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Biogeochemistry of Aromatic Surfactants in Microtidal Estuaries

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Abstract: This paper summarizes several studies on input, distribution, and fate of two different types of aromatic surfactants, the anionic linear alkylbenzene sulphonates (LAS) and nonionic alkylphenol polyethoxylates (APnEO), in a karstic estuary characterized by sharp salinity gradients. A combination of reversed-phase and normal-phase HPLC analysis followed by spectrofluorimetric detection allowed detailed characterization of homologue and oligomer compositions of aromatic surfactants in wastewater and estuarine waters. The distribution patterns of homologues and/or oligomers in the wastewater plume reveal a significant alteration of the original composition of both surfactants by biotransformation and physicochemical partitioning. The vertical distribution of surfactant residues in the estuarine water column is characterized by pronounced maxima at the air–brackish water and brackish water–sea water interface. The brackish water–sea water interface is a very efficient barrier, which prevents spreading of surfactants into the deeper layers. In addition, the interface seems to be a site of high biological activity, including biotransformation of surfactant residues. As a consequence, this layer can contain enhanced levels of lipophilic metabolites of APnEO. Biotransformation is the main mechanism for the removal of both surfactant classes from the water column. However, our studies suggest that a significant percentage of surfactant residues discharged into the stratified estuaries reaches the coastal sea due to the incomplete removal in the estuary. This situation is a consequence of a rather fast flushing of the brackish layer and a very slow biotransformation kinetics in the saline layer, especially in the winter period.

Keywords: Alkylphenol polyethoxylates · Biotransformation · Estuaries · Linear alkylbenzene sulphonates (LAS) · Wastewater

Introduction

Aromatic surfactants, including linear alkylbenzene sulphonates (LAS) and alkylphenol polyethoxylates (APnEO) with estimated annual worldwide production rates of about 3×10^6 and 6×10^5 tons, respectively, belong to the most popular surfactant classes. Due to their widespread use in such high volumes and considerable toxicity to aquatic life, LAS and APnEO should be considered potentially important environmental contaminants [1][2]. There is a great deal of structural similarity between LAS and APnEO in the hydrophobic parts of their molecules, both containing branched long-chain alkylbenzenes, while the hydrophilic parts are rather different (Fig. 1). The solubility of LAS in water is assured by a strongly polar sulphonic group substituted in *para* position, while the solubility of APnEO depends on the length of the polyethoxylate chain. Such structural features determine similarities and differences in abiotic and biological processes, which govern the environmental behavior and fate of LAS and APnEO.

It was shown in model laboratory experiments as well as in sewage treatment plants and various types of ambient waters that both LAS and APnEO exhibit a complex metabolic behavior, yielding a variety of relatively stable metabolic products. The biotransformation of LAS seems to be simpler, resulting in more polar intermediates formed by successive β- and ω-oxidations of the alkyl chain [3]. In contrast, biotransformation of APnEO is more complex and usually starts at the hydrophilic part of the molecule, resulting in a rather unique metabolic pathway, which is characterized by the formation of various stable intermediates [2]. Some of these metabolites are more lipophilic and therefore more toxic to aquatic life than the parent molecules [4]. In the last decade, interest in ecotoxicological effects of these lipophilic metabolites has dramatically increased because of their proven endocrine-disrupting potential [5][6]. The most recent studies have shown that APnEO intermediates with shorter EO-chains are further transformed by oxidation of the highly branched alkyl-chain, yielding a suite of more polar metabolites, which also exhibit significant persistence [7][8].

