International Council for the Exploration of the Sea.

CM 1979/E : 14
Marine Environmental Quality Committee

Phenolic compounds in the water and marine organisms off the Belgian coast.

Monique Baeteman and W. Vyncke.
Ministry of Agriculture, Fisheries Research Station, Ostend, Belgium.

Abstract.

Phenolic compounds were determined in water and in Asterias rubens, Ophiura spp., Macropipus holsatus, Pleuronectes platessa, Merlangius merlangus and Sprattus sprattus during an eight months' survey.

Individual data for sea-water varied between 2 and 15 µg/l with an average of 6 µg/l. Average values in marine organisms ranged from 46 to 90 µg/kg, reflecting the influence of pollution in coastal waters. Indeed, results of control analyses carried out on fish from Arctic waters were below the determination limit of 5 µg/kg.

Résumé.

La teneur en composés phénolés a été déterminée dans l'eau et dans Asterias rubens, Ophiura spp., Macropipus holsatus, Pleuronectes platessa, Merlangius merlangus et Sprattus sprattus pendant une période de huit mois.

Les concentrations dans l'eau de mer variaient entre 2 et 15 µg/l avec une moyenne de 6 µg/l. Les teneurs moyennes des organismes marins se situaient entre 46 et 90 µg/kg, reflétant l'influence de la pollution dans les eaux côtières. En effet, les résultats d'analyses effectuées sur du poisson de l'Arctique étaient inférieurs à la limite de détermination de 5 µg/kg.

Introduction.

Phenolic compounds are found as contaminants in almost all surface waters, including marine coastal waters. Potential sources of these substances include industrial waste water effluents (16) and municipal sewage (9). In addition the natural decomposition of organic matter may give rise to phenolic metabolites (8).

Phenolic substances were shown to be responsible for the tainting of fresh water fish flesh and, at higher concentrations, may provoke a toxicological responce in certain aquatic organisms (4) (15). The average lethal concentration LC_{50}^{24} of phenol was 21,5 mg/l for grey mullet (Mugil saliens) (20) and 8,2 mg/l for rainbow trout (Salmo gairdneri) (13). Gill and digestive tract epithelia of clams were damaged at concentrations of 1 mg/l for 24 h or longer (5). Data on sublethal effects of phenol are scarce. Decreased numbers of erythrocytes, lower amounts of serum proteins, lesions in gills, degenerative changes in skin, intestines, muscles and liver werd recorded in several fresh water species (10) (15). Avoidance reactions of fish to phenols were also studied by several authors but controversial results were obtained (15).

As industrial wastes containing 1,5 % phenols are discharged regularly off the Belgian coast (figure 1) a survey of the phenol content of the sea water and representative marine organisms was carried out.

Materials and methods.

- Samples.
- water : water samples were taken at 1 m from the bottom and kept untreated in ice.
  They were analyzed within 24 h.

- organisms : six marine organisms characteristic for Belgian coastal waters were taken (table 1). With the exception of plaice and whiting, where only the muscle tissue was analyzed, whole animals were taken for the phenol determination. At least five organisms were taken to obtain one combined sample. The samples were kept frozen at -30° C until analysis.

- Determination of phenolic compounds.

The colorimetric 4-aminoantipyrine method as outlined by APHA (1) was used with some modifications. With sea-water, 150 ml samples were treated with 1 ml phosphoric acid 8,5 % and 5 ml copper II sulfate 10 % and steam-distilled into 10 ml of ammonium chloride solution 5 % until 500 ml distillate was obtained. The colorimetric reaction was carried out on this distillate. Measurement was made at 460 nm with a spectrophotometer Hach DR/EL 2 (Hach Chemical Company, Ames, Iowa, U.S.A.) with long path attachment using a 5 cm (15 ml) chloroform layer.

With marine organisms the same procedure was used taking 25 g of minced material.

The results were expressed as the equivalent of phenol in µg/l or µg/kg.

Recovery tests gave an average of 93 % with a standard deviation of 4,8 %.

The determination limit was calculated on the blanks according to the method of Gabriels (6) and was 5 µg/kg for organisms and 1 µg/l for water.

- Procedure.

Sea-water and marine organisms were taken from two areas off the Belgian coast whilst carrying out a bimonthly physico-chemical and biological monitoring programme on sand extraction sites (Western area) and dumping grounds for industrial wastes (Eastern area) (figure 1).

Ten samples of marine organisms were taken at random in each area during an eight months' period (september 1978-april 1979). Nine water samples were taken three times during the same period in each area on fixed locations (figure 1).

- Results.

Table 1 - Phenolic compounds in marine organisms from Belgian coastal waters (µg/kg) (a).

