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TRENDS OF BACTERIAL CONTAMINATION INDICATORS IN MARINE SEDIMENTS, WATER AND MOLLUSKS FROM MAMAIA BAY – ROMANIAN BLACK SEA COAST

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ABSTRACT

Between 1991-2002, in Mamaia Bay, the contamination level of seawater, sediments and mussel (*Mytilus galloprovincialis*) with heterotrophic anaerobic bacteria, total coliforms and species of genera *Aeromonas*, *Pseudomonas* and *Vibrio* were pursued. Beginning 1996, a general tendency of contamination reduction in marine environment and mussels including those from more contaminated shallow water, compared with offshore ones in the past has been noticed. However, the risk of relatively high bacterial concentration, especially in the marine sediments and mussel flesh, with potential pathogenic germs pertaining to genera *Aeromonas* and *Vibrio* (10,000 germs/sediment, g flesh) keep maintain.

KEY WORDS : contamination, bacteria, species, germs, marine environment, potential pathogenic

INTRODUCTION

In the marine environment, numerous microorganisms exist, such bacteria occupying an important place, with an essential role in trophodynamics, productivity and purification processes of this environment (SOROKIN, 1999).

The bacterial diversity is larger. There are autotrophic and heterotrophic, aerobic and anaerobic, saprophytic and parasitic bacteria, bacteria implicated in nitrogen, carbon, sulfur, iron and manganese cycles. The research performed during the last years evinced new discoveries, such as the activity of anaerobic bacteria which oxidize the ammonium and methane gas, bacteria which degrade the hydrocarbons and bacteria which produce bioactive compounds (GIULIANO, 2003).

The horizontal and vertical distribution of bacteria in the marine environment is directly linked to life conditions, mainly by the salinity, temperature, pressure and food resources.

In the shallow and upper water layers, the number of bacteria is much higher then in offshore and bottom ones. The sediments from the shallow areas are richer in bacteria comparatively with seawater.

The heterotrophic aerobic saprophytic bacteria, which participate in the decomposition of the organic matter, in the first stages, represent a very important group, resulting in interim products, which in their term are taken over by the microorganisms and mineralized up to CO_2 and H_2O. The abundance of heterotrophic saprophytic bacteria represents an indicator of saprobic level in sanitary monitoring of marine environment (SOROKIN, 1999; LUTTERBACH, 2001).

Another very important bacteria group for assessing the environmental quality, health of the marine organisms, and danger for public health comprise the potential pathogenic bacteria. Among them, the coliforms bacteria are one of the main indicators of the environment quality monitoring and of the marine species dedicated for human consumption. Other species pertaining to genera *Aeromonas, Pseudomonas, Vibrio, Nocardia, Cytophaga,* can gravely affect the health of marine organisms, including the bivalve mollusks. In the Romanian coastal waters, as well as in the mollusks (flesh) inhabiting these zones, faecal bacteria (total coliforms, coliform faecal, faecal streptococci), as well as the bacteria of genera *Aeromonas* and *Vibrio* have been occasionally signaled (MIHNEA, 1980; DUMITRESCU, 1985, STOICA, 2002).

The bivalve mollusks constitute a peculiar category of marine organisms, with filtering capacity, once with the fodder elements (plankton), retaining and concentrating in their flesh the environmental pollutants, including bacteria. The bacteria accumulated in the mollusks can cause their

disease, constituting in the same time a real danger to mollusk consumers. Such bacteria are enterobacteria and pathogenic species of genera *Vibrio*, signaled both in marine waters and mollusks for human consumption (PRIEUR, 1990; PREVOT, 1991; ORTIGOSA, 1995; DUMITRESCU, 1998; ARIAS, 1999, PUJALTE, 1999).

Results regarding the bacterial loading of the marine environment and the bivalve mollusks (mussels) in the Mamaia Bay, and its evolution between 1991-2002 are presented.

Mamaia Bay represents a specific zone for the Romanian littoral; the quality oft his marine environment is influenced by the activities developed at Petrochemical Platform Midia (for oil processing) and Midia Port, the tourism and recreational activities in Mamaia resort, the waste water discharges from Constantza Nord Waster Treatment Plant and polluted freshwaters from Tabacarie Lake.

MATERIAL AND METHODS

The determination of the bacterial contamination of the marine environment and mollusks was achieved in Mamaia Bay, situated between Navodari and Constanta, between 1991-2002.