Owing to their hydrophobic moiety, both LAS and APnEO show a significant affinity for suspended particles, which results in a widespread presence of surfactant residues in aquatic sediments and biota [9]. The adsorption onto sediment and bioaccumulation are particularly pronounced for the lipophilic degradation products of

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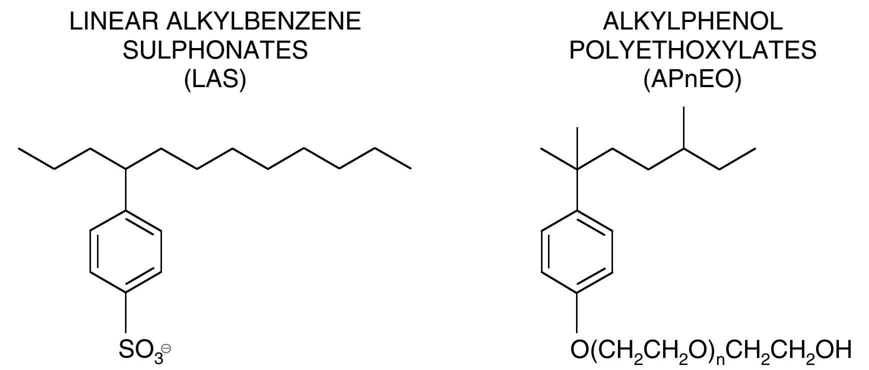

	LAS	APnEO
Homologues:	R = $C_9 – C_{14}$	R = C_8 , C_9
Isomers:	4–6 positional isomers/homologue	more than 10 differently branched isomers/oligomer
Oligomers:	–	n = 3–20
World production	3000 000 t/year	600 000 t/year

Fig. 1. Structures and acronyms of linear alkylbenzene sulphonates and alkylphenol polyethoxylates

APnEO such as nonylphenol (NP), nonylphenol monoethoxylate (NP1EO), and nonylphenol diethoxylate (NP2EO).

Coastal and estuarine waters receive large quantities of aromatic surfactants, either directly from municipal wastewaters or indirectly from polluted rivers. In the past decade, there have been an increasing number of reports dealing with the behavior and fate of LAS and APnEO in coastal and estuarine environments [10–16]. In estuaries, most of the published data deal with well-mixed macrotidal estuaries, while the studies in stratified estuaries, characteristic of the Mediterranean Sea, are relatively scarce. There are two major motivations to study behavior and fate of surfactants in estuarine environments. Many of the world estuaries are densely populated and represent areas of intensive and often mutually conflicting anthropogenic activities including fishery, industry, navigation and tourism. On the other hand, estuaries are well known as important regions of biological diversity and as such are potentially highly vulnerable to environmental stress imposed by different human activities, including those characterized by extensive introduction of man-made chemicals.

This paper summarizes our studies [17–24] conducted in the estuary of the Krka River, Croatia, and gives a comparison of environmental behavior and fate of LAS and APnEO in this unique system characterized by sharp salinity gradients.

Stratified Estuaries as Natural Laboratories

One of the main features of estuarine environments are pronounced gradients of physicochemical properties, notably salinity and turbidity, which strongly influence environmental behavior of chemical constituents entering the system [25][26]. As a consequence, even rather small estuarine sections usually cover wide ranges of the key environmental variables and represent therefore ideal natural laboratories to study the physical chemistry and bioreactivity of pollutants under ambient conditions.

The studies presented in this paper were carried out in the Krka River estuary situated in the middle part of the eastern Adriatic coast. The total length of the estuary is about 30 km and the depth varies from a few meters, immediately below the waterfalls, to 40 meters at the sea-end of the estuary (Sibenik Harbor). Owing to its unique hydrographic features, the Krka River estuary belongs to the most intensively investigated estuaries of the Mediterranean Sea with a special emphasis on the processes that occur at the estuarine boundaries. The Krka River estuary is a typical karstic estuary characterized by low terrigenous input of suspended materials and conspicuous vertical salinity gradients, resulting in a very stable stratification throughout the year (Fig. 2). The upper, freshwater, and lower, seawater layers are separated by a visible interface situated at the halocline, which contains an organic film formed mainly by accumulation of plankton-derived organic matter [26]. The depth of the halocline fluctuates seasonally, depending on the river flow, between 0.2 and 6 m.

