess the availability of heavy metals in solution, different types of organisms may be used, such as seaweeds and filter-feeding molluscs. It is known that mussels can accumulate inorganic elements from sea-water, phytoplankton and suspended materials [8]. Since heavy metals in water are usually present in a very low concentration, their dosage presents considerable analytical problems, which require sophisticated methodologies and involving laborious pre-treating and sample concentration procedures [9-11]. The fact that mussels accumulate metals from the marine environment, allows a much simpler analytical evaluation of marine metal pollution since the concentration of metals in mussels is higher than in the surrounding water.

This paper reports the results of mercury, cadmium and lead levels found in mussel samples collected together with water specimens, sediments, algae and fishes during a coastal monitoring program of Department of Merchant Navy. The sampling was performed by WWF in the ambit of the program "The sea must live".

Materials and methods

Sample collection. - In the period between August 1986-April 1987 mussel collection was made at a series of stations along the Italian coasts (Fig. 1). Marine organisms were caught near the following locations: Genoa, La Spezia, Piombino, Montecristo, Porto S. Stefano, Olbia, Fiumicino, Terracina, Naples, Ischia, Salerno, Gioia Tauro, Taranto, Brindisi, Bari, Manfredonia, Termoli, from a total of 41 sampling sites. At each site, about 60 specimens of *Mytilus galloprovincialis Lmk.* were hand-collected. The mussels were externally cleaned and maintained in clean seawater for twelve hours to empty their guts, and frozen at - 20 °C. Samples were analyzed for mercury, cadmium and lead in pools of 40-50 individuals. Length ranged from 3 to 5 cm and the mean weight resulted 1.6 ± 0.6 g.

Sample preparation. - Upon thawing, mussels were placed over a nylon net on their edges to allow the water inside the shells to drain away. The soft parts were carefully removed with a plastic knife and homogenized in a mixer to make up the samples from each sampling site. To avoid contamination, the mixer was home-covered with teflon in all parts where it touched the sample, except for blades of a disposable stainless steel type. Sub-samples from each site were done in triplicate.

Fig. 1. - Sampling localities.

Total mercury analysis. - Mercury analysis was performed on fresh tissues. After weighing, ranging from 5 to 6 g, the organisms were digested in concentrate Suprapur (Merck, Germany) sulphuric and nitric acids. Total mercury was then determined by cold vapour AAS technique after reduction with stannous chloride, according to the Official Italian Method [12]. Measurements were made with a Perkin-Elmer model 603 atomic absorption spectrophotometer equipped with a deuterium background corrector and EDL lamp.

Cadmium and lead analysis. - Prior to analysis subsamples (about 5 g) were dried at 105-110 °C to determine the water content. The mean value of the ratio fresh/dry weight resulted about 5.4. Dried tissues were digested in quarz Erlenmayer flasks using the following method: samples were mixed with 20 ml of doubly-distilled water and initially placed on a termoregulated hot-plate at 90-100 °C for about 20 min. After that, digestion was carried out by adding several aliquots of a Suprapur nitric/perchloric/sulphuric acid (25+25+1 v/v/v) mixture, to a total added volume of 12 ml. Further aliquots of nitric acid were added until a complete colorless solution occurred. Successively, the temperature of the hot-plate was increased to 150 °C and the solution evaporated avoiding dryness. The residue was dissolved in 10 ml of water together with 1 ml of concentrate Suprapur chloridric acid at 100 °C. Finally, the volume was made up to 25 ml with water.

Determinations of cadmium and lead were performed by stabilized temperature graphite furnace atomic absorption spectrometry (Perkin-Elmer model 5100 Zeeman equipped with a HGA 600 graphite furnace and an AS-60 autosampler) using the furnace programs reported in Table 1. A mixture of magnesium nitrate 2 g/l and ammonium dihydrogen phosphate 20 g/l was used as matrix modifier.

Performance tests (whole procedure). - Reagent blank values were 10 ± 2 ng for mercury, 4.5 ± 2.1 ng for cadmium and 150 ± 50 ng for lead. Mean coefficients of variation in the determination of the mercury, cadmium and lead were 3.8%, 3.9% and 6.1%, respectively. Detection limits resulted 3.3 µg/kg for mercury, 1.8 µg/kg for cadmium and 60 µg/kg for lead. Certified reference material from NIES, Japan (mussels, no. 6) was employed to check the methodology accuracy. The mean recovery values were 97.8% for cadmium and 103% for lead, respectively. Mercury recovery, performed on samples prepared in laboratory, resulted 97.2%.

