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ECOLOGICAL CHARACTERISTICS OF SEAWATER INFLUENCED BY SEWAGE OUTFALL

Patricija MOZETIČ, Vlado MALAČIČ & Valentina TURK
National Institute of Biology, Marine Biological Station, SI-6330 Piran, Fornače 41

ABSTRACT

The impact of sewage discharge from the treatment plant at Piran through submarine outfalls approx. 3500 m off the coast was studied. During the years 1998-1999 six surveys of oceanographic parameters, nutrient concentrations and faecal coliforms were carried out at the central station between the two outfalls and at the reference station. The spreading of the sewage in the water column was detected through observing small vertical salinity oscillations (~0.1 PSU) in layers of thickness less than 1 m, a few meters above the bottom. Sewage dispersal was confirmed by the presence of faecal coliforms. Their number increased (max. 1160/100 ml) in the layers with highest salinity oscillations. The ecological impact of submarine outfalls on the surrounding environment was highest in the case of ammonium, while for other nutrients the impact was almost negligible. This was confirmed by the relatively high correlation between faecal coliforms and ammonium (ρ=0.58) in the near-field sewage plume, while there was no correlation (ρ=-0.05) between these properties in the sewage at the treatment plant's outlet. The reference station proved to be unsuitable for comparison due to periodic bacterial contamination.

Key words: sewage, submarine outfall, faecal bacteria, nutrients, coastal sea, Gulf of Trieste

INTRODUCTION

Near-shore marine areas are susceptible to many different land-based sources of pollution, among which direct, untreated wastewater discharge seems to be one of the main causes of increased eutrophication. This problem is even more evident in coastal areas where economically important activities, such as tourism and aquaculture, set high standards of seawater quality.

Many legislative acts have been successfully implemented in order to control the quality of the water body receiving municipal wastewaters and for the protection of human health. Slovenia started to monitor some pollution parameters within the frame of UNEP/MED-POL Program Phase II (Tušnik *et al.*, 1989) in 1983. Among several "hot spots", referred as land-based sources of pollution, sewage effluents at treatment plants were analysed from the very beginning.

In comparison to this well-developed net of pollution control on the land, the seawater in the vicinity of sewage discharges along the Slovenian coast did not gain such attention. Up to now, the majority of research work concerning the effect of sewage dispersal in the marine environment was focused on the Piran sewage outfall (Gulf of Trieste, Adriatic Sea). The municipality of Piran, which is one of the most touristically developed Slovenian areas, solved the problem of sewage disposal in the late 70's by constructing a submarine sewage outfall 3450-m off the coast. Eleven years later, in 1987, another submarine outfall was constructed parallel to the old one at a horizontal distance of less than 200 m and 3250 m off the coast (Vuković & Malačič, 1997). A mechanical treatment plant produces a sludge that is periodically removed from digestion tanks to an onshore dumping-ground, and an effluent, that is discharged by gravity through the outfalls into a depth of 21 m (Malej, 1980; Malačič, 1997). The old and new outfalls terminate with diffusers that are 108 m and 185 m long, respectively. They are placed 1 m above the bottom and are drilled alternately with 11 to 17 lateral holes on both sides of the pipes. Up to now these diffusers are the only ones in Slovenian waters. Average effluent flow is about 10360 m^3 per day and may increase during the season due to increased population (D. Kleva - Švagelj, *pers. comm.*).

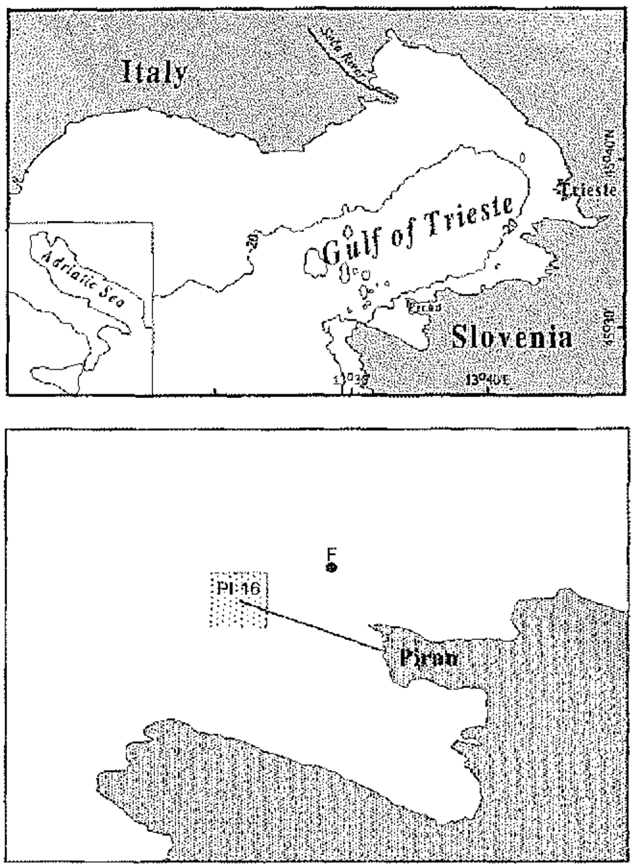


Fig. 1: Location of the Piran submarine outfall, station grid and sampling stations in the southern part of the Gulf of Trieste. Sampling station PI-16 is located in the centre of the station grid.
Sl. 1: Lokacija piranskega podvodnega izpusta, mreže postaj in vzorčevalnih postaj v južnem delu Tržaškega zaliva. Postaja PI-16 leži v sredini mreže postaj.

Early studies comprised an overview of environmental conditions in the water column and at the sea floor before (1974/75) and after (1978/79) the construction of the first submarine outfall (Avčin *et al.*, 1978; Malej, 1980; Faganeli, 1982). The authors reported a minimal effect of the outfall upon the surrounding marine ecosystem, suggesting a very rapid dispersal of the effluent and thus the high effectiveness of the Piran treatment plant. No studies were done after the construction of the second outfall until recently.

In the last three years an extensive project has been undertaken in order to analyse the dispersion of the sewage plume from the Piran submarine outfall (Malačič, 1997, 1998; Vukovič & Malačič, 1997). The dynamics of sewage plume dispersion is controlled by stratification, shear currents, turbulence and the Coriolis force. Nutrients and faecal bacteria were occasionally monitored along with oceanographic parameters at the central station located between the two diffusers.

The purpose of this paper is to estimate the extent of sewage pollution in the water column based upon ecological parameters, and to extract the most representative parameters (*i.e.* indicators) of such pollution. Furthermore, we'll try to compare these new data with the data collected 20 years ago in order to assess possible deterioration of the marine environment and the effectiveness of the sampling strategy designed for such studies.

