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Heavy Metals and Polycyclic Aromatic Hydrocarbons (PAH's) in Marine Organisms from the Ionian Sea (Italy)

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To evaluate sea water quality along the Italian coastal areas, many studies have been carried out during recent years (Crisetig et al.1985; Giaccio & Coli 1986; Benetti et al.1988; Giordano et al.1991). Information about the Ionian Sea, however, remains sparse.

The Ionian Sea, along the southern coast of Italy, is adjacent to the regions of Puglie, Basilicata and Calabria. Although tourism is important in the coastal regions, the major industrial centers are located on the Ionian coast, at Crotone, on the eastern coast of Calabria, and at Taranto, in Puglie.

In the last few years, particularly along the coast north of Crotone , industrial activities have increased considerably, and now, in a small area of about 5 square kilometers, between the rivers Esaro and Passovecchio (Fig.1), are located several large chemical and metallurgic plants and a number of smaller factories (food, mechanic, ceramic, firing, building, woodpulp, plastic, etc.). Waste waters from the largest factories and the town of Crotone are treated before flowing into the sea, while those from the small factories flow directly into rivers or the sea.

This study was carried out to evaluate the level of industrial contamination in marine organisms in the Crotone area by measuring metal and polycyclic aromatic hydrocarbon (PAH) concentrations.

MATERIALS AND METHODS

Representative samples of molluscs, shellfish and fish from the Crotone area were randomly collected from November 1989 to December 1990 both <150m (A) and >1500m (B) from the coast of the industrial area and outside the harbour (Figure 1) (Table 1). Specimens of shellfish were hand-collected, and the fish were caught with bow-nets, gill nets and trawls. The specimens were placed in clean polyethylene bags, immediately stored on ice, and taken to the laboratory to be weighed and identified.

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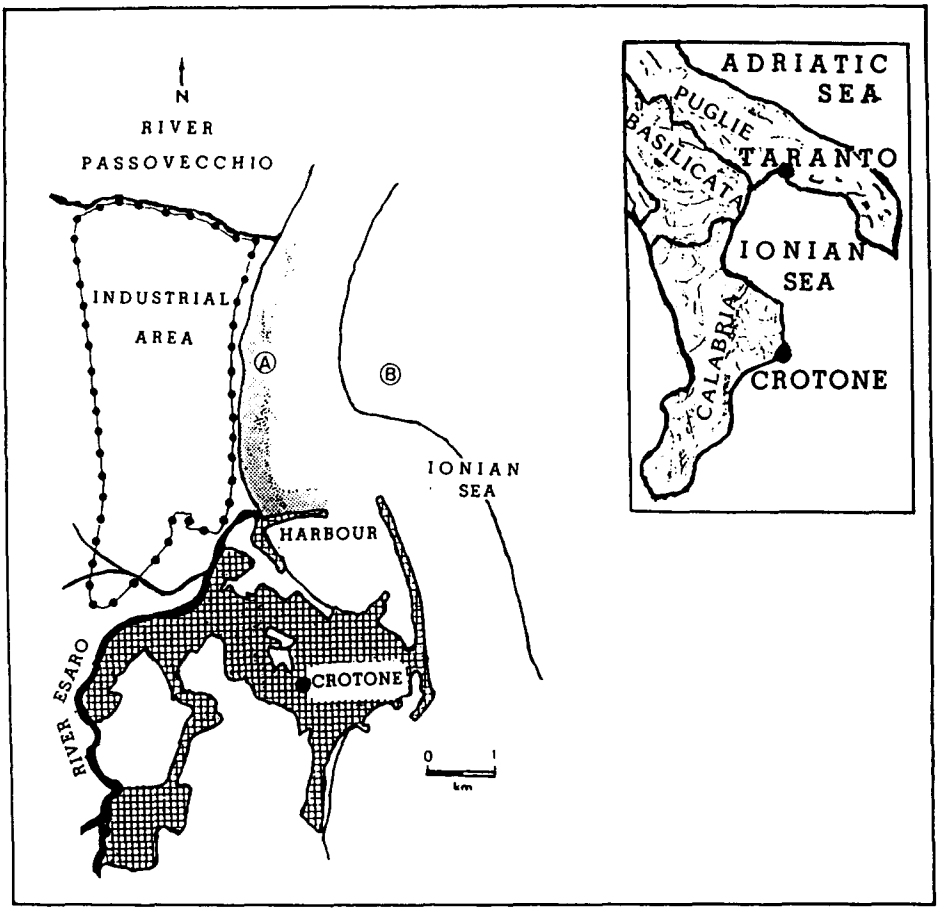

Figure 1. Sampling areas in the Ionian Sea.