Results and discussion

The choice of organisms examined in the present study depended on their systematic identification, availability throughout the year, common use for human consumption and wide geographical distribution. General results for mercury, cadmium and lead are reported in Table 2. The

Table 1. - *Furnace programs*

Parameters	Drying	Ashing	Atomization	Cleaning	Cooling
			Cadmium		
Temperature (°C)	120	800	1800	2650	20
Ramp time (s)	10	30	0	1	1
Hold time (s)	20	20	5	5	20
Internal flow (argon, ml/min)	300	300	0	300	300
			Lead		
Temperature (°C)	130	850	2000	2650	20
Ramp time (s)	30	20	0	1	1
Hold time (s)	10	20	7	5	20
Internal flow (argon, ml/min)	300	300	0	300	300

Sample volume: 10 µl; signal mode: peak area

Table 2. - *Mercury, cadmium and lead concentrations in mussels*

Sampling localities		Wet/dry factor	Mercury (µg/kg) w.w.	Mercury (µg/kg) d.w.	Cadmium (mg/kg) w.w.	Cadmium (mg/kg) d.w.	Lead (mg/kg) w.w.	Lead (mg/kg) d.w.
Genoa	S1	3.41	18	61	0.11	0.36	1.23	4.19
(Harbour)	S2	5.62	54	303	0.14	0.77	4.30	24.2
	S3	5.72	107	612	0.14	0.81	2.58	14.8
	S4	6.21	56	347	0.16	0.99	11.8	73.4
		5.24	59	325	0.14	0.73	4.98	29.1
		± 1.25	± 37	± 276	± 0.02	± 0.27	± 4.72	± 30.6
La Spezia	S1	3.11	30	94	0.29	0.92	1.43	4.45
(Outside harbour)	S2	5.93	49	291	0.22	1.29	2.62	15.5
	S3	4.73	31	147	0.14	0.65	1.11	5.24
	S5	5.00	35	175	0.10	0.50	0.96	4.80
		4.69	36	177	0.19	0.84	1.53	7.50
		± 1.17	± 9	± 83	± 0.09	± 0.35	± 0.75	± 5.34
Piombino	S1	5.12	33	169	0.14	0.69	2.36	12.1
(Harbour)								
Porto S. Stefano	S1	3.97	39	154	0.17	0.66	0.47	1.85
(Outside harbour)	S4	4.37	99	433	0.16	0.68	1.35	5.90
		4.17	69	294	0.17	0.67	0.91	3.88
Montecristo	S2	4.39	32	140	0.48	2.11	0.60	2.63
(Cala Maestra)								
Olbia	S1	3.70	12	44	0.062	0.23	0.15	0.55
(Punta Filo)								
Fiumicino	S1	3.65	22	80	0.12	0.42	0.41	1.51
(Fiumara grande)	S2	5.91	35	206	0.12	0.68	0.51	3.00
	S3	5.72	27	154	0.11	0.62	0.56	3.23
	S5	5.31	24	127	0.10	0.55	0.77	4.11
		± 5.14	27	142	0.11	0.57	0.56	2.96
		1.03	± 6	± 53	± 0.01	± 0.11	± 0.15	± 1.08

Table 2. - *(continued)*

Sampling localities		Wet/dry factor	Mercury (μg/kg) w.w.	Mercury (μg/kg) d.w.	Cadmium (mg/kg) w.w.	Cadmium (mg/kg) d.w.	Lead (mg/kg) w.w.	Lead (mg/kg) d.w.
Terracina	S3	4.44	17	76	0.09	0.39	0.46	2.06
(Outside harbour)	S4	4.78	16	76	0.08	0.38	0.50	2.39
	S5	6.04	23	147	0.10	0.60	0.90	5.78
		5.09	19	100	0.09	0.46	0.62	3.41
		± 0.84	± 4	± 41	± 0.01	± 0.13	± 0.24	± 2.1
Ischia	S1	3.36	10	34	0.13	0.44	0.40	1.34
(Punta Alaca)								
Naples	S1	3.61	20	72	0.14	0.49	0.65	2.35
(Castel dell'Ovo)	S2	6.57	40	263	0.15	1.00	0.98	6.47
	S3	6.42	44	282	0.15	0.95	1.10	7.09
	S4	6.58	35	230	0.16	1.09	1.37	9.02
	S5	6.72	22	148	0.16	1.05	2.64	17.7
		5.98	32	199	0.15	0.92	1.35	8.52
		± 1.33	± 11	± 87	± 0.01	± 0.24	± 0.77	± 5.67
Salerno	S1	5.95	19	113	0.16	0.91	0.65	3.89
(Punta Fuenti)	S2	7.09	28	198	0.15	1.04	0.77	5.47
		6.52	24	156	0.16	0.98	0.71	4.68
Gioia Tauro	S2	7.53	16	120	0.18	1.38	0.21	1.56
(S. Ferdinando)	S4	5.91	13	77	0.15	0.86	0.50	2.97
		6.72	15	99	0.17	1.12	0.36	2.27
Taranto	S2	6.28	42	263	0.14	0.87	0.79	4.96
(Capo S. Vito)	S3	5.89	43	253	0.15	0.85	1.12	6.61
		6.08	43	258	0.15	0.86	0.95	5.79
Brindisi	S4	5.91	52	307	0.093	0.55	0.97	5.73
(Outside harbour)								
Bari	S2A	6.03	16	96	0.12	0.72	0.99	5.97
(Outside harbour)	S2B	5.84	15	88	0.14	0.82	0.94	5.47
		5.94	16	92	0.13	0.77	0.97	5.72
Manfredonia	S1	5.82	19	111	0.20	1.14	0.66	3.84
(Gulf)	S2	6.66	20	133	0.18	1.19	0.33	2.20
	S3	5.65	16	90	0.19	1.09	0.24	1.37
		6.04	18	111	0.19	1.14	0.41	2.47
		± 0.54	± 2	± 21	± 0.01	± 0.05	± 0.22	± 1.26
Termoli	S1	5.05	18	91	0.16	0.81	0.34	1.73
(Bay)	S2	5.84	20	117	0.16	0.94	0.38	2.20
	S3	5.85	16	94	0.13	0.76	0.32	1.85
		5.58	18	101	0.15	0.84	0.35	1.93
		± 0.46	± 2	± 14	± 0.02	± 0.09	± 0.03	± 0.25