MATERIALS AND METHODS

Sampling strategy and field survey

Sampling was carried out at station PI-16 which is located between the submarine outfalls of Piran and at station F (Fig. 1) which is defined as the reference station. The central station PI-16 was chosen from a grid of 31 stations (Malačič, 1999a). During the years 1996-1998 several oceanographic surveys of the water column were undertaken. However, only four samplings from 1998 are presented in this paper. Biological and chemical parameters were sampled in addition to the CTD casts (conductivity, temperature, depth). An additional two samplings were performed in 1999. Surveys, labelled from 1 to 6, correspond to the following days: 2 April 1998 (1), 18 June 1998 (2), 31 August 1998 (3), 12 October 1998 (4), 24 May 1999 (5), and 21 July 1999 (6). Samplings were performed in calm weather and during low tidal currents - the tidal range of sea-surface elevation (peak-to-peak) was less than 0.6 m in the port of Koper.

The bottom at stations PI-16 and F is 21 m deep. The number and position of sampling depths varied from one sampling to another, and were defined each time separately with regard to the vertical salinity profile of station PI-16. The spreading of the sewage plume was detected from slightly lower salinity (for about 0.1 PSU) in thin layers within the water column. The number of sampling depths varied from 9 to 14, while the sampling depth interval was smaller than 2 m in the layers with slightly lower salinity. The reference station F was sampled only at a few depths, the number of which did not exceed five - the number of standard oceanographic depths (0.5, 5, 10, 15 m and just above the bottom). The exception was Survey 1 when sampling depths at station F corresponded to those at station PI-16.

Information about the input load was gained from the bacteria and nutrient analysis of sewage samples that were collected at the outflow of the treatment plant before each survey. The samples were collected about three to four hours before the sampling at station PI-16. It takes about this amount of time for the sewage to reach the sea (station PI-16).

On the basis of several oceanographic surveys of the water column near the sewage plume during different stratified conditions, a station grid was designed (Malačič, 1997). The grid with a diameter of approx.

900-m covers 31 stations around both outfalls and enables a fast dynamic survey (within one hour) of the plume extent. At grid stations vertical profiles of temperature, salinity, oxygen and fluorescence were obtained using the fine-scale multiparameter CTD probe, designed at the Centre for Water Research, University of Western Australia. The vertical resolution of the probe is of about 2.5 cm for a conventional drop speed of about 1 m/s.

Analyses

Nutrients. Concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4^{4-}) were measured in unfiltered seawater and filtered sewage samples using standard colorimetric procedures (Grasshoff, 1983). Sewage samples were filtered through glass fibre filters (Whatman GF/F). Total nitrogen (N-tot) and total phosphorus (P-tot) were analysed in unfiltered samples. Inorganic nitrogen ($\Sigma N_{in.}$) was calculated as $NH_4^+ + NO_3^- + NO_2^-$.

Faecal coliform bacteria. The number of faecal coliforms was determined following the recommendations of UNEP/WHO (1994). Water samples were filtered through the 0.45 µm pore-size Millipore filters and incubated on m-FC agar medium at 44.5±0.2°C for 24 hours.

Statistical analyses. Data from nutrient concentrations and faecal bacteria counts measured in the sewage at the outlet of the treatment plant and at the station PI-16 were statistically elaborated. Cross-correlation of standardised data was used to calculate correlation coefficients (ρ) in order to examine the linear relationship between properties (*i.e.* nutrients and faecal bacteria). Data were standardised using the following equation:

$$(\langle X\rangle - X_i)/SD$$

where X_i represents measured value, $\langle X\rangle$ mean value and SD is the standard deviation of the specific parameter.

RESULTS

Sewage composition

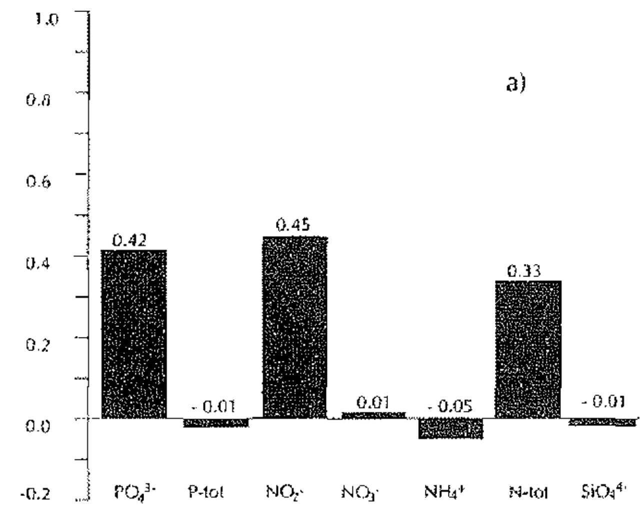
Nutrient analyses and bacterial counts were performed on sewage effluent that was treated mechanically (Tab. 1). Almost all inorganic nitrogen was present as ammonium. Concentrations of ammonium, nitrite and nitrate varied substantially: from below the detection limit up to 243 µmol l^{-1} in the case of nitrate. Except for samples collected on 12 October 1998 and 21 July 1999 the inorganic nitrogen (51-98% of total nitrogen) prevailed over organic forms.

More than 50% of total phosphorus was in the form of phosphate (PO_4^{3-}), while other forms that are normally present in sewage, *e.g.* polyphosphate and organic phosphate, were not analytically separated. The highest concentrations of ammonium, phosphate, total nitrogen and phosphorus were measured during the summer months (31 August 1998 and 21 July 1999), while in the case of nitrate and nitrite it was just the opposite: during the summer they were at their minimum. Average count of faecal bacteria was around 7.3×10^6/100 ml with a standard deviation of 2.7×10^6.

Tab. 1: Composition of sewage water at the outflow of the Piran treatment plant: concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), inorganic nitrogen ($\Sigma N_{in.}$), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), total nitrogen (N-tot) and phosphorus (P-tot) and number of faecal coliform bacteria (FC). Mean values of the parameters (< X >) and standard deviations (SD) are shown.

Tab. 1: Sestava odpadne vode na iztoku iz piranske čistilne naprave: koncentracije nitrata (NO_3^-), nitrita (NO_2^-), amonija (NH_4^+), anorganskega dušika ($\Sigma N_{in.}$), fosfata (PO_4^{3-}), silikata (SiO_4^{4-}), celotnega dušika (N-tot) in fosforja (P-tot) ter število fekalnih koliformnih bakterij (FC). Podane so srednje vrednosti (< X >) in standardne deviacije (SD) merjenih parametrov.

date	NO_3^-	NO_2^-	NH_4^+	$\Sigma N_{in.}$	PO_4^{3-}	SiO_4^{4-}	N-tot	P-tot	FC
	(µmol l^{-1})	(µmol l^{-1})	(µmol l^{-1})	(µmol l^{-1})	(µmol l^{-1})	(µmol l^{-1})	(µmol l^{-1})	(µmol l^{-1})	(No./100 ml)
02/04/98	17.96	3.40	825.93	847.29	38.75	139.44	1651.17	62.69	3.90E+06
18/06/98	43.16	16.76	841.87	901.79	40.36	249.16	924.41	61.48	1.03E+07
31/08/98	<0.01	0.04	1916.30	1916.34	46.71	388.27	1998.59	109.69	4.48E+06
12/10/98	20.92	14.32	299.30	334.54	24.16	201.60	920.50	37.29	8.00E+06
24/05/99	242.51	6.32	952.72	1201.55	58.22	220.43	1895.30	90.18	7.00E+06
21/07/99	3.04	0.07	1836.60	1839.71	102.28	262.28	5275.80	115.97	1.02E+07
< X >	54.60	6.82	1112.12	1173.54	51.75	243.53	2111.05	79.55	7.26E+06
SD	93.33	7.19	634.44	613.27	27.13	830.02	1619.34	30.80	2.68E+06



