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MICROBIAL SELFPURIFICATION PROCESSES IN THE RIVER YBBS (LOWER AUSTRIA) AS MEASURED BY DIFFERENT METHODS

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Zusammenfassung: Selbstreinigungsprozesse in der Ybbs, erfasst über verschiedene mikrobiologische Parameter.- Ein Längsprofil der Ybbs wurde untersucht, um anhand verschiedener mikrobiologischer Parameter die Selbstreinigungsvorgänge in der fliessenden Welle zu erfassen. Es zeigte sich, daß die bakterielle Aktivität, gemessen über die Inkorporation mit Tritium markierten Thyimidins, in Übereinstimmung mit den Gesamtbakterienzahlen und dem DOC hohe Werte zeigte, während die Koloniezahl der saprophytischen Keime deutlich schneller abnahm. Während die Koloniezahl also ein guter Indikator für die Verunreinigung mit leicht abbaubaren organischen Substanzen darstellt, scheinen hohe Thymidininkorporationswerte die Selbstreinigungsprozesse an sich anzuzeigen. Die mikroskopisch bestimmte Teilungshäufigkeit der Zellen (FDC) korrelierte mit keinem der übrigen Parameter und kann kurzfristige Aktivitätsänderungen oder Selbstreinigungsvorgänge nicht anzeigen. Die Methode, mittels der Teilungshemmung durch Nalidixinsäure den Prozentsatz aktiver Zellen zu bestimmen, korrelierte mit den Koloniezahlen, ergab jedoch sehr niedrige Werte. Dies ist möglicherweise auf die kurze Inkubationsdauer zurückzuführen.
Vergleicht man den hier erhobenen Gütezustand mit der Situation vor der Errichtung der Kläranlagen (WENINGER, 1988), ergibt sich eine eindeutige Verbesserung der Wassergüte. Trotzdem ist der Unterlauf noch deutlich belastet, wobei die Güte bedingt durch die stark schwankenden Abflußbedingungen und unregelmäßige Abwassereinleitung starken Schwankungen unterliegt.

Introduction

Microbial decomposition of organic material constitutes one of the most important parts in self-purification processes in river ecosystems. In the past, microbiologists concentrated their attention mainly on detecting pollution and waste water input using different bacteriological indicators. Apart from the indicators of fecal contamination (eg. fecal coliforms, fecal streptococci), mainly the colony count (also referred to as saprophytic bacteria, plate count, standard plate count or standard count) has been used as indicator of pollution with organic substances. These "saprophytic" bacteria were supposed to be specialized on the degradation of organic pollutants (UHLMANN, 1975). Recently, the introduction of new reliable methods allows good estimates of the total bacterial count, and of bacterial activity and production (e.g. FUHRMAN & AZAM, 1980, 1982; HOBBIE et al. 1977). On the other hand, since the work of RAZUMOV (1932) and others it is well known that the different types of colony counts represent only a small percentage (about 0,1 %) of the total bacterial count. Therefore, it seems

probable, that not only the saprophytic bacteria counted as colony counts play a role in degrading the organic substances, but an important percentage of the total bacterial count is responsible for this degradation, and thus for the self-purification potential of the river water.

To evaluate this, a longitudinal profile of the Ybbs (a river 155 km in length in lower Austria), was examined twice regarding the following microbial parameters: a) total bacterial count and biomass, b) the frequency of dividing cells (=fdc), c) the living cells as estimated by the nalidixic acid inhibition method (KOGURE et al., 1979), d) the colony count of saprophytic bacteria and e) bacterial activity by the tritiated thymidine incorporation method. The data obtained were compared to an other set of data from the same sampling stations carried out in 1989 and April 1990 (KASIMIR et al. 1990; and unpublished results). The objective of the present study was to compare the response of different methods to the changing pollution level of the River Ybbs. Only the surface water was taken into account in the present study due to time and difficulties inherent in bed sediment examinations. The authors are conscious of the fact, that in fast flowing rivers most of the bacterial biomass and consequently the selfpurification capacity is to be found in the bed sediments (KASIMIR, 1990).

