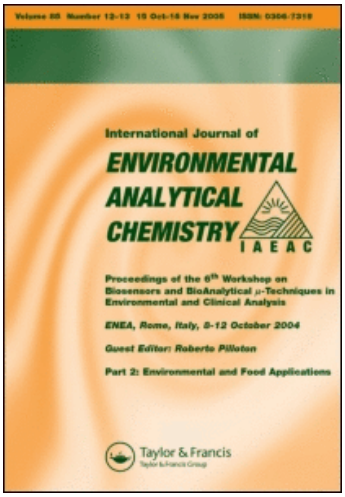


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# A Comparative Study of Chemical and Microbiological Monitoring of Pollutant Hydrocarbons in Urban Aquatic Environments

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# A Comparative Study of Chemical and Microbiological Monitoring of Pollutant Hydrocarbons in Urban Aquatic Environments†

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Conventional chemical and microbiological methods—aromatics by UV-fluorescence and the number of oil-degrading microorganisms, respectively—have been used for the monitoring of pollutant hydrocarbons in three different aquatic systems: two rivers, one harbour and three marine coastal areas. An evaluation of the first year data of such study is presented.

Relative populations of total heterotrophic microorganisms and those of degrading hydrocarbons correlate satisfactorily with hydrocarbon concentrations in marine areas, where chronic pollution situations occur, whereas unreliable results were obtained in the river systems. The water temperature seems to have a positive influence on the response of microorganisms to oil pollution.

KEY WORDS: Oil pollution, marine pollution, aromatic hydrocarbons, oil-degrading microorganisms, UV-fluorescence.

## INTRODUCTION

Anthropogenic hydrocarbons are ubiquitous in nature and primarily in aquatic environments as a consequence of the accidental or intentional discharges of petroleum products into either fresh waters or sea waters.

The ecological impact produced in these environments by acute oil spills

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is actually well documented both from the chemical and microbiological standpoints. In such situations, the identification of pollutant sources and the ultimate fate of hydrocarbons are questions of major concern.[1]

On the contrary, less attention has generally been paid to areas receiving chronic inputs (estuaries, rivers, etc.) in which the conditions, although apparently less severe, are not necessarily less harmful.[2] The emphasis in these cases should be placed in long term monitoring surveys. Questions of interest are the intercomparability of the information provided by the different methodologies used in different places and, particularly, the assessment of to what extent the parameters measured reflect the historical exposure of the environment to hydrocarbons. In this respect indigenous microorganisms play an important role in determining the capability of the systems for handling these pollutants.

During the last decade scientific efforts have gone toward the development of analytical techniques sensitive enough for pollution baseline studies and capable of making distinction between natural and anthropogenic hydrocarbons.[3] On the other hand, significant progress has been made on the microbiology and ecology of the latter mainly on the occasion of large marine oil spills or simulated acute discharges.[5,6] However, the information available from chronic or apparently unpolluted situations and especially from waters nearby urban areas, including distributions of hydrocarbons and oil degrading microorganisms, is rather scarce.[7,8] Therefore, a long term monitoring of petroleum hydrocarbons by conventional chemical and microbiological methods was undertaken in the vicinities of the highly populated area of Barcelona. Three different systems were selected, namely a harbour, rivers and marine coastal sites. The first year data will be presented and evaluated below.



## EXPERIMENTAL

### Sampling

The water sampling was carried out at a series of stations located in the surroundings of Barcelona as indicated in Fig. 1. Shoreline water samples were collected in stations 1, 4 and 6, river samples were obtained from stations 2 and 5, and the harbour samples from station 3.

These stations were visited fortnightly during the period of October 1980–September 1981 with weighted dark glass bottles. The bottles were immediately sunk to a depth of 20 cm, where they filled, as a small buoy prevented them from sinking further.[9]

Bottles for hydrocarbon analysis (2·51 of capacity) were thoroughly cleaned with spectro-grade carbon tetrachloride before use. Immediately after sampling, 40 ml of the same solvent was added to prevent

degradation and then were stored at 4°C until analysis. This was performed within the first 24 hours.

Water samples for microbial enumeration were collected asseptically in sterile 250 ml glass bottles and used for analysis directly.