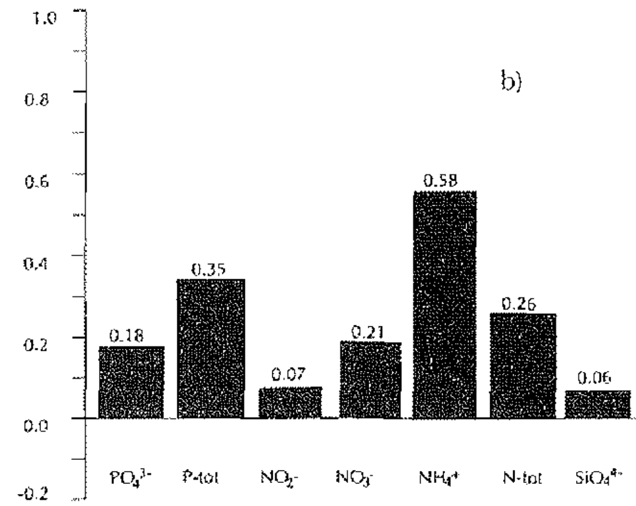


Fig. 2: Coefficients of cross-correlation analyses between faecal bacteria and nutrients.
a) data from the sewage at the outlet of the treatment plant
b) data from station PI-16

Sl. 2: Koeficienti korelacijske analize med fekalnimi bakterijami in hranilnimi snovmi.
a) podatki iz odpadne vode na iztoku iz čistilne naprave
b) podatki s postaje PI-16

Tab. 2: Coefficients of cross-correlation analyses performed on nutrient concentrations and faecal bacteria counts, which were measured in the sewage at the outlet of the Piran treatment plant (N = 6).
Tab. 2: Korelacijski koeficienti, izračunani za koncentracije hranilnih snovi in števila koliformnih bakterij (N = 6). Analize so bile opravljene v odpadni vodi na iztoku iz piranske čistilne naprave.

	PO_4^{3-}	P-tot	NO_2^-	NO_3^-	NH_4^+	N-tot	SiO_4^{4-}	FC
PO_4^{3-}	1	0.80	-0.60	0.03	0.71	0.96	0.22	0.42
P-tot	0.80	1	-0.81	0.05	0.95	0.76	0.64	-0.01
NO_2^-	-0.60	-0.81	1	0.11	-0.79	-0.68	-0.33	0.45
NO_3^-	0.03	0.05	0.11	1	-0.24	-0.17	-0.21	0.01
NH_4^+	0.71	0.95	-0.79	-0.24	1	0.71	0.74	-0.05
N-tot	0.96	0.76	-0.68	-0.17	0.71	1	0.21	0.33
SiO_4^{4-}	0.22	0.64	-0.33	-0.21	0.74	0.21	1	-0.01
FC	**0.42**	-0.01	**0.45**	0.01	-0.05	0.33	-0.01	1

Cross-correlation analyses (Tab. 2) of nutrient concentrations and faecal bacteria in sewage show variable relationships among parameters. Some nutrients are highly correlated among each other (e.g. PO_4^{3-}, P-tot, NH_4^+, and N-tot), while faecal bacteria are only slightly correlated to phosphate and nitrite (Fig. 2a). There is no meaningful correlation between the bacteria and ammonium in the sewage.

Vertical structure of the water column

CTD casts were performed at every survey and sampling depths at station PI-16 were determined using the salinity vertical profile. In this work only one vertical profile of temperature, salinity, fluorescence and dissolved oxygen will be presented as an example (24 May 1999) of the fine vertical structure of the water column that is affected by the sewage plume (Fig. 3). Warming of the atmosphere in late spring contributed to the rise of seawater temperature in the upper layers. Consequently, a weak stratification of the water column with a thermocline at approx. 12 m was re-established, separating the upper warmer layers (17.0-18.8°C) from the colder bottom ones (12.4-13.4°C). Salinity gradually increased with depth from the value at the surface (36.27 PSU) towards the value at the depth of the thermocline (37.04 PSU). In the layer between 11 and 16 m slight oscillations of salinity ranged from 0.03 to 0.12 PSU. They indicate an intrusion of less saline water presumably originating from the outfalls. Below 16 m depth the salinity increased again and reached the maximum (37.69 PSU) at the bottom. The fluorescence peak and highest oxygen concentration were detected at a depth of around 16 m. The salinity vertical profile was crucial for

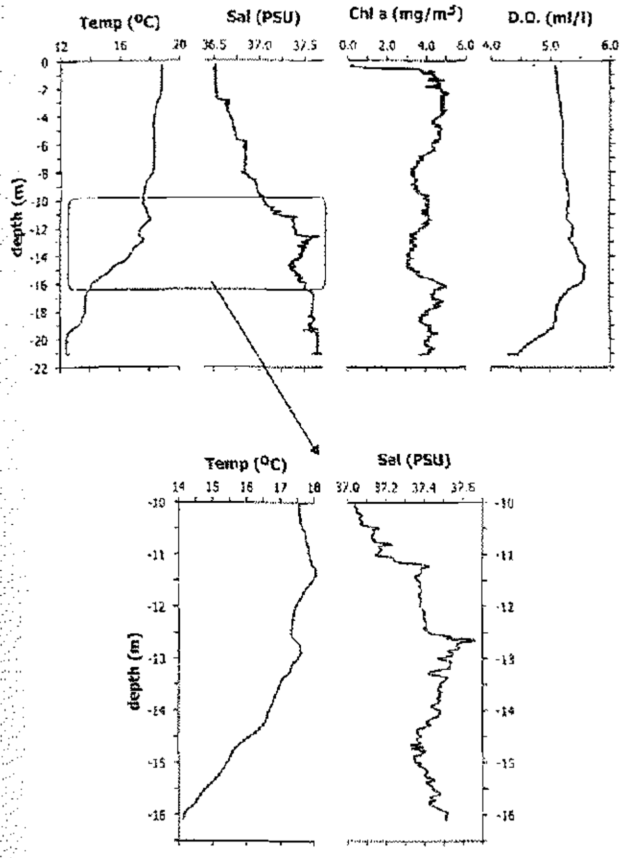


Fig. 3: Temperature, salinity (Sal), in situ fluorescence (Chl a) and dissolved oxygen (D.O.) vertical profiles recorded on May 24, 1999 using CTD fine-scale probe. A detail of vertical profiles of temperature and salinity between 10 and 16 m depth is shown in the lower figure.