Organism	Western area			Eastern area		
	Average (b) (n=10)	s	v (%)	Average (b) (n=10)	s	v (%)
Sea star (Asterias rubens)	51(2)	33,2	65	46(3)	29,0	63
Brittle star (Ophiura spp.)	85(2)	53,5	63	79(3)	73,1	92
Swimming crab (Macropipus holsatus)	76	35,4	47	59(2)	55,4	94
Plaice (Pleuronectes platessa) (0-I year)	74(1)	42,7	58	62	33,7	54
Whiting (Merlangius merlangus) (0-I year)	79	67,1	85	59	39,0	66
Sprat (Sprattus sprattus)	90(1)	60,3	67	63	34,8	55

(a) s = standard deviation ; v = coefficient of variation.

(b) number of analyses under the determination limit of 5 µg/kg in brackets ; these concentrations were considered to be 0 for further calculations.

The average results of the three phenol determinations on water are reported in figure 1. Individual data varied between 2 and 15 µg/l (Western area) and 2 and 13 µg/l (Eastern area). Total averages for both areas were 6 µg/l with standard deviations of respectively 3,1 (W) and 2,4 (E). The difference between these two data was not significant (F-test). The pooled standard deviation was 2,7 corresponding with a variation coefficient of 45 %.

The content of phenolic compounds in marine organisms is reported in table 1. Highest reported value was 225 µg/kg in a sea star. Although average values were higher in the Western area, t-tests showed these differences not to be significant, undoubtedly due to the high standard deviations. Application of the Hartley test (7) showed these deviations not to be significantly different.

- Discussion.

In the water, phenolic compounds could be detected in the whole coastal area under survey. The average value of 6 µg/l is higher than the value reported for sea-water in general i. e. 1-3 µg/l (2) but is low when considering concentrations occurring in some inland surface waters. The values reported here are in accordance with previous Belgian investigations carried out in 1971-75 which showed the phenol content of the sea water to be lower than 13 µg/l from a distance of 6 km from the coast on wards (17).

As most harmful effects on marine life are occurring only with concentrations in the mg/l region (4) (15) damage to living ressources of the sea is rather unlikely. It has moreover been shown that several groups of micro-organisms, especially Pseudomonas and Bacterium, are able to degrade phenol compounds thus preventing excessive accumulation in the water (3) (11) (14) (18) (19). It should also be mentioned in this respect that fish are able to dispose of phenol through biliary excretion from the liver, probably after detoxication by sulfate conjugation (12).

The concentration of phenolic compounds in the marine organisms tested showed important variations. Averages on the other hand were fairly similar, situated mostly around 60-80 µg/kg. Although these values may be considered to be low, they nevertheless reflect the influence of pollution in coastal waters. Indeed, results of control analyses carried out on redfish (Sebastes marinus) and cod (Gadus morhua) from Arctic waters were below the determination limit of 5 µg/kg. It should however be stressed that frequent organoleptic assessments of several fish species caught in Belgian coastal waters did not indicate the presence of flavour-imparting substances in the flesh.

Finally, these investigations allowed to conclude that the dumping of organic waste containing phenols in the area reported in figure 1 did not influence significantly the content of these compounds in the water or in the organisms. Other sources apparently contribute more to the amounts found. It should be stressed that the concentrations mentioned in this paper relate to aminoantipyrine-reactive substances only. The limitations of this method should be taken into account (1).

Literature.

(1) American Public Health Association (1975) : Standard Methods for the Examination of Water and Wastewater, 14th Ed., Washington, D. C. p. 577.

(2) Degens, E., Reuter, J. and Shaw, K. (1964) : Geochim. Cosmochim. Acta 28 (1) 45.

(3) Ermolaev, K. and Mironov, O. (1975) : Mikrobiologiya 44, 928.

(4) European Inland Fisheries Advisory Commission (1973) : Water Res. 7, 929.

(5) Fries, C. and Tripp, M. (1976) : Mar. Fish. Rev. 38 (10) 10.

(6) Gabriels, R. (1970) : Anal. Chem. 42, 1439.

(7) Hartley, H. (1950) : Biometrika 37, 308.

(8) Hoak, R. (1962) : Int. J. Air Water Pollut. 6, 521.

(9) Hunter, J. (1971) : in : Organic Compounds in Aquatic Environment, S. Faust and J. Hunter (Ed.), Marcel Dekker, Inc., New York.

(10) International Council for the Exploration of the Sea (1978) : On the Feasibility of Effets Monitoring, Cooperative Research Report No. 75, ICES, Copenhagen.

(11) Kaplin, V., Semenchenho, L. and Ivanov, E. (1968) : Gidrokhim. Mater. 46, 199.

(12) Kobayashi, K., Akitake, H. and Kimura, S. (1976) : Bull. Jap. Soc. Sci. Fish. 42, 45.

(13) McLeay, D. (1976) : J. Fish. Res. Board Can. 33, 1303.

(14) Mikryakova, T. and Klajn, N. (1975) : Tr. Inst. Biol. Vnutr. Vod (30), 130.

(15) Mitrovic, V. (1972) : in : Marine Pollution and Sea Life, M. Ruivo (Ed.), Fishing News (Books) Ltd, London.

