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Bacterial biomass and bacterial uptake of glucose in polluted and unpolluted groundwater of sandy and gravelly deposits

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With 2 figures and 2 tables in the text

Investigations on the bacteria in organically polluted and unpolluted groundwater of sandy and gravelly deposits were carried out in the river Fulda valley near the town of Fulda (Fed. Rep. of Germany) as a part of a research project on the ecology of groundwater including influences of infiltrating substances (HUSMANN 1974/75, 1978). The uppermost layers in the area are of clay, poor clay and fine-grain sand ranging in depth from 0.8 to 3.4 m. This is followed by sandy and gravelly deposits, 1.6 to 3.2 m deep. A layer of clay, impervious to water begins at 3.8 to 5.0 m below the surface. Detailed description of the geological situation is given by HUSMANN (1974/75) and MARXSEN (in press).

Groundwater samples were collected from the layer of sandy and gravelly deposits, about 0.5 m above the layer of clay, by means of pumping tubes installed in the area. The bacteria were counted and classified on the basis of their shape and size by means of epifluorescence microscopy after staining with acridine orange. Methods described earlier (MARXSEN in press) were modified as suggested by WATSON et al. (1977) by prestaining the filters (polycarbonat membrane, 0.2 μm pore size) with irgalan black GBL (Fa. Geigy, 24 h, 2 g · l^{-1} in 2 % acetic acid). In addition an objective "Planapo 63/1.40 Oel" was used for counting. For each size-fraction an average volume was estimated and this value was used for calculation of the total cell volume. The heterotrophic activity was measured by determining uptake kinetic parameters with ^{14}C-labelled glucose (method of WRIGHT & HOBBIE 1966). Experimental conditions were the following: Natural water temperature, 1 h incubation time, and 5 concentrations from 30 to 600 μg glucose per l. The methods are described in detail by MARXSEN (in press), where also results of investigations from January 1979 are published as well as maps on the location of sampling stations (pumping tubes), groundwater level, flow directions, and chemical parameters. In 1979, from each of 16 pumping tubes in different areas 4 samples were taken at 3 months interval to record yearly variations in bacteriological parameters.

The only sampling station with nearly natural conditions is no. 28 (oxygen: 6.8—7.4 mg $O_2 \cdot l^{-1}$; COD ($KMnO_4$): maximum 0.2 mg $O_2 \cdot l^{-1}$). The number of bacteria per ml varies only little (2.0—3.7 · 10^6) as well as the total bacterial volume (0.22—0.43 $mm^3 \cdot l^{-1}$). These values are relatively low as compared to other groundwater samples (see Fig. 2, Table 1), but high compared to surface waters. Similar values are found in eutrophic lakes (OVERBECK 1975). The V_{max}-values during the whole year were about 0.1 μg glucose · l^{-1} · h^{-1}, with small fluctuations. Such low heterotrophic potentials are only rarely found in eutrophic lakes. In neighbouring small unpolluted streams similar values were measured only at some places during short periods in winter (see Table 2). Also the specific activity is low (0.03—0.06 · 10^{-9} μg glucose · h^{-1} · $cell^{-1}$ and 0.3—0.6 μg glucose · h^{-1} · mm^{-3}). In the streams mentioned above such low values were found on a few

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Table 1. Minimum and maximum values of the determined bacteriological parameters.

total number of bacteria B_n (10^6 bacteria · ml^{-1})	1.5 — 15
total volume of bacteria B_v ($mm^3 \cdot l^{-1}$)	0.19 — 1.6
medium volume of bacteria (μm^3)	0.076— 0.21
maximum uptake velocity, uptake potential V_{max} (μg glucose · $l^{-1} \cdot h^{-1}$)	0.09 — 3.3
sum of substrate affinity and natural substrate concentration $K_t + S_n$ (μg glucose · l^{-1})	18 — 530
turnover time T_t (h)	27 —1400
specific uptake potential V_{max}/B_n (10^{-9} μg glucose · $h^{-1} \cdot cell^{-1}$)	0.03 — 0.38
specific uptake potential V_{max}/B_v (μg glucose · $h^{-1} \cdot mm^{-3}$)	0.2 — 3.2

occasions in winter. WRIGHT (1978), during his investigations of Essex estuary, recorded such low values only at offshore sampling stations.

The most polluted part of the investigation area is at pumping stations 7 and 25. Here sometimes no oxygen was found (maximum: 1.5 mg $O_2 \cdot l^{-1}$; COD at 25: 2.5—4.3 mg $O_2 \cdot l^{-1}$). Total bacterial counts and volumes were at a medium range if related to other sampling stations (25: 3.6—11 · 10^6 bacteria · ml^{-1}; 0.48—0.91 $mm^3 \cdot l^{-1}$). Probably because of insufficient oxygen conditions in this part of the investigation area, often patterns of glucose uptake were recorded which did not agree with MICHAELIS-MENTEN kinetics or any other usual type.