Sl. 3: Vertikalni profili temperature, slanosti (Sal), in situ fluorescence (Chl a) in raztopljenega kisika (D.O.), posneti s CTD sondo 24. maja 1999. Na spodnji sliki je prikazan izsek vertikalnih profilov temperature in slanosti na globini 10 do 16 m.

determination of 12 sampling depths. Between the depth range 11.5-16.5 m the samples were collected at depths with an interval smaller than 1 m.

Water quality of the recipient

Nutrient concentrations and the sanitary quality of the seawater were measured in the near-field of the sewage plume (station PI-16), as well as at the reference station (station F), in order to examine the impact of sewage on the surrounding media. Fine-scale vertical distributions of chemical and microbiological parameters during six surveys at station PI-16 are shown in figures 4a and 4b.

Faecal coliforms are the fundamental indicators of sewage pollution. Therefore, we first followed the distribution of bacteria in the water column. Secondly, we compared nutrients to bacterial distribution in order to determine the most representative nutrient(s) of sewage dispersion. The number and vertical distribution of faecal coliforms differed substantially among the six surveys (Fig. 4a, upper panel). On 18 June 1998 we observed no sewage at station PI-16, while on other occasions the number of bacteria varied from less than 1 to 1160/100 ml. A peak value of 1160 counts/100 ml was counted on 2 April 1998 at depth of 12 m, with the second highest 890 counts/100 ml at depth of 14 m on 24 May 1999. Low values, apart from the situation of zero counts on 18 June 1998, were counted during late summer (31 August 1998). Then, the highest count of 215/100 ml was reached at the bottom.

The vertical distribution of faecal coliforms also varied substantially. During spring surveys (2 April 1998 and 24 May 1999) the bacteria were present only in a narrow layer of a thickness of 3 m between depths of 11 and 14 m. An approx. 15-fold increase was detected at depths of 12 m and 14 m with respect to counts at other depths. During other periods faecal coliforms were more evenly distributed throughout the water column. Bacteria were found in a thicker layer between 7 and 13 m, with peak values at the bottom (31 August 1998) and at depths between 11 and 16 m (12 October 1998 and 21 July 1999).

With a few exceptions vertical profiles of ammonium followed faecal vertical distributions (Fig. 4a, upper panel). Peaks of ammonium (4.08 - 7.33 $\mu mol\ l^{-1}$) were measured at the same depths as bacterial maximums, and also at the bottom (3.23 $\mu mol\ l^{-1}$). Concentrations of other nitrogen forms, especially nitrate, were generally higher in the upper layers of the water column (1.83 - 6.01 $\mu mol\ l^{-1}$) and again at the bottom (7.72 $\mu mol\ l^{-1}$; Fig. 4b, lower panel). Phosphate and total phosphorus (Fig. 4b, upper panel) showed a similar vertical pattern. Extremely high phosphate concentrations were measured on 31 August 1998 at the surface (0.30 $\mu mol\ l^{-1}$), at the bottom (0.92 $\mu mol\ l^{-1}$) and at 15 m depth (0.41 $\mu mol\ l^{-1}$). In other cases the concentrations of phosphate were below 0.10 $\mu mol\ l^{-1}$. Increased phosphate and total phosphorus values were only occasionally observed in the layers where faecal coliforms were present. Silicate concentrations varied from 1.85 to 34.72 $\mu mol\ l^{-1}$ (Fig. 4a, bottom panel). Similarly to nitrate, the highest values of silicate concentrations were generally measured in the bottom layers (except for 2 April and 31 August 1998).

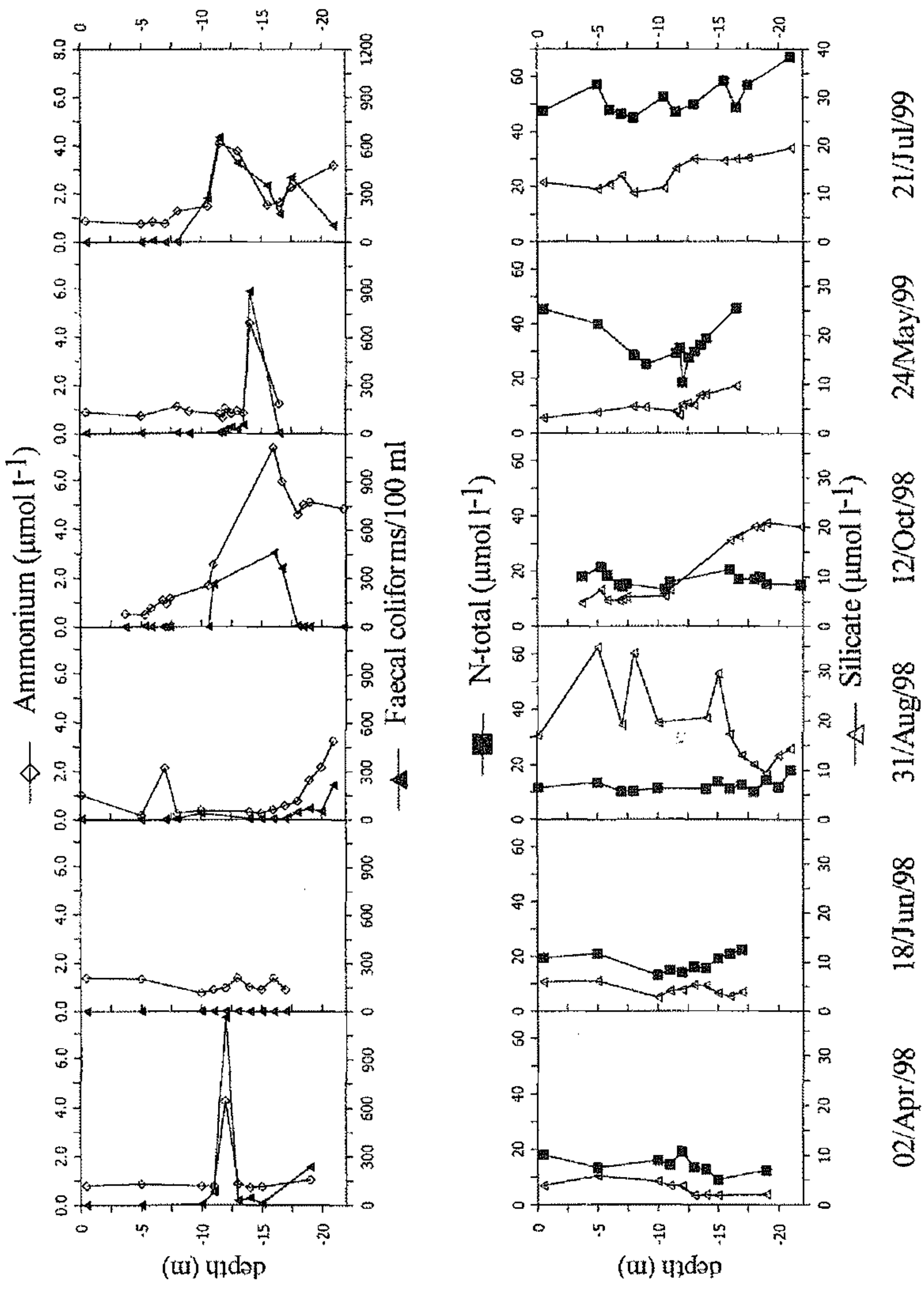


Fig. 4a: Vertical distribution of faecal coliform bacteria, ammonium, total nitrogen (N-total) and silicate at station PI-16 during six surveys during the years 1998-1999.
Sl. 4a: Vertikalna porazdelitev fekalnih koliformnih bakterij, amonija, celotnega dušika (N-total) in silikata na postaji PI-16 tekom šestih vzorčevanj med leti 1998-1999.