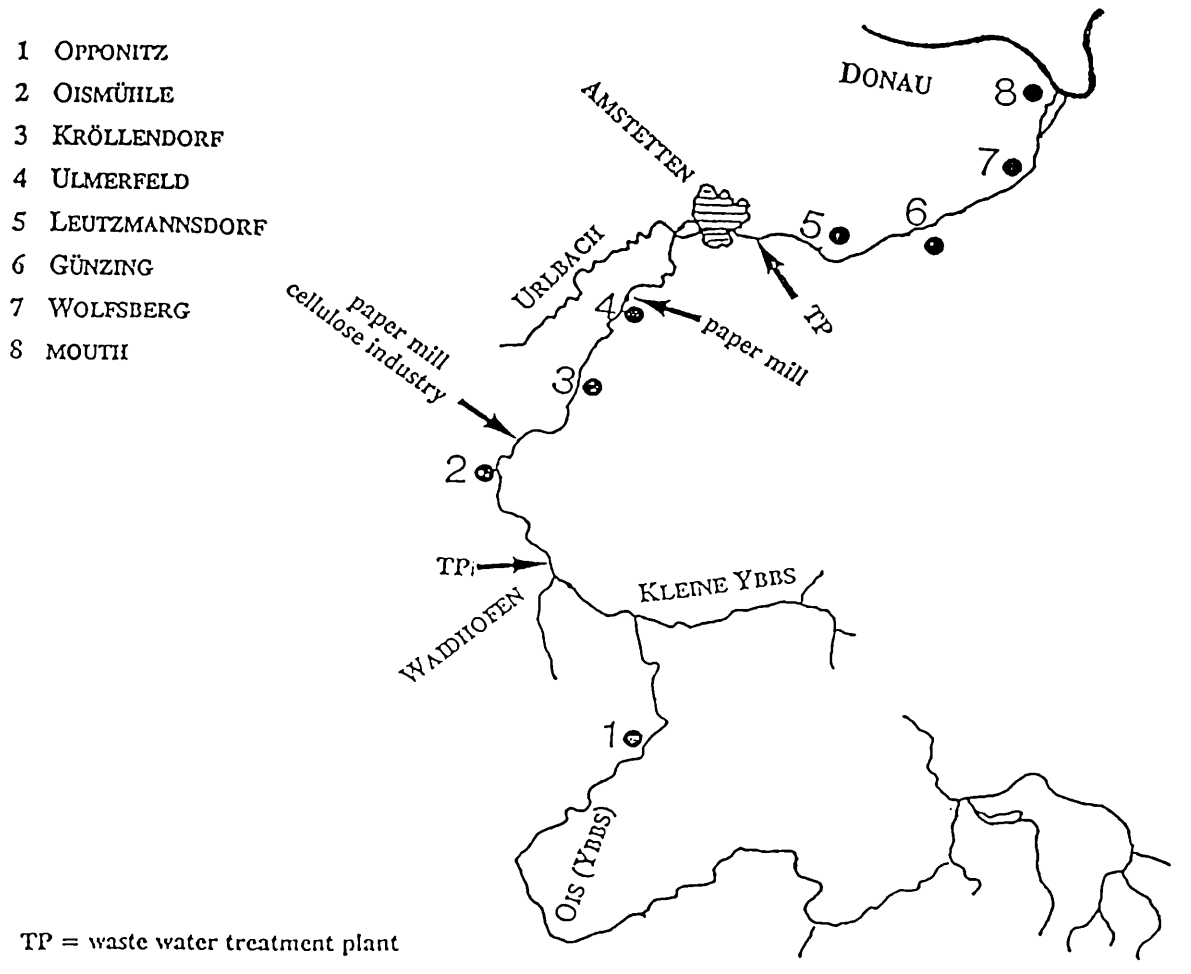
Abbreviations used in text and figures

AODC = acridine orange direct count; DP = degree of participation; FDC = frequency of dividing cells; TCA = trichloroacetic acid; TTI = tritiated thymidine incorporation; HNF = heterotrophic nanoflagellates.

Investigation area

The River Ybbs was chosen because in a relative short flow distance of 100 km, it is possible to see a great variety of water quality classes, from very clean to polluted ones. Similarly, communal and industrial waste water with or without treatment are clearly contaminating the Ybbs. Fig.1 shows the River Ybbs with the different sampling points and the most important towns and industries.

Fig.1 The River Ybbs and the sampling stations



In Waidhofen and Amstetten the effluents of waste water treatment plants are discharged into the Ybbs. In this area most of the industrial plants of the Ybbs region are situated (paper mills and cellulose producing industry and others, most of them connected with a sewage treatment plant).

The first sampling took place during low water level (mean water temperature: 16°C) on 25.8.90; the second sampling on 13.9.90 (average water temperature: 11,3°C) was done after heavy rainfalls and fast growing water level.

Methods

The total bacterial count was done using acridine orange epifluorescence microscopy according to HOBBIE et al. (1977), using a final AO-concentration of 0,01% and black polycarbonate filters (NUCLEPORE or PORETICS) with a pore size of 0,2 μm. The samples were preserved with formalin (final concentration of 2%). The frequency of dividing cells was counted according to HAGSTRÖM et al. (1979). Between 25 and 50 fields (200-1000 bacteria) were counted per filter. On the same filters, the number of heterotrophic flagellates (HNF: 4-10 μm diameter), the percentage of particle-attached bacteria, the number of particles (4-30 μm) and three different size classes (>4μm,1-4μm,<1 μm) were recorded. The mean variation coefficient of the direct counts was 25%.

The chemicals used were filtered (0,1 μm) before preparing the samples, controls (without adding samples) were counted to check if the solutions were bacteria-free.

The following procedure was used for the nalidixic acid inhibition method (modified after KOGURE et al., 1979): a final concentration of 20 μg/ml of nalidixic acid and respectively of yeast extract, was added to 10 ml of sample. The samples were incubated for 4 h at room temperature, and the incubation was stopped with a 2% final concentration of formalin. The samples were then counted using the acridine orange procedure as described above. Cells with a very big size were recorded as NAL positive cells.

Bacterial activities were measured via the DNA-synthesis rates using the tritiated thymidine incorporation method according to FUHRMAN & AZAM (1980, 1982). Triplicate 10 ml samples and formalin killed blanks (2% final concentration), were incubated in the laboratory with 10 nM of tritiated thymidine at room temperature between 18°C -20°C for 1 hour. This incubation was terminated by adding formalin. After extraction in ice - cold 20% TCA for 20 minutes, the samples were filtered onto 0.2 μm pore size cellulose nitrate filters (diameter 25 mm [SARTORIUS] prewashed with 2 ml of 5% TCA). The sample vials and the filters were rinsed twice with 4 ml ice-cold 10% TCA. Then the filters were dissolved and radioassayed in FILTER COUNT scintillation cocktail in a PACKARD CA 2000 or a Tricarb 300 counter. Counting efficiency , determined by the channel ratio method using a set of quenched standard samples (PACKARD), was between 48 and 55 %.

