The influence of the association patterns of phosphorus–substrates and xylene–substrates on the degradation of xylenes in an alluvial aquifer

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Abstract: The association patterns of xylene and of total phosphorus with sediment constituents in an alluvial aquifer consisting of clays and sands which had been contaminated by a spill of technical grade xylene were investigated with the aim of assessing the dependence of *in situ* xylene degradation on the P-availability. The investigated alluvial sediments of the Makiš Plain (Sava river alluvion, Quaternary age, Serbia and Montenegro) are relatively poor in P. The most reliable associations were proposed by analyzing the quality of the correlations of xylene- and of P-contents with 32 sediment characteristics. The association patterns depend on the mineralogical and granulometrical composition of sediments, the structural characteristics of the organic matter (N/C-ratio) and the redox-conditions. Differing association patterns in the clays and the sand had been developed, and these characteristics influence the intrinsic xylene degradation. The main proof for the occurrence of xylene degradation is the percentul share of bacteria which oxidize xylene as a single carbon-source.

Keywords: alluvial clays, alluvial sands, minerals, phosphorus association patterns, xylene association patterns, bacterial degradation.

INTRODUCTION

Alkyl-benzenes can be degraded in aerobic environments,[1,2] as well as by much slower anaerobic mechanism, including, denitrification,[3] dissimilatory Fe(III) or sulphate reduction[3–5] and methanogenesis.[3] The principal limiting factors on

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the degradaton of hydrophobic organic substances in an alluvial sediment could be the following: 1) sorption of organic substances on inorganic or organic sediment particles, achieving thereby a higher stability against microbial degradation,[6–8] 2) immobilization of exoenzymes and/or of bacteria by clay minerals,[9] 3) adverse effects of degradation products on microbiota,[2] 4) poor availability of nutrients, such as nitrogen,[10] or phosphorus.[1,10]

The limited accessibility of phosphours in aquatic sediments is a result of many factors – such as (*i*) the formation of sparingly soluble compounds or stable complexes, with Ca–, Fe(III)–, Al–, Mg–ions,[11] (*ii*) sorption on clay minerals,[12,13] (*iii*) sorption on Fe(III)–oxides or hydroxides,[13–15] (*iv*) sorption on OM,[11,16] (*v*) occlusion of P-compounds by solid phases of Fe(III), Ca, Al or OM.[17] Therefore, in alluvial sediments, characterized by an intensive microbial activity as well as by groundwater, river and atmospheric waters mediated electron-acceptor and -donor cycling, the sorption processes could be highly competitive, *i.e.*, P-binding on the most active substrate will occur. As a consequence, the intrinsic degradation of organic pollutants may depend on the accessibility of P.

The aim of this investigation was to assess the association patterns of xylene and of phosphorus with the individual sediment constituents in a P-poor alluvial sedimentary sequence, which has been polluted with xylene by an accidental spill, and to estimate the possible effects of these associations on *in-situ* xylene degradation. Comparative P– as well as xylene–association studies in clay and sandy environments and their effects on the degradation of xenobiotics are rather rare.

SITE DESCRIPTION

The research area, the Makiš Plain near Belgrade, is a part of the Sava river alluvion of Quaternary age (Fig. 1). The Makiš alluvion has a complex lithologic structure, showing frequent alternations of different sandy, silty and clay sediments with depth.[18] Some of the Belgrade Waterworks Raney- and tube-wells are situated on the Sava riverbank of the Makiš Plain (Fig. 1). As a consequence of the inclination of the sedimentary sequences, the groundwaters, as well as infiltrated atmospheric and surface waters, migrate towards the wells.[18] Therefore, the water quality in the wells is affected by pollution and diagenetic processes in these alluvial sediments.

In 1984, a railway-accident caused a spill of 45 tons of technical grade xylene at the location of the borehole P-10 (Fig. 1; Table I.). Xylene was detectable in the groundwaters until the year 1994.

EXPERIMENTAL

Sampling and sample processing. Sediment samples: 9 clays and 10 sands, from 7 research boreholes were investigated (Fig. 1; Table I). The samples were taken between September 13 and 27, 1994, as the middle-parts of each lithologic member, during the drilling of research-boreholes. The native samples were divided into two sub-samples, one for granulometrical and mineralogical analysis and the other, the main amount of 2–4 kg, was immediately put in polyethylene bags and
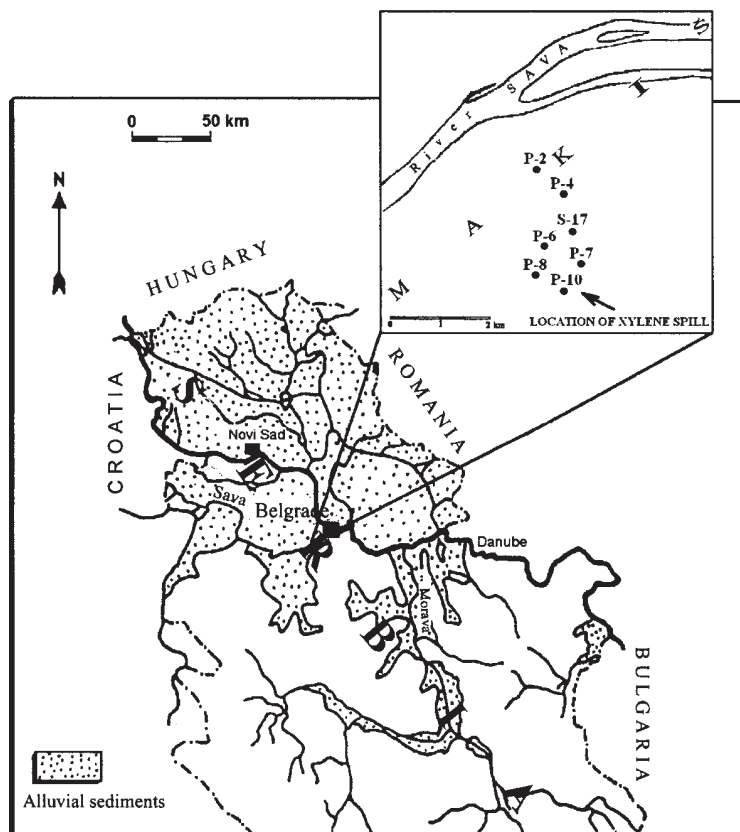
Fig. 1. Makiš Plain within the alluvial formations of North Serbia, and the sector with the bore-hole locations.

