Internat. Rev. Hydrobiol.	**88**	2003	3–4	372–384

WAIRIMU A. MUIA[1], GERNOT BRETSCHKO[2] and GERHARD J. HERNDL[3]

[1]Egerton University, P. O.Box 536, Njoro, Kenya; e-mail: wairimu@copy.com.ke
[2]Biological Station Lunz, A-3293 Lunz am See, Austria
[3]Dept. of Biological Oceanography, Netherlands Institute for Sea Research (NIOZ), P. O. Box 59, 1790 AB Den Burg, Texel, The Netherlands; e-mail: herndl@nioz.nl

A Study of Biofilm in a Second Order Tropical Stream, Njoro River, Kenya: First Results

key words: tropical river, biofilm, carbohydrates, exopolysaccharides, sediment organic matter

Abstract

Between January and December 1999 the extent of biofilm development has been studied at two sites (muddy sediment site [A] and sandy sediment site [B]) in Njoro River, Kenya. Grain size, organic matter and bacterial abundance were determined at different depth layers of the sediment. Particulate carbohydrate concentrations were measured as an indicator of biofilm development in the sediment. Additionally, the development of the biofilm was investigated by placing glass slides at rock and sediment surfaces and in different sediment depth layers. Our first results for sediment samples collected in January 1999 and growth on slides exposed for 28 days in March 1999 is presented. Grain size analysis revealed that sediment particles < 1 mm contributed between 82 to 91% at site A and 45 to 67% at site B depending on the sediment layer. At site A, total organic matter (% weight loss on ignition) and total organic carbon (TOC) were more or less constant with depth while both parameters significantly decreased with depth at site B. Total organic nitrogen (TON) was detectable at all depth layers at site A but only in the top 40 mm layer at site B. Bacterial numbers were highest in the top 40 mm layer at both sites. Bacterial abundance of $3.95 \pm 5.96 \times 10^8$ and $4.10 \pm 3.88 \times 10^8$ cells g^{-1} dry sediment were recorded at site A and B, respectively. For both sites, highest concentrations of colloidal carbohydrates occurred in the top 40 mm layer, but concentrations were more or less constant below. Bacterial abundance on glass slides was higher on slides placed on rock surfaces than on fine sediment surfaces or within fine sediments.

1. Introduction

In low order streams the main source of energy is allochthonous organic matter (CUMMINS, 1974; MEYER, 1994). This organic matter is mainly in dissolved form (CUMMINS *et al*., 1983) and represent 30–70% of the dissolved organic carbon (DOC). Particulate organic matter (POM) of terrestrial vegetation entering streams is leached resulting in significant portion of its organic matter as dissolved compounds. The principle-processing site for the DOC and POM is the streambed (LOCK and HYNES, 1976; DAHN, 1981). Here microorganisms (especially bacteria and fungi) form a biofilm (MARSHALL, 1984; LOCK, 1993) i.e., a complex assemblage of cells embedded in a gelatinous matrix of exopolymeric substances (EPS). The functions of the matrix range from attachment off cells to surfaces, protection from dehydration and other environmental stresses (LOCK, 1993).

The biofilm bacteria in the sediment dominate stream metabolism by contributing the majority of systems respiration (EDWARDS *et al*., 1990). Bacteria rapidly degrade the labile components of the DOM in a matter of days (WETZEL and MANNY, 1972). The resistant plant materials of the POM are first degraded by fungi and the solubilized products are utilized by the bacteria. The enzymically transformed organic matter is more readily available to benthic animals (BOTT *et al.*, 1984) which also colonize the sediments (BRETSCHKO, 1992).

 1434-2944/03/3-407-0372 $ 17.50+.50/0

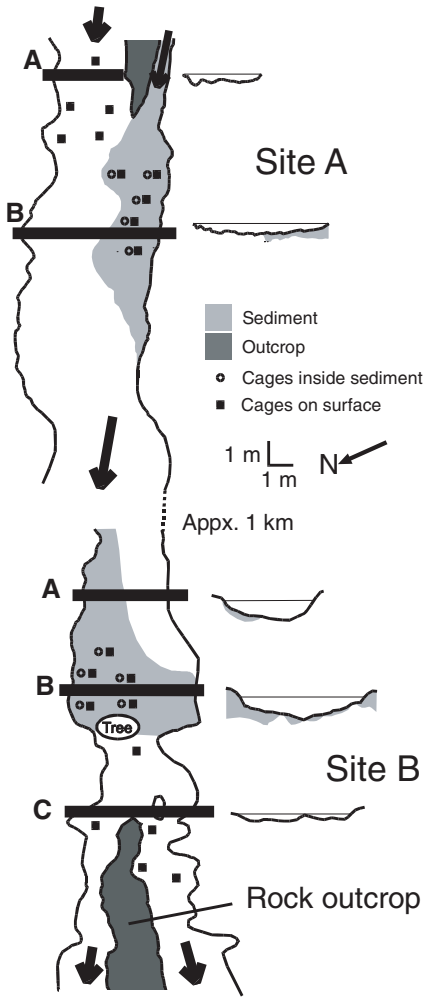

Figure 1. Sampling sites in a section of Njoro River within Egerton University campus. Site A is a muddy sediment site while B is a sandy sediment site.

The zoobenthos also utilizes biofilm microbes directly as a food source (ARMSTRONG and BÄRLOCHER, 1989; SCHALLENBERG and KALFF, 1993; BORCHARDT and BOTT, 1995).

Biofilm accumulations in the riverbed or on suspended particles (BERGER *et al*., 1996) contribute largely to the removal of contaminants from the water (BOUWER, 1989; WILDERER and CHARACKLIS, 1989). Thus the biofilm enhances self-purification of surface water.

