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Determination of heavy metals in biofilms from the River Elbe by total-reflection X-ray fluorescence spectrometry[1]

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Abstract

Heavy metal contents of aquatic biofilms isolated from stones collected from, and ceramic plates exposed in, the River Elbe were determined by total-reflection X-ray fluorescence spectrometry (TXRF). The fractions of several elements (K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Pb) referred to the dry mass are 100 to 60 000-fold higher in the biofilm matrix compared with the bulk water phase. Biofilms grown on the exposed plates have a mass fraction of the determined elements higher by a factor of 2–3 compared with the biofilms derived from stones. These differences may be attributed to the different ages of the biofilms.

Keywords: Aquatic biofilms; Heavy metals; River water quality; Total-reflection X-ray fluorescence (TXRF)

1. Introduction

To understand and describe the processes in aquatic ecosystems, knowledge about the partitioning and accumulation of toxic elements, e.g. heavy metals, is a necessity. One important part of aquatic biocoenosis is represented by microbial interfacial films. These biofilms are water-rich aggregates of such microorganisms as algae, bacteria and protozoa and their extracellular polymeric substances. They cover the interfaces in natural systems and are able to concentrate and bind ions from the passing water [1–4].

In the present paper, we present some preliminary results on heavy metal mass fractions in natural aquatic biofilms grown in a polluted stream. Problems arising from the small dry mass of biofilm samples can be overcome by employing total-reflection X-ray fluorescence (TXRF). TXRF is a highly sensitive method for determining trace elements in the low absolute μg range and is therefore applicable to small sample sizes. This method was applied to determine heavy metals in biofilm samples collected from the most polluted river in Germany, the Elbe. Previous results had shown the high potential of TXRF for elemental analysis in various biological environmental samples [5,6]. Stones or water plants are naturally occurring substrates for growing biofilms. Depending on the substrate, the composition of microbial biofilms may vary significantly. Additionally, man-made constructions such as bollards, bridges and other stream-related buildings

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provide sources for the settlement and development of biofilms.

2. Experimental

2.1. Sampling

The experiments were carried out with natural biofilms sampled from their original substrates (stone samples) and from ceramic plates exposed in the stream for several weeks at one sample site. We chose an automatic water quality monitoring station located at km 318 (left side) upstream, south of the city of Magdeburg, for different sampling purposes. Firstly, we sampled biofilms from stones on the left bank of the river and from the station pontoon itself [6]. Secondly, we exposed five ceramic plates (diameter 140 mm) for about 4–5 weeks. The plates were fixed in a row with a nylon rope and anchored 0.5 m below the water surface. The plates with the microbial films grown of them were transported to the laboratory in a plastic bowl covered with Parafilm within 1 h after sampling. In the laboratory the biofilm was scraped off inside a clean bench with the aid of a Teflon spatula. During the experiment, flooding led to the erosion of groyne fields and transport of large tree trunks, which destroyed some of the exposed plates.