Isotope dilution of tritiated thymidine by non-radioactive thymidine was determined for several sampling stations by adding different concentrations (10, 20, 40, 60, 80 nM) of unlabelled thymidine to a 10 nM concentration of tritiated thymidine according to MORIARTY & POLLARD (1981).

Results

The results show the increasing pollution of the river in the longitudinal profile. Beginning from Opponitz, the "clean water" reference site, total bacterial numbers, numbers of heterotrophic flagellates, colony counts and thymidine incorporation increased. After the city of Amstetten, there is no great waste water inflow and a selfpurification stretch can be observed with decreasing load of pollutants.

Table 1 shows the range of the data and the average values of the parameters measured.

Oxygen concentration varied between 6.7 and 13.8 mg/l, the minimum values were always reached at Ulmerfeld, after the inflow of paper mills and cellulose industry waste water.

Table 1 Averages and range of data (5 sampling days)

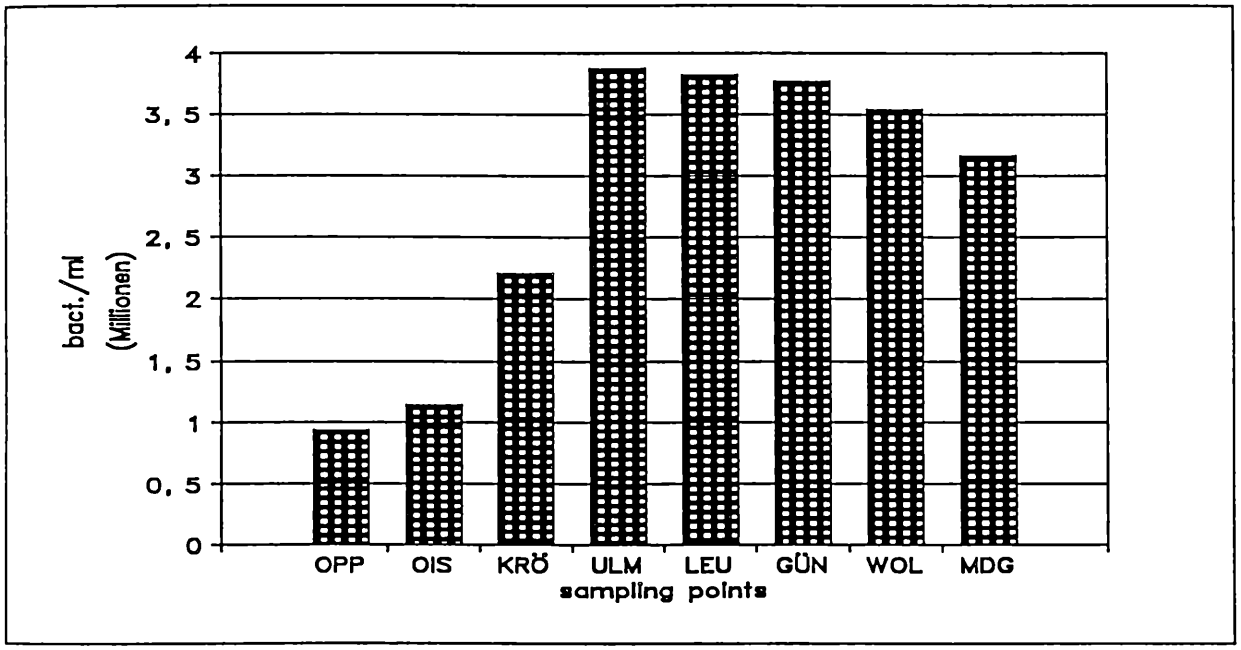
Parameter	Average	Minimum	Maximum
AODC (b./ml)	2700000	388000	7700000
attached bacteria (%)	8.8	0	31.4
FDC (%)	4.5	0.6	14.5
HNF (Ind./ml)	2230	220	7600
particles/ml	9000	0	42000
living cells (NAL+) (%)	2.6	0.96	5.71
colony count (cfu/ml)	10300	450	58000
TTI (nmol/l*h)	0.041	0.003	0.239
spec.act. (nmol/b.*h)	$1.5*10^{-11}$	$2.5*10^{-12}$	$8.3*10^{-11}$
suspended solids (mg/l)	9.8	0.9	104
oxygen (mg/l)	10.5	6.7	13.8
BOD_5 (mgO/l)	3.2	1.2	5.8
Razumov coefficient	540	52	2667
TOC (mgC/l)	15.7	2.7	32.5
DOC (mgC/l)	5.1	2.5	6.8
water temperature (°C)	11.7	8.4	18

The conductivity as a measure of the salt content was always more than 300 μS, mostly between 340 and 380 μS.

Direct counts

The total bacterial count varied between 388000 and 7700000 bacteria/ml. Fig.2 shows the average values of 5 sampling days. The low values (Opponitz) correspond to the concentrations found in unpolluted mountain brooks (KASIMIR, 1990) while the higher values downstream Kröllendorf are similar to the concentrations in big rivers like the Danube (KASIMIR & KAVKA, 1989). Up to >30% (mean: 8,8%) of the bacteria were attached to particles.