It was shown that such stratified estuaries are particularly suitable for studies of environmental behavior of different constituents since the entire range of estuarine master variables can be accessed at one single station, which significantly simplifies the required sampling strategy. On the other hand, the Krka River estuary shows a conspicuous change of anthropogenic pressure along its longitudinal profile. The largest part of the estuary is a pristine environment situated within the borders of a National Park area with only few smaller settlements and very limited industrial activities, while the main source of anthropogenic input are wastewaters of the city of Sibenik (40,000 inhabitants), which enter the estuary in its lower part, *i.e.* in the area of Sibenik Harbor. Municipal and industrial wastewaters are discharged into the estuary without pretreatment through several outlets along the northern shoreline of the Sibenik Harbor basin.

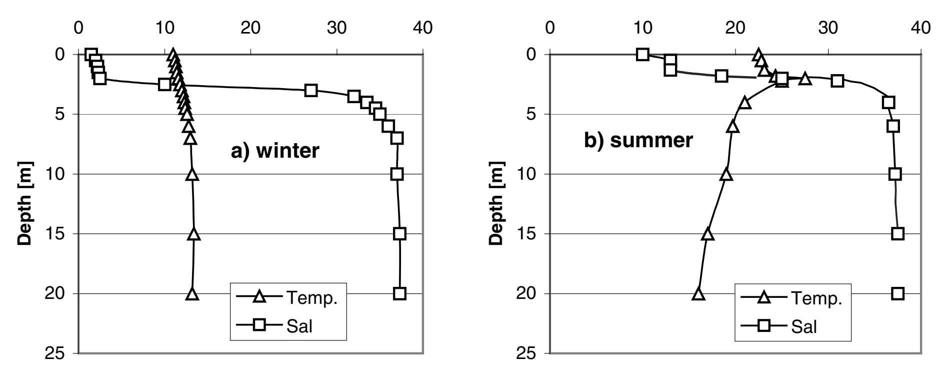

Fig. 2. Typical vertical profiles of temperature [°C] and salinity [PSU] in the water column of the Krka River estuary in winter and summer [27]

Since 1989, several sampling campaigns were performed, comprising sampling of wastewaters and the estuarine water column. In order to determine the spread of the wastewater plume into the Sibenik Harbor, water samples were collected at different distances from the major sewage outlets at depths characteristic of brackish (0.5 m) and saline (6 m) layers. A more detailed sampling on the vertical profile of the water column, with special emphasis on the brackish water–seawater interface, was performed at the station E4A, which is situated in the middle part of the Sibenik Harbor.

Methods

LAS and NPnEO, including their stable metabolic products, were determined using an approach based on HPLC separation followed by spectrofluorimetric detection, which involved both reversed phase (separation of homologues and isomers) and normal phase (separation of oligomers) systems [28–31]. High-resolution gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) were used for identification and confirmation purposes. In order to investigate the partitioning behavior of surfactant residues, water samples were previously filtered through glass fiber filters (GF/F) and the dissolved and particulate fractions were further processed separately. Briefly, the dissolved fraction of both classes of aromatic surfactants was enriched from wastewater and estuarine waters using C_{18} solid-phase extraction [10], while the particulate fraction was extracted using an ultrasonically enhanced extraction. Lipophilic metabolites of NPnEO (NP, NP1EO and NP2EO) were determined by an alternative enrichment procedure, using a continuous steam distillation/solvent extraction in a special apparatus, followed by a direct analysis of the cyclohexane extracts by normal phase HPLC [28].

In addition to the determination of aromatic surfactant residues in the estuary, the die-away biodegradation experiments were performed to assess the behavior of and fate of the target compounds [18][24]. The experiments were performed using autochthonous microbial populations, which were sampled in different estuarine compartments as well as in different seasons. The biodegradation media were always kept at temperatures identical to those found in the ambient water at the time of sampling.