2.2. Sample preparation

In order to avoid both contamination and loss of trace elements, the whole procedure was performed with great care. Only sub-boiled reagents and ultra-pure deionized water were used for preparation. Homogeneity of the slimy biofilm mass was produced by stirring at 10 000 rpm in an Ultra-Turrax unit equipped with a Ti shank precleaned by rinsing in hot HNO_3. Subsequently, the homogenized biofilm was evaporated to dryness (105°C) inside the clean bench. The dried and homogenized biofilm powder (6–15 mg) was weighed into small PTFE beakers (30 ml, Saivillex, Canada) using an analytical balance (MC1 RC 250 S, Sartorius, Germany). After adding 2.5 ml of HNO_3:H_2O_2 (4:1) mixture, the beakers were closed with screw caps and placed on a hot plate. Digestion was performed within 6 h at 150°C, yielding clear solutions. The beakers were cooled to room temperature and the solutions were diluted to 5 ml with ultra-pure water after the release of nitrous gases. With this kind of digestion, sample/acid mixture ratio has to be carefully chosen because of the resulting internal pressure caused mainly by nitrous gases (NO_x), which is compensated for only by the ductility of the PTFE material. An aliquot of 1000 μl of the sample solution was spiked with 100 μl of internal standard solution (10^4 μg l^{-1} yttrium), giving a final concentration of $\approx$ 1000 μg l^{-1} Y. From this mixture, a 20 μl portion was transferred to the highly polished quartz sample carrier and treated with 5 μl of polyvinyl alcohol (PVA) solution. Subsequently, the liquid on the sample carrier was evaporated by gentle heating to obtain a small amount of residue. For each sample solution, the mass of the residue on the sample carrier was determined by three weighings on a microbalance (MC 5, Sartorius, Germany), yielding 57 $\pm$ 3 μg. To investigate sample homogeneity and the size of the particles remaining on the sample carrier, one biofilm sample was analyzed by scanning electron microscopy (SEM). The microscope (DSM 942, Zeiss-Jena, Germany) was equipped with an Oxford CT 1500 Cryotrans system and an Oxford-Link ISIS micro-analysis system, which consists of an energy dispersive X-ray fluorescence detector (EDX). Scanning electron micrscope (SEM) photographs (Fig. 1) show good sample homogeneity and particles of size $<$ 10 μm remaining on the sample carrier. Sample homogeneity is also confirmed by analyses for calcium and iron by EDX, which reveal a homogeneous pattern on the sample carrier (Fig. 2).

2.3. Instrumentation

An EXTRA IIA total-reflection X-ray fluorescence spectrometer (Atomika Instruments Ltd., Oberschleissheim/Munich, Germany) was used. The spectrometer was equipped with an 80 mm^2 Si(Li) detector having a resolution of 168 eV at 5.9 keV, two 2 kW fine-focus tubes (Mo and W) operating at 50 kV and 38 mA maximum, and a computer controlled multichannel analyzer system combined with a spectrum deconvolution program. Only Mo excitation was used. Measurement time was 500–1000 s.