## Hydrocarbon analysis

No separation was made between particulate and soluble hydrocarbons. Approximately 2 litres of water were extracted twice with 40 ml of spectro-grade carbon tetrachloride. The joined extracts were dried over $Na_2SO_4$ and evaporated.

The resulting residue was dissolved in 5 ml of spectro-analyzed *n*-hexane and then analyzed by UV-fluorescence (excitation at 300 nm) with a Perkin–Elmer MPF-3 spectrophotometer. Quantitation was performed on the emission spectra at 330 nm, using a Kuwait diesel-oil reference sample (b.p. 250–350°C).

Alternatively, control extracts were fractionated in a 30 cm × 1 cm i.d. silica-alumina column (1:1), 4% water deactivated. One column volume of *n*-hexane-methylene chloride (1:1) was eluted and analyzed for hydrocarbons as described previously. No significant differences were found in the results when compared with the direct quantitation of the extracts.



## Microbiological methods

The quantitative determination of microorganisms was carried out using the most-probable number technique (MPN) in three tubes series. MPN procedure have been suggested as a substitute for plate count procedures for enumerating oil-degrading microorganisms.[10,11]

The liquid medium used for enumeration of the total heterotrophic microorganisms (in g/l) consisted of 2.0 g glucose, 5.0 g casamino acids and 1.0 g yeast extract dissolved in sterilised artificial marine water (Biolife D-1141-505) or distilled water, according to the sample origin. Tubes were incubated one week at 22°C, at which time growth, as evidenced by turbidity, was scored.

Hydrocarbon-utilizing microorganisms were also enumerated. The basal medium was that of Bushnell-Haas Broth (Difco 0578-01),[12] supplemented with 3% ClNa, when the sample consisted of seawater. Two sets were used, one to which no carbon source was added, as a control, and the other with 1% sterilized kerosene as the sole source of carbon and energy.[13] Incubation was for 14 days at 22°C and 120 r.p.m. in an orbital shaker, after which relative growth was scored. A kerosene, containing 35% alkanes, 50% naphtenes and 15% aromatics was chosen as substrate,

because it provides an adequate range of hydrocarbons to which the microbes could respond and is more representative of the products that might pollute the sampling area.

## RESULTS AND DISCUSSION

The area of Barcelona, we have selected for study, is adequate for stating the problem we are dealing with, as the comparability of the information given by chemical and microbiological methods regarding hydrocarbon chronic pollution. It is well representative of an urban-industrial area, inhabited by 2.5 million persons, with a suspected chronic incidence on

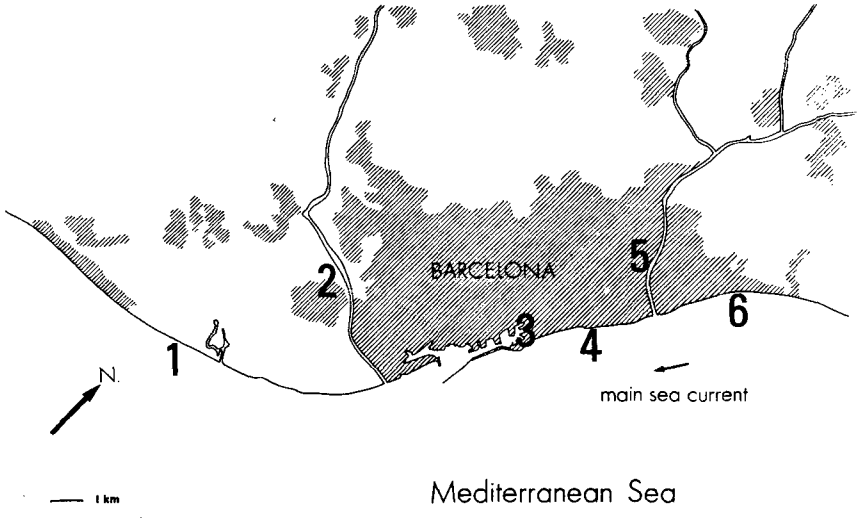



FIGURE 1 Location of sampling sites: (1) Gavá beach, (2) Llobregat river, (3) Barcelona harbour, (4) Barceloneta beach, (5) Besós river, (6) Badalona beach.

contiguous fluvial and marine coastal systems.