Characterization of Surfactant Input

The input of aromatic surfactants into the Sibenik Harbor was determined by analyzing wastewaters from all major outlets of the city of Sibenik [22][23]. The homologue- and oligomer-compositions of LAS and APnEO (Fig. 3) found in wastewater extracts revealed the presence of common commercial mixtures of the target compounds, which have been partially changed due to the physico-chemical partitioning and biotransformation in the sewer system. It should be stressed that a significant percentage of LAS (11–59%) and NPnEO (6–60%) entering Sibenik Harbor *via* municipal wastewaters is bound to sewage particles. This fact markedly influences their fate in the estuary. The average chain length of the total LAS in the wastewater samples of the city of Sibenik was 11.47 ± 0.17. The more lipophilic higher homologues were enriched in the particulate- and depleted in the dissolved phase, as indicated by a significantly altered corresponding chain lengths of 11.93 ± 0.14 and 11.2 ± 0.14. The determination of aromatic nonionic surfactants in wastewater extracts by reversed-phase HPLC [29] indicated a strong predominance of nonyl homologues, while the presence of octyl homologues was insignificant (<5%). The oligomer composition of NPnEO in the total wastewater samples displays a great deal of similarity to those commercial mixtures that are commonly used in detergent formulations, which are characterized by an oligomer distribution maximum at NP10EO.

The comparison of distribution patterns in the dissolved and particulate phases shows a pronounced selectivity in the partitioning of individual oligomers. The oligomer composition in the particulate phase is characterized by an enhanced abundance of lower oligomers, resulting in oligomer distributions that show typical maxima between NP6EO–NP9EO. This situation can be explained by decreased water solubilities [32] and, consequently, increased lipophilicities [33] of the lower oligomers. The observed change in the oligomer distribution patterns depends on the concentration of suspended solids in the wastewater sample. The typical maximum of oligomer distribution in the dissolved phase displays a shift of the maximum towards higher oligomers (NP11EO–NP12EO). It should be noted that the oligomer distributions presented in Fig. 3 show only the distribution of higher oligomers (nEO> 3), which are considered parent compounds [34]. In addition to these parent oligomers, their lipophilic metabolites, including NP, NP1EO and NP2EO, were detected in all samples in lower but significant concentrations (typically below 10 µg/l of the individual metabolite). Their over-proportional presence in the wastewater extracts as compared to that in commercial surfactant mixtures indicated an ongoing change of the original NPnEO composition by biotransformation. Due to a rather short sewer system (residence time is estimated to be less than 1 h), only a minor part of the parent compounds is biotransformed and the contribution of the lipophilic metabolites to the total concentration of nonylphenolic compounds does not exceed 5%.

The characteristics of physicochemical partitioning and biotransformation processes described above do not depend on the total concentration of LAS and NPnEO and are applicable as a general feature describing the input composition of target compounds. However, it is interesting to note that the concentration of aromatic surfactants varied significantly during the period covered by these investigations (1989–2003). In the late eighties, the average levels of the total LAS and NPnEO were rather similar (approximately 0.5 mg/l), suggesting an extensive usage of NPnEO in that period. Assuming an aver-

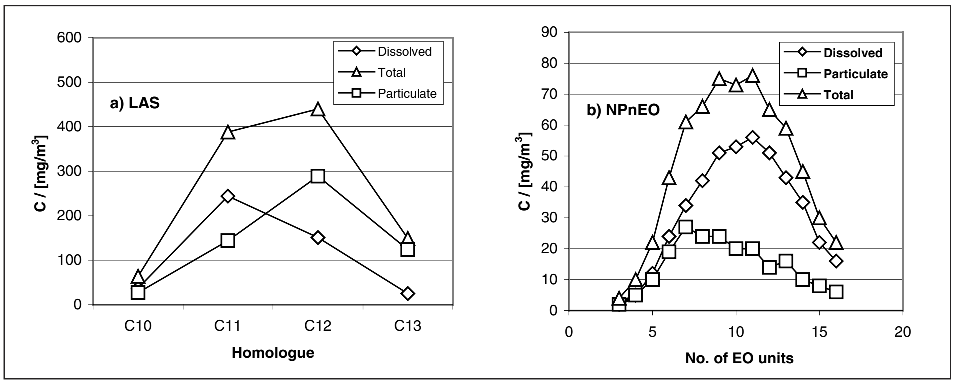

Fig. 3. Distribution of (a) homologues of linear alkylbenzene sulphonates (LAS) and (b) oligomers of nonylphenol polyethoxylates (NPnEO) in dissolved and particulate fractions of municipal wastewaters entering Sibenik Harbor