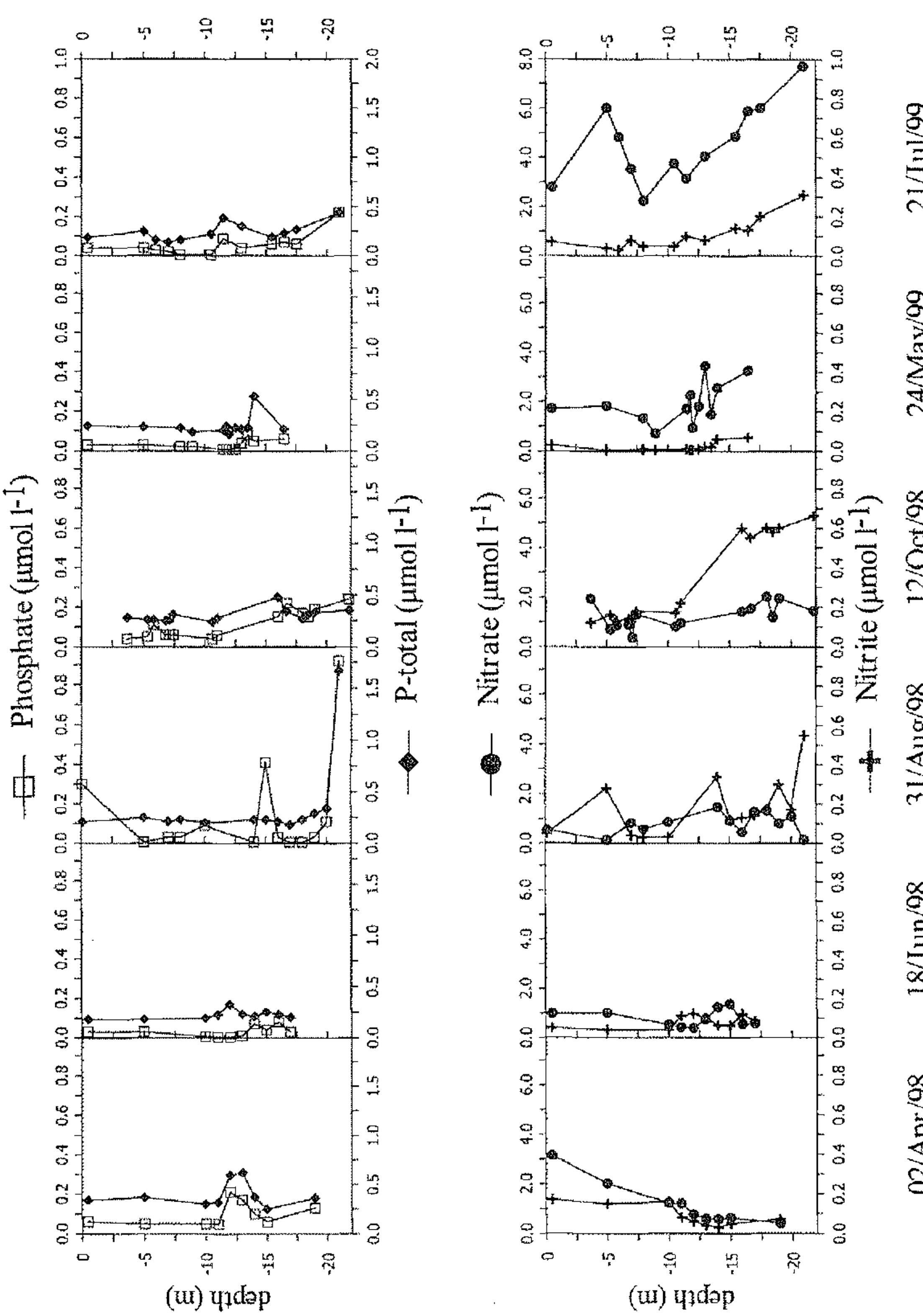


Fig. 4b: Vertical distribution of phosphate, total phosphorus (P-total), nitrate and nitrite at station PI-16 during six surveys during the years 1998-1999.

Sl. 4b: Vertikalna porazdelitev fosfata, celotnega fosforja (P-total), nitrata in nitrita na postaji PI-16 tekom šestih vzorčevanj med leti 1998-1999.

Tab. 3: Coefficients of cross-correlation analyses performed on nutrient concentrations and faecal bacteria counts, which were measured in the seawater at station PI-16 (N = 70).
Tab. 3: Korelacijski koeficienti, izračunani za koncentracije hranilnih snovi in števila koliformnih bakterij (N = 70). Analize so bile opravljene v morski vodi na postaji PI-16.

	PO_4^{3-}	P-tot	NO_2^-	NO_3^-	NH_4^+	N-tot	SiO_4^{4-}	FC
PO_4^{3-}	1	0.82	0.49	-0.10	0.37	-0.12	0.27	0.18
P-tot	0.82	1	0.40	-0.11	0.37	-0.09	0.02	0.35
NO_2^-	0.49	0.40	1	-0.02	0.72	-0.19	0.47	0.07
NO_3^-	-0.10	-0.11	-0.02	1	0.14	0.87	0.19	0.21
NH_4^+	0.37	0.37	0.72	0.14	1	0.09	0.29	0.58
N-tot	-0.12	-0.09	-0.19	0.87	0.09	1	0.07	0.26
SiO_4^{4-}	0.27	0.02	0.47	0.19	0.29	0.07	1	0.06
FC	0.18	**0.35**	0.07	0.21	**0.58**	0.26	0.06	1

Nutrients and faecal bacteria measured at station PI-16 were statistically elaborated in a way similar to that for the sewage effluent. Data from all six surveys collected at all depths were cross-correlated (Tab. 3). The strongest relationships were observed between PO_4^{3-} and P-tot, and NO_3^- and N-tot (ρ = 0.82 and 0.87, respectively). However, the impact of sewage discharge on the recipient is revealed with the correlation between faecal bacteria, indicators of such pollution, and nutrients (Fig. 2b). The highest correlation coefficient between bacteria and nutrients was calculated in the case of ammonium (ρ = 0.58). A relatively low correlation coefficient was observed for total phosphorus (ρ = 0.35). In all other cases (faecal coliforms *vs.* nitrite, nitrate, total nitrogen, phosphate and silicate) there was no meaningful correlation, indicating no effect of sewage input on these nutrient forms.

Nutrients and sanitary quality at the reference station

Station F was chosen as a reference station because we expected no impact of sewage pollution at that station. We therefore compared the nutrient data from both stations (PI-16 and F).