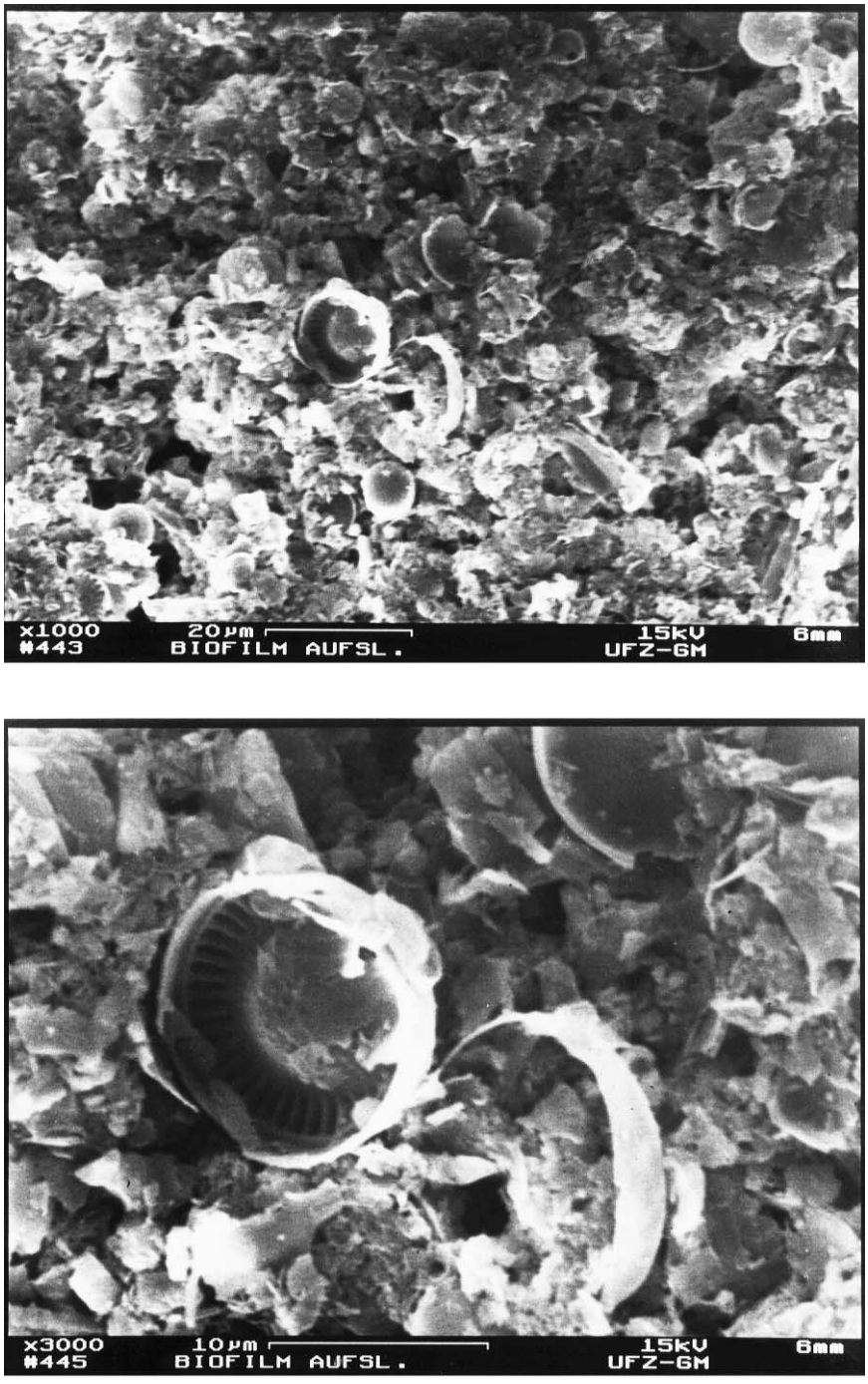

Fig. 1. SEM photographs of a biofilm sample on a sample carrier magnified 1000 times (above) and 3000 times (below). The particle size is below 10 μm.

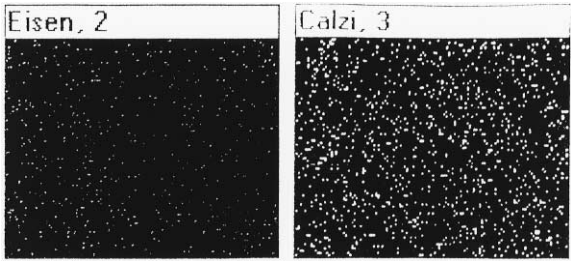

Fig. 2. Distribution pattern of iron (left part) and calcium (right part) on the sample carrier measured by SEM-EDX, showing good sample homogeneity.

2.4. Analytical performance

The precision of the method was determined by triplicate analysis of the homogenized dry samples (preparation of three independent sample carriers for each sample solution). The standard deviation (1σ) was less than 10% for most elements. The accuracy of the method was checked by parallel measurements of certified material CRM 414 (trace elements in plankton) and NIST 1643c (trace elements in water). The results are given in Table 1.

3. Results and discussion

Table 2 shows mass fractions for the biofilms sampled from different sources and during varying climatic conditions. The measured mass fractions for all elements were very high for the biofilm matrices compared with the typical values for River Elbe water. ''Enrichment'' or ''bioconcentration'' factors (BCF) were determined as the ratio of the mass fraction of a given element in the biofilm and the concentration of the same element in the total water phase. They range from 96 to 6×10^4 depending on the element, with the lowest factor for Ca and the highest factor for Mn (Table 3). By the concentration of an element in the total water phase we mean the value obtained after pressure digestion of the bulk water in a microwave system with the same acid mixture as used for digestion of the biofilms. This was done because biofilms incorporate contaminants adsorbed onto solids, such as suspended particulate matter from the passing water, which may contribute to the element mass fractions of the biofilm samples to an unknown extent. This effect can be overcome by comparing the results of the biofilm with the concentrations in the total water phase. It is noteworthy that the mass fractions of nearly all heavy metals in the River Elbe are correlated with the mass fractions of suspended particulate matter. With respect to the variations of element concentrations in the River Elbe, which were monitored on a weekly basis, no marked variation of the BCF of a given element was obtained between the maximum and minimum values. The BCF were calculated for only two biofilm samples grown on exposed plates. These samples had been exposed to a range of known heavy metal concentrations in the water phase. Nevertheless, weekly analyses neglect short term peaks of higher