Therefore, six stations representing different situations were chosen accordingly (see Fig. 1). The results of the first year monitoring, based on the determination of hydrocarbon concentrations and microbiological populations, are presented in Figs. 2 and 3 and summarized in Table I.

Hydrocarbon concentrations have been estimated by UV-fluorescence using a middle distillate (Kuwait diesel-oil) as a reference standard. Although this procedure exhibits some limitations as a means of quantitating oil in water, basically due to the arbitrary nature of the selected standard,[14] it was nevertheless adequate in this case, bearing in mind the sensitivity and simplicity of the technique and that only the

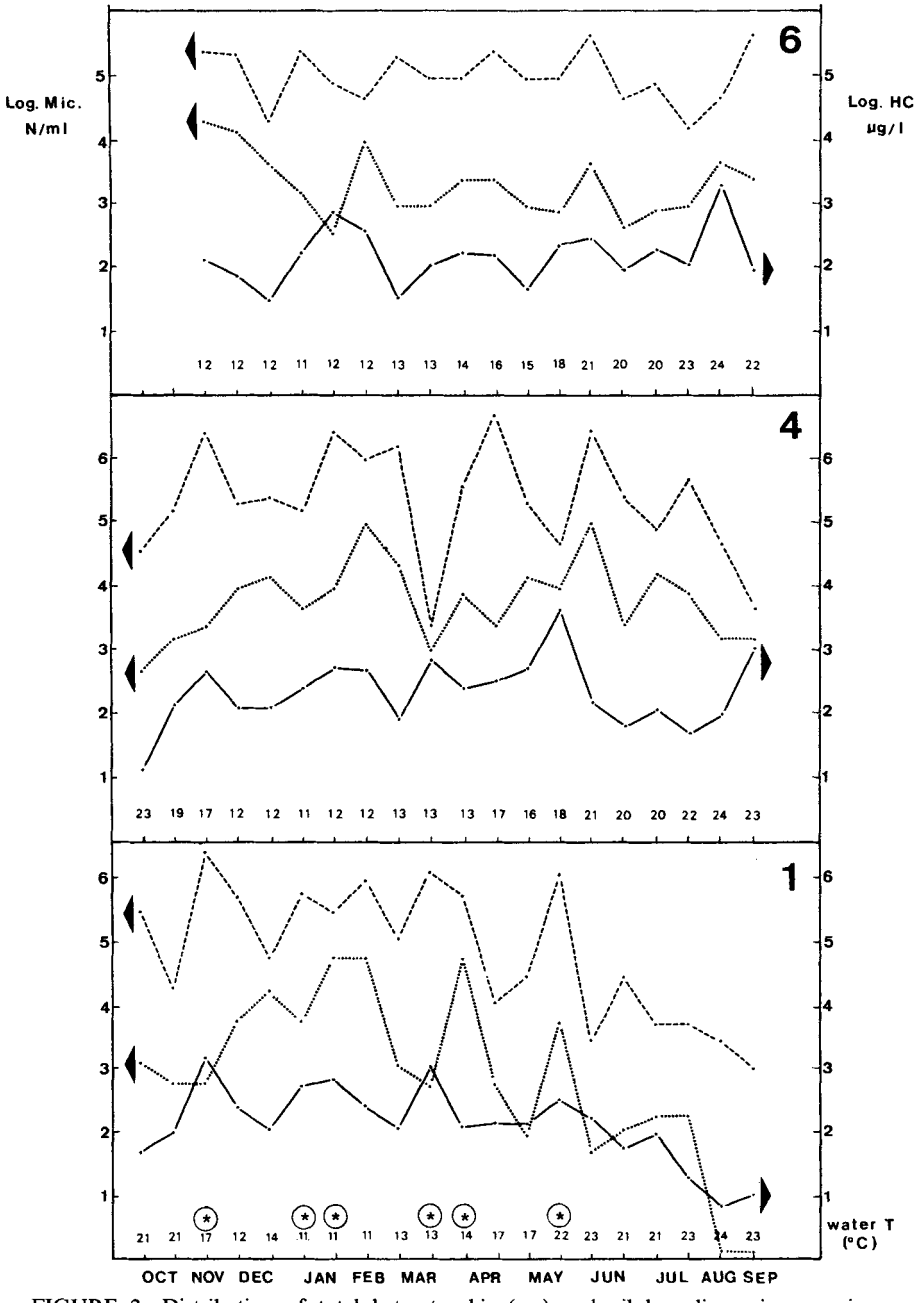



FIGURE 2 Distribution of total heterotrophic (- - -) and oil-degrading microorganisms (. . .), and hydrocarbon concentrations (———) in marine stations (1, 4, 6).

