ACCUMULATION OF INORGANIC AND ORGANIC POLLUTANTS BY BIOFILMS IN THE AQUATIC ENVIRONMENT

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Abstract. In a partly urbanized catchment to the south of Trier, Germany, short term variations in river sediment compounds as well as the bioaccumulation of pollutants on surface associated microbial coatings (biofilms) were investigated weekly during a period of six months. Concentrations of selected heavy metals (Cu, Zn, Pb), polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and for microbial characterisation protein, carbohydrate and uronic acid were analyzed. Sorption processes on biofilms were determined by temporal variations in pollutants and microbial parameters and through the comparison of sorbed substances in biofilms and sediments. The results show, that sorption events on biofilms play an important and dynamic role in spring and summer for transport and accumulation of the investigated pollutants in the aquatic environment. The amount of pollutants sorbed on sediment particles is not only dependent on the particulate bound or solved pollutants in the river water, but is strongly controlled by the changing conditions of the biofilms.

Key Words: River sediments, biofilms, organic micropollutants, heavy metals, temporal variations, biosorption, accumulation processes

1. Introduction

The adsorption on particle surfaces plays a decisive role in the transport of hydrophobic pollutants in river water (Karickhoff, 1981; Means *et al.*, 1980). Therefore, suspended particles and sediments are not only important sinks, but also a potential source of pollutants. Investigations carried out in 1992 (Schorer *et al.*, 1994; Symader *et al.*, 1994) have shown that in river sediments, temporal variations of heavy metals and organic micropollutants in the sediment surface layer, occurred during the year. These variations were not controlled by random processes. The observed phenomena can be explained in part by the different sources which supplied these pollutants at different times as well as mixing and dilution processes.

In the aquatic environment, the surfaces of mineral particles are, for the most part, covered with organic coatings which can drastically change the sorption behaviour of the particles. These organic coatings are made from humic substances and biofilms (Flemming *et al.*, 1996; Characklis & Marshall, 1990). A considerable amount of data concerning adsorption on humic substances is available from the literature (e.g. Frimmel & Christman, 1988). In contrast to this inanimate matrix, biofilms can actively influence the sorption, desorption and decomposition of pollutants. It must be expected, that a part of the accumulation of pollutants in river sediments is due to sorption on microbial coatings (Baughman & Paris, 1981; Urey *et al.*, 1976; Tsezos & Bell, 1989). The importance of sorption and desorption processes on biofilms with respect to temporal variations in pollutant contents in river sediments is complex and not understood (Evans *et al.*,

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1990; Flemming *et al.*, 1996). Nevertheless, sorption data on the accumulation of pollutants by biofilms are available only from lab studies with defined conditions and as a result, without the competition of a natural ecosystem. Unfortunately, investigations on natural river ecosystems are missing. This process could be an important key to the understanding of loadings, transport and sink of pollutants in the aquatic system. The sorption of pollutants on biofilms plays a very dynamic role, because they are not chemically inert. Changing conditions in the environment can induce changes both in the microbiota and their physiology. The sloughing off of biofilms and the breakage of binding sites in the extracellular matrix can result in remobilization and in a new source of pollutants (Flemming *et al.*, 1990, Tsezos & Bell, 1989).

In our study, the importance and influence of the sorption properties of biofilms contributing to the accumulation of pollutants in river sediments were investigated in more detail. Short temporal variations in the sediment compounds as well as the bioaccumulation of pollutants on surface associated biofilms were determined. At two fixed sampling stations, concentrations of different inorganic and organic micropollutants in river sediments and in biological growth were analyzed weekly during a period of six months.

2. Study area

The study area represents the drainage basin of the Olewiger Bach with an area of 39 km^2. It is located to the south of Trier in the western part of Germany. Devonian shales of the Hunsrück mountains with quartz and diabas veins form the bedrock geology. In the northern part of the drainage basin, pleistocean terraces of the river Mosel lie on the geological underground (Wagner, 1983). Land use is predominantly agricultural with grassland and arable farming. Settlements take up 10 % of the whole area. Several roads, small industries and waste water from solitary farms, with no connection to a sewage plant, influence the quality of the river water.