Sampling station 26 is situated in a peripheral area of the polluted water stream and is influenced at different times with different intensities of pollution which can be seen from the chemical data (oxygen: 3.3—7.1 mg $O_2 \cdot l^{-1}$; COD: 0.4—2.1 mg $O_2 \cdot l^{-1}$; see Fig. 1). The bacterial biomass values are among the highest recorded in this investigation (5.1—15 · 10^6 bact. · ml^{-1}; 0.72—1.6 $mm^3 \cdot l^{-1}$), as well as the V_{max}-data (0.6—3.3 μg glucose · $l^{-1} \cdot h^{-1}$) which at some occasions reached levels which would be high for eutrophic lakes (see Table 2). The specific uptake potential values are also among the highest in the entire investigation area (0.12—0.22 · 10^{-9} μg glucose · $h^{-1} \cdot cell^{-1}$; 0.84—2.1 μg glucose · $h^{-1} \cdot mm^{-3}$). Comparisons with data from other ecosystems are given in Table 2.

The seasonal fluctuations of B_n, B_v, V_{max}, V_{max}/B_n, V_{max}/B_v, $K_t + S_n$ (Fig. 1) showed highest values in spring when nearly the whole valley had been flooded a few weeks earlier. The highest values of V_{max} were found at all sampling stations influenced by the flood except one pumping tube which was influenced throughout the year by river water. However, at most sampling stations maximum values were not so distinctly high as at sampling station 26. Bacterial biomass maxima did not occur in the water of all sampling stations, more often no maxima of V_{max}/B_n and V_{max}/B_v were found. Despite some deviations the reported seasonal fluctuations of the bacteriological parameters of pumping tube 26 are typical for the moderately polluted part of the investigation area where anaerobic conditions do not occur, but the levels of the fluctuations differ between the sampling stations. The reported flood influence was confirmed by an additional investigation of some sampling stations in winter 1980.

The pumping tubes 28-26-24-9, situated in a line at right angle to the flow direction of the groundwater in rather similar sediment layers, represent conditions from nearly natural (28) to a medium pollution area (24, 9). At sampling station 28 uptake potential and specific uptake potential values are lowest (see Fig. 2). The highest bacterial biomass and V_{max} values were found in the samples

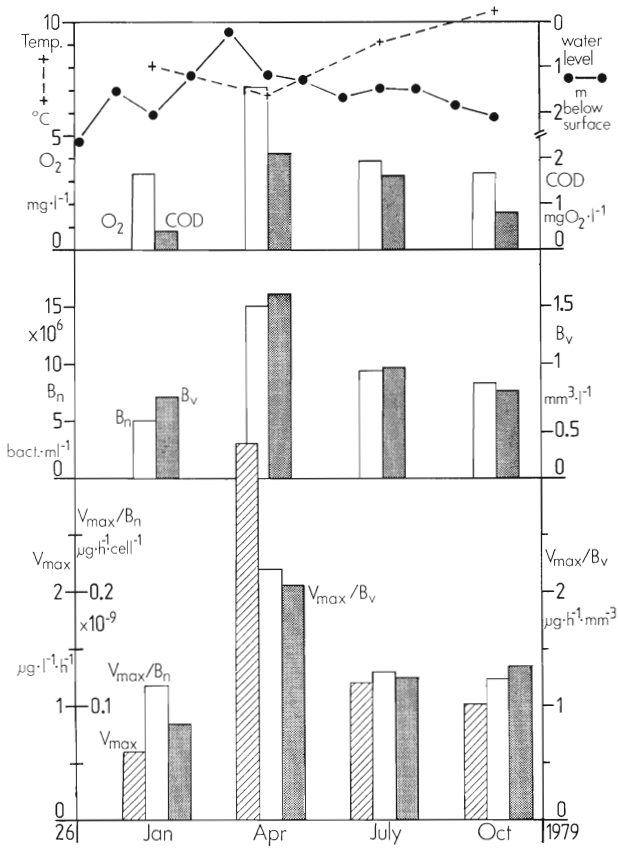

Fig. 1. Bacteriological parameters in the groundwater from pumping tube 26 during 1979. For explanation of abbreviations see Table 1.

Table 2. Values of V_{max} (µg glucose · l^{-1} · h^{-1}) and V_{max}/B_n (10^{-9} · µg glucose · h^{-1} · $cell^{-1}$).