kept in a refrigerator at a temperatutre below 4 °C before being further processed. Independently of these, samples for microbiological analysis were taken under sterile conditions. Successively, as the main samples arrived in the laboratory, they were further processed. The sandy samples were homogenized on a glass plate and divided into three subsamples, (*i*) for moisture determination, (*ii*) for extraction and determination of the xylene content, and (*iii*) the rest for chemical analyses, was wrapped in aluminium foil previously washed with dichloromethane, put in polyethylene bags and kept in a deep-freezer at about – 22 °C. Successive chemical analyses were then performed. The cores of clay samples were cut into several sections on a glass plate and than subdivided into three parts, analogous to the sands. Immediately after the performed subdivision, the corresponding subsamples for moisture content (at 105 °C), and the extraction of xylene were further processed. All results are given on the basis of dry matter.

Total phosphorus. The native samples were digested with $HClO_4+HNO_3$. The phosphorus was determined by a molybdenum-blue method using a glycerol solution of $SnCl_2 \cdot 2H_2O$ as the reducing agent.[19]

Xylene. In order to assure the total removal of moisture and quantitative extraction of xylenes, the native samples (about 300.00 g), were extracted more than 36 h with CH_2Cl_2 in a Soxhlet apparatus. The aqueous phase was separated and the xylene content was determined in the dichloromethane solutions by GC-analysis without concentrating. The GC-apparatus was equipped with a BP-5 capillary column and FID, using nitrogen as the carrier gas (flow rate 1 ml/min). The column

temperature program was started at 70 ºC, held for 4 min., and then ramped at 4 ºC/min to 150 ºC. The column was isothermically held at 150 ºC for 1 min. The xylene peaks were identified by coinjection of xylenes, and standard solutions of xylenes were used for calibration.

TABLE I. List of samples and granulometric composition

Sample	Borehole	Depth m	Lithologic characteristic	CFS %	MFS %	FFS %	Fine sand %	Silt %	Clay %
1	P-2	3.75	Sand	16.4	52.1	31.4	–	–	–
2	P-2	7.50	Sand	29.8	61.5	8.5	–	–	–
3	P-2	10.50	Sand	11.0	52.6	36.2	–	–	–
4	P-4	7.25	Sand	15.9	43.9	40.1	–	–	–
5	P-6	3.90	Sand	ND	ND	ND	–	–	–
6	P-6	7.50	Sand	21.4	39.6	38.9	–	–	–
7	P-6	11.50	Sand	37.4	43.8	18.6	–	–	–
8	**S-17**	**3.30**	**Clay**	–	–	–	**17.0**	**48.3**	**34.5**
9	S-17	7.50	Sand	45.6	43.3	11.0	–	–	–
10	**P-7**	**11.50**	**Clay**	–	–	–	**14.5**	**50.8**	**34.7**
11	**P-7**	**3.30**	**Clay**	–	–	–	**23.5**	**35.8**	**40.6**
12	**P-7**	**7.50**	**Clay**	–	–	–	**9.4**	**51.3**	**39.3**
13	P-7	11.50	Sand	35.5	30.7	33.8	–	–	–
14	**P-8**	**2.50**	**Clay**	–	–	–	**15.4**	**53.3**	**31.3**
15	**P-8**	**5.50**	**Clay**	–	–	–	**20.4**	**48.3**	**30.9**
16	**P-8**	**7.50**	**Clay**	–	–	–	**ND**	**ND**	**ND**
17	P-10	1.50	Sand	47.1	46.6	6.3	–	–	–
18	**P-10**	**2.50**	**Clay**	–	–	–	**24.7**	**41.4**	**33.6**
19	**P-10**	**3.25**	**Clay**	–	–	–	**30.6**	**40.2**	**29.2**

Legend: CFS – Coarse fraction of sands (1.000 - 0.600 mm); MFS – Middle grain fraction of sands (0.400 – 0.200 mm); FFS – Fine fraction of sands (0.100 – 0.050 mm); Clay samples are marked in bold. ND = not determined

Organic carbon, (C_{org}) was determined by elemental microanalysis of the samples by the Pregl technique, after destroying the carbonates by treatment with HCl.[20]

Extractable organic substance (bitumen) was determined gravimetrically after a 36 h successive extraction of the native samples with CH_3OH and CH_2Cl_2, in a Soxhlet apparatus.