3. Methodology

3.1 SAMPLING PROCEDURE

Sampling biofilms in their natural aquatic habitat from surfaces like mineral particles, stones or plants is impossible, because the biological coatings cannot be separated from the surface of the substrate without changing or disturbing their original sorbing properties (MacNicol & Beckett, 1989). Therefore, the biofilm samples were obtained from a chemically inert artificial silicate material, which is similar to natural surfaces because of its roughness and its chemical properties. The silicate substrates were packed into nets and were exposed in the river water near the sediment surface. Because the lag time for developing biofilms on surfaces takes several days to weeks, the silicate substrates were left in river water for five weeks to allow sufficient time for biofilms to develop and pollutants to accumulate. To observe short term variations, five week old silicate substrates with grown biofilms were taken out and examined weekly over a period of six months. Sediments were sampled weekly at the same site.

3.2 ANALYTICAL PROCEDURE

Material, which was lying loosely on the silicate substrates was carefully removed with deionized water. Then the adhering biofilm was dried. These samples were analyzed together with the substrate, since separation of the biofilm material is impossible. However, this procedure was not a hindrance, as the silicate substrate turned out to be chemically inert for our analyzing programme. The biofilm mass was calculated as the difference between the whole sample material and the cleaned substrate after analysis.

The biofilm and sediment samples were analyzed for selected heavy metals (Pb, Cu, Zn, Fe, Mn), nutrients (Ca, Mg, K), polychlorinated biphenyls (PCB), and polycyclic aromatic hydrocarbons (PAH). In addition proteins, carbohydrates, uronic acids, and organic matter (loss on ignition) were determined. After decomposition under pressure with concentrated nitric acid, heavy metals and nutrients were analyzed with an atomic absorption spectrometer. For the analysis of PAHs and PCBs the samples were spiked with internal standards and solvent extracted with acetone/hexane (1:1) in a Soxhlet system for eight hours. After rotary evaporation to near dryness, the solvent extracts were purified by column chromatography. Identification and quantification were achieved by gas chromatography/mass spectrometry operating in the selected-ion-monitoring mode. The protein, carbohydrate and uronic acid content were analyzed photometrically (Lowry *et al.*, 1951; Bailey, 1958; Blumenkrantz & Asboe-Hansen, 1973).

4. Results and Discussion

The concentrations of carbohydrates and organic matter (OM) in the biofilm samples are described in Fig. 1. The OM content of the sediment samples is shown for comparison. The content is about three times higher in the biofilms than in the sediments. This indicates, that compared to the sediment samples, a higher accumulation of biological substances like biofilms was achieved in the sampled material. The OM content of the biofilm samples increases strongly in May and for the rest of the sampling period, the concentrations remain at a lower level and only vary slightly. The carbohydrate contents, however, show greater variations during the sampling period and the temporal development between OM and carbohydrate content is quite different. The variations in carbohydrates, proteins and uronic acids (both not shown) indicate the continuous changing conditions of nutrient supplies and population development.

At the beginning of May lower hydraulic forces, lower turbidity with little suspended solid contents caused by lower discharge (Fig. 2), a good nutrient supply and an increased irradiation result in fast biological growth. At this stage nutrients are fixed in the biomass. This limited supply results in a decrease in the amount of biological growth in the middle of June. The changes in the contents of carbohydrates, proteins and uronic acids exhibit a change in the biomass in the sampling period. A further important factor is that over the year the discharge controls the levels of concentrations. Depending on the height and temporal length of the discharge peak, the discharge causes a drop in OM content in the sediments.

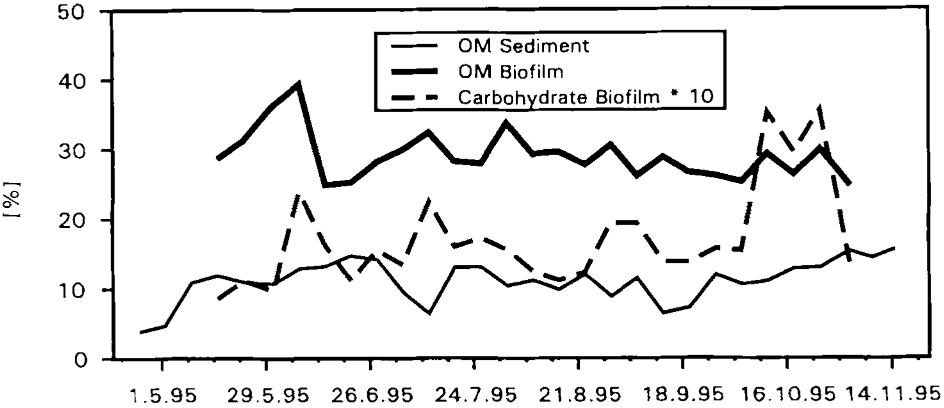

Fig. 1. Weekly organic matter (OM) and carbohydrate content in biofilms and weekly organic matter (OM) content in sediments (dry weight).