Despite the importance of the ecological roles played by bacteria in aquatic environments, a considerable gap in our knowledge on the ecology of bacteria in lotic systems exists (LEFF, 1994). This is even more pronounced in the tropics where microbial studies have widely been omitted in limnological investigations (JACKSON and SWEENEY, 1995). Although some comprehensive ecological studies on tropical rivers have been carried out (BISHOP, 1973) few of these are in tropical Africa. Interest in ecological studies of streams in Kenya is now emerging. A few investigations have been conducted in some Kenyan rivers (MATHOOKO,

Table 1. Characteristics of data on study sites. Triplicate readings were made for the physical-measurements. Mean ± SD.

Location	Most abundant riparian plant species	Altitude m	Conductivity μS cm^{-1}	pH-value	Oxygen % saturation	Temperature °C	Discharge m^3 s^{-1}	Susp. Solids g l^{-1}
SITE A	*Syzigium cordatum* *Rhus natalensis*	2210	185.3 ± 0.6	8.10 ± 0.01	51.0	14.7 ± 0.1	0.002	0.36 ± 0.16
SITE B	*Syzygium cordatum* *Acacia abyssinica*	2195	186	7.85 ± 0.01	57.5 ± 0.7	14.2	0.009	0.31 ± 0.05

1996; SHIVOGA, 1999; MWANGI, 2000). These studies focused on macro- invertebrates or organic matter dynamics.

Lack of information regarding stream bacterial assemblages continues hindering our ability to understand stream ecosystems in a holistic manner. Consequently, this paucity of information is a stumbling block in efforts geared to make sound management in the conservation of the quality and quantity of the declining freshwater resources in Kenya.

The present study describes the concentrations of the major biofilm parameters at different depths at a muddy and a sandy sediment sites in Njoro River, Kenya. Specific emphasis was put on the determination of bacterial abundance and its relation to sediment organic matter. To gain a better insight into the colonization rate of sediment bacteria, glass slides were introduced at different sites with contrasting grain size distribution of the sediments. Furthermore, the concentration of carbohydrates to describe biofilm development in stream sediment was determined.

2. Study Site

River Njoro is a second order tropical river in the Rift Valley province of Kenya (for map of the river and catchment description, see MAGANA, this volume). Two study sites (Fig. 1, Table 1) were selected along the river section within Egerton University campus. The muddy sediment site (Site A) is about 16 m long. It is characterized by fine muddy sediments deposited in a pool near the left bank. Sandy sediment site (Site B) is about 1 km downstream of Site A. It is characterized of sandy deposits in a pool formed by damming effect of a tree (*Syzygium cordatum*) growing in the active channel.

3. Material and Methods

3.1. Surface Water Sampling and Basic Parameters Measured

The concentration of suspended solids was determined by filtering 100 ml of water through tared membrane filters (Sartorius, cellulose nitrate, 0.45 μm pore size), drying at 80 °C for 48 hr and weighing. Conductivity was measured with a WTW-LF90, temperature and pH with WTW-pH91 and oxygen with OX192. The mid section method was used to calculate discharge from water velocity measurements taken with a flow meter (General Oceanics Inc. Florida, Model 2030 R). These measurements were made at 60% depth from the surface over a transect every 0.5 m. Water samples for determining bacterial density were preserved in 0.2 μm filtered 3.7% formaldehyde.

3.2. Sediment

Five sediment cores were collected with a plastic coring tube (ca. 50 mm inner diameter) on 12th January 1999. The cores were stored in a cool box at 4 °C and brought back to the nearby laboratory for processing. The water layer above the sediment core was carefully siphoned off and the sediment was extruded and sliced into 0–40, 40–80, 80–140 and 140–200 mm layers, aseptically mixed thoroughly to ensure uniform distribution of the sediment. From each layer, a wet sample portion of approximately 30 g was dried at 80 °C for 4 days and then reweighed. The dry sample was then used for determining total organic carbon (TOC), nitrogen (TON) and weight loss on ignition (WLI). Sediment for carbohydrate analyses was frozen at −70 °C and lyophilized. About 1 g wet sediment from each layer was preserved in 0.2 μm filtered 3.7% formaldehyde for the enumeration of bacteria.

3.3. Redox Potential

Redox potential (E_h) was measured as an operational parameter to indicate oxic and anoxic conditions at the study site. The measurements were taken in a nearby laboratory less than one hour after sampling. Readings were made at 1 cm intervals in the coring tubes using a method similar to that described by PARKES *et al.* (1979). The platinum and calomel electrodes (Ingold) were connected to a WTW – pH91 meter and pre-calibrated with ZoBell solution (0.003 M $K_3Fe(CN)_6$ and 0.003 M $K_4Fe(CN)_6$ in 0.1M KCL) as suggested by MUDROCH and AZCUE (1995).

3.4. Granulometric Analysis

Sediment samples from each layer were wet sieved on a mechanical shaker fitted with sieves (Retsch) of mesh sizes 10, 6.3, 1, 0.5, 0.1, and 0.063 mm. The fractions were oven-dried (80 °C for 3 d) and the percentage weight distribution for each grain size class plotted as cumulative curves for calculations of median grain size (Md, mm) sorting coefficients (S_O) 25% (Q1) and 75% (Q3) quartiles (MÜLLER, 1964).

3.5. Weight Loss on Ignition

Approximately 1 g of dry sediment from each sediment layer was ignited in a muffel oven (Labotherm® L3/C6) at 500 °C for three hours. The loss in weight expressed as a percentage of the weight of the original sample was used as a describer of organic matter in the sediment.