Sulfides. The native samples were treated with HCl in presence of Sn(II)-chloride and the released H_2S was absorbed in a solution of $Zn(CH_3COO)_2$. The absorbed H_2S was determined spectrophotometrically with *p*-amino-dimethylanilinesulfate.[21]

Sulfates. The native samples were digested in a mixture of hydrochloric and phosphoric acid. The sulfates were determined in the obtained solutions by a semimicro complexometric method consisting of the precipitation of the sulfates with a $BaCl_2$ solution, then dissolving the $BaSO_4$ precipitate in an EDTA-solution and titrating the excess EDTA with a $MgCl_2$ solution.[22]

Metals. The native samples were treated with 2 M HCl in a waterbath for six hours and the metals were determined from the solution by AAS.

Particle size distribution. The sand samples were fractionized on a DIN-series of Standard sieves, whereas a decantation method based on the determination of the settling times according to the Stokes' law was used for the clay samples.

Minerals. The main minerals in the samples were determined semiquantitatively by mineral microscopy using the phase contrast technique.

Microorganisms. A series of solution up to 10^6 dilutions were prepared from the water extracts of samples obtained under sterile conditions. Agar-plates containing the appropriate nutrients and/or substrates for the individual trophic species of microorganisms (MO) were inoculated with these solutions, as a follows (only the procedure for the more specific xylene oxidizing bacterial consortia are given): a) the Xylene Oxidizing Mixotroph Bacteria (XOMB), on nutritive agar under the addition of 1 g/l of a mixture of isomeric xylenes; b) the Xylene Oxidizing Bacteria, which utilize xylene as the single Carbon source (XOBC), on a nutritive substrate containing only a mixture of xylene isomers (1 g/l), as the C-source, and an inorganic N-source. The results represent the mean value for two runs of MO-development and are expressed as the number of MO per g dry sediment.

Aparatus. Spectrophotometric determination of phosphorus and sulfides were performed on a Zeiss Specol 11 spectrophotometer. GC analysis of xylene was performed on Perkin–Elmer 3920 instrument. Metals were determined using a Varian SpectraAA 55 instrument.

Glassware. All glassware was washed with detergent, rinsed with tap water, distilled and redistilled water and finely, after drying in an oven, rinsed with dichloromethane.

Chemicals. All the employed chemicals were commercially available products of analytical reagent grade. Additionally, the organic solvents, methanol and dihchloromethane, as well as hydrochloric acid, were redistilled before use. Exclusively, redistilled water was used for the preparation of the reagents.

Used abbreviation: ACCM – Accesory minerals; ACM – Agglomerated clay minerals; BIT – Bitumen (extractable organic matter); C_{org} – Organic carbon; CFS – Coarse fraction of sands (1.000 – 0.600 mm); FFS – Fine fraction of sands (0.100 – 0.050 mm); FID – Flame ionisation detector; GC – Gas chromatograph; ILL – Illite; Me – Metal ions; MFS – Middle grain fraction of sands (0.400 – 0.200 mm); MIC – Micas; MNT – Montmorillonite; MO – Microorganisms; OM – Organic matter; XOBC – Bacteria oxidizing xylene as the single carbon source, XOMB – Xylene oxidizing mixotroph bacteria; XYL – Xylene.

RESULTS AND DISSCUSSION

An attempt to identify the most reliable substrates of xylene and of phosphorus in the aluvial sediments was made by analyzing the quality of the correlations of XYL- and of P-concentration with 32 constituents or characteristics of the clays and the sands.

Sandy and clay sediments have many differing physical and chemical characteristics resulting in a certain degree of selectivity in the accumulation and transformation of substances with enter these environments.[11,23] Therefore, the association of xylenes and of phosphorus with constituents of the sediments will be discussed for clays and sand separately.

TABLE II. Chemical and mineralogical characteristics of the samples (on a dry weight basis)

Sample	Xylene µg/kg	C_{org}[a]	Bitumen µg/g	Sulfides/a	Sulfates[a]	P[a]	N[a]
1	31	290	39	0.00	2.2	9.3	50.0
2	26	346	126	0.51	3.4	8.9	9.3
3	832	234	230	0.33	19.6	8.4	14.3
4	17783	230	61	0.02	12.5	6.7	10.7
5	46	252	29	0.04	15.7	8.7	6.4

TABLE II. Continued

Sample	Xylene μg/kg	C_{org}[a]	Bitumen μg/g	Sulfides/a	Sulfates[a]	P[a]	N[a]
6	49	226	117	1.42	17.6	7.7	20.7
7	41	425	85	0.55	20.6	7.2	20.0
8	**246**	**177**	**57**	**0.01**	**14.6**	**2.7**	**27.1**
9	32	223	141	4.92	17.9	4.9	23.6
10	**166**	**449**	**91**	**0.18**	**30.6**	**3.8**	**47.9**
11	**87**	**510**	**148**	**10.01**	**21.3**	**5.4**	**59.3**
12	**54**	**336**	**84**	**7.86**	**ND**	**5.0**	**17.1**
13	129	196	57	1.32	13.2	2.9	17.8
14	**347**	**346**	**67**	**0.06**	**16.8**	**7.7**	**36.4**
15	**9772**	**556**	**276**	**16.31**	**16.4**	**9.0**	**55.0**
16	**46**	**425**	**130**	**8.38**	**25.8**	**7.2**	**32.9**
17	10715	338	ND	0.35	11.3	ND	13.6
18	**71**	**977**	**249**	**0.12**	**26.2**	**ND**	**107.9**
19	**87**	**510**	**117**	**0.24**	**18.4**	**ND**	**42.9**