Faecal coliforms were absent at the station F (Fig. 5) during four samplings in 1998 except for the slight increase in the bottom layer on 31 August 1998. On the contrary, they were found on both 1999 samplings at depths below 10 m with relatively high numbers on 24 May 1999 (450 and 165 counts/100 ml). Concentrations of all nutrients were in a range similar to the range of nutrients measured at station PI-16. On average, nitrate and total nitrogen were higher at station F (2.30 and 36.51 μmol l^{-1}, respectively) while all other mean values were higher at station PI-16. It should be stressed, however, that the number of sampling depths at station F was lower than the number of depths at station PI-16. Moreover, half of the samplings at station F were taken only in subsurface or middle layers. A thorough comparison is therefore limited.

DISCUSSION

The sewage composition from the Piran treatment plant shows a high variability of parameters during six samplings (Tab. 1). High concentrations of nitrate and nitrite in the sewage were measured in spring, while during the summer period they were close to the detection limit. These forms of nitrogen are normally absent from fresh sewage as they are products of the biological oxidation processes within the treatment plant (Masters, 1998). Therefore, high oscillations of nitrite and nitrate in Piran sewage might be attributed to the different age of the sewage at the time of sampling. We have to consider also that the Piran sewerage system is of a combined type, meaning that during heavy rainfalls surface drainage waters coming from roads, roofs and paved areas are collected in the treatment plant. This leads to fluctuations in both the volume and concentration of sewage. It was shown in a previous work (Malej *et al.*, 1997) that the main nitrogen form in the rainwater that was collected at the local meteorological station was nitrate. A considerable fraction of nitrogen was bound in the organic form.

The concentration of ammonium could also, to some extent, be an indicator of the age of the sewage as this form of nitrogen is the final product of the decomposition processes of organic nitrogen and hydrolysis of urea (Masters, 1998). Higher concentrations of ammonium during the summer might also be attributed to the increased tourist population and not only to the age of the sewage. The same conclusion might be drawn about orthophosphate, total nitrogen and phosphorus. Faganeli (1982) already observed this in a previous work.

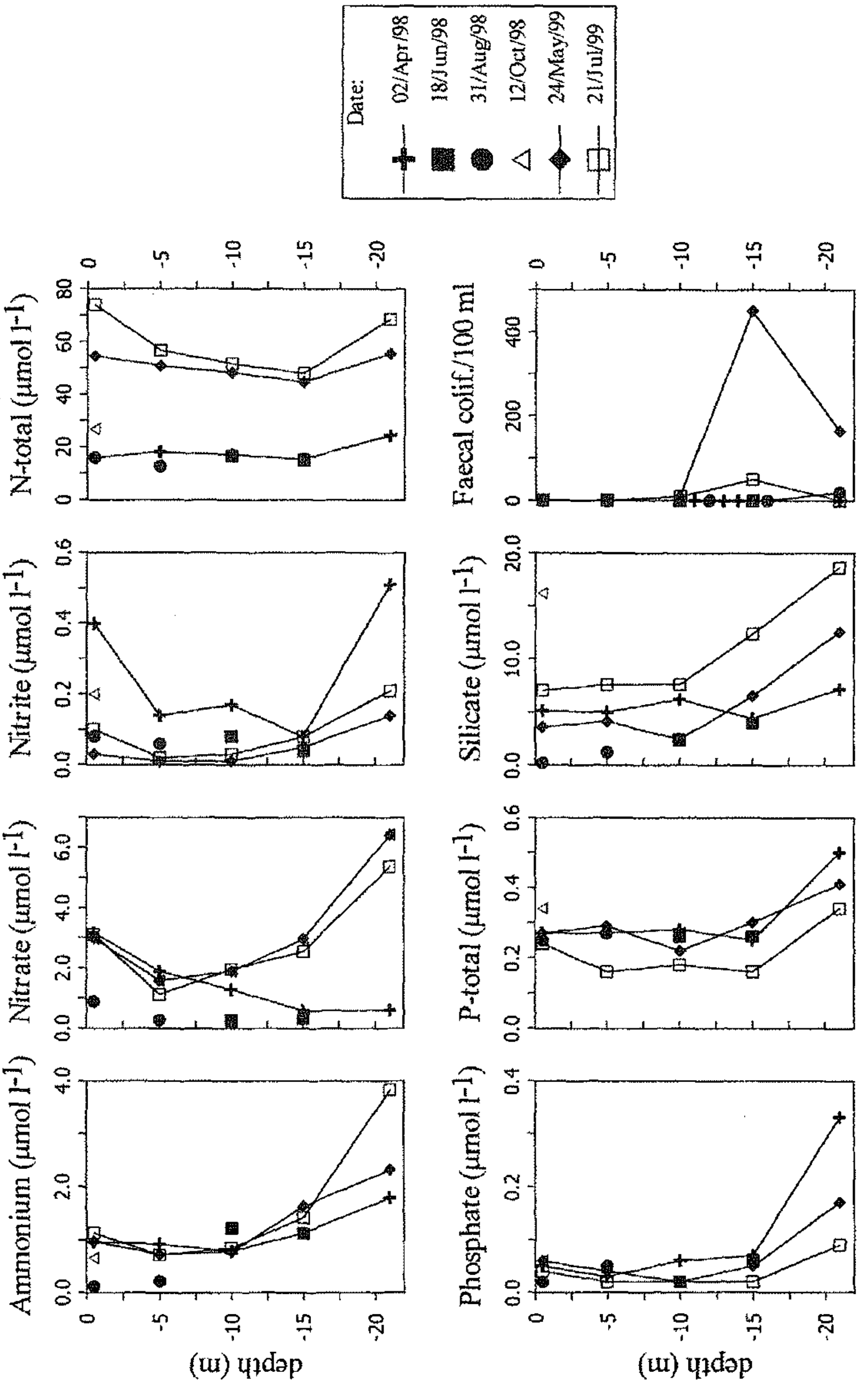


Fig. 5: Vertical distribution of nutrients and faecal coliform bacteria at reference station F during six surveys during the years 1998-1999.
Sl. 5: Vertikalna porazdelitev hranilnih snovi in fekalnih koliformnih bakterij na referenčni postaji F tekom šestih vzorčevanj med leti 1989-1999.

After mechanical treatment the sewage is gravitationally released to the sea through the two outfalls. Oceanographic properties of the recipient at station PI-16 on 24 May 1999 were shown as an example. At that time the less saline water, presumably originating from the two diffusers, was retained below the thermocline at a depth of 12 m. The sewage plume was identified by small salinity oscillations in the range of 0.03 to 0.12 PSU in thin layers of a thickness of less than 1 m at depths between 13 and 15 m. The sampling strategy was arranged in such a way that the sewage plume was detected as much as possible through frequent samplings that were separated along the vertical by less than 1 m. On 24 May 1999 frequent sampling was performed in depths between 11.5 and 16.5 m.