3.6. Total Organic Carbon and Total Nitrogen

TOC and TON were determined according to the method described by BRETSCHKO and LEICHTFRIED (1987). The samples were ground and homogenized. Inorganic carbon in approximately 150 mg sub samples were transformed to CO_2 with 0.1 M HCl and removed by bubbling pressurized air through the sample. The samples were filtered through combusted glass fibre filters (Whatman GF/F) and then analyzed for TOC and TON in a LECO CHN analyzer. The results are expressed as mg TOC. g^{-1} and mg TON. g^{-1} dry sediment, respectively. Two sub samples from each sediment layer from four or three replicate cores for site A and B respectively were used.

3.7. Bacterial Abundance

The sediment and water samples were homogenized by shaking the sample after adding in tetra sodium pyrophosphate (PPi) to a final concentration of 10 mM. There after the sample tubes were put on crushed ice and sonicated in pulsed mode (60 s, 45% duty cycle, power setting 3.2 in a Branson 250 sonifier)

with a tapered microtip. One-5 ml of diluted sample was stained with DAPI solution (5 μg ml^{-1} final concentration) in the dark for 15 min (VELJI and ALBRIGHT, 1993). The stained sample was filtered through a black 0.25 μm polycarbonate filter (Millipore) which was washed with 1 ml of 0.2 μm filtered water and subsequently covered in paraffin oil on a glass slide and examined under an epifluorescence microscope. At least 300 bacterial cells were counted in 30 randomly selected fields. Preparations were made from 3 replicate water samples from each site and from each depth layer from three sediment cores.

3.8. Carbohydrate Determination

Carbohydrates in sediment were determined using a method similar to that of UNDERWOOD *et al.* (1995) for determination of the amount of EPS within sediments. For total carbohydrates (TCHO) analysis, three sub samples, 10–15 mg each of freeze dried sediment from each depth layer in the three sediment cores from each site, were mixed with water followed by addition of aqueous phenol (5%) and concentrated H_2SO_4. After centrifugation absorbance of the supernatant was measured against the reagent blank at 485 nm (DUBOIS *et al*., 1956; LIU *et al*., 1973). Glucose was used as a standard. Colloidal EDTA carbohydrates (Coll-CHO) is defined as the material remaining in suspension after aqueous extraction and centrifugation of sediment (UNDERWOOD *et al*., 1995). About 20 to 38% of coll EDTA extract is polymeric (UNDERWOOD *et al.*, 1995). Coll-CHO were determined by extracting a known amount of dry sediment with 10 mM EDTA at 20 °C for 15 min. After centrifugation, the amount of Coll CHO in the supernatant was determined using the phenol sulfuric acid assay. Polymeric carbohydrates (EPS-CHO) in the EDTA extracted fraction were precipitated with cold ethanol (70% final concentration, in the refrigerator). The extract was centrifuged at 5,000 rpm ($3430 \times g$) and the pellet resuspended in 1 ml of water. Then, the phenol sulphuric acid assay was repeated. CHO concentrations were determined after subtracting the sediment blank as suggested by GERCHAKOV and HATCHER (1972). Results were expressed as μg glucose equivalents per gram dry sediment. Sample size is similar to that of TCHO.

3.9. Determination of in situ Biofilm Development on Glass Slides

Biofilm development was studied on sterile glass slides exposed for 28 days in five replicate metallic cages pushed randomly in the fine sediment, placed on rock or fine sediment surfaces at both study sites (Fig. 1). After retrieval, the glass slides were gently cleaned with 0.2 μm filtered water, fixed with 3.7% formalin and stained in 1 μg ml^{-1} DAPI solution for 20 min. The number of bacteria in at least 30 randomly selected fields was counted under an epifluorescence microscope.

3.10. Data Analysis

Because of the heterogeneity of variances of some of the measured variables non-parametric tests were chosen for statistical data analysis using SPSS 8.0 for Windows. Within site comparisons of the parameters analyzed from sediments were done using Kruskal–Wallis test. The Mann-Whitney U-test was used to test for the differences the variations between sites. Relationships between parameters were determined using Spearman's rank correlation coefficients.

4. Results

4.1. Redox Potential

Highest E_h values were recorded in the water layer (Fig. 2). There was a decrease in E_h at the water sediment interface down to 30 mm sediment depth. Below this depth, down to 200 mm, E_h fluctuated between 150 to 250 mV at both sites.

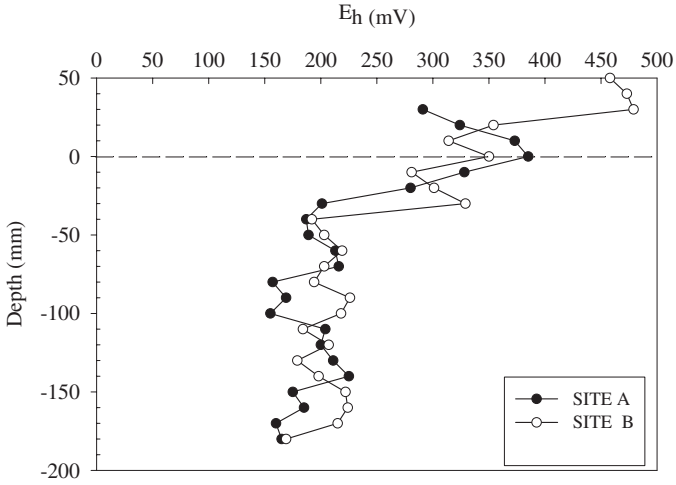

Figure 2. Redox Potential (E_h) recorded in sediment cores taken from Site A and B on 12 January 1999 in Njoro River.