Muscle tissue of fishes and octopus and entire body of shellfish were removed and frozen at -20° until analysis.

Analytical samples were prepared as follows: 10 specimens of the same shellfish species were pooled and homogenized; for fishes less than 100 grams 3 specimens of the same species were pooled and homogenized; fishes more than 100 grams and octopus specimens were analyzed individually.

Samples for heavy metals analyses were lyophilized and a portion of the lyophilized sample was dried at 110°C to constant weight for about 24h; 0.5g of lyophilized sample were closed-vessel digested in concentrated nitric acid, allowed to settle for some 8 h, and finally heated at 130-140°C for 1 h. The cooled solution was transferred to Pyrex tubes with teflon closures, diluted with distilled water to 15 ml and analyzed for Cd, Cr and Pb, with a Perkin-Elmer atomic absorption spectrophotometer 1100 equipped with a HGA-300 heated graphite furnace system. Mercury was

Table 1. Characteristics (species, weight, number of samples) of marine organisms analyzed.

Name		zone A			zone B		
Common	Taxonomic	N° of specimens	Average weight (g)	N° of analytical samples	N° of specimens	Average weight (g)	N° of analytical samples
Octopus	*Octopus vulgaris*	4	545	4	5	421	5
Common mussel	*Mytilus galloprovincialis*	160	-	16	-	-	-
Wart venus	*Venus verrucosa*	240	-	24	-	-	-
Grey mullet	*Mugil cephalus*	10	218	10	8	190	8
Rock goby	*Gobius paganellus*	50	30	16	47	18	16
Conger eel	*Conger conger*	6	180	6	5	200	5
Lug brick	*Merluccius merluccius*	6	100	6	6	93	2
Pandora	*Pagellus erythrinus*	5	190	5	6	135	6
Salema	*Boops salpa*	4	306	4	5	360	5
Scorpion fish	*Scorpaena scrofa*	7	370	7	9	282	9
Common sole	*Solea solea*	13	36	4	10	49	3
Streaked weever	*Trachinus draco*	10	70	3	10	37	3
Striped mullet	*Mullus barbatus*	30	120	30	23	189	23

analysed with a MHS-10 mercury/hydride system (Medina et al. 1986). Several blanks were included with the reagents used to check for possible contamination. For quantitation of Cd, Cr and Pb, the standard addition method was applied, while Hg concentration was calculated by direct comparison with a standard calibration curve (Normex AAS heavy metal solutions, Farmitalia Carlo Erba). The minimum detectable levels, in mg kg^{-1} wet weight, were: 0.0025 (Cd); 0.025 (Cr); 0.05 (Pb); 0.01 (Hg). Experiments undertaken with heavy metal standard fortified samples showed recovery rates of 78% for Pb and Cd, 82% for Cr and 74% for Hg.

For PAH analyses 30-50 g of the original sample homogenates were weighed and digested in an alkaline mixture (200 ml ethanol, 35 ml 50% aqueous KOH - 2 g $Na_2S.9H_2O$) refluxing on water bath 2h; extracted, after cooling to 40°C, by water - n-hexane (1:1 v/v) three times; n-hexane combined extracts were dried over anhydrous Na_2SO_4 and concentrated by amber evaporator to ca 3-5 ml. Purification of the samples was carried out by a silica-gel clean up column (Takatsuki et al. 1985). HPLC analyses were carried out using a Beckman Liquid Chromatograph equipped with a Model 165 variable wavelength UV detector at 254 nm, a Shimadzu C-R3A calculator-integrator and a SupelcoSil LC-PAH 25 cmx4.6 mm column. Elution was carried out at a flow-rate of 2 ml min^{-1} using a gradient of mobile phase acetonitrile-water starting from 35:65 (V/V) to 100% acetonitrile over 14 min. Sixteen PAHs were investigated: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, indeno(1.2.3-cd)pyrene.

Experiments undertaken with PAH standard fortified samples showed a recovery rate ranging between 55% and 95%, with an average of 86%; detection limits varied from 1 mcg kg^{-1} (anthracene) to 20 mcg kg^{-1} (acenaphthylene) of wet weight. PAH concentrations were determined comparing the peak area obtained from the samples with those obtained from the reference standards (PAH mixture 610-M Supelco Inc., Bellefonte, PA, USA).