Sample	Quartz %	Micas %	Illite %	MNT %	ACM %	ACCM %	Ca/a	Mg/a	Fe/a	Al/a
1	46.4	11.0	-	-	12.5	5.0	1530.9	254.6	161.9	226.8
2	58.2	11.0	-	-	2.5	5.0	2502.5	423.3	169.4	244.6
3	40.9	14.3	-	-	14.4	5.0	1630.7	299.5	175.1	289.8
4	38.3	16.7	-	-	10.0	7.9	1645.2	292.1	183.9	274.3
5	ND	ND	ND	ND	ND	ND	2968.1	329.5	119.1	273.5
6	38.1	23.9	-	-	1.9	5.0	2975.0	411.8	174.9	313.2
7	31.6	17.2	-	-	0.9	11.1	2953.6	357.1	161.5	138.6
8	**21.1**	**4.2**	**18.5**	**4.9**	**-**	**2.3**	**1636.7**	**191.7**	**461.6**	**446.6**
9	47.2	13.0	-	-	1.1	0.7	1453.6	256.7	178.7	129.0
10	**8.8**	**5.5**	**14.5**	**18.1**	**-**	**1.4**	**3315.9**	**308.5**	**297.9**	**477.8**
11	**16.5**	**4.7**	**18.6**	**21.9**	**-**	**-**	**2545.2**	**336.5**	**316.6**	**308.7**
12	**11.6**	**1.4**	**17.6**	**27.7**	**-**	**0.5**	**ND**	**ND**	**ND**	**ND**
13	44.2	18.1	-	-	3.4	5.1	2093.3	313.5	271.1	207.9
14	**8.0**	**3.1**	**23.4**	**21.6**	**-**	**1.5**	**3319.4**	**347.2**	**261.4**	**343.2**
15	**16.8**	**4.0**	**29.6**	**13.0**	**-**	**0.5**	**2161.7**	**357.1**	**274.3**	**258.7**
16	**ND**	**ND**	**ND**	**ND**	**-**	**ND**	**2664.7**	**383.0**	**266.1**	**238.7**
17	46.8	10.4	-	-	-	1.1.	1140.2	170.7	171.5	85.6
18	**20.1**	**2.1**	**15.2**	**3.0**	**-**	**0.6**	**919.7**	**143.2**	**467.5**	**765.4**
19	**20.6**	**3.5**	**22.4**	**1.1**	**-**	**0.2**	**3460.1**	**217.2**	**173.7**	**461.1**

Legend: MNT – Montmorillonite; ACM – Agglomerated clay minerals; ACCM – Accessory minerals; a – μmol/g; ND – Not determined; Clay samples are marked in **bold**

Xylene and phosphorus associations in clays

Amongst the clay population, two groups differing in the nature of their reducing environments can be distinguished. Group A (samples 8, 10, 14, 18 and 19), from depths of 1.75 – 3.30 m, with a low iron monosulfide content of 0.12 ± 0.09 μmol/g – a fact which indicates unstable reducing conditions (URC-clays). Group B (11, 12, 15, 16), from depths of 4.35 – 5.70 m, with a high FeS content of 10.64 ± 3.89 μmol/g, having, therefore, rather steady sulfate-reducing conditions (SRC-clays). As early diagenetic processes greatly depend on the intensity of the sulphate-reducing conditions,[24,25] XYL- and P-associations in these groups will be discussed separately. In the clays with URC, the xylene concentration ranged between 71 and 347 μg/kg (median: 166 μg/kg). The clays with SRC showed a broader xylene concentration range: 46 – 9772 μg/kg (median: 70 μg/kg).

The clays with URC. Xylene molecules, being of relatively low polarity, could be sorbed on many sediment constituents. Of the 12 minerals and 4 metal-species investigated, positive but statistically insignificant correlations with xylene were obtained for the following sediment constituents: the micas ($r = 0.363, p = 0.549$), ilite ($r = 0.368, p = 0.542$), Mg ($r = 0.696, p = 0.192$), montmorillonite ($r = 0.717, p = 0.173$) and the accessory minerals ($r = 0.818, p = 0.091$). This points to a partition of xylene mainly between the 4 mineral species and sorptive Mg-species. Illite and montmorillonite, directly, or indirectly *via* their humic coatings,[26] are known sorptive substrates for organic molecules.[6–8] On hand of these results, an association of xylene with the mentioned clay constituents can be proposed. In fact, the best correlation, and with the smallest intercept on the *y*-axis, was obtained with the sum of their concentrations (Fig. 2a, Eq. (1)):

$$\log [XYL, \mu g/kg] = 0.0212 [MIC + ILL + Mg + MNT + ACCM, \%] + 1.48 \quad (1)$$
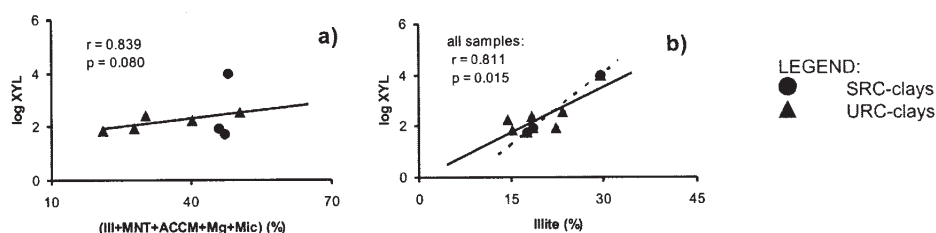

Fig. 2. Correlations of xylene in the clays: a) clays with unstable reducing conditions, log XYL (μg/kg) *vs.* the sum of the contents of the micas, illite, Mg, montmorillonite and the accessory minerals (%); b) log XYL (μg/kg) *vs.* illite (%) for all clays (full line) and clays with stable reducing conditions (dashed line).