Fig.2 acridine orange direct count, average values (n = 5)



The bacterial biomass calculated from direct counts using an average bacterial volume of 0,2 μm^3 and a volume to carbon ratio of 1,21 $pgC/\mu m^3$ (WATSON et al., 1977), varied from 10 mgC/m^3 to 200 mgC/m^3.

The bigger size classes of bacteria had a tendency to increase in absolute number and as percentage, but due to the low number of large cells, and because of the resulting variance, this tendency can not be seen clearly.

Colony counts

Regarding the colony counts (fluctuating between 450 and 58000 cfu/ml), the water quality of the Ybbs can be described as follows: a) From Opponitz (class I to II) to Kröllendorf water quality of class II dominates; b) between Ulmerfeld and Wolfsberg water quality of class II-III dominates and sometimes water quality class III can be

reached. Decreasing colony counts and therefore increasing water quality can be observed in the selfpurification stretch after Günzing. The last sampling station, before the effluent of the Ybbs into the Danube, has a water quality class II considering the average colony counts (Fig.3). These findings correspond to the classification map of the Federal Institute of water quality (BmLF, 1989).

Fig.3 colony counts of the River Ybbs, averages (n = 5)

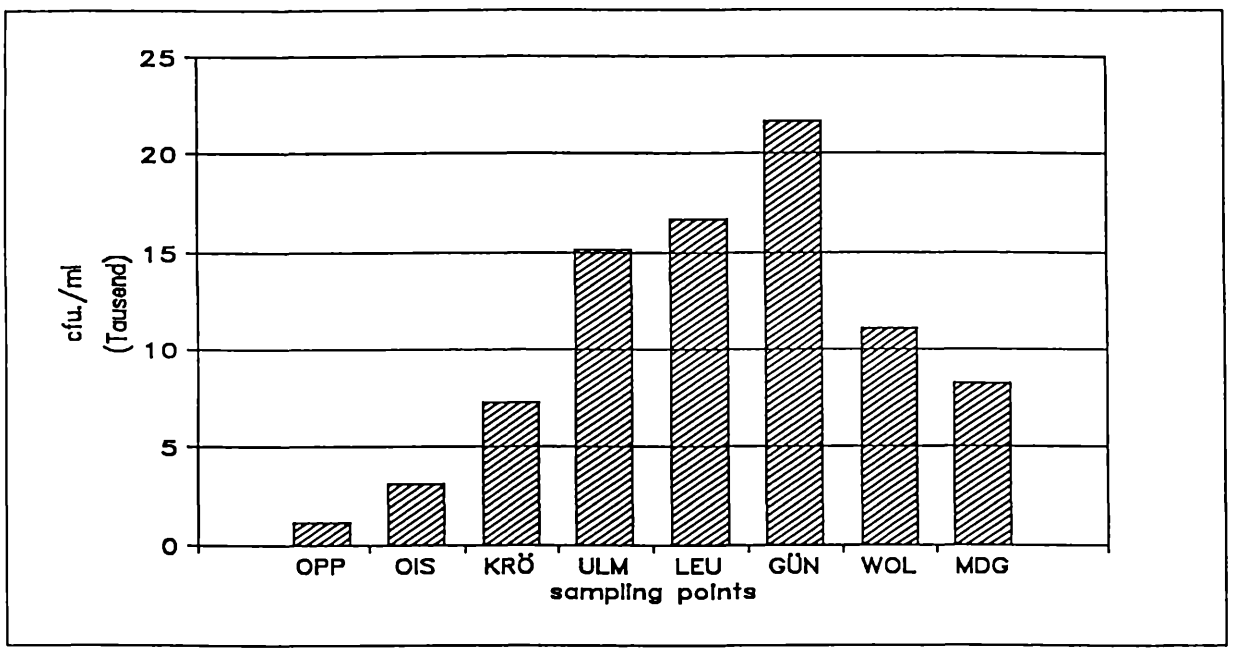
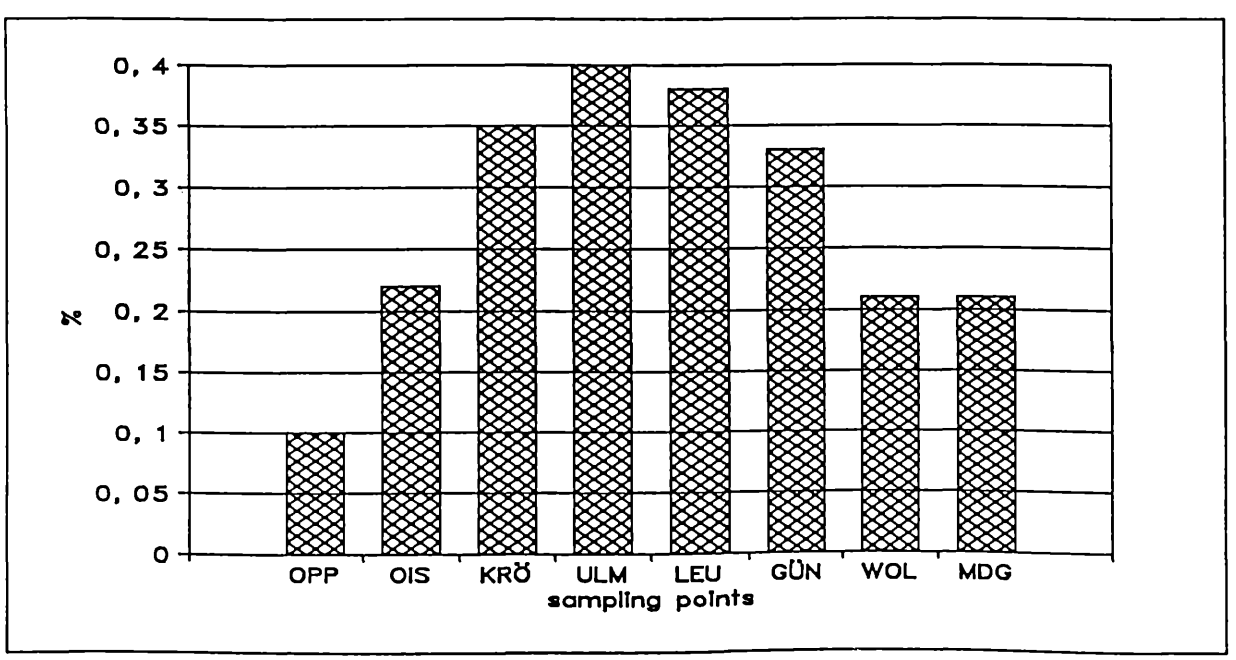
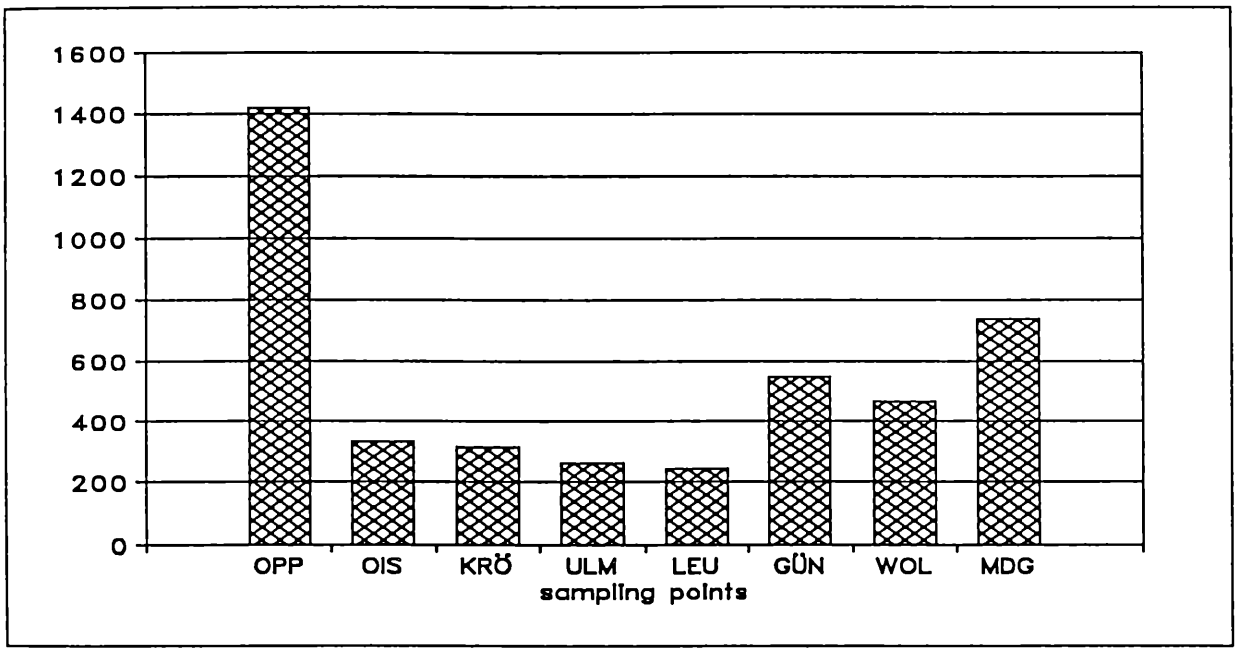