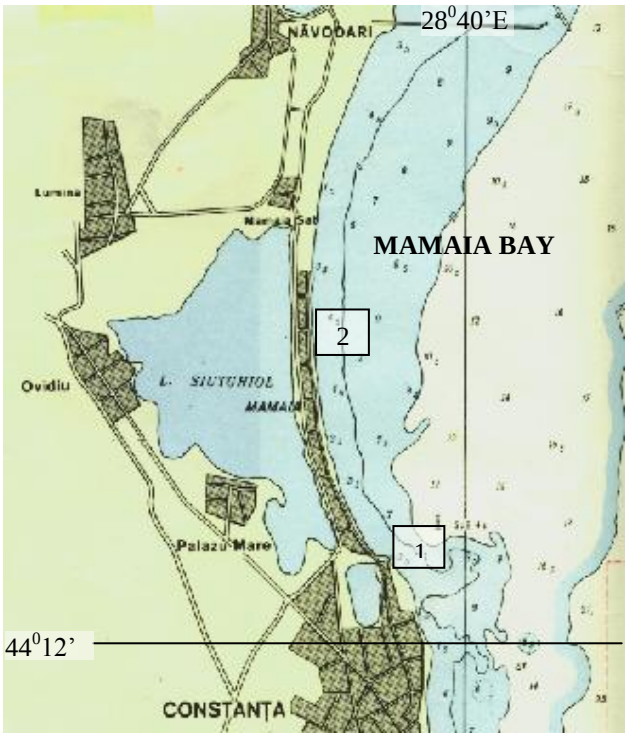

Fig.1 – Sampling station from Mamaia Bay

The water and sediment samples were collected monthly, from April to September, in two near shore sites: first (1)in the vicinity of discharging site of urban wastewater from Constanta Nord Wastewater Treatment Plant; second(2) in front of Casino Mamaia (Fig.1). Offshore, the water and sediment samples from 5, 8 and 10m depths, were collected once a year (June-August).

The mussels (*M. galloprovincialis*), from the southern part of Mamaia Bay, attached on dams and rocky bottoms, were analyzed (from seashore up to 10m depths).

The bacteria groups analyzed were the saprophytic aerobic heterotrophic germs, total coliforms, *Aeromonas, Pseudomonas* and *Vibrio*.

Classical the work methods were used, meaning insemination in dilutions on usual and selective cultural media, the bacteria being identified through their morphological and bio-chemical characteristics in culture (PREVOT, 1961; HOLT, 1985).

The saprophytic heterotrophic aerobic bacteria were identified through inseminations in dilutions on PETRI glasses, on agar peptone, incubation on 22-24^{o}C and read after 24-28 hours.

The total coliforms were determined through multiple pipes, insemination of positive pipes on confirmation medium, and numbering of most probable number, after 24-28 hours of incubation at 37^{o}C.

The bacteria pertaining to *Aeromonas, Pseudomonas* and *Vibrio* were identified through inseminations in dilutions on usual (agar) and selective media (Mac Conkey, Meitert Istrati, Levine, VSA, TGBS), morphologic characteristics and biochemical tests (Gram coloration, oxidase, catalase, sugars, gelatine, indol, lysin, arginine, urea etc.)

RESULTS AND DISCUSSIONS

1. The heterotrophic bacteria densities in the marine waters did not oscillate too much with time (Fig. 2). However, in 1994 and 1998, lower concentrations were determined: the maximum number did not exceed 200,000 germs/ml. In 1991-1992 end 2000-2002, the maximum densities ranged from 500,000 to 850,000 germs/ml.

The sediments were much more loaded with heterotrophic bacteria. The more value was registered in 1991, with 20 mil. bacteria/g sediment, after that the contamination had reduced very much, up to maximum of 2.5 mil. germs/g in 2002 (Fig. 3).

In the mussels flesh, the number of heterotrophic bacteria was much higher then in water and sediments. The maximum contamination was registered in 1991, with 30 mil. germs/g flesh (Fig. 4). In 1992, a high value was registering too - 10 mil. /g flesh, then the contamination level decreased considerably, the values being around 1 mil. germs/g flesh after 1994.