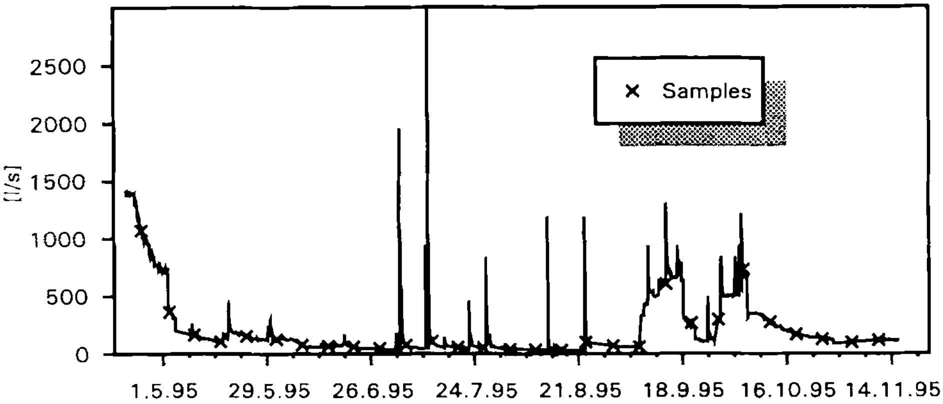

Fig. 2. Discharge Olewiger Bach 1995.

Fig. 3 shows the content of copper, lead and zinc in the sediments and biofilms. The heavy metals in sediments have a similar temporal structure to the OM in sediments. At the beginning of the sampling period in 1995 the pollutant contents are dominated by hydrodynamic conditions. At the end of a flood in April (Fig. 2) the sediments were replaced by mineral particles with very low contents of pollutants and OM. The low discharge in May leads to a strong increase in concentrations in the river sediments. For example, zinc increases from 100 mg/kg up to 270 mg/kg within two weeks. However, the short, but high discharges in July (2000 and more than 3500 l/s) partly eroded the sediment, which was rich in OM and pollutants. The content of heavy metals decreases. Both transport forces and time are insufficient to wash out the whole sediment from the river bed. Afterwards, the variations in heavy metal content can be more or less related to increases in the discharge. It is remarkable, that after the extreme flood waves in July the heavy metal content increases more than the OM content. It is the quality, rather than the quantity of OM that seems to be responsible for the sorption of heavy metals. The reason could be that a change in the composition of the biofilm within the sediment occurred. This is indicated by the change in the protein/carbohydrate-ratio.