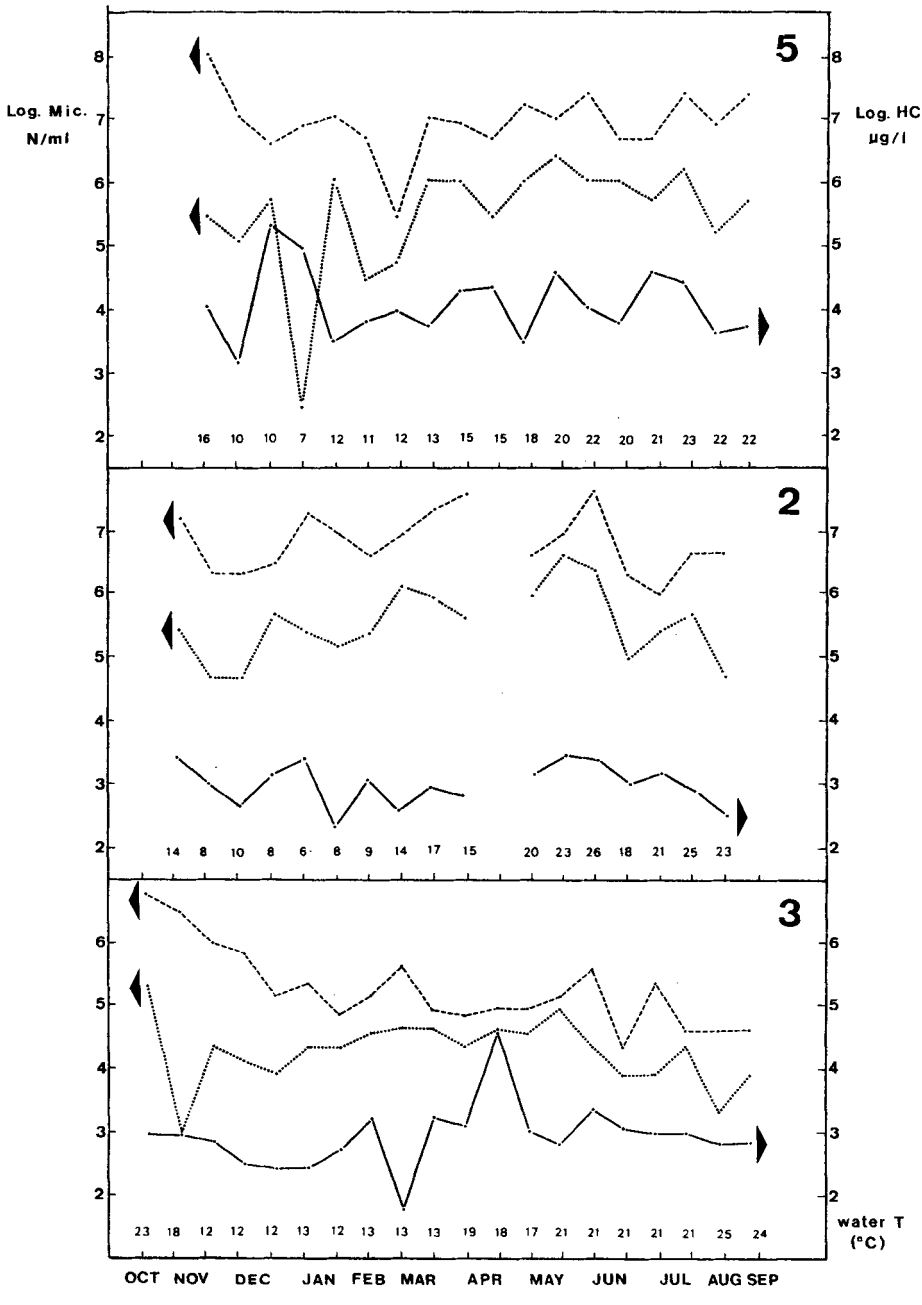



FIGURE 3 As Fig. 3 for river (2, 5) and harbour (3) stations.