On the other hand, for the preferentially expected xylene substrates, the whole OM expressed as C_{org},[26] and the extractable OM[27] (bitumen), an inverse proportionality in the URC-clays was observed:

$$\log [XYL, \mu g/kg] = -0.001 [C_{org}, \mu mol/g] + 2.580,$$
$$(r = -0.815, n = 5, p = 0.093) \quad (2)$$

$$\log [XYL, \mu g/kg] = -0.003 [BIT, \mu g/g] + 2.547,$$
$$(r = -0.826; n = 5, p = 0.085) \tag{3}$$

This result is unexpected because the sedimentary OM cannot behave as a non-sorbent for hydrophobic organic substances.[27] Most probably, this negative correlation between OM and xylene is an effect of the complex partitioning of the sedimentary OM itself (associations with clay minerals, silica-species, oxides and hydroxides of Al, Fe, Mn, microbiota[6–8,26,28]) and at least a partial complementarity between the xylene and the OM-substrates. In fact, the whole OM as well as bitumen correlate significantly with the content of acid-extractable Al, demonstrating that the OM of URC-clays is mainly associated, directly or indirectly, with Al spices:

$$[C_{org}, \mu mol/g] = 1.687[Al, \mu mol/g] - 349.75, (r = 0.892, n = 5; p = 0.042 \tag{4}$$

$$[BIT, \mu g/g] = 0.469[Al, \mu mol/g] - 117.58, (r = 0.949, n = 5; p = 0.014) \tag{5}$$

This preferentially binding of the sedimentary OM on Al-species is most probably the consequences of their high pH of zero charge. According to Sverjensky[29] and Schoonen,[30] the pH_{zpc}-values for α-Al_2O_3 and γ-AlOOH are 9.1 and 8.2 respectively. These values show that at the usual pH range of alluvial sediments, the mentioned Al-species will be positively charged and, thus, attract the anionic structures of humic and fulvic acid, which represent a major part of sedimentary OM. Simultaneously, almost all relevant silicates, oxides and hydroxides, with the exception of amorphous $Fe(OH)_3$, are negatively charged[29,30] and, thus will repulse humic substances but not hydrophobic organics such as xylene. In other words, as a result of such a selectivity in sorption, a complementarity between the main substrates of xylene and of OM in these clays probably exists. This explanation is consistent with the obtained negative correlations of all xylene substrates with the contents of C_{org} and bitumen, as well as by the negative correlations of the mentioned substrates and the aluminium contents (the values are not given). All this, however, does not exclude the occurrence of sorption of a smaller part of the OM on the xylene substrates, followed by sorption of xylene directly onto this already sorbed part of the OM.

It is well known[13,16,17,31,32] that phosphorus in aquatic sediments and soils shows the following operational distribution pattern: 1) loosely adsorbed and exchangeable P; 2) Al-associated P, 3) several types of Fe- and Mn-associated P, 4) occluded P, and 5) several types of Ca-associated phosphorus. For the clays of both, the URC- and SRC-environments together, two statistically significant positive correlations for the total P were found: (*i*) with illite (Fig. 3a), and (*ii*) with Mg (Fig. 3b). In addition, relatively good positive, although statistically not significant, correlations were found for C_{org} ($r = 0.606, n = 7; p = 0.149$) and for bitumen ($r = 0.673; n = 7; p = 0.098$).

The other sediment constitutents with known strong affinity for binding phosphorus[11,13–17,32] gave weak correlations (Ca), or showed good but inverse proporti-
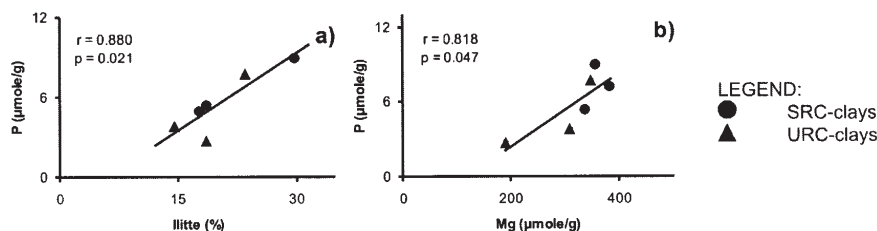
Fig. 3. Correlations of the total phosphorus (μmol/g) in all clay samples:
a) with illite (%), b) with Mg (μmol/g).

onalities (non-sulfidic Fe: $r = -0.810$; $p = 0.050$, and Al: $r = -0.830$; $p = 0.038$). These significant negative correlations are most probably the consequence of an inverse proportionality, *i.e.*, complementarity between the extractable non-sulfidic Fe- and of Al-species and the proposed main P-substrates, the mineral illite and extractable Mg-species, as is indicated by their negative correlations: (*i*) non-sulfidic Fe *vs*. Illite: $r = -0.558$, $p = 0.193$; (*ii*) Al *vs*. Illite: $r = -0.684$, $p = 0.090$, (*iii*) non-sulfidic Fe *vs*. Mg: $r = -0.617$, $p = 0.103$; and (*iv*) Al *vs*. Mg: $r = -0.878$, $p = 0.004$). The observed weak correlation between the total P and Ca is probably a consequence of the partitioning of phosphorus in the investigated Ca-rich clays (3.7 – 13.8 %) between many constituents of the sediment as has already been proposed.[31]