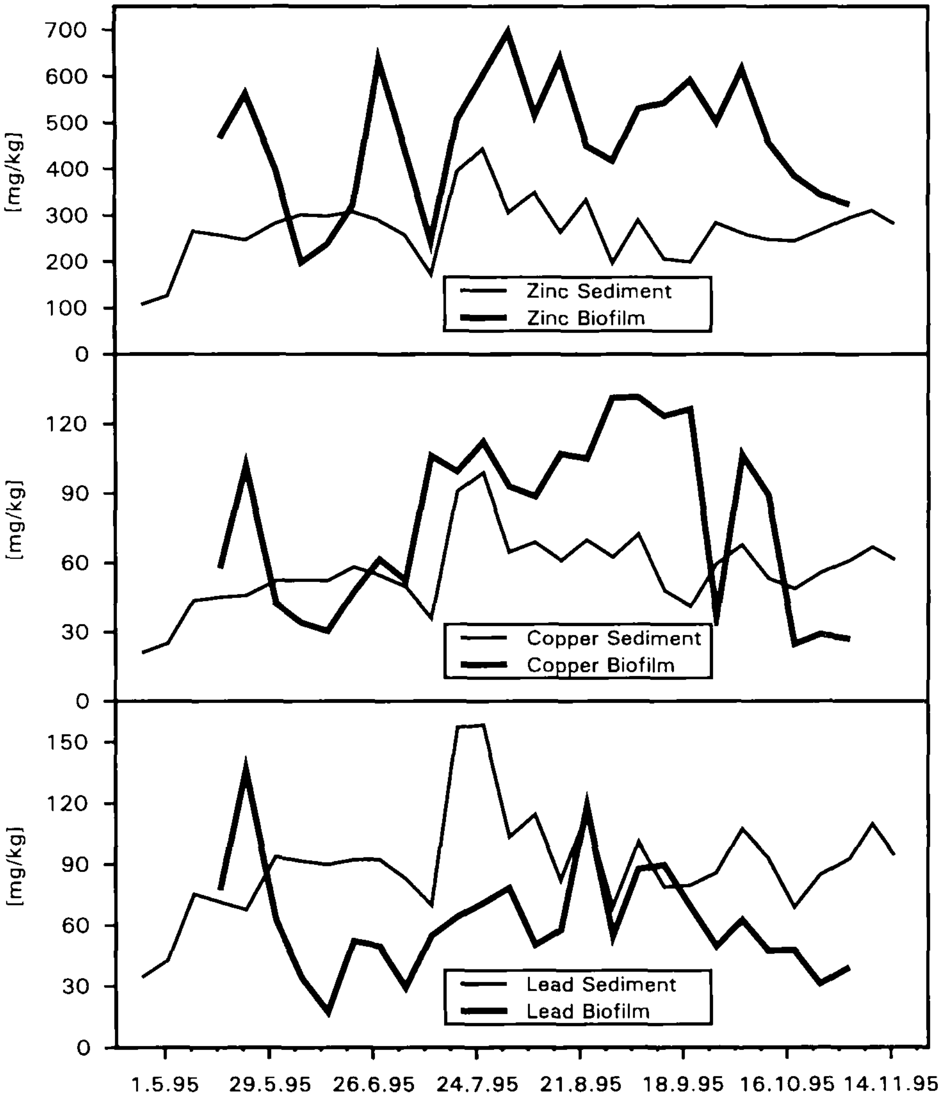

Fig. 3. Temporal variations in zinc, copper and lead in biofilms and sediments.

In general, concentrations of heavy metals in the biofilms show high temporal variations. In the middle of June a strong decrease of zinc, lead and copper follows a maximum content in May. The amount of OM and, for example, of carbohydrate shows quite a different pattern. With the sorption, the amount of biological material is not the decisive factor. During the course of summer, the contents generally increase but are interrupted at specific times by decreases, which are marked by the different heavy metals. On July 3rd, a flood wave (2000 l/s) causes a short term decrease in heavy metals. In spite of the next extreme flood (more than 3500 l/s) one week later, lead and copper increase, but zinc decreases. Ensuing peaks of individual heavy metals (e.g. Pb 23.8.:119 mg/kg) indicate inputs of polluted material into the river system. Sudden decreases (e.g. Cu 29.9.: 36 mg/kg) are difficult to understand. Desorption processes or a change in the sorption properties of the material may be the reason.

The high variations show, that sorption is not only dependent on the amount of sorbed substances available, but also on the conditions of the biofilms. Carboxyl- and hydroxyl-groups of the extracellular polymer substances (EPS) have a very high capacity for the sorption of heavy metals. A small amount of EPS could theoretically bind a large amount of a given metal (Geesey & Jang, 1989), but the affinities of EPS vary depending on the specific metal and micro-organisms involved (Harvey & Luoma, 1985). The variations in EPS composition, which were indicated by the biological parameters, seem to influence the sorption and are responsible for a part of the variance. A fast decrease after maximum concentrations shows, that desorption processes may also play an important role.

Compared to sediment contents, zinc and copper are strongly enriched in biofilms. Lead however, is not found in high quantities in biofilms. This must be due to the different mechanisms of enrichment and the sorption properties of the biofilm. In summer, copper content shows lower variations than lead and zinc (with the exception of 29.9.). More stable fixing by the biofilm matrix is the reason for lower desorption. When low water-levels occur, the biofilm seems to have an important influence on the level of concentrations of pollutants in sediments. At this time sorption of heavy metals by biofilms causes an increase in pollutant levels in sediments. If this sediment is resuspended or if the biofilm is disturbed by external conditions, the sediment can be a potential source for pollutants.