metal levels found in mussels are generally quite low, with the exception of some sites where the concentrations for cadmium and lead resulted higher than the mean values.

Mercury. - Results obtained agree with those found by some authors (Majori *et al.*, Camoni *et al.* and Medina *et al.*) [13-15] who carried out similar experiments, whereas they are lower than those found by others (Leonzio *et al.* and Lyngby) [16, 17]. The concentration levels are in the interval ranging from 10 μg/kg wet weight (w.w.), found in the Ischia area, to 107 μg/kg w.w. found in the Genoa S3 site. The mean concentration found in the bay of Naples (32 ± 11 μg/kg w.w.) agrees with the one obtained in a monitoring program previously made in the same area some years ago [14].

Generally, the values obtained are lower than the concentration limits established by several countries for fish, including shellfish, crustaceans and molluscs (FAO Fisheries Circular no. 764, 1983): 0.5 mg/kg w.w. (Australia; Brazil - no. 18/75 by National Committee of Alimentary Norms and Standards; France - Ministry of Agriculture, 21 December 1976; Spain - Resolution of the General Health Division, 30 March 1973); 0.7 mg/kg w.w. (Italy - EEC origin through Ministerial Decree of 27 April 1978); 1.0 mg/kg w.w. (Holland; USA-Ref. CPG 7108.07 June 1978).

Cadmium. - The cadmium concentration values varied between 0.062 mg/kg w.w. (Olbia) and 0.48 mg/kg w.w. (Montecristo). Results are similar to those reported by other authors in various areas of the Tyrrhenian sea and the Gulf of Trieste [13, 16]. On the contrary, the concentrations were greater than the ones found by Medina *et al.* [15] in the area of Valencia-Castellon, Spain. All values found in the localities examined are lower than the limits established by some countries such as Australia and Hong Kong: 2 mg/kg w.w.; Tasmania: 2.5 mg/kg w.w.; Holland and New Zealand: 1.0 mg/kg w.w.; Federal Republic of Germany: 0.5 mg/kg w.w. The mean concentration found in the area of Naples (0.15 mg/kg w.w.) is as the one reported in a previous paper [14].

Lead. - Lead concentrations found in the organisms examined are in the 0.15 mg/kg w.w. (Olbia) - 11.8 mg/kg w.w. (Genoa S4) range. The results agree with those reported in previous papers [7, 13, 16] and for the most part are lower than the limit of 2 mg/kg w.w. established both by the Italian legislation (D.M. 27 April 1978) and by other countries (Chile, Finland, New Zealand, Holland, Venezuela). Only in the localities of Genoa (sites S2, S3, S4), La Spezia (site S2), Piombino and Naples (site S5) values were higher than the mean. In 13 of out 41 collection sites (31.7%), the lead concentrations were higher than the limit of 1 mg/kg w.w. established by some countries such as Switzerland, Sweden and Thailand. On the contrary all values, with the exception of those found in Genoa S4, were lower than 10 mg/kg w.w. limit adopted in the United Kingdom. Only the Genoa area presents higher mean concentration values with a wide dispersion of data (4.98 ± 4.72 mg/kg w.w.). In other localities, the mean concentration values were lower than the 2 mg/kg w.w. limit. The particular collection point (inside harbour) could explain the higher concentrations found in Genoa. For lead, results are also in agreement with those previously reported for the bay of Naples [14].

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