TABLE I

Range and mean values of the parameters determined in the monitored stations

| Station | Identification | Total hydrocarbons ($\mu$g/ml) | Total heterotrophes (N/ml) | Oil-degrading microorganisms (N/ml) | Oil-degrading total heterotrophes (%) |
|---|---|---|---|---|---|
| 1 | coastal | 1186–6 (av. 231) | $2.0 \times 10^6 - 9.0 \times 10^2$ (av. $3.4 \times 10^5$) | $4.5 \times 10^4 - 1.1 \times 10^0$ (av. $8.3 \times 10^3$) | 4.29 |
| 6 | coastal | 1922–32 (275) | $4.5 \times 10^5 - 1.5 \times 10^4$ ($1.5 \times 10^5$) | $2.0 \times 10^4 - 3.5 \times 10^2$ ($3.1 \times 10^3$) | 4.80 |
| 4 | coastal | 4452–13 (491) | $4.5 \times 10^6 - 3.5 \times 10^3$ ($8.2 \times 10^5$) | $9.5 \times 10^4 - 4.5 \times 10^2$ ($1.6 \times 10^4$) | 8.02 |
| 3 | harbour | 42300–64 (3546) | $6.5 \times 10^6 - 2.5 \times 10^4$ ($7.1 \times 10^5$) | $1.1 \times 10^5 - 1.1 \times 10^3$ ($3.1 \times 10^4$) | 22.79 |
| 2 | river | 3005–214 (1248) | $4.5 \times 10^7 - 2.0 \times 10^6$ ($1.2 \times 10^7$) | $4.5 \times 10^6 - 4.5 \times 10^4$ ($7.7 \times 10^5$) | 9.77 |
| 5 | river | 176570–1153 (24357) | $9.5 \times 10^7 - 2.5 \times 10^5$ ($1.4 \times 10^7$) | $2.5 \times 10^6 - 2.5 \times 10^2$ ($7.1 \times 10^5$) | 8.23 |



measurement of relative concentrations of hydrocarbons in the samples was intended.

It is apparent from Figs. 2 and 3 that hydrocarbon concentrations have a large variability between stations, depending on the vicinity of industrialized or populated areas. Open marine stations (No. 1, 4 and 6) are the less polluted (av. 231–491 $\mu$g/l) because most of the urban waste waters receive appropriate treatment in depuration plants. However, concentrations in station 4 are slightly higher (av. 491 $\mu$g/l), probably due to the influence of the Besós river outflow, which follows the main sea current parallel to the coast in a NE-SW direction (Fig. 1).

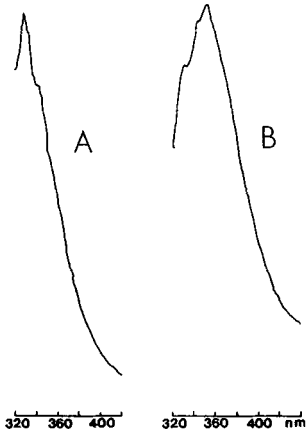
In any case the observed levels are approximately two orders of magnitude higher than those generally found far from the coast,[15,16] pointing to the definite influence of continental activities on marine coastal pollution. The two rivers (stations 2 and 5), which are small streams (715 and $98 \times 10^6$ m$^3$/year, respectively) running through very industrialized areas, are contributing more significantly to the coastal pollution in this area (av. 1.2–24.3 mg/l).

Finally, the harbour, as a typically commercial port, (about 8000 vessels/year) exhibits a rather constant and elevated concentration (av. 3.5 mg/l).

It is interesting to note that the hydrocarbons variability is not only

quantitative but qualitative. Figure 4 shows the types of spectra displayed by the samples from the different stations. Type A, which is largely predominant in river samples, is produced by petroleum products containing one or two ring aromatic compounds, such as diesel or lube oils. On the other hand, type B, which is mainly observed in marine samples, corresponds to petroleum products containing three and four rings, as crude oils or heavy residues (tanker washings, fuel-oils, etc.).