age discharge of municipal wastewaters of about 0.3 m^3/s, the inputs of LAS and NPnEO were estimated at 5 tons per year each. As a consequence of risk reduction measures, which were introduced in Western Europe, as well as reduced industrial activities during and after the war in Croatia (1991–1995), the usage pattern of aromatic surfactants has changed dramatically. The concentration of NPnEO decreased below 0.1 mg/l, while the concentration of LAS in wastewater increased to 5 mg/l.

Distribution in the Wastewater Plume

The spread of the wastewater plume in the Sibenik Harbor was investigated by sampling at different distances from the main outlet at two different depths characteristic of the brackish compartment (0.5 m; salinities <20 PSU) and marine compartment (6 m; salinities >37 PSU) of the estuary. The results indicate that spreading of wastewaters occurs almost exclusively in the brackish layer, while the underlying saline layer remains hardly affected. Fig. 4 shows the distribution of LAS and NPnEO in the wastewater plume. As can be seen, there is a strong reduction (down to 1–10 µg/l) of the surfactant concentrations in the closest proximity (25 m) of the outlet. After a distance of only 100 m the concentration of both LAS and NPnEO in the plume dropped down below 2 µg/l. The observed effect depends strongly on the wind conditions, which can enhance the velocity and direction of surface currents as well as mixing of the brackish and saline layers [35]. Such a fast reduction of surfactant concentration with an increasing distance from the outlets can mainly be assigned to a very fast dilution, however, differences in the distribution patterns of individual LAS homologues suggests the importance of some other mechanisms that lead to the elimination of parent compounds. The same conclusion was reached from the changing distribution patterns of lipophilic metabolites of NPnEO, which exhibit a much lower concentration decrease than the parent compounds. If the relative contribution of lipophilic metabolites in the total nonylphenolic compounds is plotted *versus* distance from the outlet (Fig. 5), one can see that their percentage in the plume significantly increases (up to 54%). This indicates the possible importance of some oligomer-specific elimination processes in the plume, most probably biotransformation of the parent NPnEO. An alternative mechanism, *i.e.* fast sedimentation of surfactant molecules with sewage-derived particles, is less likely since this process would tend to decrease the relative percentage of lipophilic metabolites.

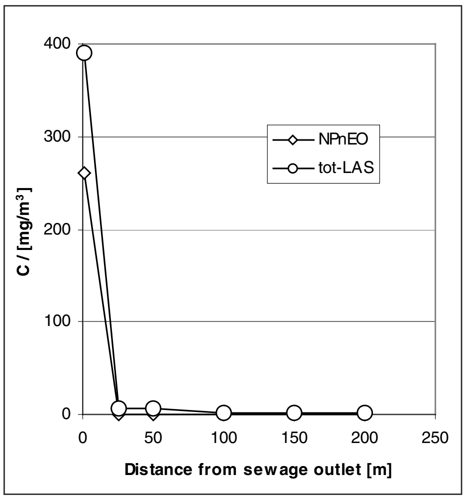

Fig. 4. Dilution of linear alkylbenzene sulphonates (LAS) and nonylphenol polyethoxylates (NPnEO) in the wastewater plume near the outlet in the Sibenik Harbor

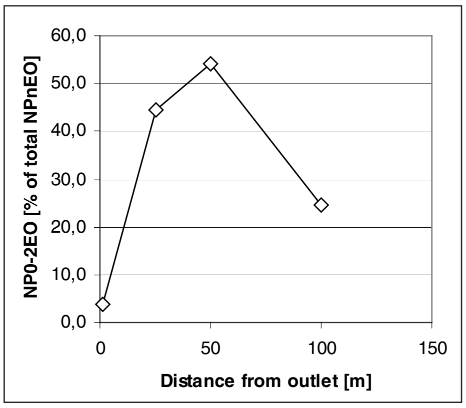

Fig. 5. Relationship between the parent nonylphenol polyethoxylates (NPnEO) and their lipophilic metabolites (NP0-2) in the wastewater plume in the Sibenik Harbor