	V_{max}	V_{max}/B_n	
Rohrwiesenbach, woodland stream	0.05 — 3.0	0.06 —0.68	MARXSEN 1976, 1980
Breitenbach, open grassland stream	0.09 — 1.1	0.18 —1.35	MARXSEN 1976, 1980
Essex estuary, mouth — 4 km upriver	0.81 — 1.31	0.098 —0.262	WRIGHT 1978
Essex estuary, 1.5—14 km offshore	0.0017— 0.75	0.0028—0.15	WRIGHT 1978
Fulda, river at slightly polluted part	2.3 —12.7	1.3 —2.2	

from tube 26. There, too, clearly higher concentrations of COD were observed than at 28. At sampling stations 24 and 9 mostly higher COD values compared with those for 26 were recorded, but oxygen concentrations were lower. Corresponding to oxygen concentrations, bacterial biomass and uptake potential is decreased. So, bacterial biomass and heterotrophic uptake potential is highest at a sampling station with increased concentrations of organic substances and medium oxygen level. If the oxygen level decreases further, below about 3 mg · l^{-1}, there is a clear limiting influence on bacterial biomass and heterotrophic activity.

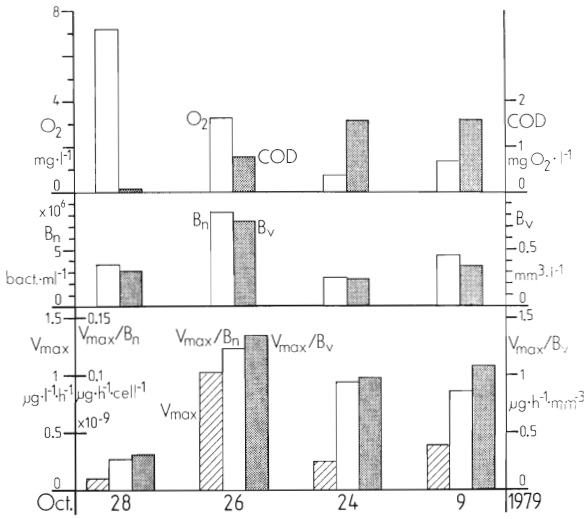

Fig. 2. Bacteriological parameters for October 1979 in the groundwater from the pumping tubes 28-26-24-9 which are situated in a line at right angle to groundwater flow. For explanation of abbreviations see Table 1.

Although an attempt was made to compare bacteriological parameters at various locations along the direction of groundwater flow, such comparisons have only a limited significance because of the following reasons. First, the sediment layers along groundwater flow, unlike those across the line where the stations 28-26-24-9 are located, are more variable. Second, there is no constant and continuous infiltration of polluting substances in the groundwater, and as such samples taken from several tubes following each other in direction of flow do not represent different decomposition levels of the same initial conditions. Keeping these limitations in mind, the investigations during 1979 confirmed earlier recorded trends (MARXSEN in press) of decreasing bacterial biomass and heterotrophic potential with flow direction of groundwater.

It must be emphasized that the values of the bacteriological investigations of the groundwater samples, which were collected through pumping tubes, are only relative. Most groundwater bacteria are "Aufwuchs" bacteria on sand particles, whereas the samples in the present study contained only those bacteria which are either free—living in the interstitial water, or are separable from particles by the pumping suction, or which may be attached onto those smaller particles that were pumped up. These bacteria are only a small part of the unknown total number. However, it is presumed that because of the uniformity of sampling technique and the similarity of sediment (at least at the sampling stations described in detail) always a comparable proportion of the bacterial population was obtained in the samples.

In considering these sampling difficulties the specific uptake data are of special importance. They are indicators of the physiological state of the bacteria independent from their total biomass. It might be that relatively many bacteria from sampling station 28 are, because of nutrient starvation, rather inactive (dormant;

waiting cells), as reported from other ecosystems (e. g. JANNASCH 1955; WRIGHT 1978), whereas at sampling station 26 the bacterial population is rather active because of sufficient nutrient and oxygen conditions. Only a few results of specific uptake investigations are available (Table 2). Results obtained in the present study for groundwater from an unpolluted area were in the upper range of those obtained by WRIGHT (1978) from offshore areas. The maximum values found in peripheral areas of the polluted groundwater stream (pumping tubes 26, 24) were similar to the maximum values recorded by WRIGHT (1978) from Essex estuary, but lower than the highest values from unpolluted streams (MARXSEN 1976, 1980) or the results from the slightly polluted river Fulda.

Recently experiments were started to find out the real number of bacteria by retrieving sand that had been sterilized, was contained in stainless steel nets and was placed for varying time periods in the pumping tubes. Initial results show that the number of bacteria per ml of the groundwater samples amounts to less than 10 % of the total number of bacteria per ml sediment.

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