As far as microbial populations is concerned, it can be seen from Table I that the numbers and types of microorganisms reflect to some extent the nature and degree of water pollution.




| | % OCURRENCE | |
|---|---|---|
| STATION | TYPE A | TYPE B |
| 1 | 56 | 44 |
| 2 | 96 | 4 |
| 3 | 95 | 5 |
| 4 | 65 | 35 |
| 5 | 72 | 28 |
| 6 | 50 | 50 |

FIGURE 4 Types of emission fluorescence spectra displayed by the analyzed samples. The table indicates the occurrence of each type in the different stations.

Total heterotrophes exhibit a large variability within and between the stations, paralleling that of the hydrocarbon concentrations, as indicative of the organic pollution. The numbers are of the same order of magnitude as those observed in other coastal areas, e.g., Mulkins *et al.*[13] reported values in the range of $3.0 \times 10^5 - 2.2 \times 10^7$ in the Northwestern Atlantic and Gunkel *et al.*[7] of $5.3 \times 10^4 - 4.0 \times 10^7$ in the North Sea.

A rough correlation also exists between the concentrations of hydrocarbons in water and those of oil-degraders, whose occurrence in aquatic environments has been recently reviewed by Atlas.[6] However, it appears that these microorganisms have only a response capacity when hydrocarbons increase up to approximately 500 $\mu$g/l as in shoreline stations. The relative importance of oil-degraders within total microorganisms, is also increasing with hydrocarbons concentrations, to the point that for the marine sites (stations 1, 6, 4 and 3) the correlation

shows a regression coefficient of 0.9887, so providing a reliable microbiological criteria for evaluating hydrocarbon pollution in these systems.

In fact, Walker and Colwell[17] suggested that counts of petroleum-degrading microorganisms be expressed as a percentage of the total population rather than a total number of petroleum degraders per se. Subsequently, the above mentioned correlation was observed by several authors.[2,18] Le Petit, *et al.*[19] reported that hydrocarbon-utilizing microorganisms accounted for 10% of the total heterotrophes in the area of a refinery effluent, whereas it was only of 4% in areas unrelated with hydrocarbon pollution. In the present study the less polluted marine stations (No. 1 and 6) exhibit only 4.2–4.8% of oil-utilizing microorganisms, by contrast with the harbour which has 22%. This feature is not followed by the river stations, where a much higher increment of the number of total heterotrophes against oil-degraders takes place, despite the considerable amount of hydrocarbons present. Unlike the case of the harbour, where hydrocarbons are the major pollutant type, the total organic charge of the rivers is composed of a large variety of pollutants, probably exhibiting different toxic or bacteriostatic effects for the indigenous microorganisms and particularly for the oil-degraders. This will explain the behaviour deviation of the rivers in comparison with the other sites.

The comparison of the chemical and microbiological monitoring profiles obtained for each station permits one to approach another important problem such as the autodepuration capacity of the systems or the response capability of the microbiological populations to an input of hydrocarbon pollutants.

Several studies have shown that within a few days after an oil spill the population of hydrocarbon-utilizing microorganisms rises by several orders of magnitude,[20] thus contributing to the degradation of the oil.

When we observed in Fig. 2 the profiles corresponding to stations No. 1, 6 and 4 it can be realized that an increase in hydrocarbons correspond to an increase in oil-degraders. However, from October–May there is a time-lag of response for the latter which is minimized during June–August. Environmental factors are most probably the responsibles for such a behaviour. Particularly, the temperature has been recognized to have a marked effect on microbial growth and enzymatic activities,[21] being the limiting factor for the degradation of dissolved hydrocarbons. In these stations the response delay of the microorganisms is clearly shortened when the water temperature reaches to about 20°C.

The autodepuration capacity is particularly significant in station 1, where periodical inputs of urban and industrial waste waters were known.

These inputs are indicated by asterisks (*) and they coincide with an increase in hydrocarbon concentration. At the end of the monitoring period the declination of the hydrocarbons produces also a diminution in the microbial populations, clearly pointing to the close correlation between both parameters.

The situation in the river systems (stations 2 and 5, Fig. 3) is more random. However, during summer the correlation between hydrocarbons and microorganisms seems to improve slightly.

In conclusion it can be said that microbiological methods are suitable for monitoring hydrocarbon pollution in ecosystems where continuously small amounts of organic substances or much higher, but predominantly hydrocarbons, are introduced.

These systems also exhibit a higher degradation potential. The microbial response seems to be faster when the water temperature increases.



## Acknowledgement

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