The maximum values of the water, sediment and mussels flesh contamination were determined in Mamaia Bay shallow waters, where the urban waste waters from Constanta Wastewater Treatment Plant and

freshwaters from Tabacarie Lake are discharged. The minimum values occurred in offshore waters, at 10m depths.

This contamination reduction tendency of the marine environment and mussels inhabiting Mamaia Bay was evinced between 1998-2002, when the highest values did not exceed 800,000 germs/ml water, 2.5 mil. /g sediment and 1 mil. /g flesh, owed mainly to reduction in urban wastewaters, as well as to the cleaning of the bathing areas of Mamaia resort. Thus, Mamaia bay shows a tendency from a previously polluted area (1992-1994), to one with a limited pollution level.

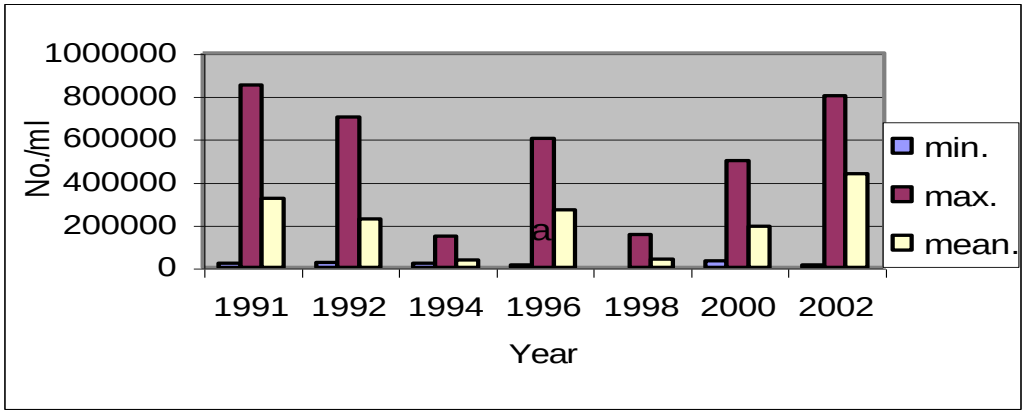

Fig.2 - Contamination with heterotrophic germs of Mamaia Bay water

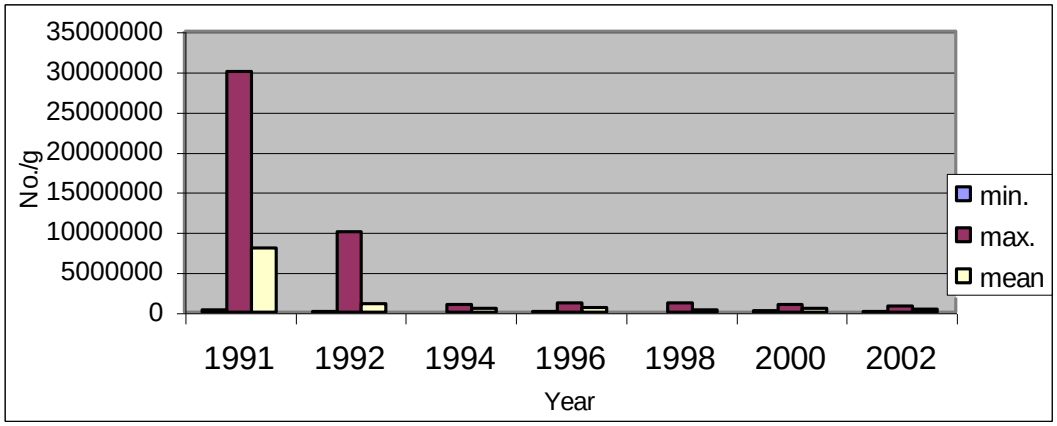

Fig. 3 – Contamination with heterotrophic germs of Mamaia Bay sediments

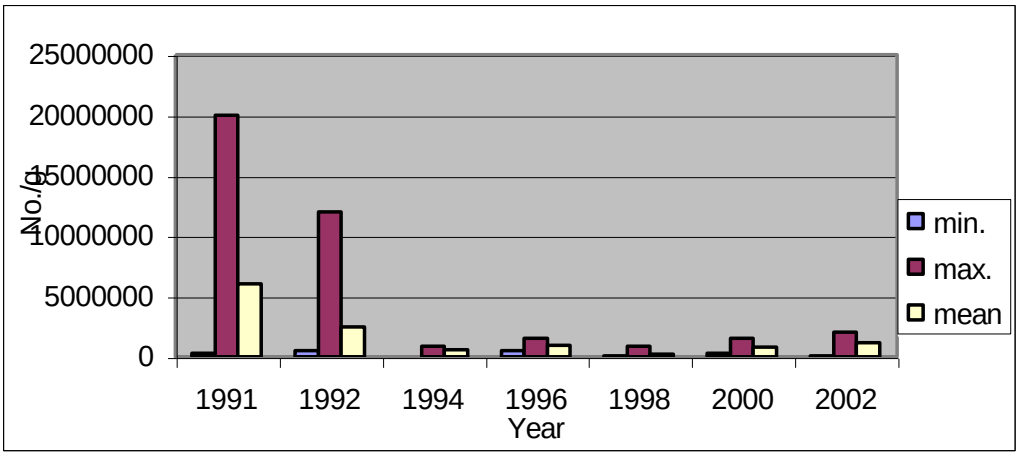

Fig. 4 - Contamination with heterotrophic germs of mussels inhabiting Mamaia Bay

2. Total coliforms were present in seawater, sediments and mussels flesh (Table 1).

In seawater, the maximum number of coliforms reached 1,609 germs/ml, especially between1991-1994. After 1996, it has considerably decreased, so that the value did not exceed 160 germs/ml water in 2000-2002, while the lower ones were up to 2 germs/ml water.

The sediments were 10 times more loaded with coliforms then the seawater. The maximum level was determined between1991-1994, with 16,090 germs/g; then, a substantial diminishing was noted in 2002, up to 348 germs/g sediment.

Beginning 1996, the minimum content of coliforms was 24 germs/g, except 2000 with 911 germs/g.