Table 1
Comparison of certified and measured element mass fractions and concentrations in two reference materials, CRM 414 ''trace elements in plankton'' and NIST 1643c ''trace elements in water''. Values for the reference materials given without a standard deviation are not certified. The measured values are the result of three independent analyses of CRM 414 and ten independent analyses of NIST 1643c. The 1σ standard deviation is given

Element	CRM 414, plankton		NIST 1643c, water	
	Certified/(μg g^{-1})	Measured/(μg g^{-1})	Certified/(μg l^{-1})	Measured/(μg l^{-1})
K	7.55	6.53 ± 0.23	2.3	1.98 ± 0.19
Ca	65	61 ± 1	36.8 ± 1.4	34.3 ± 2.7
Cr	23.8 ± 1.2	27.5 ± 3.2	19 ± 0.6	19 ± 1.5
Mn	299 ± 12	256 ± 6.1	35.1 ± 2.2	33 ± 2.5
Fe	1.85	1.75 ± 0.11	106.9 ± 3	106 ± 4
Ni	18.8 ± 0.8	17.9 ± 1.6	60.6 ± 7.3	59 ± 3.4
Cu	29.5 ± 1.3	26.9 ± 1.6	22.3 ± 2.8	22 ± 2.3
Zn	112 ± 3	119 ± 1.5	73.9 ± 0.9	81 ± 2.5
Pb	3.97 ± 0.19	< 5	35.3 ± 0.9	36 ± 2.2

Table 2
Element mass fractions in biofilms from different sources in the River Elbe. All samples are from the left side at km 318. Samples Stone A, Stone B and Pontoon were taken during September 1994 [6]. All values given are related to dry mass. The standard deviation is the result of three independent analyses of each sample solution

Element	Stone A	Stone B	Pontoon	Plate, Aug 1995	Plate, Nov. 1995	Plate, May 1996
K/(mg g^{-1})				12.33 ± 0.23	13.3 ± 0.62	12.4 ± 0.17
Ca/(mg g^{-1})				10.8 ± 0.26	16.8 ± 0.31	6.6 ± 0.2
Cr/(μg g^{-1})	40 ± 13	30 ± 11	20 ± 10	89 ± 12	98 ± 6	70.7 ± 6.1
Mn/(μg g^{-1})	910 ± 5	856 ± 5	796 ± 7	3030 ± 32	5570 ± 386	2270 ± 23
Fe/(mg g^{-1})	16 ± 0.04	13 ± 0.03	6 ± 0.02	23.9 ± 0.25	27 ± 1	18.4 ± 0.3
Ni/(μg g^{-1})	34 ± 4	25 ± 3	22 ± 4	33 ± 5.1	49 ± 6.4	40 ± 3.6
Cu/(μg g^{-1})	54 ± 3	42 ± 2	33 ± 3	88 ± 5.6	82 ± 5.5	79.3 ± 4.9
Zn/(μg g^{-1})	531 ± 4	437 ± 3	487 ± 4	1040 ± 8.1	1100 ± 89	514 ± 8
Pb/(μg g^{-1})	34 ± 6	26 ± 5	78 ± 6	103 ± 2.6	92 ± 8.5	41 ± 1

pollution. Calculation of BCFs for biofilms sampled from stones is not possible, as the exposure time is unknown. However, the results are useful for comparison with those for the exposed ceramic plates. This can be shown by comparing the differences in mass fraction of heavy metals which are obvious between the biofilms sampled from their original substrates and from the exposed plates. Such differences apply to all the elements determined.

In general, the elemental mass fractions of newly grown biofilms on ceramic plates are two to three times higher than those in biofilms from stones (Fig. 3). Apart from the different substrates, the different seasons could have also contributed to the differences in mass fractions. A more reliable explanation for these differences may be the fact that the biofilms grown on the exposed plates represent a time span of only 4–5 weeks. This may have resulted in a quite different microbial community compared with that on the stones. It is well known from experimental results that biofilm growth is accompanied by a change in composition of bacterial species [7–9]. Data on the bioaccumulation of the measured elements in bacteria occurring in nature can be found in different fields of research (e.g. technical bioengineering or bioremediation). However, some work has been reported on the interactions of bacterial communities or individual species in streams or lakes with the heavy metals iron, manganese, copper, zinc and lead [10–15]. Dean-Ross [13] found a remarkable decrease in diversity of the culturable bacterial community and the occurrence of more tolerant species when a natural microbial community was exposed to different levels of Zn. It is obvious that the microbial structure of the biofilm grown on the stones has adapted to the environmental