It is interesting to note that the partitioning of LAS into the particulate phase can be enhanced at higher salinities due to stronger ionic interactions of LAS with the cations present (Fig. 6). The percentage of particulate LAS in the saline layer (salinity 30–37 PSU) is therefore significantly higher than that in the brackish layer (salinities <20 PSU). A similar effect was reported by Rubio *et al.* [36], who studied partitioning of LAS between water and estuarine sediments.

Distribution on the Vertical Profile

The most characteristic feature of the surfactant distribution in a microtidal estuary is the distribution pattern on the vertical profile of the water column (Fig. 7). The maximal concentrations of both LAS and NPnEO are observed at estuarine phase boundaries, *i.e.* in the surface microlayer (air–brackish water interface) and at the brackish–seawater interface. The tendency of aromatic surfactants to accumulate at the phase boundaries is in agreement with their amphiphilic nature. The enrichment factors in the surface microlayer (sampled with the Garrett net) were in the range from 3.2–19.5, while the apparent enrichment at the brackish water–seawater interface was smaller. However, comparatively lower enrichment factors at the brackish water–seawater interface could be simply a consequence of insufficient resolution of the procedure that was used for the sampling of organic films at the brackish water–sea water interface (2 cm), as compared to the well-known Garrett net technique. It should be noted that the concentrations of both LAS and NPnEO in the underlying seawater layer are very low, indicating that their transport through the brackish water–saline water interface is greatly reduced. The interface prevents both the mixing of the two layers as well as a vertical flux of suspended particles [26]. It is interesting to note that a similar vertical concentration profile of NPnEO-derived compounds, characterized by a pronounced maximum at the boundary of two physically different water masses, was observed in Lake Geneva with the

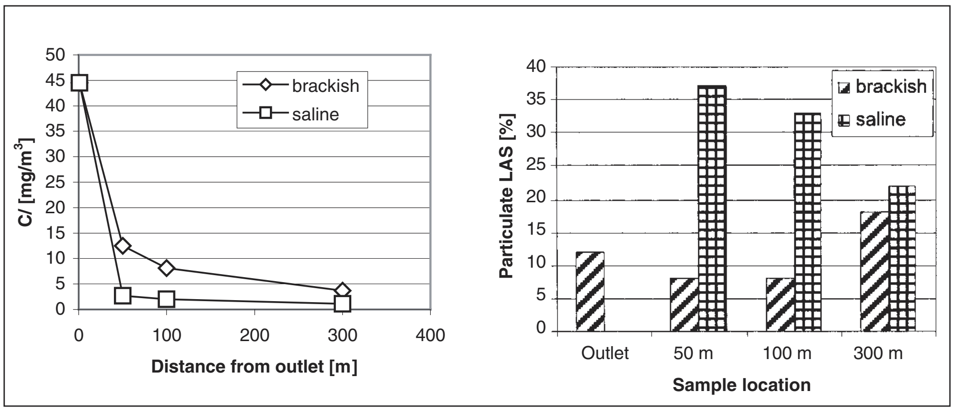

Fig. 6. Distribution of linear alkylbenzene sulphonates (LAS) in the brackish and saline layers near the outlet in the Sibenik Harbor