Fig.4 Percent colony count of direct count



The percentage of colony counts was 0.04-0.76% of direct counts (considering the averages 0.1 - 0.4%). This percentage increased between Opponitz and Ulmerfeld and decreased downstream Ulmerfeld, reflecting self-purification processes (Fig.4.). The Razumov coefficient (direct count/colony count), sometimes used as an indicator of pollution, showed values between 52 and 1417. The average values of this coefficient are shown in Fig.5.

Fig.5 Razumov coefficient, averages (n = 4)



Bacterial activity

The bacterial activity, measured by the thymidine incorporation method, showed a similar pattern for the longitudinal profile: low values (between 0,003 and 0,005 nmol/l*h) were measured in Opponitz, while maximum values between 0,03 and 0,24 nmol/l*h could be registered in the self-purification zone between Leutzmannsdorf and Wolfsberg (Fig.6). The last sampling station showed no significant decrease of this parameter, indicating the continuation of degradation processes, while colony counts were decreasing constantly. The increase of thymidine activities does not directly follow the waste-water and nutrient input. When comparing Fig.6 to Fig.7 (the dissolved organic carbon, measured on 2 sampling days), it can be seen that the main DOC input takes place after Kematen, where treated waste-water of the cellulose-industry and paper mill of Kematen flows into the Ybbs-River. Only after two to three sampling

points downstream the bacterial activities increased, thus reflecting this nutrient input. This delay could be due to the adaptation of the enzymatic systems of most of the bacteria constituting the total bacterial count to the new nutrient situation.