Table 3
Range of typical element concentrations in the bulk water phase of the River Elbe and calculated bioconcentration factors (mean values only are given). For the sampling date in August 1995, no data on heavy metals in the River Elbe were available. The data for September 1994 are given only for comparison and are not used in calculation

Element	Bulk water phase				
	September 1994	November 1995	May 1996	BCF, Nov. 1995	BCF, May 1996
K/(mg l^{-1})		7.2	6.9	1.9×10^3	1.8×10^3
Ca/(mg l^{-1})		91	69	1.9×10^2	9.6×10^1
Cr/(μg l^{-1})	< 2	7.2	5	1.4×10^4	1.4×10^4
Mn/(μg l^{-1})	105	96	150	5.8×10^4	1.5×10^4
Fe/(μg l^{-1})	465	849	1930	3.2×10^4	9.6×10^3
Ni/(μg l^{-1})	6.7	9.7	6.3	5.0×10^3	6.4×10^3
Cu/(μg l^{-1})	5	6.7	8.1	1.2×10^4	9.9×10^3
Zn/(μg l^{-1})	79	60	59	1.8×10^4	8.7×10^3
Pb/(μg l^{-1})	3.9	3.6	6	2.6×10^4	6.8×10^3

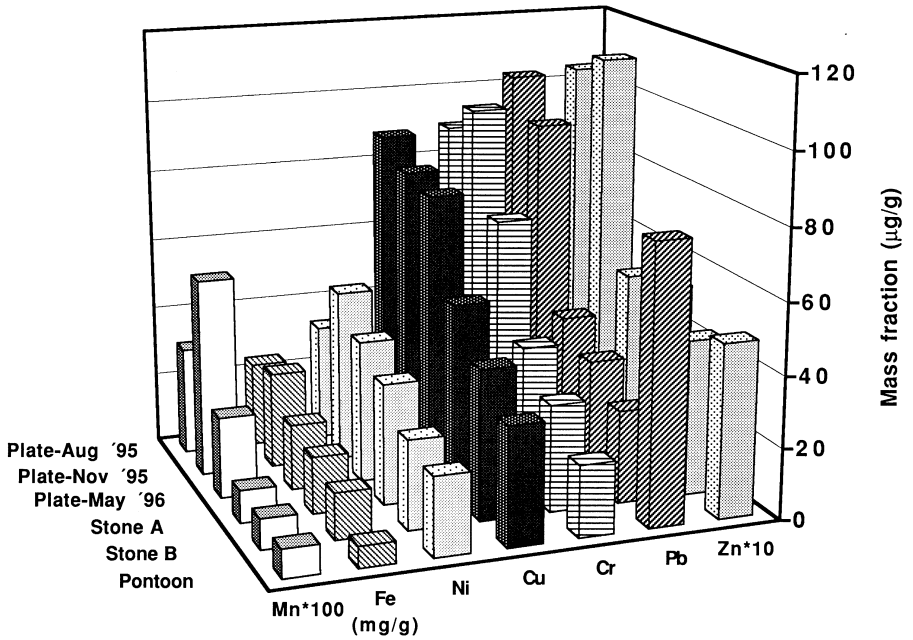

Fig. 3. Heavy metal mass fractions in biofilms from the River Elbe originating from stones or exposed plates. Plotted values should be multiplied by a factor of 100 for Mn and by a factor of 10 for Zn. The mass fraction for Fe is given in mg g^{-1}.