The sorption of organic hydrophobic compounds onto biofilms is a well known phenomenon (Decho, 1990). However, little research has been done on the binding mechanisms and sites of sorption. Fig. 4 shows the contents of two representative PAHs and one PCB in biofilms and sediments. While the PCBs of the sediments have the same temporal patterns as the OM and the heavy metals, the PAHs show a slightly different behaviour. In comparison to the heavy metals, the same mixing and dilution processes of different loaded materials and the enrichment of OM, influence the temporal course of PCB and PAH. The temporal pattern of the PAHs is difficult to recognize, because there are three exceptions of high PAH concentrations, which suppress the other variances: the first exception takes place over a period of two weeks after the high floods in July when concentrations of PAHs increase abruptly; the second and third divergences begin in October and November respectively with two weaker concentration peaks. Waste water effluents must be responsible for these peaks.

The PAH-and PCB-content in biofilms show high variations, with a general increase from May to September, and a sudden decrease in October. The variations between the individual PAHs are similar, but differences can be seen in the detailed structures. Although similar processes control the sorption, they occur to a different extent depending on the physico-chemical properties. The variations in PCB-content are lower and until August are similar to the PCB-content of the sediments. The temporal behaviour of organic micropollutants in biofilms is distinguished from that of heavy metals.

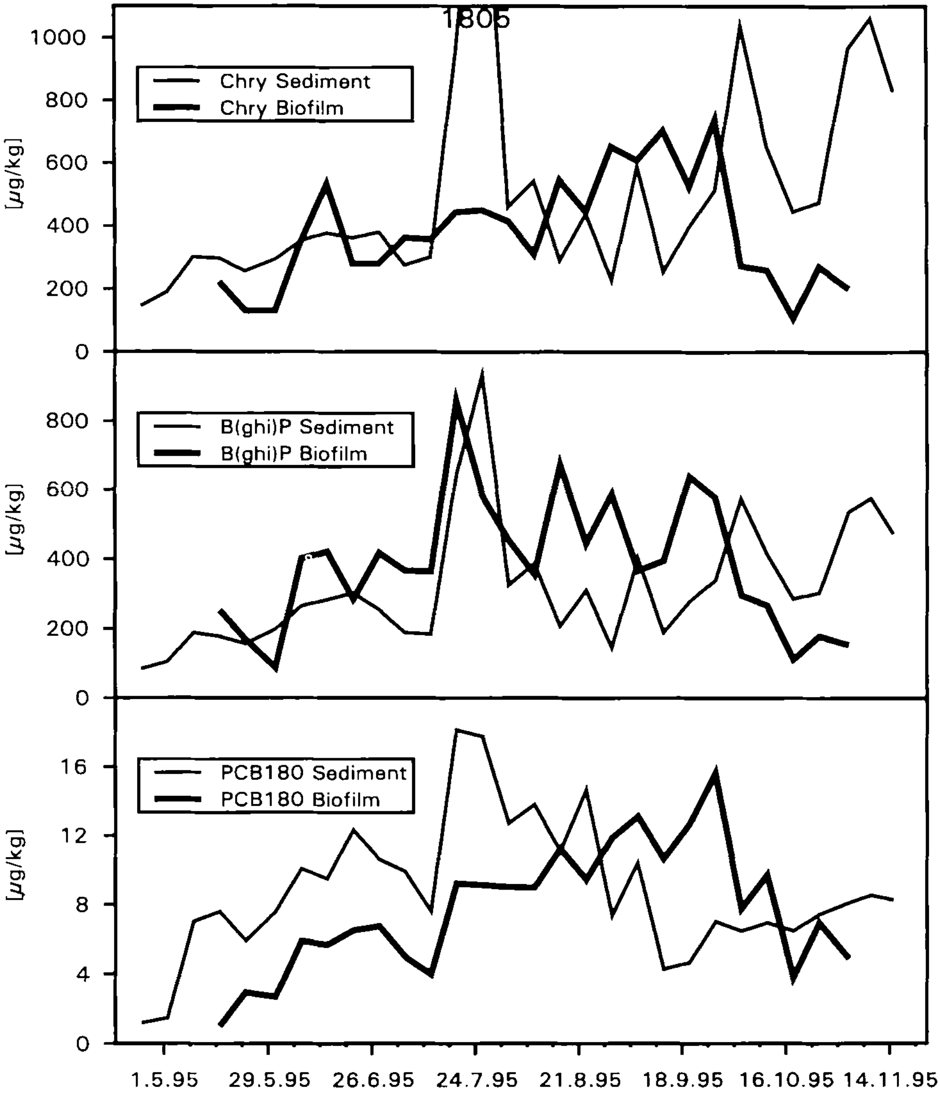

Fig. 4. Temporal variations in selected PAH and PCB in biofilms and sediments.