Results are expressed as mg kg^{-1} ww for heavy metals and mcg kg^{-1} ww for PAH's, and corrected on the basis of their recovery rate. Results are summarized as the average of the concentrations of pollutants found in samples from either A or B zones. The range was included only for the highest mean values computed on a number of analytical samples more than 4.

RESULTS AND DISCUSSION

Concentrations of heavy metals and total PAH's by species are shown in Table 2, while Table 3 shows the prevalence of positives and average concentration for each PAH.

All the analytical samples showed measurable concentrations of heavy metals.
Cadmium concentrations in fish specimens collected at <150 m (A)

Table 2. Mean concentrations of heavy metals (Cd, Cr, Pb and Hg mg kg^{-1} wet weight) and total PAH's (mcg kg^{-1} wet weight) (range in brackets) in marine organisms from zones A and B in the Ionian sea (Italy).

Name	Cd		Cr		Pb		Hg		Total PAH's	
	A	B	A	B	A	B	A	B	A	B
Octopus	0.03	0.02	0.21	0.35	0.27	0.47	0.09	0.10	129 (92-146)	28
Common mussel	0.65 (0.28-0.99)	-	0.44 (0.20-0.63)	-	7.60 (5.80-11.90)	-	0.01	-	580 (256-920)	-
Wart venus	0.57 (0.22-0.86)	-	0.45 (0.15-0.73)	-	1.05	-	0.08	-	37	-
Grey mullet	0.01	0.01	0.70 (0.30-1.05)	0.27	0.19	0.20	0.11	0.08	40	77
Rock goby	0.01	0.01	0.08	0.47	0.22	0.07	0.15 (0.05-0.35)	0.05	86	96
Conger eel	0.07	0.03	0.79 (0.42-0.98)	0.34	0.34	0.40	0.07	0.09	53	84
Lug brick	0.02	0.02	0.63	0.49	0.08	0.10	0.04	0.14 (0.10-0.24)	77	88
Pandora	0.01	0.03	2.20 (0.91-4.10)	0.22	1.48 (0.56-2.25)	0.18	0.15 (0.06-0.22)	0.07	86	340 (110-442)
Salema	0.01	0.06	0.31	0.62	0.18	0.39	0.08	0.04	120	37
Scorpion fish	0.01	0.06	0.36	0.85 (0.19-1.45)	0.32	0.39	0.12	0.11	72	61
Common sole	0.01	0.02	0.58	0.59	0.10	0.07	0.18	0.07	48	28
Streaked weever	0.03	0.01	0.84	0.24	0.88	0.18	0.07	0.12	22	86
Striped mullet	0.01	0.02	0.50	0.77	0.10	0.49	0.08	0.05	25	128 (80-195)

and >1500 m (B) from the coast were quite similar, varying between 0.01 and 0.07 mg kg^{-1} (wet wt). Cadmium concentrations in fish were lower than those reported by Arru et al. (1979), Giaccio & Rabitti (1982), Tesei & Nana (1984), Sapunar et al. (1989). In shellfish, concentrations of 0.65 and 0.57 mg kg^{-1} (wet wt) in the common mussel and wart venus, respectively, were considerably higher than that found in common mussel coming from other sites of Calabria and Puglie (Giordano et al. 1991), and from the Marche coast in the Adriatic sea (Benetti et al. 1988).

Chromium concentrations varied between 0.08 and 2.20 mg kg^{-1} (wet wt) in samples from (A) and between 0.22 and 0.85 mg kg^{-1} in those from (B). On average, grey mullet, conger eel. pandora and streaked weever had higher Cr concentrations when collected near the coast. Cr concentrations were much higher than those found by Giaccio & Cichelli (1985) and by Giaccio et al. (1987) in fish (<0.05-0.60 mcg g^{-1}) and shellfish (<0.05-0.45 mcg g^{-1}) from Tremiti Islands in the Adriatic Sea.The average Cr concentration in common mussel, 0.44 mg kg^{-1} (wet wt), was lower than that determined in mussel from the eastern coast of Greece (Nicolaidu & Nott 1990), but similar to findings of Medina et al. (1986) on shellfish collected along the Mediterranean coast of Spain.