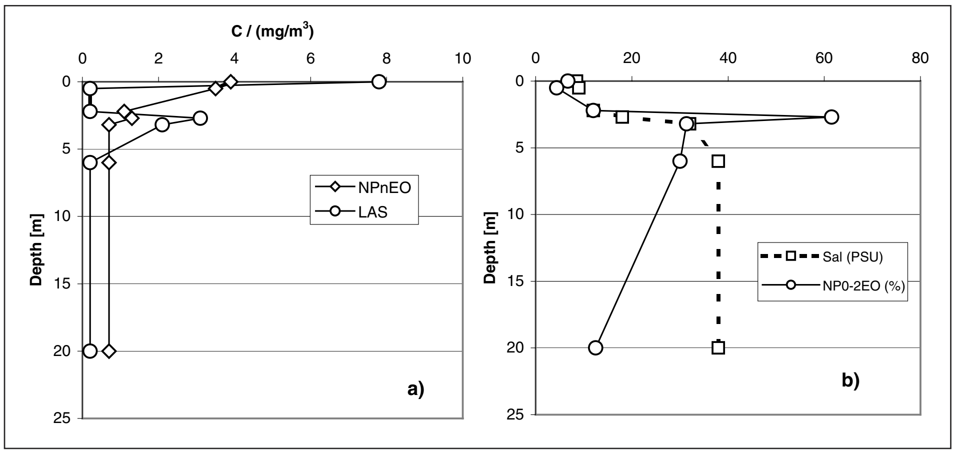

Fig. 7. Vertical profiles of surfactant residues in the water column of a stratified estuary: (a) distribution of linear alkylbenzene sulphonates (LAS) and nonylphenol polyethoxylates (NPnEO) and (b) percentage of lipophilic metabolites in the total NPnEO

maximum that was situated at the thermocline [37].

The homologue and oligomer compositions of aromatic surfactants vary along the vertical profile, suggesting that significant changes occur during the passage through the interface. As indicated in Fig. 7, the concentration of lipophilic metabolites of NPnEO shows a very sharp maximum at the halocline, reaching a high share of over 60% of the total nonylphenolic compounds. This indicates a very efficient transformation of NPnEO at the brackish water–sea water interface.

Biotransformation of Aromatic Surfactants

The biotransformation processes were shown to be seasonally dependent and the seasonal differences are more clearly reflected in the oligomer composition than in the homologue patterns. It should be stressed that it is difficult to decouple the effect of biotransformation on the LAS homologue composition from the changes induced by physico-chemical partitioning, since in both cases the aqueous phase is preferentially depleted in higher oligomers. To assess the effect of biotransformation on LAS composition one should look at the isomer composition, which shows preferential degradation of external isomers [11][18]. For NPnEO oligomers, one could clearly separate the effects of biotransformation and adsorption, since biotransformation is more effective for the higher oligomers, while the lower oligomers exhibit a stronger affinity for adsorption. The corresponding average number of EO units in summer and winter are typically found in the ranges of 7 to 9 and 9 to 11, respectively, which indicates more efficient degradation during summer. Field observations on seasonally dependent transformation were confirmed by controlled laboratory experiments, which were conducted using natural microbial populations, originating from both brackish and saline estuarine compartments. The results presented in the Table show the estimated biotransformation rates of NPnEO and LAS under different temperature conditions. The biotransformation rate constants for the total LAS ranged from 0.030 to 0.269 d^{-1}, corresponding to half-lives of 2.8 to 23 d. Estimated overall biotransformation rate constants for NPnEO (0.01 to 0.28 d^{-1}) are generally in a similar range to those for LAS, however, a more detailed analysis shows that NPnEO are more resistant to biodegradation by microbial populations from the saline layer and/or under lower temperatures. In addition, the model experiments have shown that biotransformation of NPnEO results in the formation of persistent metabolites, including NP1EO and NP2EO as well as corresponding carboxylic acids [24]. Since both surfactant classes are biotransformed several times faster by estuarine populations from the brackish layer than those from the underlying saline layer, it was suggested that the present surfactant-degrading bacteria were predominately of wastewater origin [19].

The effect of temperature on the biotransformation rate is rather pronounced. At the temperatures representative of summer (23 °C) the transformation rate of LAS is approximately 2 times faster than under winter temperature (14 °C) conditions. This effect is even more pronounced for NPnEO, mainly because of its very slow biotransformation under winter temperature conditions. The biotransformation is particularly inhibited at low temperatures in the saline layer, resulting in very long half-lives in that layer.

