Occurence of enteroviruses in the West-Istrian Coastal Area of the Northern Adriactic, Croatia

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Viral contamination of seawater occurs mainly through discharges of sewage effluents into rivers that ultimately find their way to the sea, or through offshore disposal of sewage outfalls.

A control of the presence of viruses in seawater, mussels and sediments collected in the Limski Kanal (mariculture area) and in the touristic centers along the west-istrian coast was performed during the 1989-1990 period. Simultaneously, a sanitary quality control of recreational and mariculture waters as well as mussels was conducted. The concentration of viruses in seawater was done by adsorption in the quartz sand (SCHWARTZBROD and LUCENA, 1978). The elution of viruses from sediment particles was done according to GERBA *et al.* (1977). The minced mussel meat was centrifuged in a buffered solution, followed by a supernatant inoculation on GMK cell line and precipitate reconcentration for hepatitis A virus antigen detection. The enteroviruses level was determined by plaque-assay using GMK cell line (LENETTE and SCHMIDT, 1979). Hepatitis A virus was detected by immune enzyme test (ELISA). The sanitary quality of waters and mussels was assessed according to WHO/UNEP (1977) guidelines.

In water samples only the hepatitis A virus was detected. Meanwhile, in controlled mussels several types of viruses were found (Hepatitis A virus, Coxsackievirus B3, Echovirus types 4 and 11). The following viruses appeared in the sediments: Hepatitis A virus, Coxsackievirus types B2 and B3 and Poliovirus type 3.

Concerning the proportion of virus positive samples (Table 1) waters and sediments from recreational areas were more polluted compared to Limski Kanal area. Meanwhile, a higher viral contamination of mussels was recorded in Limski Kanal area compared to recreational coastal waters.

Table 1. Occurrence of enteroviruses in different matrices.

Study area	Sea water N	(+)	%	Sediments N	(+)	%	Mussels N	(+)	%
Limski Kanal area	27	4	14.8	17	6	35.3	19	6	31.6
Coastal recreational areas	23	7	30.4	33	15	45.5	40	11	27.5

N - number of tested samples, (+) - number of virus-positive samples

Concerning the seasonal aspect of viral contamination the highest number of virus' positive samples was recorded in the mariculture area (35.7 %) as well as in recreational areas (58.3 %) during springtime (Table 2). Due to the karstic type of the Istrian Peninsula and rainy winter-spring period an increased number of virus positive samples during the springtime is expected. The fall increase of viral contamination in recreational areas probably resulted from quadruple effluents enlargement of the touristic centers during the summer-fall period. Most touristic and municipal centers along the coast dispose their domestic wastewaters by traditional methods, which are quite inadequate to destroy viruses. Viruses survive in the marine environment for a prolonged time, especially if protected by particulate organic matter.

Table 2. Enteroviruses occurrence during the seasons of the 1989-1990 period.

Study area	Spring N	(+)	%	Summer N	(+)	%	Fall N	(+)	%	Winter N	(+)	%
Limski Kanal area	28	10	35.7	22	4	18.2	6	1	16.7	7	1	14.3
Coastal recreational areas	12	7	58.3	66	20	30.3	18	6	33.3	-	-	-

N - number of tested samples, (+) - number of virus-positive samples

The most frequent virus detected in water samples was the hepatitis A virus, particularly in Limski Kanal area. Due to the influence of polluted groundwaters from the nearby springs,periodically the internal part of this area did not meet adequate sanitary quality of water and shellfish (FUKS and DEVESCOVI, 1989). A simultaneous sanitary ambient quality control and virus presence detection revealed, that although the water sanitary quality was acceptable according to WHO standards (1983), viruses were detected in the water, mussels and sediments, respectively. The inevitable conclusion, based on an initial screening for detection of enteroviruses, particularly in Limski Kanal, is that the mariculture area is surrounded by potential sources of viral contamination. Undoubtedly, the present finding on the Kanal's utilization for shellfish and fish raising purposes should cause an alarm among all interested and responsible for preventing its contamination. Consequently, water and food sanitary surveillance, besides the present bacterial control, become inadequate. The introduction of virological tests should be considered, especially in cases where the present knowledge indicates that they are indispensable.