The mussels accumulated concentrations of total coliforms up to 16,090 germs/g flesh. Although the presence of coliforms was significantly lower after 1994, the mussels contamination was maintained at risky levels for consumer's health. In 2000 and 2002, the mean values were high, 636 and 170 germs/g flesh, respectively. The lower values of 50 germs/g, for 1991-2002 period, pointed out that, in this area, there were periods and locations where the mussels were suitable for human consumption.

The highest values of the total coliform concentrations were identified in seawater, sediments and mussels from the zone up to 50m depths; the lowest ones were registered offshore.

Table 1

Contamination with total coliforms of Mamaia Bay waters, sediments and mussels

No.	Year/Value		Water no/ml	Sediments no/g	Mussels no/g
1	1991	Max.	**1609**	**16090**	**16090**
		Min.	75	120	20
		Mean	823	8613	620
2	1992	Max.	**1609**	**16090**	**16090**
		Min.	91	43	50
		Mean	819	7951	652
3	1994	Max.	**1609**	**16090**	**16090**
		Min.	0	33	0
		Mean	771	8352	523
4	1996	Max.	**900**	**3480**	**1609**
		Min.	40	24	46
		Mean	621	2388	578
5	1998	Max.	**1609**	**1720**	**1609**
		Min.	0	24	2
		Mean	813	489	320
6	2000	Max.	**160**	**3480**	**1609**
		Min.	0	911	54
		Mean	61	1726	636
7	2002	Max.	**160**	**348**	**430**
		Min.	2	9	16
		Mean	57	124	170

3. Bacteria pertaining to *Aeromonas, Pseudomonas* and *Vibrio* genera were identified in more concentrations in sediments and mussels flesh like the heterotrophic bacteria and total coliforms (Table 2).

The maximum values in sediments were registered in 1991, 1992 and 1994 up to 26,000 germs *Aeromonas*/g, 10,000 germs *Pseudomonas*/g, and 12,000 germs *Vibrio*/g. In the same years, in mussels flesh, the concentrations reached 40,000 germs *Aeromonas*/g, 10,000 germs *Pseudomonas*/g and 100,000 germs *Vibrio*/g. Under these circumstances, the mussels presented signs of being taken ill, manifested through wounds on their gills, mantle and hepatopancreas. Death mussels were found near the shores.

Beginning 1996, the *Aeromonas* and *Vibrio* bacteria got reduced step by step, so that in 2000 and 2002 they did not exceed 1,500/ml water and 1,000/g sediment and flesh.