A detailed three-year study of the oceanographic properties around the submarine outfall confirms that within the turbulent wastewater field the fluctuations of temperature are in general of the order of 0.1°C, and of salinity less than 0.1 PSU (Malačič, 1999a). During periods of stratified water column the sewage plume generally lies at depths between 13 and 18 m - a few meters above the bottom, while during the homogeneous water column (winter), sewage can emerge at the surface (Vukovič & Malačič, 1997; Malačič, 1999b). The sewage plume spreads horizontally in thin lenses of a thickness of less than 1 m in windless, stratified conditions (Malačič, 1999a). Small vertical variations of temperature and salinity that characterise these lenses indicate very fast dilution of sewage close above both diffusers. The study also showed that without seasonally-oriented measurements (*i.e.* CTD casts) it is very difficult to separate the influences of ambient conditions (*e.g.* currents, winds, tide) on the spreading and rising of sewage. One possibility, although expensive, would be to use dye tracers (*e.g.* rhodamin) (Proni *et al.*, 1994; Adams *et al.*, 1998; Yilmaz *et al.*, 1998).

The spreading of a sewage plume was confirmed by measurements of faecal coliforms. Faecal coliform bacteria such as *Eschericia coli* are traditionally used as indicators of sewage contamination because they appear to be specific to sewage pollution. They are present in large numbers, relatively easy to quantify (Nichols *et al.*, 1993), and are taken as a sign of potential pathogen presence. Domestic sewage discharged in coastal waters contains an unhealthy mix of both harmless and infectious microorganisms, such as faecal streptococci (*Streptococcus faecalis*) and human pathogenic viruses, which are more resistant to environmental stress and thus survive longer in the marine environment (days-weeks). Recent studies indicate that, instead of faecal coliforms, sterol biomarkers could also be a suitable parameter to detect sewage (Mudge & Gwyn Lintern, 1999), especially when a historic measure of sewage contamination is required. The comparison between the measurements of 24 May 1999 (Fig. 4a, upper panel) with the CTD cast (Fig. 3) showed that the occurrence and high number of faecal coliforms coincided with the layer of highest salinity oscillations along the vertical. Similar vertical distribution of salinity and bacteria was also observed during other months with the peak values of bacteria always below 10 m. The only exception was 18 June 1998 when no faecal coliforms were detected. Absence of bacteria was also observed during other surveys performed between 1996 and 1997 (Vukovič & Malačič, 1997). Faecal coliforms have been found also in the upper layers (above 10 m), but only during the homogenous water column (Vukovič & Malačič, 1997; *unpubl. data*). A possible explanation for the bacterial absence at station PI-16 observed on 18 June 1998 and reported previously by Vukovič & Malačič (1997) can be attributed to the fact that the sampling was not performed exactly at station PI-16. At this station the sampling usually takes half an hour. Since anchoring is prohibited at that position the research vessel drifted away from station PI-16 approx. 400 m due to winds and surface currents. Besides ineffective sampling there are other plausible factors which may considerably reduce the survival rate of faecal bacteria in the marine environment - primarily solar radiation, as well as salinity, pH changes, the presence of toxic substances, predation and parasitism, lysis by bacteriophage, suboptimal water temperature, and nutrient deficiencies (Cheryl *et al.*, 1995; Šolić & Krstulović, 1992). The effect of another factor, the osmotic stress due to moving from fresh to saline waters, is even more intensified when the sampling is performed outside the near-field of the sewage plume (*i.e.* drifting of the vessel).

The highest number of faecal coliforms (from 215 to 1160/100 ml) is above the regulation limit for bathing waters set by the State Department for Public Health, which require less than 100 coliforms/100 ml (Official Gazette, 1988). The area around the Piran submarine outfall is not designed for bathing or aquaculture activity. On the contrary, no activity should be allowed in the zone around the diffusers. According to UNEP (1995) recommendations "... as a precaution against damage by anchors or fishing gear, submarine outfalls should be marked with clear buoys at the end at every bend of the unprotected part, fitted with clear signs prohibiting anchoring and fishing in a 200 m radius around it, and warning against swimming or wind-surfing in the vicinity." These high coliform numbers should be a major concern from the point of view of the treatment plant operation. Taking into account the number of faecal coliforms at the treatment plant outlet (Tab. 1) and in the sea, the abatement factor of the microbial load was between 10^4 and 10^7 in most cases (97%) which is beyond the range of UNEP recommendations for submarine outfalls (UNEP, 1995). Only in the two highest cases of bacterial contamination (890 and 1160/100 ml) the abatement factor was around 10^3

which is in agreement with UNEP recommendations (1995). This abatement factor is usually a combination of initial hydraulic dilution over a submarine pipe and of bacterial decay. UNEP (1995) recommends an outfall system that ends with a diffuser of appropriate length at a certain distance from sensitive areas (*e.g.* bathing areas). This recommendation is met for the outfall at Piran. However, it is planned that the submarine outfall will be combined with the chemical disinfection of sewage sludge to decrease bacterial abundance and nutrient load even more.

Bacterial numbers were correlated with nutrient concentrations, and nutrient measurements at both stations (station PI-16 and reference station F) were compared in order to detect other indicative parameter(s) of sewage pollution. Vertical distribution of analysed substances (Figs. 4a, 4b), and calculated correlation coefficients between them (Tab. 3, Fig. 2b) show that only ammonium could be an indicator of sewage dispersion ($\rho = 0.58$). Interestingly, this relationship between ammonium and faecal bacteria cannot be detected in the sewage effluent (Tab. 2, Fig. 2a) where other nutrient species (phosphate and nitrite) are more linearly correlated to bacteria than ammonium, although this correlation is relatively weak. All other nutrient concentrations at the station PI-16 are comparable to the concentrations at the reference station (Fig. 5) and to concentrations that are generally measured in the southern part of the Gulf of Trieste (Vukovič *et al.*, 1997-1999). Thus, only a slight influence of sewage dispersal on the pelagic environment was detected. In the Gulf of Trieste maximal concentrations of ammonium are generally measured at the bottom (Vukovič *et al.*, 1997-1999). They result from degradation and sedimentation processes. In the water column above the submarine outfall the ammonium peak values (4.56-7.33 µmol l^{-1}) were up to two times higher than those measured at the reference station (max. 3.83 µmol l^{-1}) and were found in the layers of highest bacterial contamination. Sometimes the peak was detected at the bottom. Concentrations and vertical distribution of remaining nutrients are most likely greatly influenced by other external inputs (*e.g.* rain water, river water; Malej *et al.*, 1995, 1997) and processes (biological uptake, bacterial degradation, sedimentation, entrapment at the bottom). This hypothesis is even more plausible when variable sewage composition and fast dilution at the diffuser's outlet is taken into account. Except for ammonium, and to a lesser extent for total phosphorus, station PI-16 reflects similar nutritional conditions as station F. Similar findings were described for more polluted areas than the Bay of Piran, with higher external nutrient loads (municipal wastewater, industry, rivers). A water quality study of Tampa Bay in Florida (Wang *et al.*, 1999) showed that external loading of ammonium comprised less than 1% of total ammonium flux and that major seasonal variations can be attributed to mineralisation of organic nitrogen (algal death and respiration) and to phytoplankton uptake.