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Microbiological investigation of the surface water of the Laguna Veneta (North Italy) in relation to light gas-oil biodegradation

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Pollution by hydrocarbons is a serious problem in the Laguna Veneta (North East Italy) because one of the busiest oil port in Italy is located in this area; also, the lagoon receives polluted waters from an heavily industrialized and populated hinterland (FOSSATO, 1990).

Microbial degradation of hydrocarbons, universally recognized as a natural remediation, is the most important biological mechanism in oil decontamination of marine waters.

The aim of this study has been: 1) to assess the distribution of oil-degrading microorganisms in the lagoon; 2) to evaluate the oil degradative potential of surface waters; 3) to isolate strains with efficient oil degradative activity and 4) to test the hydrocarbon-degrading activity of axenic cultures in laboratory condition.

Surface waters from three different stations from the central area of the lagoon (A,B,C, from outer to inner part) were sampled aseptically in November 1990 and in June 1991 with a Schomaker sampler.

Basic physical and chemical parameters were determined in each station and in each period.

Enumeration of total aerobic heterotrophic bacteria was carried out by direct epifluorescence method (HOBBIE *et al.*, 1977). Oil degrading bacteria were determined by the MPN technique (MILLS *et al.*, 1978) and by the Spread Plate Technique. To isolate oil degrading strains, colonies which were different for colour, size and morphology were streaked twice and stored at -80°C in 25 % glycerol.

The microbial activity was determined by oxygen consumption in presence of hydrocarbons. Water samples from each station with and without 0.2 % of light gas-oil were incubated at 22°C. Oxygen consumption was determined by Winkler's method after 7 and 14 days in November 1990, and after 5, 10 and 15 days in June 1991.

To determine the oil degrading activity, at each station 8 flasks with 200 ml of water were spiked with 0.2 % of light gas-oil. Four flasks were sterilized before this addition. The percentage of oil consumed after 5, 10, 15 and 30 days of incubation at 22°C was determined by FIP gas chromatography, after extraction with n-hexane.

To test the oil degrading activity of axenic cultures, the strains best growing on plates were cultured in flasks until stationary phase, then the consumption of gasoil was determined by gas chromatography. The microbial activity was also tested by oxygen consumption measured by Clark's probe.

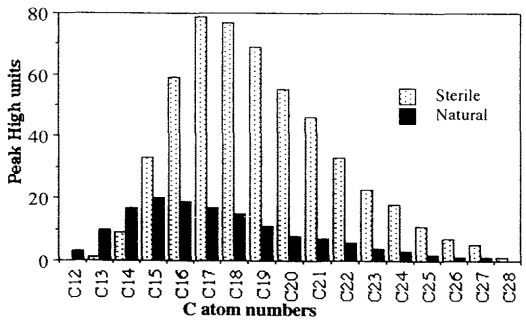
The concentration of heterotrophic and oil degrading bacteria was higher in June than in November. A higher percentage of oil degrading bacteria has been also found in June, probably due to better temperature conditions (ATLAS and BARTHA, 1972). GALASSI and CANZONIER (1977) also found a higher hydrocarbon degrading activity in the Southern part of the Laguna Veneta in warmer season.

The percentage of oil degrading bacteria, considered as a biological index of hydrocarbon pollution (WALKER and COLWELL, 1976) was not significantly correlated with the expected pollution gradient from station A to station C. The threshold of hydrocarbon concentration for induction of oil-degrading activity in bacteria has not been well investigated and no significant correlation has been often found in the environment between the two parameters (LEAHY and COLWELL, 1990).

The oxygen consumption in water samples spiked with light gas-oil was significantly enhanced in all samples, with the highest oxygen depletion in surface water of the station C (June 1991).

The highest oil degradation activity was also found in the same station after 30 days of incubation, resulting in more than 80 % depletion of the n-paraffins (Fig.1).

Fig.1 Degradation pattern of the n-paraffins, determined by gas chromatography, in natural and sterile water samples from the Laguna Veneta after 30 days of incubation at 22°C.



In total, sixty three strains of bacteria were able to grow in presence of oil, the number of isolates being higher in June and mainly in the station C. The strains were mostly gram-negative.

Among the four strains (B1, B4, C3, C4) tested for *in-vitro* light gas-oil degradation, the best hydrocarbon degrading activity has resulted in a depletion of 44.6 % of the gas-oil n-paraffins, i.e. in a consumption of 0.77 µg/mg protein/ml and 5.5 µl $02/10^9$ cells/h by strain C3.

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