(16) Nemerow, N. (1971) : Theories and Practices of Industrial Waste Treatment, Addison - Wesley Publ. Comp., Reading, Mass., USA.

(17) Nihoul, J. and Boelen, C. (Ed.) (1975) : Niveaux de pollution du réseau hydrographique et de la zône côtière belges - Projet Mer, Rapport Final, Vol. 11C, Services du Premier Ministre, Bruxelles.

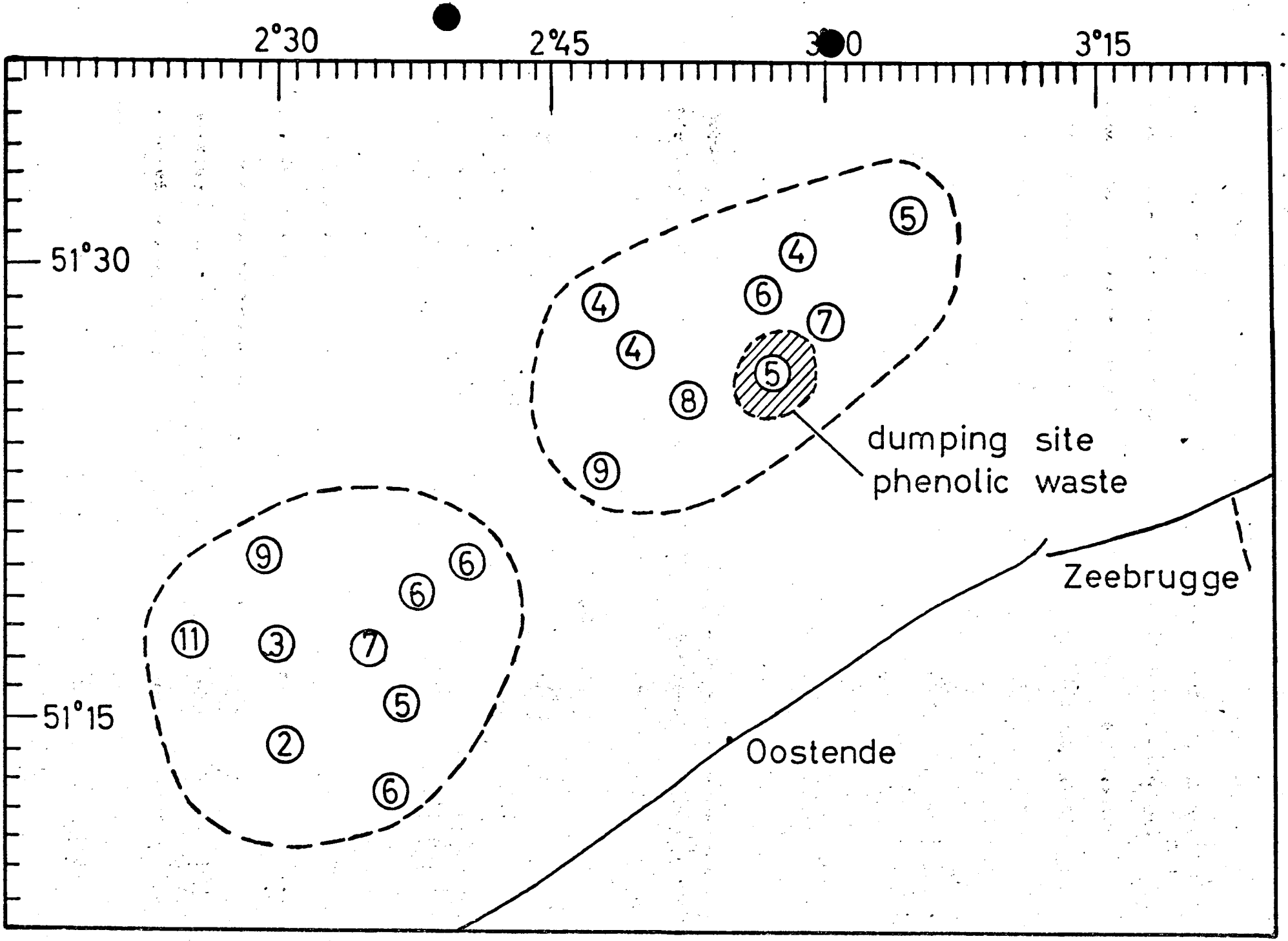
(18) Polisois, G., Tessier, A., Campbell, P. and Villeneuve, J. (1975) : J. Fish. Res. Board Can. 32, 2125.

(19) Thielemann, H. (1975) : Acta Hydrochim. Hydrobiol. 3, 191.

(20) Zambriborshch, F. (1977) : Gidrobiol. Zh. 13(2), 91.

Legend to fig. 1.

Western and Eastern sampling areas showing the average concentrations of phenolic compounds in the water (in µg/l).


2°30
2°45
3°15
51°30
51°15
dumping site
phenolic waste
Zeebrugge
Oostende