Stone and Mudroch[15] evaluated the clays as the second most important substrates of inorganic P in river sediments. Among the clay minerals, illite has the strongest affinity for sorbing orthophosphate ions:[12] the adsorption capacity for orthophosphate being 64.6 – 77.5 μmol/g in the pH range of 4 – 7. Comparing these values with the average P/Illite ratio found in the clays, amounting to only 31.4 ± 7.5 μmol/g, and bearing in mind the good correlations P *vs*. illite (Fig. 3a), it is very likely that illite carries a substantial amount of phosphorus, perhaps *via* metal bridges[12] – in the present case – *via* Mg-bridges. However, the four simultaneously existing correlations of P, (*i*) significantly with illite and with Mg and (*ii*) fairly good with C_{org} and bitumen, suggest the occurence of a more complex association feature involving systems such as OM–Mg–phosphate and Illite–Mg–phosphate. A similar association feature existing in the system *Organic matter–Metal ion–Orthophosphate* has already been proposed by Boers and DeBles,[11] and theoretically developed by Sigg *et al.*[33]

Sumarizing the most reliable obtained association patterns of xylene and of phosphorus, the following associates could be proposed (the major contributions are marked in bold):

a) XYL(1)---MIC; b) XYL(2)---ILL(1); c) XYL(3)---Mg(1); d) XYL(4)---ACCM
e) P(1)---Mg(2)---ILL(2);   f) P(2)---Mg(3)---OM(1);
g) P(3)---Mg(3)---ILL(3)---XYL(5):   h) OM(2)---Al

The clays with SRC. This sedimentary environment is characterized by rather permanent reducing conditions with the occurence of sulfate reduction, but still containing on average 24.0 ± 8.8 μmol/g sulfates. Xylene appears to be associated mainly with illite and bitumen (Fig. 2b, Eqs. (6) and (7)). The bituminous fraction

of OM, being the most hydrophobic constituent of the sediments has a high affinity for sorbing organic hydrophobic pollutants:[27]

$$\log [XYL, \mu g/kg] = 0.188 [ILL, \%] - 1.560; (r = 1.000; n = 3, p = 0.006) \quad (6)$$

$$\log [XYL, \mu g/kg] = 0.013 [BIT., \mu g/g] + 0.255; (r = 0.958; n = 4, p = 0.042) \quad (7)$$

These all demonstrate a quite different partition feature of xylene in the SRC-clays in comparison to the URC-clays. Moreover, the partition feature of OM itself in this environment differs: no correlation with Al, but relatively good coorelations with quartz and the micas were observed. It was not possible to assess the reason for these differences because of the small number of SRC-samples. As a consequence of all these facts a partition of xylene between illite, OM, quartz and the micas was proposed. For example, for the sums (illite + C_{org}) and (illite + C_{org} + quartz) high correlation coefficients were obtained (Eq. (8) and (9)):

$$\log [XYL, \mu g/kg] = 0.181 [ILL + C_{org}, \%] - 1.602$$
$$(r = 1.000; n = 3; p = 0.003) \quad (8)$$

$$\log [XYL, \mu g/kg] = 0.1356 [ILL + C_{org} + Q, \%] - 2.536$$
$$(r = 0.964; n = 3; p = 0.170) \quad (9)$$

The association pattern of phosphorus in the SRC-clays, as was shown, is similar to that in the URC-clays.

On the basic of the more reliable xylene and P-associations, several associates, *i.e.,* the partition pattern of xylene and phosphorus can be proposed:
a) XYL(1)---ILL(1); b) XYL(2)---OM(1); c) XYL(3)---OM(2)---Q;
d) XYL(4)---OM(3)---MIC; e) P(1)---Me---ILL(2); f) P(2)---Me---OM(4);
g) XYL(5)---ILL(3)---Me---P(3); h)XYL(6)---OM(5)---Me---P(4);
i) XYL(7)---OM(6)---P(5); j) XYL(8)---ILL(4)---P(6).
A general characteristic of this pattern seems to be the occurrrence of associations of xylene and of phosphorus with identical substrates.

Xylene degradation and limiting factors in the clays. As xylene occurs in the clays in a sorbed status, it could be greatly protected against microbial degradation,[6–8] however, even in strong reducing environments, the occurrence of xylene degradation is well documented.[3–5] In each case, the association pattern of xylene could be one of the factors influencing its degradation kinetics. In the investigated clays, the substantial presence of xylene-oxidizing bacteria represents a proof for a slow, yet occuring degradation, Hence, in the URC-clays, the average abundance of the xylene as a single carbon cource oxidizing bacteria (XOBC) was 6.7 ± 8.8 % (median 6.4 %), whereas in the SRC-clays their percentual share was, as expected, lower: 0.9 ± 0.7 % (median 1.0 %) (Table III). The percentual share of the xylene oxidizing mixotroph bacteria (XOMB) in these two environments was 45.3 ± 16.4

% and 16.7 ± 8.8 %, respectively. The primary oxidation products of xylenes, the xylenols, were not detected (detection limits were between 10 and 20 μg/kg dry sediment). This could be the consequence of either, a fast incorporation of the formed reactive xylenols into the humic substances or of their rapid further oxidation.