Fig.6 Bacterial activity, TTI (average, n = 5)

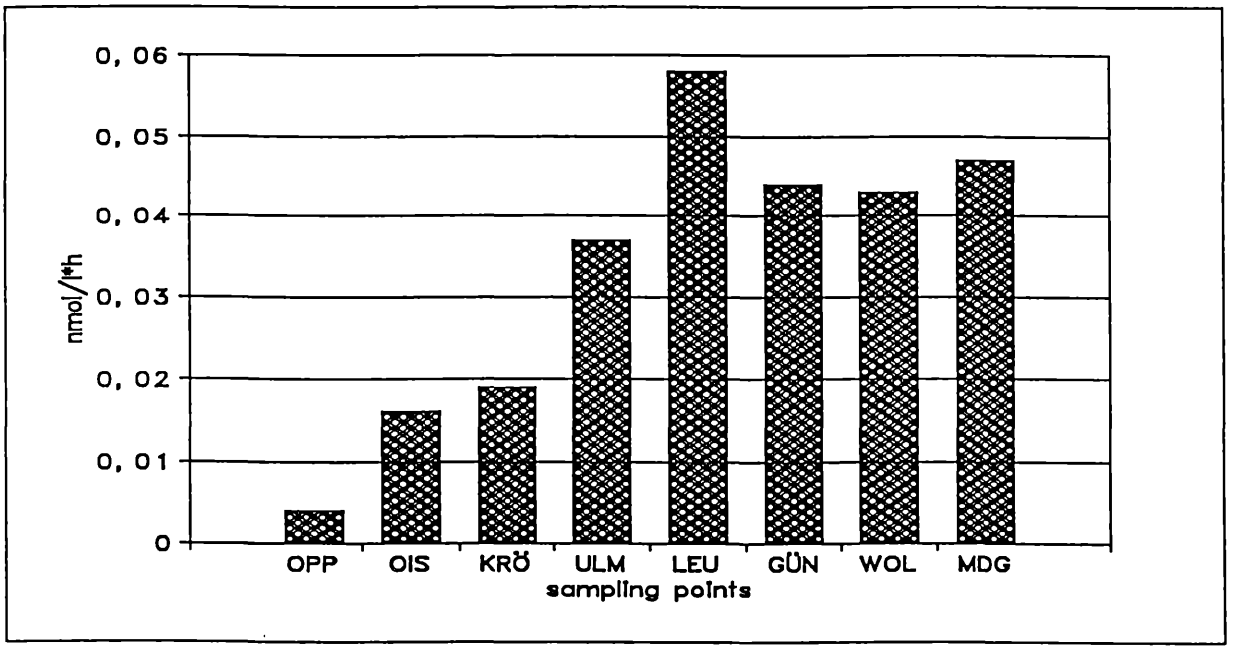
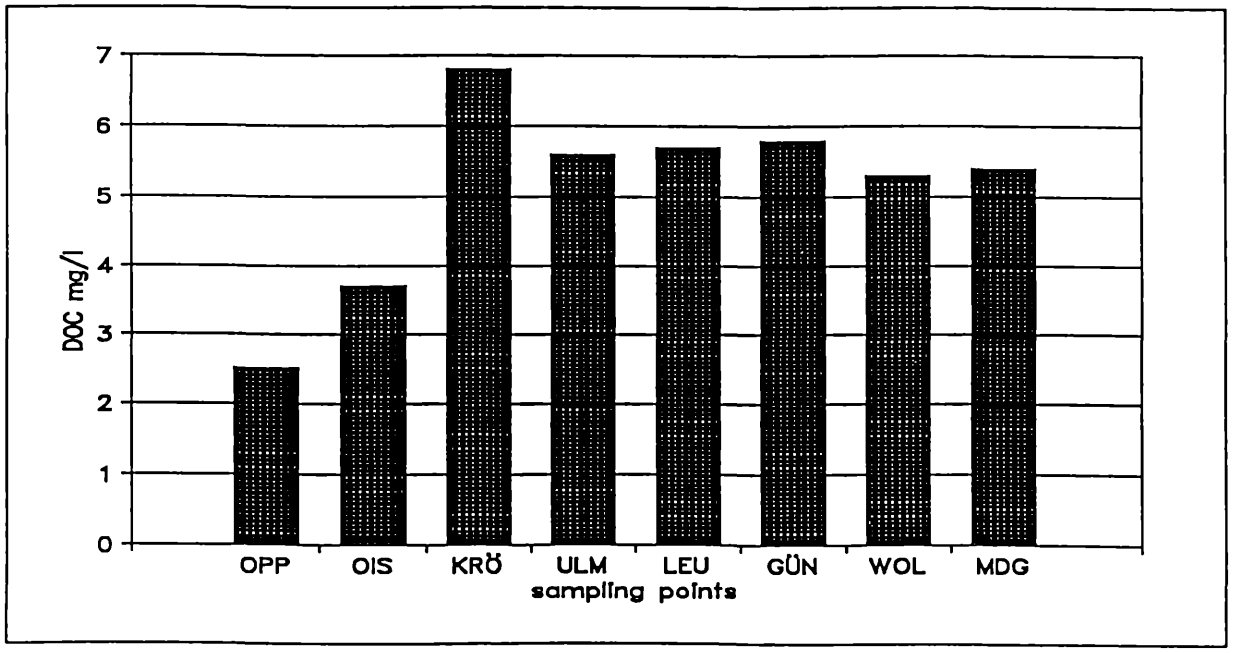


Fig.7 Dissolved organic carbon (average, n = 2)