4.2. Sediment Water Content and Grain Size Distributions

The sediment water content at site A ranged between 31–37% while at site B, the range was 21–27% (Table 2). Between – site variations were statistically significant (Mann-Whitney U-test, $n = 40$, $P < 0.001$) but within site variations were not significant.

80 to 91% of the sediment particles were <1 mm at site A and between 45 and 67% were <1 mm at B. The median grain size diameter at site A varied between 0.23–0.33 mm and between 0.61–1.16 mm at site B. The sediments were poorly sorted as S_O was always >2.0.

Table 2. Water content (%) and grain size distribution for different depth layers of sediment core samples obtained from muddy (A) and sandy grained (B) sites in Njoro River. Data for January 1999. Median grain size (Md), Quartiles (Q1 = 25%, Q3 = 75%), substrate heterogeneity or sorting coefficient, $S_o = (Q3/Q1)^{1/2}$. Mean ±SD, n = 5.

SITE	Depth (mm)	Water content (%)	Grain size <1mm (%)	Md (mm)	Q1 (mm)	Q3 (mm)	S_o
A	0–40	36.3 ± 6.0	91.5 ± 5	0.23 ± 0.04	0.09 ± 0.02	0.49 ± 0.10	2.35 ± 0.23
	40–80	31.1 ± 7.0	82.0 ± 23.6	0.33 ± 0.26	0.15 ± 0.11	0.74 ± 0.55	2.37 ± 0.34
	80–140	35.8 ± 11.2	88.7 ± 4.9	0.26 ± 0.14	0.09 ± 0.05	0.58 ± 0.16	2.65 ± 0.54
	140–200	37.2 ± 10.0	85.4 ± 7.1	0.28 ± 0.13	0.11 ± 0.04	0.60 ± 0.15	2.44 ± 0.34
B	0–40	27.0 ± 7.0	67.4 ± 8.0	0.61 ± 0.27	0.16 ± 0.06	1.78 ± 0.66	3.40 ± 0.22
	40–80	21.6 ± 5.3	63.8 ± 14.1	0.82 ± 0.20	0.24 ± 0.09	1.80 ± 0.76	2.95 ± 1.19
	80–140	24.5 ± 8.3	48.2 ± 10.7	1.16 ± 0.33	0.45 ± 0.16	2.74 ± 0.76	2.62 ± 0.73
	140–200	22.8 ± 5.0	45.5 ± 14.8	1.21 ± 0.49	0.54 ± 0.22	2.43 ± 0.98	2.27 ± 0.81

4.3. Sediment Organic Matter

The organic matter content of the sediments are shown in Fig. 3 and 4. A significant positive correlation ($P < 0.01$, $n = 28$) was found between WLI (%) and TOC suggesting TOC data can be derived from WLI (%) if TOC concentrations are not available. There was an apparent increase in WLI (%) with depth at site A but TOC in this site remained more or less constant. Within site comparisons of either WLI (%) or TOC did not reveal any significant differences. At site B, WLI (%) and TOC decreased with depth, and within site comparisons were significant for both parameters (Kruskal Wallis test, $n = 20$, $df = 3$, $P < 0.05$). Comparison between sites for the two parameters indicated significant spatial variations (Mann-Whitney U-test, $n = 28$, $P < 0.01$).

TON increased slightly with depth at site A but was only detected in the upper sediment layer in site B (Fig. 4). Spatial variations for this parameter were not statistically significant. C : N ratios obtained ranged between 43 and 167 and are indicative of relatively low quality food.

4.4. Bacterial Abundance and Carbohydrate in Sediments

Higher bacterial abundance in the top 40 mm layer of the sediment was detectable for both sites (Fig. 4). Within site variations were only significantly different at site A (Kruskal Wallis test, $n = 12$ df 3, $P < 0.05$), whereas site comparisons yielded significant differences (Mann-Whitney U-test, $n = 24$, $P < 0.01$). The mean number of bacteria in surface water was 4.9 ± 0.91 and $4.6 \pm 0.7 \times 10^5$ ml^{-1} at the muddy and the sandy sites but these were not statistically significantly different.

The CHO concentrations are depicted in Table 3. Spatial variations within sites for carbohydrates fractions were not significant. Significantly higher concentration fractions of TCHO, Coll-CHO and EPS-CHO were recorded at site A compared to site B (Mann-Whitney U-test, $n = 24$, $P < 0.05$ and $P < 0.01$ and $P < 0.001$, respectively).

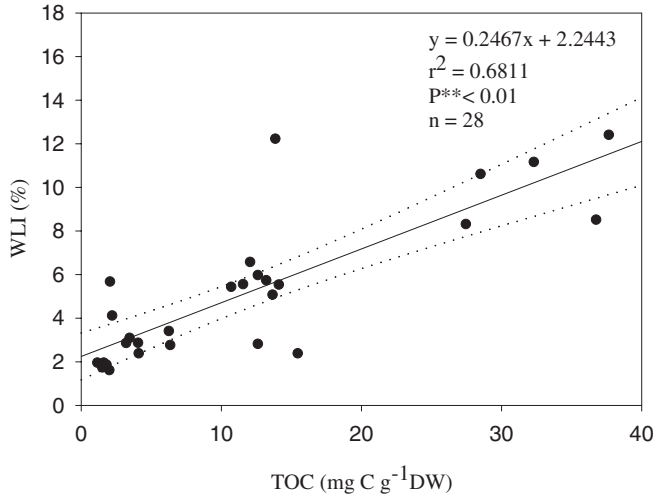

Figure 3. Relationship between total organic carbon (TOC) and weight loss on ignition (WLI) from sediment samples collected two sites in Njoro River in January 1999. Dashed lines indicate 95% CL.