Assessment of Elimination Mechanisms and Residence Times

To assess the elimination and residence time of the two surfactant classes, a simple calculation has been performed assuming that biodegradation and transport to the bottom sediment are the main elimination mechanisms. Taking into account the rather low concentration of aromatic surfactants in the surface sediments (<5 mg/kg), the transfer of surfactants to estuarine sediments was calculated to be less than 1% of the total amount of 5 t/year that enters the Sibenik Harbor [22][23]. Therefore, this mechanism is not expected to be dominant for the removal of aromatic surfactants from the water column. The reason is, most probably, the hydrographic structure of the stratified estuary, which prevents an efficient transport of sediments to the bottom. Consequently, biotransformation should be considered the most important removal mechanism. The efficiency of elimination of aromatic surfactants by biotransformation was estimated using the one-box steady-state estuarine model proposed by Morris [38]. The original expression was modified to suit an irreversible first-order reaction as follows:

$$\text{Biodegradation (\%)} = kT / (1+kT)$$

where k and T represent the biotransformation rate constants and hydrodynamic residence time (flushing time), respectively. The average residence time of water in the brackish layer of the Sibenik Harbor was estimated at 1.2 d, while the residence time

Table. Overall biotransformation rate constants for linear alkylbenzene sulphonates (LAS) and nonylphenol polyethoxylates (NPnEO) in brackish and saline compartments of the Sibenik Harbour.

Estuarine compartment	Temperature [°C]	LAS Rate constant (d^{-1})	NPnEO Rate constant (d^{-1})
Brackish	13–14	0.161	0.02–0.03
	22.5–23	0.247	0.17–0.28
Saline	13–14	0.020	0.01–0.02
	22.5–23	0.156	0.02–0.05

of the saline layer is much longer and varies from 6.2 d in winter to 18 d in summer [39]. Taking into account the overall biotransformation rates given in the Table, it was estimated that the expected removal rate of LAS in the brackish layer ranges from 16–23%. Such a relatively low removal is mainly due to a very short residence time of water in that layer. Removal rates in the saline layer range from 11% in winter and 74% in summer. For NPnEO, the estimated elimination in the brackish layer varies between 10% in winter and 25% in summer. In the saline layer, the elimination of NPnEO in winter is rather restricted (6%), but reaches 47% in summer. Based on these estimates it is apparent that most of the surfactant residues entering the estuary are discharged into the coastal waters, though in a very dilute form.

Comparison of the Krka River estuary with other estuaries characterized by larger tidal ranges [40] indicates that the elimination processes are less intensive in stratified estuaries. The most probable explanation for the incomplete elimination of aromatic surfactants in the brackish layer is a very short flushing time of that estuarine compartment, which does not provide enough time for the action of active bacterial populations. On the other hand, the biotransformation kinetics of surfactants in the saline water layer are very slow, particularly in winter.

Conclusions

The behavior of aromatic surfactants in microtidal estuaries reveals some interesting features, which are specific for such aquatic systems and, consequently, a careful ecotoxicological assessment is required. The wastewater plumes, which enter permanently stratified estuaries, spread almost exclusively in the upper (brackish) layer, while the underlying saline layer remains much less affected due to the existence of a well-defined brackish water–seawater boundary, which prevents pollutant transport into the deeper layer. This organic-rich interface has the ability to accumulate surfactant residues. As a result surfactant concentrations in that layer are one order of magnitude higher than in the bulk of the water column. Since the interface represents a site of intensive biological activity, such enhanced exposure concentrations can be of major environmental concern.

On the other hand, the removal of surfactants from the water column of the stratified estuaries is relatively inefficient, which is a consequence of a rather low sedimentation rate and relatively slow biodegradation, especially in the saline compartment of the estuary. Therefore, the residence time of surfactant residues in stratified estuaries greatly depends on flushing time of the brackish layer.

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