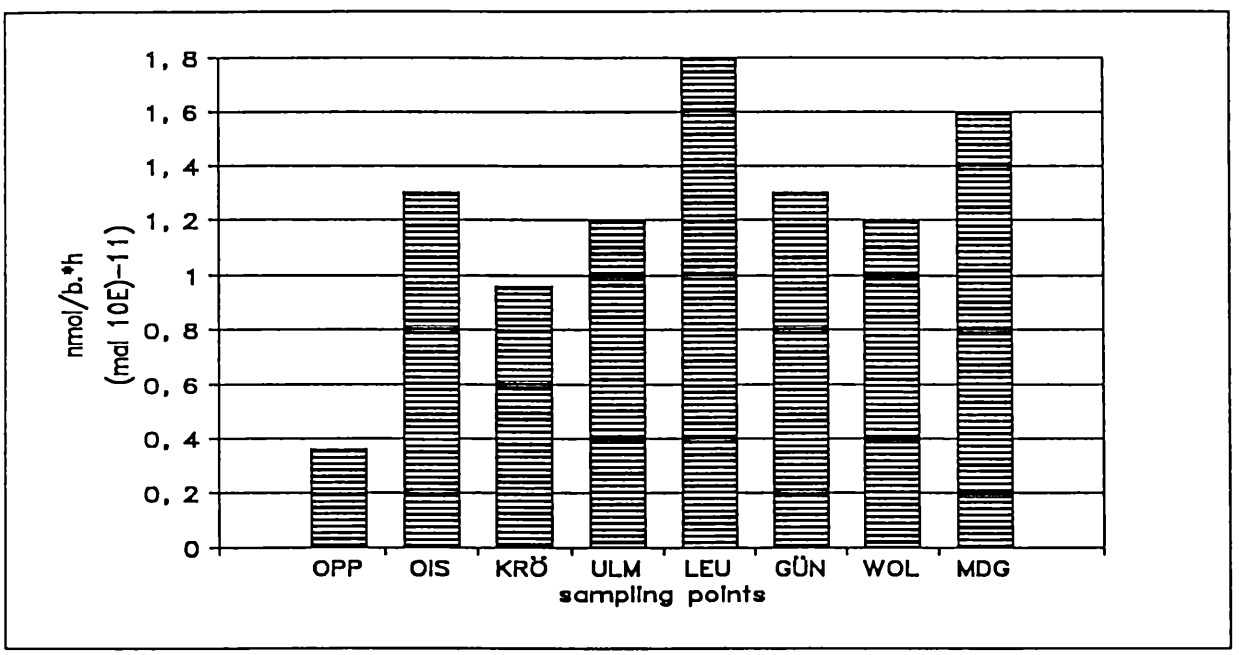


The specific activity, i.e. the thymidine incorporation rates per cell, varied from $2,5*10^{12}$ to $8,3*10^{-11}$ nmol/cell*h. The average values are shown in Fig.8.

Frequency of dividing cells

The FDC values fluctuated between 0,2 and 14,52 % (Fig.9) and showed no correlation (Spearman correlation coefficient) to any other parameter. This finding confirms the statement, that the frequency of dividing cells does not reflect actual activities in fast flowing river ecosystems with a high amount of allochthonous bacteria (KASIMIR, in press).

Fig.8 TTI, specific activity, average (n = 5)



Nalidixic acid inhibition

Between 0.96% and 5.71% of the cells were NAL positives. Their number was markedly higher than the colony count, but still much lower than the percentage of active cells. Although the nalidixic acid inhibition method was only performed on one sampling day (13.9.90, Fig.10), the pattern of NAL-positive cells seems to be similar to the pattern of the percentage of the colony count of direct count (see Fig.4).

Toxicity as measured by the Microtox method with luminescent bacteria could not be detected [only measured on 25.8.90] (KAVKA, pers.comm.).

Fig.9 frequency of dividing cells (average n = 5)