In terms of bacterial contamination, station F was found unsuitable as a reference site. The distance between the two stations is 0.85 Nm. Historically, station F has a recognisable status as a reference station for the non-polluted, open-waters of the Gulf of Trieste (Fanuko, 1986). On both 1999 surveys faecal coliforms were found at this station, indicating the impact of sewage discharge on the environment, presumably due to specific oceanographic properties at that time. High bacterial numbers were counted again in November 1999 at depths between 8 and 10 m (*unpubl. data*). This fact necessitates simultaneous measurements of currents at different depths.

Tab. 4: Comparison of the mean nutrient concentrations measured 20 years ago (Faganeli, 1982), and mean nutrient concentrations from this work at the station above the submarine outfall at Piran.
Tab. 4: Primerjava srednjih vrednosti koncentracij hranilnih soli v površinskem in pridnenem sloju, izmerjenih pred 20 leti (Faganeli, 1982) in tistih, izmerjenih v tej študiji, na postaji v bližini piranskega podvodnega izpusta.

Parameter (µmol l^{-1})	Surface layer		Bottom layer	
	Faganeli (1982)	this work	Faganeli (1982)	this work
NO_2^-	0.23	0.08	0.29	0.33
NO_3^-	2.18	1.84	1.95	2.05
NH_4^+	2.18	0.92	2.97	2.62
$\Sigma N_{in.}$	4.59	2.84	5.41	5.00
PO_4^{3-}	0.18	0.08	0.16	0.31

Finally, a comparison was made between recent nutrient data and the data measured 20 years ago (Malej, 1980; Faganeli, 1982) when the treatment plant of Piran started to operate (Tab. 4). Concentrations measured only in the surface and bottom layers were considered for the comparison. Mean nutrient concentrations presented in this work are generally lower than those reported by Faganeli (1982), especially in the surface layer. Only in the case of phosphate in the bottom layer are mean concentrations of recent measurements higher than those measured two decades ago. These slight differences in nutrient concentrations within the last 20 years indicate no major deterioration of the pelagic environment, although a continuous monitoring of water column properties is missing for an affirmative conclusion in this direction (*i.e.* long-term studies). Prompt monitoring of the sediment is even more crucial - no information about the deterioration of the sediment over the last 20 years is available. To date only one survey of the bottom organisms in the vicinity of the sewage outfall has been performed since the treatment plant started its operation (Avčin *et al.*, 1978). The need for an overall survey of the sediment structure and benthic communities is even more profound if we consider the higher susceptibility of the sediment to pollution over time as compared to the pelagic environment (Gray *et al.*, 1990). The impact of sewage discharge on the sediment has to be considered also in view of its greater potential for faecal contamination. A recent study (Pommepuys *et al.*, 1992) has shown that sediments can accumulate a variety of microorganisms, such as faecal coliforms, faecal streptococci, *Clostridium perfringens* spores, and viruses. Sediments may contain 100 to 1000 times as many faecal indicator bacteria as the overlying water and may provide a favourable, nonstarvation environment for the bacteria (Cheryl *et al.*, 1995; Ashbolt *et al.*, 1993).

Comparison between recent and past studies of the impact of sewage discharge also brings up the shortcomings of sampling design, which in turn is reflected in the results. During her study Malej (1980) observed faecal coliforms only at the bottom of station PI-16, whereas at a depth of 10 m and at the surface no bacterial contamination was reported. It must be pointed out that in both studies (Malej, 1980; Faganeli, 1982) only the layers at the surface, at the bottom and at 10 m depth were sampled. Our study showed that the past sampling strategy was inadequate since much important information can be lost. All recent studies (Vukovič & Malačič, 1997; Malačič, 1999a, 1999b) also demonstrated the importance of a small-scale vertical sampling strategy in such an extremely variable environment, where the indicator parameters, and furthermore, undesirable eutrophication effects are difficult to follow.

CONCLUSIONS

Our results showed a relatively high correlation between faecal bacteria and ammonium at the station above the submarine outfalls. Thus, in addition to faecal coliforms, ammonium can be identified as an indicator of the faecal pollution in the near-field sewage plume. Almost no correlation was observed between bacteria and other nutrients. Except for the ammonium no meaningful impact of the sewage discharge on the surrounding environment was observed when compared with nutrient concentrations at reference station F. Bacterial contamination was sometimes detected at station F, which was therefore found unsuitable as a reference site.

A small-scale vertical sampling scheme with unevenly distributed sampling depths proved to be a successful strategy for the detection of sewage spreading in the water column. Drawbacks of the survey are related to the long sampling time and technical/human limitations (not enough sampling depths in the upper water column, lack of measurements of currents). The sampling scheme was based on small salinity fluctuations in the range of 0.1 PSU along the vertical. These vertical oscillations indicate thin lenses of sewage.

A comparison between nutrient concentrations, measured in the vicinity of the outfalls in this study and concentrations measured 20 years ago, did not demonstrate a deterioration of the pelagic environment. However, continuous sewage discharge over the last two decades has probably affected the sediment - its chemical structure and bottom organisms. The lack of these data strongly necessitates further studies of the sediment.

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EKOLOŠKE ZNAČILNOSTI OBALNEGA MORJA V BLIŽINI PODVODNEGA KANALIZACIJSKEGA IZPUSTA

Patricija MOZETIČ, Vlado MALAČIČ & Valentina TURK
Nacionalni inštitut za biologijo, Morska biološka postaja, SI-6330 Piran, Fornače 41

POVZETEK

Predstavljen je vpliv odplak piranske čistilne naprave, ki se stekajo vzdolž dveh podvodnih izpustov v obalno morje okoli 3500 m pred Piranom. V obdobju 1998-1999 je bilo opravljenih šest vzorčevanj, ki so vključevala oceanografske meritve, analize hranilnih snovi ter fekalnih koliformnih bakterij na postaji, ki leži sredi obeh izpustov in na referenčni postaji. Širjenje odplak v morski vodi je bilo zaznano nekaj metrov nad dnom z majhnimi, vertikalnimi spremembami slanosti (red velikosti ~0,1 PSU) v manj kot meter debelih slojih. Analize fekalnih bakterij, indikatorjev fekalnega onesnaženja, so potrdile lego odplak v vodnem stolpcu. Število fekalnih bakterij (max. 1160/100 ml) se je povečalo v slojih z največjimi variacijami slanosti. Največji vpliv podvodnega izpusta na obalno morje je bil opažen v primeru amonija, na ostale hranilne snovi pa širjenje odplak nima večjega vpliva. Relativno visok korelacijski koeficient je bil izračunan med fekalnimi bakterijami in amonijem (ρ=0,58) v bližini podvodnega izpusta, medtem ko ta povezava ni bila opažena v odplakah v sami čistilni napravi (ρ=-0,05). Referenčna postaja se je izkazala kot neustrezna, ker so se tudi tam občasno zadrževale odplake.

Ključne besede: odplake, podvodni izpust, fekalne bakterije, hranilne snovi, obalno morje, Tržaški zaliv

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