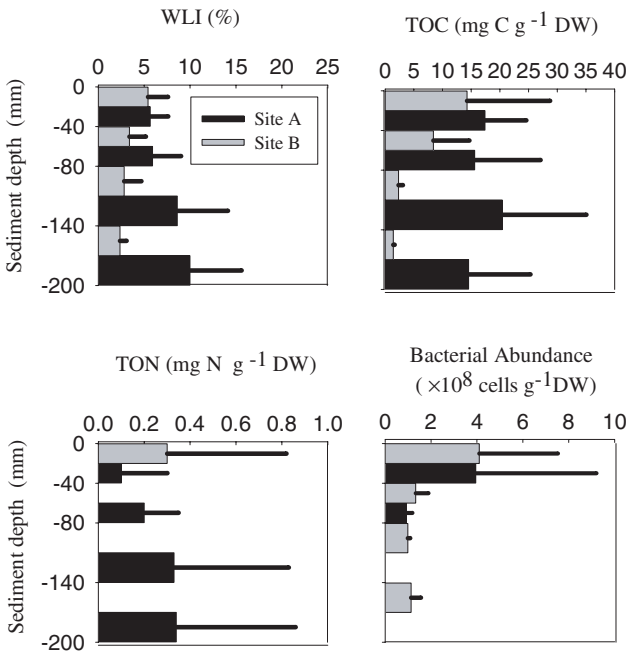

Figure 4. Distribution of organic matter parameters and bacteria at different sediment depth layers at muddy (A) and sandy (B) sites in Njoro River. Data for January 1999. Means ±SD

At site A, a moderate positive correlation was detected between TOC and TON ($P < 0.005$) (Table 4). Moderately strong, albeit significant positive correlations were also found between TOC and Coll-CHO and between TOC and EPS ($P < 0.05$ in both cases). TCHO and bacterial abundance were not significantly correlated to other parameters. At site B, modest but significant positive correlations ($P < 0.05$) were obtained between TCHO and TOC, Coll-CHO and EPS-CHO and between Coll-CHO and bacterial abundance while there was no correlation between TCHO and EPS. All other possible relationships were not significant.

When the results from the two sites are combined, Coll-CHO was tightly correlated to EPS-CHO and TOC ($P < 0.01$) and was moderately and strongly positively correlated to TON ($P < 0.05$). EPS-CHO was strongly positively correlated to TOC ($P < 0.01$) and moderately

Table. 3. Concentration of carbohydrates [expressed as mg gluc. eq. g DW of sediment^{-1}] at different depth layers at muddy and sandy sites (A and B respectively) of Njoro River. Mean and SD, n = 3.

Depth mm	Site A			Site B		
	Total CHO	Colloidal CHO	EPS CHO	Total CHO	Colloidal CHO	EPS CHO
0–40	5.1 ± 3.4	0.71 ± 57	0.33 ± 0.11	3.02 ± 1.5	0.56 ± 0.17	0.27 ± 0.24
40–80	6.9 ± 1.9	0.54 ± 0.17	0.21 ± 0.11	3.4 ± 3.2	0.12 ± 0.04	0.04 ± 0.01
80–140	6.5 ± 5.4	0.49 ± 0.21	0.14 ± 0.05	2.1 ± 0.82	0.12 ± 0.03	0.04 ± 0.02
140–200	2.2 ± 1.4	0.51 ± 0.14	0.23 ± 0.05	1.4 ± 0.60	0.08 ± 0.03	0.03 ± 0.02

Table 4. Correlation matrix (Spearman's rank correlations) for some variables measured in sediment from Njoro River, Kenya. Variables expressed per g^{-1} DW. Values of r are significant at *P < 0.05 and P** < 0.01. For comparisons within Site A and B n = 12 except for TOC and TON in site A, where n = 16. For combined data, n = 24 except for TOC and TON where n = 28.

SITE		Coll CHO	EPS CHO	TOC	TON	Bacteria
A	TCHO	0.20	−0.12	−0.28	−0.16	0.25
	Coll CHO		0.49	0.66*	0.24	0.16
	EPS CHO			0.66*	0.38	0.58
	TOC				0.74**	0.12
	TON					0.29
B	TCHO	0.21	0.00	0.64*	0.22	0.46
	Coll CHO		0.62	0.37	0.48	0.64*
	EPS CHO			0.29	0.48	0.31
	TOC				0.48	0.38
	TON					0.39
A and B combined	TCHO	0.37	0.25	0.36	0.35	−0.07
	Coll CHO		0.83**	0.72**	0.62**	−0.07
	EPS CHO			0.76**	0.61**	−0.09
	TOC				0.66**	−0.23
	TON					−0.05

correlated to TON (P < 0.01) and TOC was moderately correlated to TON (P < 0.01). Non significant correlations were found between TCHO and other parameters. Correlations between bacterial cells with all other parameters were not significant.

4.5. Biofilm Development

Highest bacterial abundances on exposed glass slides was generally recorded for slides placed on rocks, followed by those on sediment surface, 0–10 cm sediment depth, and the lowest abundance was 10 –20 cm depth horizon (Fig. 5). Within site variations were significant for both sites (Kruskal Wallis test, df 3, P < 0.01) while variations between sites were not significant.