TABLE III. Abundance of the trophic species of microorganisms (n/g dry sediment) and the mean values of the percentual share of the MO in the different environments

Samples	Nonspecific microorganisms	XOBC	XOMB
1	1960	1120	2560
2	22290	60	1490
3	33090	<10	3530
4	24890	410	750
5	6760	<10	820
6	18500	1380	1380
7	26520	110	4510
8	**1450**	**110**	**2190**
9	36240	630	3800
10	**910**	**220**	**2330**
11	**1440**	**<10**	**290**
12	**6760**	**130**	**1670**
13	13510	<10	1660
14	**630**	**10**	**350**
15	**4820**	**90**	**1760**
16	**9870**	**<10**	**440**
17	24600	760	4740
18	**7500**	**120**	**3420**
19	**8630**	**4340**	**6860**
URC-clays	**48.0±17.5**	**6.7±8.8**	**45.3±16.4**
SRC-clays	**82.4±9.8**	**0.9±0.7**	**16.7±9.2**
Sands I	89.3±5.8	0.9±1.1	9.8±5.5
Sands II	75.0±26.8	7.0±9.0	18.0±18.3

Legend: XOMB – xylene oxidizing mixotroph bacteria; XOBC – xylene as a single C–source oxidizing bacteria; URC – clays with unstable reducing conditions; SRC – clays from stable reducing conditions; Sands I – samples with N/C < 0.09; Sands II – samples with N/C > 0.09. Clays are marked in **bold**

It was interesting to access the existence of limiting factors for this intrinsic bacterial xylene degradation, such as the deficiency of nitrogen or phosphorus.[1,6,10] For nitrogen, the highly significant linear correlation with C_{org} (Eq. (10)), for all samples, regardless of the strength of the sulfate-reducing conditions,

demonstrates that nitrogen is predominantly organic, the content of inorganic N being very small:

$$[N, \mu mol/g] = 0.1122 \, [C_{org}, \mu mol/g] - 6.026; \, (n = 9; \, r = 0.940; \, p = 0.0002) \quad (10)$$

As a consequence, this characteristic could be a limiting factor for the abundance of the microorganisms because the availability of N depends on the degradation kinetics of the organic N-structures. In order to investigate the dependence of xylene degradation of the nitrogen content of the sediments, the abundance of XOBC was normalized by dividing the number of bacteria with the content of bitumen (n bacteria/μg bitumen). The logarithm of the in this way obtained specific abundances of xylene oxidizing bacteria, SA_{XOBC}, was correlated with the atomic N/C_{org}-ratio (Fig. 4a). Two groups of samples were obtained, differing significantly (F - and t-test, $p < 0.05$) in their log SA_{XOBC}, which for group I is 0.286 ± 0.098 and for group II 0.899 ± 0.314. For group I, a weak positive trend with increasing N/C-ratio was obtained, whereas group II showed no dependence at all. Therefore, it was concluded that the content of nitrogen was very probably not a limiting factor for the degradation of xylenes in these alluvial clays.

The average P-contents in the URC-and the SRC-clays were 4.7 ± 2.6 and 6.7 ± 1.8 μmol/g, respectively (the difference is not signficant at $p < 0.05$). By comparing these values with the average P-contents in other relevant environments,[34] such as soil – 25.8, river mud – 21.3 and suspended river material – 3.71 μmol/g, it becomes obvious that the alluvial clays of the Makiš Plain are relatively poor in P, and therefore P could represent a limiting factor in the biodiagenetic transformations of organic pollutants. Investigation of the dependence of SA_{XOBC} on the
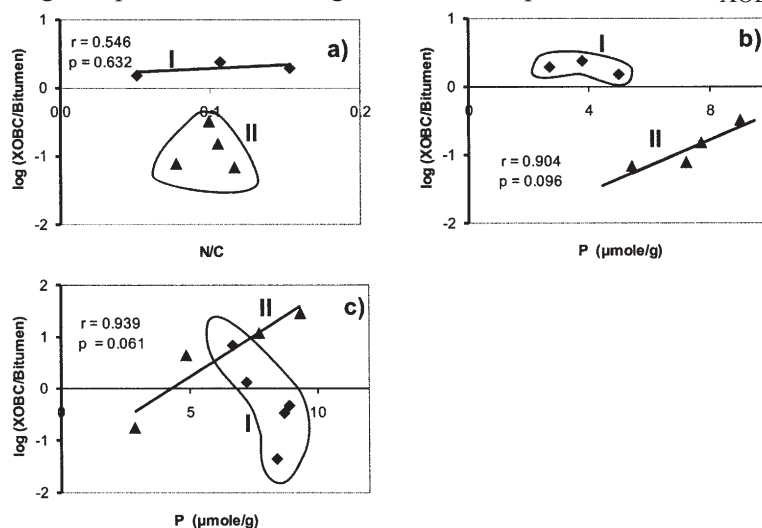

Fig. 4. Dependence of the specific abundance of bacteria which oxidize xylenes as the single carbon-source (number of bacteria/bitumen, n/μg): a) on the atomic N/C-ratio of the OM in the clays; b) on the phosphorus content in the clays (μmol/g); c) on the phosphorus content in the sands (μmol/g).

phosphorus content (Fig. 4b) did not show a dependence on the P content for the Group I samples. On the contrary, a high correlation coefficient was obtained for the Group II samples, indicating a possible dependence of xylene degradation on the P-content in these clays (Eq. (11)):

$$\log [XOBC/BIT, n/\mu g] = 0.1902 [P, \mu mol/g] - 2.292;$$
$$(r = 0.904; \ n = 4; \ p = 0.094) \tag{11}$$

The reason for this difference in the influence of P on the SA_{XOBC} in the two groups of samples is most probably due to the difference in the amount of phosphorus in associations of critical stability,[11,13–17,31,32] which determines the P-availability in the individual samples.