Except in the common mussel, Pb concentrations were in the range of 0.08 - 1.48 mg kg^{-1} (wet wt) in samples from site A and between 0.07 and 0.49 mg kg^{-1} (wet wt) from B. In three species, rock goby, pandora and streaked weever, Pb concentrations in specimens from A were considerably higher than those obtained from B. These results agree with data from other surveys carried out in the Mediterranean sea (Giaccio & Rabitti 1982; Tesei & Nana 1984; Medina et al. 1986; Giordano et al. 1991). In the common mussel the average Pb concentration, 7.60 mg kg^{-1} (wet wt), was quite similar to those found in the Gulf of Genoa by Giordano et al. (1991) and the Valencia coast (Spain) by Medina et al. (1986).

Mercury concentrations, ranging between 0.01 and 0.18 mg kg^{-1} (wet wt), were higher in rock goby, pandora, salema and common sole from A. Our data for shellfish agree with those found at other sites of the Italian coast, but are lower, both for shellfish and fish, than the average concentration of mercury in the Mediterranean, estimated at 0.3 mg kg^{-1} (wet wt) (Unep 1987). All values were also lower than the Italian limits for fish (0.70 mg kg^{-1}, wet wt) consumption.

Total PAH levels were in the range of 22-580 mcg kg^{-1} and 28-340 mcg kg^{-1} (wet wt) from zones A and B, respectively. All the analytical samples had measurable concentrations of at least one PAH, except acenaphthene and benzoperylene. Common mussel had the highest concentration (580 mcg kg^{-1} wet wt). The greatest differences between the A and B specimens were found in octopus, pandora, salema, streaked weever and striped mullet. The large variability in values among the various species confirms a previous survey carried out in the Gulf of Naples (Amodio-Cocchieri et al. 1990), in which PAH concentrations detected in common mussel were very low while in wart venus and in fish were

noticeably higher than those found in the present study.
With regard to the distribution and relative concentrations of the sixteen compounds investigated (Table 3), phenanthrene and anthracene were detected more frequently (65 - 75% of the specimens, respectively), but their concentrations were quite low (13 - 17 mcg kg^{-1}, wet wt); acenaphthylene was present in 24% of the specimens and showed the highest concentration within the range 25 - 525 mcg kg^{-1} (wet wt), with an average concentration of 135 mcg kg^{-1} wet wt; acenaphthene and benzoperylene were never detected; benzo(a)-pyrene was present in 16% of the samples at concentrations from 5 to 79 mcg kg^{-1} (wet wt) with an average of 17 mcg kg^{-1}, which is similar to that found in a previous survey (Amodio-Cocchieri et al. 1990).

Table 3. Frequency and concentrations of PAH's in analytical samples of marine organisms from the Ionian Sea (Italy).

PAH's	Positive N	Positive %	Concentration (mcg kg^{-1} wet weight) *mean ± s.d.	range
Naphthalene	4	2	46 ± 38	20 - 55
Acenaphthylene	53	24	135 ± 143	25 - 525
Acenaphthene	0	0	-	-
Fluorene	26	12	7 ± 5	3 - 14
Phenanthrene	143	65	13 ± 9	1 - 42
Anthracene	165	75	17 ± 8	2 - 34
Fluranthene	18	8	38 ± 42	10 - 100
Pyrene	4	2	50 ± 47	45 - 68
Benzo(a)anthracene	22	10	33 ± 41	5 - 100
Crysene	37	17	13 ± 8	1 - 25
Benzo(b)fluranthene	37	17	20 ± 43	1 - 133
Benzo(k)Fluranthene	53	24	26 ± 18	7 - 62
Benzo(a)pyrene	35	16	17 ± 25	5 - 79
Dibenzoanthracene	9	4	29 ± 26	25 - 33
Benzoperylene	0	0	-	-
Indeno(1.2.3cd)pyrene	40	18	84 ± 63	7 - 183

* Computed for positive samples only

According to our results contamination by heavy metals and polycyclic aromatic hydrocarbons (PAH's) in the majority of analyzed marine organisms living along the coast of Crotone is quite low and, in most cases, not different from that found in the same species collected in the open sea. In the common mussel, which is considered one of the best biological markers in defining water pollution, however, the concentrations of cadmium, lead and PAH's were similar to those found in places classified as moderately polluted (Pancirov & Brown 1977; Iosifidou et al. 1982; Rainio et al.1986; Giaccio et al. 1987). This observation suggests the need for an increasing effort in controlling sources of pollution in the Ionian Sea.

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