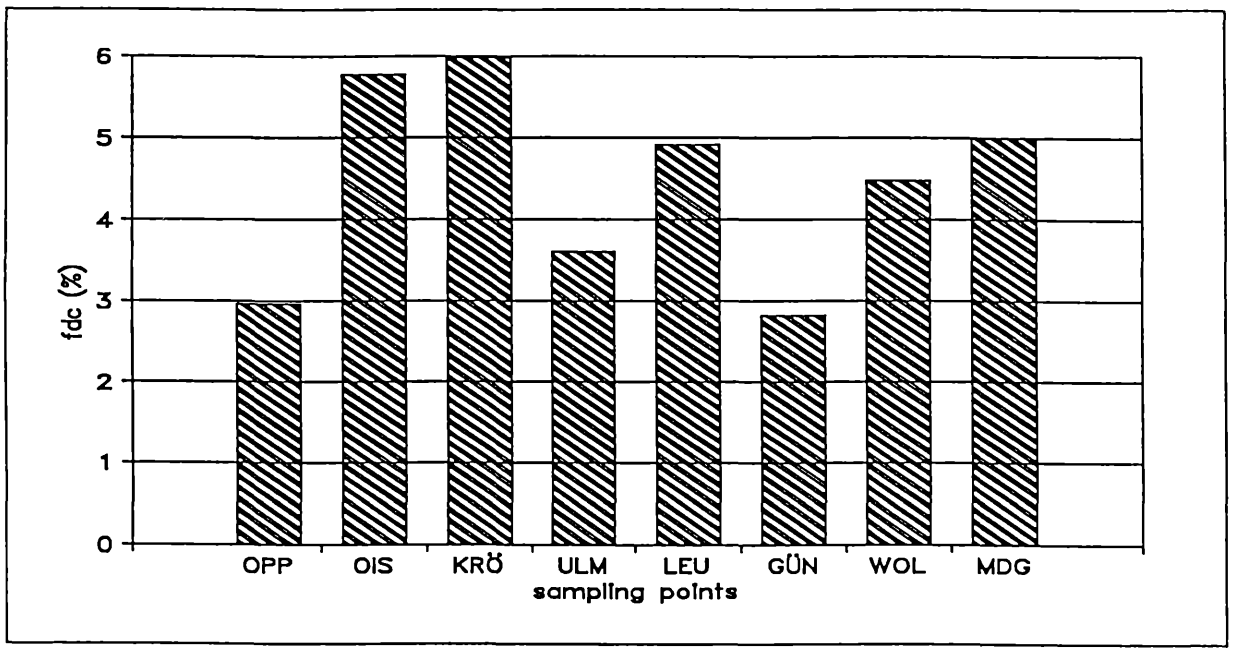
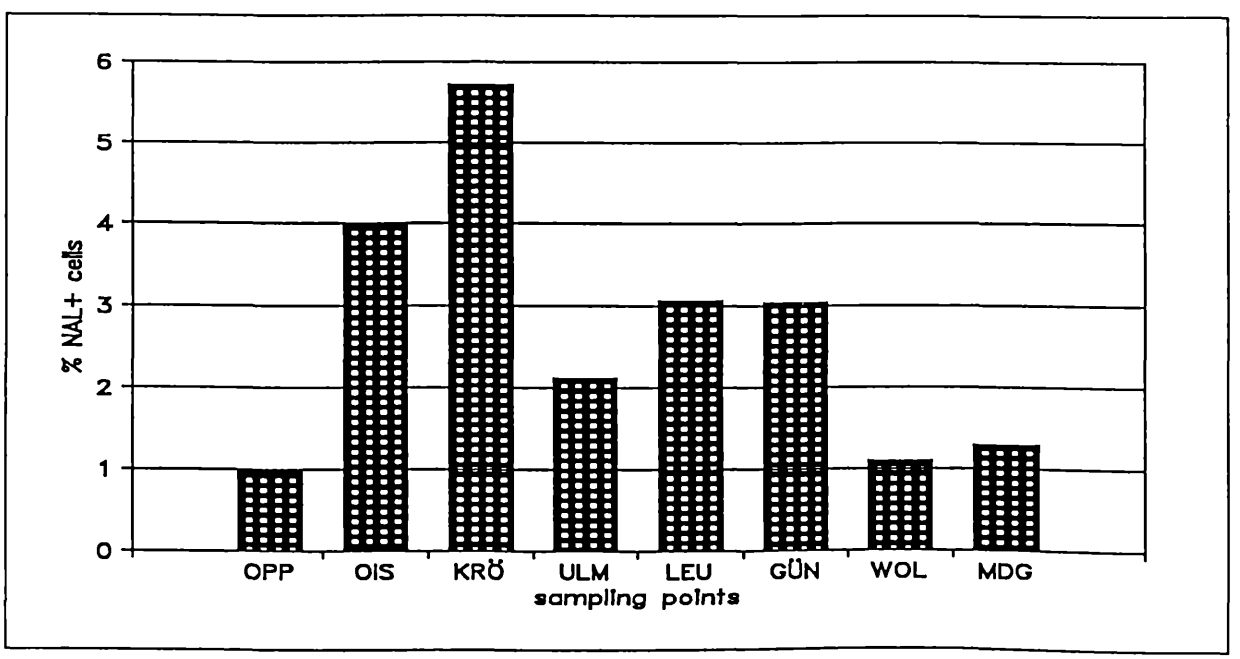


Fig.10 Active cells (nalidixic acid method, 13.9.90)



Methodological observations

AODC (epifluorescence direct counts)

For counting HNF and FDC, more than 50 fields should be counted to get a satisfactory coefficient of variation.

Tritiated thymidine incorporation

The degree of participation varied greatly (40-70%), therefore a correction of the thymidine incorporation values can be done only, if the DP has been determined for every sampling point. In one case (Opponitz), the DP could not be estimated because the calculated regression did not cross the X-axis in a realistic region. This could be due to the fact, that in low nutrient situations bacteria use thymidine as a carbon and nitrogen source. Consequently, the ^{3}H-label is found not only in the DNA, but in other macromolecules. So the values obtained should be corrected accordingly. Thus, in the case of Opponitz, the clean water site, the incorporation values may be overestimated by an unknown factor, in the other cases the values are underestimated by 30-60%.

Summary

Despite the few sampling dates and the rapidly changing conditions in riverine ecosystems, it could be shown, that bacterial activities, corresponding to the total bacterial numbers, and DOC values remained high while colony counts decreased. Whilst colony counts showed to be a good indicator of organic contamination, high bacterial thymidine incorporation values seem to indicate the self-purification process itself. The frequency of dividing cells did not correlate with any other parameter and does not seem to reflect short time activities or selfpurification processes. The nalidixic-acid inhibition method of counting active cells corresponded to the colony counts but gave low figures of active cells, perhaps due to the relatively short incubation time.

As compared to the situation before the construction of waste water treatment plants (see WENINGER, 1988), the water quality improved significantly. However, the lower part of the River Ybbs is still polluted; depending on the strongly fluctuating flow regime and discontinuous waste water inflow, the water quality is not constant.

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