The sorption of organic micropollutants by biofilms occurs by interactions with particles or the solution by partition equilibriums (Baughman & Paris, 1981). In comparison to the PAH patterns in the sediment, the PAHs with higher molecular weight and higher partition coefficient (e.g. Benzo(ghi)perylen) are more enriched in biofilms than the PAHs with lower molecular weight (e.g. Chrysen). More rigid binding and less microbial decomposition may be the reasons (Mackay *et al.*, 1992). In August and September, the high values of PAHs and PCBs in biofilms exhibit, that at this time the conditions for sorption were optimal. The high concentrations correspond with high content of proteins and uronic acids. An increased biological activity results in an increased sorption of organic micropollutants. The concentrations of heavy metals in the biofilm are also highest at this time. The decrease of all pollutants in October cannot be explained by a simple desorption process. A possible explanation is the change in the biofilm matrix which due to seasonal changes, is now made up of a large quantity of dead material.

The differences between biofilm and sediment content are remarkable. In contrast to the sediments, the biofilm samples represent a period of time up to a maximum of five weeks. At different times of exposure, the growth of and sorption by the biofilms are influenced by meteorological and hydrological conditions. At the beginning of the exposure of the porous silicate material to river water a starting phase of several days is required for first pioneers of micro-organisms to adhere and grow. At this time an enrichment of pollutants is negligible, as there is no significant biomass. On the other hand episodic flood waves, depending on their intensities, can erode parts of the mature and thicker biofilms. The thickness and shaping of the biological growth also play an important role. Further important factors include the time of pollutant enrichment in the water phase, the supply of pollutants and the physico-chemical conditions (such as the pH-value) within the water phase (Flemming *et al.*, 1996). The longer and more intensive contact the biofilm makes with the pollutants, the more the pollutants can be enriched.

5. Conclusions

In the complex system of sorption in aquatic systems, biosorption is a highly dynamic and variable factor in pollutant transport and accumulation. It is controlled partly by the availability of pollutants and partly by the biological activity of the biofilm. The dynamic nature of biofilms gives them a distinctive role in sorption processes, because immobilization in biomass can be reversible.

Sediments have a highly dynamic temporal structure throughout the year. The temporal variations of pollutants in the biofilm are mostly hidden in the sediment samples by mixing and dilution processes and by effluents from point and nonpoint sources. If the sediments are undisturbed for several weeks, the importance of bioaccumulation is not negligible. During sediment deposition, the influence of biosorption is an important factor in the accumulation of pollutants in river sediments, especially during low water periods in summer.

There are clear differences between the investigated pollutants in the biofilms. Variable stability of binding on charged functional groups in the biofilm matrix and the transport of the pollutants onto various particles are responsible for the sorption of heavy metals. Physico-chemical properties play a central role for organic micropollutants. It can be shown that with decreasing water solubility there is an increased possibility that organic micropollutants can be bound. The more persistant substances such as certain PCBs are accumulated to a greater extent than others. In comparison to laboratory studies, with fixed conditions that do not include the competition of natural ecosystems for sorption, our results show, that laboratory results can be transferred only to a limited degree to the aquatic environment.

The high content of heavy metals and hydrophobic organic micropollutants show that biofilms represent an important and dynamic sink in the aquatic environment for sorbed substances. As biofilms are not inert chemical structures, the biological binding sites for pollutants will sometimes be degraded. If the physico-chemical conditions change, for example by resuspension through flood waves or by microbial degradation processes of the biological binding sites, the pollutants can be desorbed and remobilized in a more soluble form and can assume a potentially higher bioavailability. The highly dynamic biofilm will then change from a sink to a source.

Acknowledgments

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