Table 2

Contamination of marine environment and mussels flesh in Mamaia Bay

No./ml water; g sediment; g mussel flesh

No.	Year - Value		Aeromonas			Pseudomonas			Vibrio		
			Water	**Sediments**	**Mussels**	**Water**	**Sediments**	**Mussels**	**Water**	**Sediments**	**Mussels**
1	1991	Max.	**20000**	**50000**	**100000**	**5000**	**1000**	**1500**	**30000**	**12000**	**10000**
		Min.	2000	2000	2000	500	0	100	1000	100	100
		Mean	8500	26000	30000	1500	500	600	12500	4200	3400
2	1992	Max.	**30000**	**15000**	**20000**	**5000**	**2000**	**4000**	**55000**	**120000**	**100000**
		Min.	3300	0	0	600	0	0	1700	0	0
		Mean	9616	6500	8900	1835	800	1100	14550	15500	13007
3	1994	Max.	**1500**	**10000**	**40000**	**500**	**10000**	**10000**	**3000**	**10000**	**30000**
		Min.	0	0	0	0	0	0	0	100	500
		Mean	344	3366	8333	83	1666	1666	700	5666	8750
4	1996	Max.	**1000**	**5000**	**2000**	**100**	**3000**	**500**	**1000**	**5000**	**5000**
		Min.	50	100	100	0	0	0	0	100	100
		Mean	300	1033	730	50	1000	200	200	2400	1450
5	1998	Max.	**10000**	**2000**	**2500**	**5000**	**500**	**600**	**2500**	**2500**	**3000**
		Min.	10	100	100	0	0	0	10	100	100
		Mean	1905	800	850	871	250	300	4000	1150	1200
6	2000	Max.	**100**	**1500**	**5000**	**10**	**100**	**100**	**300**	**4000**	**8000**
		Min.	0	500	100	0	50	0	50	300	50
		Mean	70	1000	1800	3	65	33	133	1930	2500
7	2002	Max.	**1500**	**5000**	**3000**	**500**	**1000**	**1000**	**1000**	**10000**	**10000**
		Min.	50	50	500	50	50	100	100	100	500
		Mean	516	2000	500	216	366	533	530	5033	4160

As for the *Vibrio* bacteria, although their concentrations have diminished between 2000-2002 comparatively with 1991-1994, the levels maintained still high, up to 1,000 germs/ml water and 10,000 germs/g sediment and flesh. The mean values assessed especially for 2002 (530 bacteria/ml water, 5,033 bacteria/g sediment and 4,160 bacteria/g flesh) reveal that *Vibrio* bacteria were frequently present in high number, both in Mamaia

Bay seawater and mussels inhabiting these waters. The situation must be an alarming signal for specialists who monitor the health status of marine mollusks from the Romanian littoral, and the quality of the mollusks designed for human consumption.

However, the reduction in bacterial contamination of the marine environment and mollusks as well, from a significantly polluted zone, emphasizes the slight tendency toward improvement of environment quality, at the Romanian littoral. This tendency was noted also at other levels of the ecosystem, through the nutrients, heavy metals, organochlorine pesticides concentration reduction, rehabilitation of some biota with ecological and economic importance.

The concentration of *Aeromonas* and *Vibrio* bacteria in still high, as well as that of the faecal bacteria, especially in sediments and mussels flesh this improvement is therefore still fragile, and a much longer period is necessary to reach a stable equilibrium of the marine environment quality in this area.

CONCLUSIONS

1. Saprophytic aerobic heterotrophic bacteria, total coliforms and *Aeromonas, Pseudomonas* and *Vibrio* bacteria were identified in the marine environment (water and sediments) and mussels from the Mamaia Bay between 1991-2002.

2. In shallow waters, up to 5 m depths, concentrations of bacteria in the seawater, sediments and mollusks flesh were 10-100 times higher then in offshore ones, at 10m depts.

3. The bacterial concentrations were frequently 10 times higher in sediments and mussels flesh then in marine water.

4. After 1996, a general tendency of reduction in bacterial concentration of seawater, sediments and mussels in Mamaia Bay, was put in evidence.

5. Although the contamination has been significantly reduced in Mamaia Bay, leading to an improvement in the environment quality, the risk for public health keeps maintained, due to a still high contamination with potential pathogenic germs of *Aeromonas* and *Vibrio* genera, especially in sediments and flesh mussels flesh.

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