5. Discussion

Various inherent problems associated with Eh measurements in natural environment have been discussed by WHITEFIELD (1969), WHITFIELD (1974) and MUDROCH and AZCUE (1995). Despite these problems, Eh has been widely used as an estimate of redox potential and can be useful in predicting anaerobic biomes where living organisms are unlikely to be found in sediments. In this study, Eh was used as an operational parameter to indicate oxic and anoxic conditions at the study sites. No attempt was made to make quantitative interpretation of Eh measurements in relation to the other parameters measured. According to WHITFIELD (1974) in well-aerated systems Eh ranges from around +500 to +300 mV. This closely agrees with the measurements recorded in the water and upper sediment layers in the present study (0–20 mm for site A and 0–30 mm for site B) where Eh was about 300 mV or higher. Most

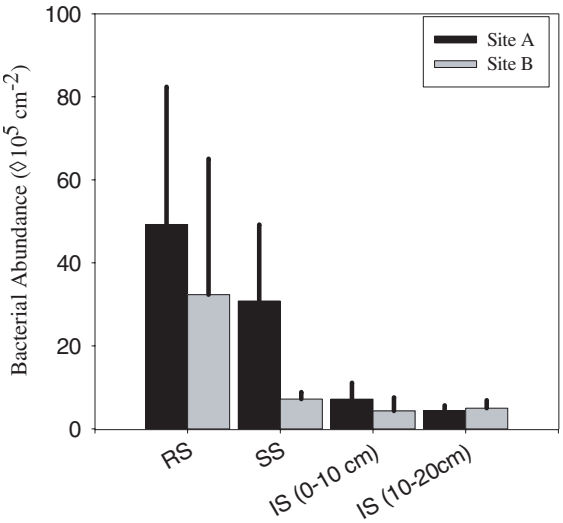

Figure 5. Biofilm development on glass slides exposed for 28 days at sites A and B in Njoro River. RS = Rock surface, SS = Sediment surface and IS = Within sediment. Means ±SD.

of the measurements recorded for our study sites were below this limit indicating poorly oxygenated sediments below 2 or 3 cm sediment depth.

Fine grained sediments in gravel streams have been found to contain more organic matter than coarser sediments and this has been attributed to the fact that finer sediments have a greater surface area for adsorption of organic matter (LEICHTFRIED, 1985). The comparison between sites for WLI (%) and TOC supports this idea since significantly higher organic matter was found at the muddy site which has higher proportion of particles less than 1 mm. Site B shows that higher TOC contents in the upper sediment layers, probably due to an accumulation of recently deposited organic matter at the sediment water interface. Although similar observation should be expected for site A there, the organic matter content remained constant over depth perhaps due to the higher proportion of small sediment particles (Table 2) which are likely to reduce the water flow through the sediments. A weak porewater flow at site A might also contribute to the more or less constant concentration of total carbohydrates at this study site. Significant inter-site variations could be due to smaller particle size and thus higher total surface area at site A sediments compared to those in site B. N was strongly correlated to TOC but sediments at both sites are of poor food quality as indicated by high CN ratios.

BATTIN and SENGSCHMITT (1999) found significant relationships between bacterial numbers and Coll-CHO and between bacterial numbers and uronic acids in sediment from an impounded section of the river Danube. The fact that EPS-CHO was not correlated to bacterial abundance in our study may indicate that, apart from bacteria, microphytobenthos could have contributed to the EPS-CHO mainly in the upper layers in addition to the bacteria. Similarly, GEESEY *et al*. (1978) showed that epilithic algae contributed the bulk of microbial biomass in a mountain streambed while bacteria contributed only a negligible fraction.

Bacterial counts in the water column were much lower than in the sediment, indicating that in small streams sediment bacteria are numerically more important than bacterioplankton (GEESEY *et al*., 1978; KASIMIR, 1990). Total bacterial numbers of surface sediments were

similar at both sites. This is possibly due to the high content of labile organic matter at the sediment surface. Significant differences between sites and low numbers of bacteria at deeper sediment horizons at site A points to the influence of smaller grain sizes; which increases the resistance to throughflow (BRETSCHKO, 1994) and thus affects oxygenation of the sediments. However, anaerobic conditions were encountered at about the same sediment depth at both sites (2 and 3 cm). Higher bacterial abundance has been found on smaller particle sizes (HARGRAVE, 1972; BOTT and KAPLAN, 1985) with larger surface area for colonization (DEFLAUN and MEYER, 1983; LEICHTFRIED, 1985). These muddy sediments are also richer in organic matter compared to larger particles (KONDRATIEFF, 1984; LEICHTFRIED, 1985; SINSABAUGH *et al.,* 1992). CLARET *et al*. (1998) showed in their study that even though bacterial abundance generally decreased with depth, if a higher proportion of fine-grained sediment is present in deeper layers, more bacteria were found. In the current study more bacteria were expected in the muddy sediment than at the sandy site; that this was not the case indicates that ecological factors other than size and organic matter content played a role in the distribution of heterotrophic bacteria in sediments. This means that apart from particle size and organic matter content, other ecological factors play a role in the distribution of heterotrophic bacterial biofilm in sediments.

One advantage for employing glass slides accompanied with epifluorescence microscopy to determine bacterial biofilm development in sediments is that microorganisms are counted on the slides and results per surface area can be directly obtained. The method is therefore quite rapid and the microbes counted have been recently active. Within site comparisons showed that at both study sites conditions on rocks, fine sediment surfaces and within sediments provided different growth conditions for bacteria. Highest bacterial abundances were reached on rocks where oxygenation was probably better than at sediment surface or within the sediment. The lack of between-site differences could be due to similar physical and chemical conditions at both sites, at least on the sediment and rock surfaces. The number of cells recorded on the artificial substrates are within the range given in related studies (BÄRLOCHER and MUDORCH, 1989; CLARET, 1998).