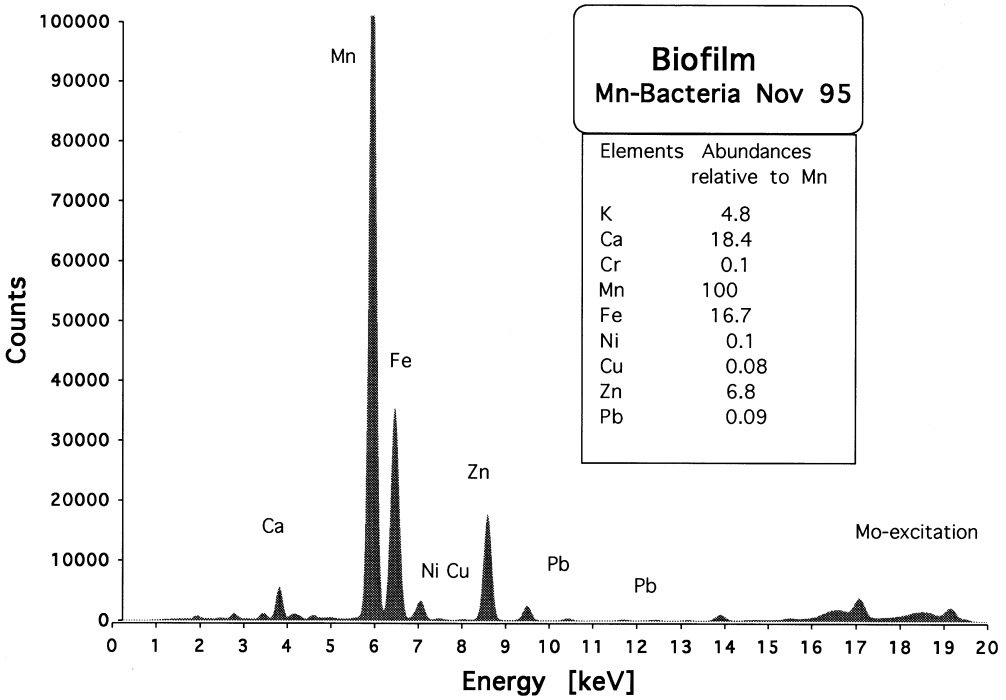

Fig. 4. TXRF spectrum of a Mn-rich biofilm grown on an exposed plate in November 1995. Abundances relative to Mn are given.

conditions of the River Elbe. This may have resulted in proliferation of more tolerant species compared with the bacterial community developing on the exposed plates. Moreover, an important part of the specific binding capacities for heavy metals in biofilms is represented by extracellular polymers. Their binding capacity can be three times higher than that of the bacterial cells themselves, as described for lead [15]. This may be an additional factor leading to higher elemental mass fractions in the biofilms from the ceramic plates, supposing that they have a higher content of extracellular polymers. The idea of varying bacterial community structures as the reason for the different element mass fractions is supported by the observation of two biofilm types of different colors which developed on a plate exposed in November 1995. On one quarter of the plate a reddish-brown biofilm was observed. It was not possible to collect material for quantitative analysis, but the TXRF technique has the advantage of providing qualitative information about the elemental composition with only a minute amount of material. In contrast to the typical greenish-brown biofilm grown on the same plate, and also the other biofilms investigated, manganese is the main metal in the reddish-brown biofilm (Fig. 4).

In conclusion, there are some indications for different bacterial community structures between the biofilms from stones and those grown on exposed plates, which result in different mass fraction levels of heavy metals. In future, we need to comfirm these findings by additional microbiological work, and we shall start with laboratory experiments to determine metal-specific bioconcentration factors.

4. Conclusions

TXRF has proved to be a powerful analytical tool for analyzing element composition in complex biofilms. It is possible to determine the mass fraction of heavy metals in small samples with good accuracy and precision. Biofilms are very diverse and, because of their heterogeneity, they contain different mass fractions of heavy metals. With respect to the variations observed in element concentrations of the bulk water phase of the River Elbe, bioconcentration factors between about 10^2 and 6×10^4 were obtained depending on the element of interest. Minor variations of a chosen element in biofilms, harvested from either stones or exposed plates, may be caused by seasonal effects. The generally higher metal binding capacity of newly grown biofilms on exposed ceramic plates is assumed to be related to microbial communities differing from those growing on stones. Other reasons such as the different sources/substrates or different climatic conditions and different pollution situations may contribute additionally to the differences in the chemical composition.

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