6. Conclusions

Poor through-flow due to higher proportions of small grain sizes may be considered as one of the most important factors determining growth of biofilm in Njoro River sediments. Through-flow may influence transport and storage of organic matter within the sediment as well as degree of aeration. In that way through flow controls the biocoenosis in the bed sediment. Nitrogen may be a growth limiting factor for bacterial growth in Njoro River since it was present only at low concentrations that resulted in high CN ratios indicating food of low quality. Artificial glass slides used to determine bacterial biofilm development in sediments is a rapid method for biofilm studies since microorganisms are counted directly on the slides. However, it is important to establish optimum exposure duration since overgrowth by microorganisms might occur. Care should be taken in interpreting EPS results in natural environments as it is difficult to exclude other sources of high molecular weight compounds detectable by the phenol sulphuric acid method.

7. Acknowledgements

The authors are grateful to the Austrian Academic Exchange Service (ÖAD) for providing a scholarship to enable A. W. MUIA to study in Austria and conduct fieldwork in Kenya. We are grateful to ALBERT BRUGGER (University of Vienna) for his initial assistance in carbohydrate analyses, the staff at

Egerton University, Kenya, and at the Biological Station Lunz, Austria, for technical support. Comments and suggestions given on an earlier version of the manuscript by two anonymous referees is acknowledged.

8. References

ARMSTRONG, S. M. and F. BÄRLOCHER, 1989: Adsorption of amino acids from epilithic biofilms in streams. – Arch. Hydrobiol. **115**: 391–399.

BÄRLOCHER, F. and J. H. MURDORCH, 1989: Hyporheic biofilms: a potential food source for interstitial animals. – Hydrobiologia **184**: 61–67.

BATTIN, T. J. and D. SENGSCHMITT, 1999: Linking sediment biofilms, hydrodynamics and river bed clogging: evidence from a large river. – Microb. Ecol. **37**: 2185–2196.

BERGER, B., B. HOCH, G. KAVKA and G. HERNDL, 1996: Bacterial colonization of suspended solids in the river Danube. – Aquat. Microb. Ecol. **10**: 37–44.

BISHOP, E. J., 1973: Limnology of a small Malayan River Sungai Gombak. Dr. W. Junk B. V., Publishers, The Hague, 485 pp.

BORCHARDT, M. A. and T. L. BOTT. 1995: Meiofaunal grazing of bacteria and algae in a piedmont stream. – J. N. Am. Benthol. Soc. **14**: 278–298.

BOTT, T. L., L. A. KAPLAN and F. T. KUSERK, 1984: Benthic bacterial biomass supported by stream dissolved organic matter. – Microb. Ecol. **10**: 335–344

BOTT, T. L. and L. A. KAPLAN, 1985: Bacterial biomass, metabolic state, and activity in stream sediments: relation to environmental variables and multiple assay comparisons. – Applied and Environ. Microbiol. **50**: 508–522.

BOUWER, E. J., 1989: Transformation of xenobiotics in biofilms. – *In*: CHARACKLIS, W. G. and P. H. WILDERER (eds.) Structure and function of biofilms. Wiley and sons ltd., Toronto, pp. 251–267.

BRETSCHKO, G. and M. LEICHTFRIED, 1987: Determination of organic matter in river sediments. – Arch. Hydrobiol. Suppl. **68**: 403–417.

BRETSCHKO, G., 1992: Differentiation between epigeic and hypogeic fauna in gravel streams. – Regulated Rivers **7**: 17–22.

BRETSCHKO, G., 1994: Bedsediment extension, grain shape and size distribution. – Verh. Internat. Verein. Limnol. **25**: 1631–1635.

CHARACKLIS, W. G., 1989: Biofilm processes. – *In*: CHARACKLIS, W. G. and K. C. MARSHALL (eds.) Biofilms. John Wiley and sons Inc. pp. 195 –231.

CLARET, C., 1998: A method based on artificial substrates to monitor hyporheic biofilm development. – Internat. Rev. Hydrobiol. **83**: 135–143.

CLARET, C., P. MARMONIER and J.-P. BRAVARD, 1998: Seasonal dynamics of nutrients and biofilm in interstitial habitats of two contrasting riffles in a regulated large river. – Aquat. Sci. **60**: 33–55.

CUMMINS, K. W., 1974: Structure and function of stream ecosystems. – BioScience **24**: 631–641.

CUMMINS, K. W., J. R. SEDELL, F. J. SWANSON, G. W. MINSHALL, S. G. FISHER, C. E. CUSHING, R. C. PETERSEN and R. L.VANNOTE 1983: Organic matter budgets for stream ecosystems: Problems in their evaluation. – *In*: BARNES J. R. and MINSHALL G. WAYNE (eds.), Stream Ecology – Applicaton and Testing of General Ecological Theory. Plenum press, New York.

DAHM, C. N., 1981: Pathways and mechanisms for removal of dissolved organic carbon from leaf leachate in streams. – Can. J. Aquat. Sci. **38**: 68–76.

DEFLAUN, M. F. and L. M. MAYER, 1983: Relationships between bacteria and grain surfaces in intertidal sediments. – Limnol. Oceanogr. **28**: 873–881.

DUBOIS, M., J. K. GILLES, P. A. HAMILTON, P. A. REBERS, and F. SMITH, 1956: Colorimetric Method for the determination of sugars and related substances. – Anal. Chem. **28**: 350–356.

EDWARDS, R. T., J. L. MEYER and S. E. G. FINDLAY, 1990: The relative contribution of benthic and suspended bacteria to system biomass, production and metabolism in a low- gradient blackwater river. – J. N. Am. Benthol. Soc. **9**: 216–228.

GEESEY, G. G., R. MUTCH and J. W. COSTERTON, 1978: Sessile bacteria: an important component of the microbial population in small mountain streams. – Limnol. Oceanogr. **23**: 1214–1223.

GERCHAKOV, S. M and P. G. HATCHER, 1972: Improved technique for analysis of carbohydrates in sediment. – Limnol. and Oceanogr. **17**: 583 –596.

HARGRAVE, T. B., 1972: Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. – Limnol. and Oceanogr. **17**: 583 –596.

JACKSON, J. K. and B. W. SWEENEY, 1995: Present status and future directions of tropical stream research – J. N. Am. Benthol. Soc. **14**: 5–11.

KAPLAN, L. A., and T. L. BOTT, 1983: Microbial heterotrophic utilization of dissolved organic matter in a piedmont stream. – Freshwater Biology **13**: 363–377.

KASIMIR, G. D., 1990: Die mikrobiellen Biozönosen eines alpinen Baches. – *In*: DAUBNER, I. (ed.), Internationales Hydromikrobiologisches Symposium. Veda, Verlag der Slowakischen Akademie der Wissenschaften Bratislava, pp. 245–250.

KONDRATIEFF, P. E. and G. SIMMONS, 1984: Nutritive quality and size fractions of natural seston. in an impounded river. – Arch. Hydrobiol. **101**: 401–412.

LEFF, L. G., 1994: Stream bacterial ecology: A neglected field? – ASM News. **60**: 135–138.

LEICHTFRIED, M., 1985: Organic matter in gravel streams (Project Ritrodat-Lunz). – Verh. Internat. Verein. Limnol. **22**: 2058–2062.

LIU, D., L. P. T. S. WONG and B. J. DUTKA, 1973: Determination of carbohydrates by a modified phenol sulfuric acid method. – Water Res. **7**: 741–746.

LOCK M. A. and H. N. B. HYNES 1976: The fate of "dissolved" organic carbon derived from autumn-shed maple leaves (*Acer saccharum*) in a temperate hard – water stream. – Limnol. Oceanogr. **21**: 436–443.

LOCK, M. A., 1993: Attached microbial communities in rivers. – *In*: FORD T. E. (ed). Aquatic Microbiology. Blackwell Scientific publications, Oxford, pp. 113–138.

MATHOOKO, J. M., 1996: Artificial physical disturbance at the surface of a Kenyan Mountain stream with particular reference to the ephemeroptera community. Ph.D thesis, University of Vienna. 224 pp.

MARSHALL, K. E., 1984: Microbial adhesion and aggregation. Dahlem Konferezen Berlin. Life science report 31. Springer Verlag

MEYER, J. L., 1994: The microbial loop in flowing waters. – Microb Ecol. **28**: 195– 199.

MUDROCH, A. and J. M. AZCUE, 1995: Aquatic sediment sampling. Lewis Publishers, Boca Raton, 219 pp.

MÜLLER, G., 1964: Sediment-Petrologie 1-Methoden der Untersuchung. Schweitzerbart, Stuttgart, 303 pp.

MWANGI, B. M., 2000: Bedsediment organic matter and macro invertebrate responses in catchment land use along a low order Tropical Stream: Sagana River, Kenya. Ph.D thesis, University of Vienna, 224 pp.

PARKES, R. J., M. J. BRYDER, R. H. MADDEN and N. J. POOLE, 1979: Techniques for investigating the role of anaerobic bacteria in estuarine sediments. – *In*: LITCHFIELD C. D. and P. L. SEYFRIED. (eds.) Methodology for Biomass determination and Microbial Activities in Sediments. American Society for Testing and Materials, Philadelphia. pp. 107–117.

SCHALLENBERG, M. and J. KALFF, 1993: The ecology of Sediment Bacteria in lakes and comparison with other aquatic ecosystems. – Ecology **74**: 919–934.

SINSABAUGH, R. L., T. WEILAND and A. E. LINKINS, 1992: Enzymic and molecular analysis of microbial communities associated with lotic particulate organic matter. – Freshwater Biology**. 28**: 393–404.

UNDERWOOD, G. J. C., D. M. PETERSON and R. J. PARKES, 1995: Measurement of microbial carbohydrate exopolymers from intertidal sediments. – Limnol. Oceanogr. **40**: 1243- 1253.

VELJI, M. J. and L. J. ALBRIGHT, 1993: Improved sample preparation for enumeration of aggregated aquatic substrate bacteria. – *In*: KEMP, P. F., B. F., SHEER, E. B. SHERR and J. J. COLE (eds.) Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, London, pp. 139–142.

WETZEL, R. G. and B. A. MANNY, 1972: Decomposition of dissolved organic carbon and nitrogen compounds from leaves in an experimental hard- water stream. – Limnol and Oceanogr. **17**: 927–931.

WHITFIELD, M., 1969: E_h as an operational parameter in estuarine studies. – Limnol. Oceanogr. **14**: 547–558.

WHITFIELD, M., 1974: Thermodynamic limitations on the use of platinum electrode in E_h measurements. – Limnol. Oceanogr. **19**: 857–865.

WILDERER, P. A. and W. G. CHARACKLIS, 1989: Structure and function of biofilms. – *In*: W. G. CHARACKLIS and P. A. WILDERER (eds.) Structure and function of biofilms. John Wiley and sons ltd. pp. 5–17.