Xylene and phosphorus associations in the sands

The FeS content in the sands ranged between 0.00 and 4.92 µmol/g (mean value and median being 0.94 ± 1.48 and 0.43 µmol/g, respectively). These data show a sulfate-reduction process had occurred in the sands. As opposed to the clays, the sands did not show a dependence of the sulfide concentration on depth and, thus, a reliable differentiation between stable and unstable reducing-environments was not possibly. This feature is most probably a consequences of the relatively coarse structure of sands, which enables a more intensive circulation of ground, river and atmospheric waters, allowing a more equilibrated partition of the solutes and, also, facilitating changes of the redox conditions.

The xylene concentrations in the sands ranged between 26 and 17783 µg/kg (Table I), the median being 47 µg/kg. In an attempt to identify the most reliable substrates of xylenes, no statistically significant correlations of xylene with the sediment constituents were found. Even C_{org} and bitumen, as well as other constituents which are known substrates for hydrophobic organic substances,[26,27] gave insignificant correlations. Therefore, it is proposed that xylene in the sand was partitioned between numerous substrates. The statistically significant positive correlation of xylene with the sum of the coarse fraction, CFS and the fine fraction of sands, FFS (Table I) is in accordance with this proposition. As can be seen from Fig. 5a, the samples can be divided into two groups differing in their atomic N/P- and N/C-ratios: Group I, with N/P < 2 and an average N/C-ratio of 0.04 ± 0.01 and Group II with N/P > 2 and also with a higher N/C-ratio amounting of 0.12 ± 0.04 (Eqs. (12. I) and (12.II)); sample 7, was disregarded in Eq. (12. I) because, according to its N/P-ratio, it shared with Group II:

$$\log [XYL, \mu g/kg] = 0.1654 (CFS + FFS, \%) - 0.490$$
$$(r = 0.998; \ n = 4, \ p = 0.002) \tag{12.I}$$

$$\log [XYL, \mu g/kg] = 0.030 (CFS + FFS, \%) - 0.025$$
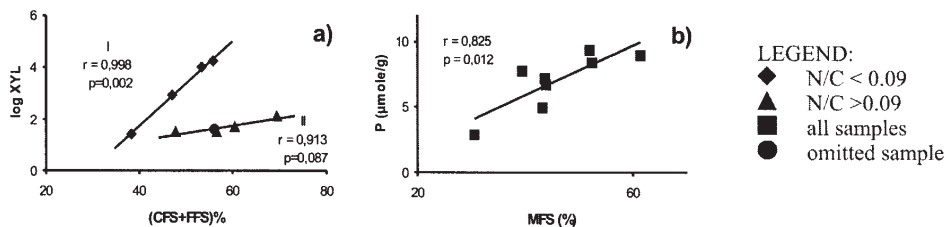$$(r = 0.913, \ n = 4, \ p = 0.087) \tag{12.II}$$

Fig. 5. Correlation diagrams for the sandy sediments: a) dependence of xylene concentration (μg/kg) on the sum of the contents of the coarse and fine fractions (%); b) total phosphorus (μmol/g) *vs*. the content of middle grain fraction (%).

These correlations show that xylene is partioned between the constituents of the coarse and fine fraction of the sands in dependence, of the N/C-ratio, which possibly represents a structural characteristic of sedimentary OM. Therefore it can be assumed that this dependence on the N/C-ratio indicates an, at least, partial association of xylenes with the OM in these sand fractions. The sands of Group I contain a slightly higher amount of C_{org} and bitumen in comparison to Group II sands, but the difference is not significant at $p < 0.05$. In addition, the OM of group I, having a lower N/C-ratio, is probably more hydrophobic than the OM of group II and, for all these reasons, the sands of group I sorbed a greater amount of xylene (Fig. 5a).

Concerning the phosphorus associations in the sands, only one statistically significant positive relation of P was found, that was with the content of the middle grain fraction, MFS (Table I, Fig. 5b). Significant dependences of the P-distribution on the particle size have already been observed.[15,35] This correlation shows that P was preferentially bound to certain components of the MFS. On hand of the positive correlation trends, such P-carrying components could be the agglomerated clay minerals (ACM), C_{org}, bitumen, magnesium, calcium and quartz. Quartz can hardly play a role as a direct substrate for orthophosphate binding because its pH_{zpc}[29,30] can be as low as 2.0 and, therefore, under natural pH conditions, the quartz surface will repulse the phosphate ions. However, according to Sigg *et al.*,[33] phosphate anions can be bound on various solid phases, including quartz, *via* the association scheme: *Solid phases with ionisable functional groups – Metal ions – Phosphate*. Boers and DeBles[11] proposed a similar scheme: *OM – Metal ions – P.* Accordingly, it can be concluded that P in the sands mainly forms associations like the above-mentioned under the participation of several components of the MFS, such as OM, quartz, ACM, Mg-, and Ca-species.

Xylene degradation and limiting factors in the sandy sediments. Despite the protecting role of its substrates,[6–8] proof for the occurrence of xylene degradation is the presence of bacteria which oxidize xylenes as a single carbon source, XOBC (Table III): the procentual share of XOBC in populations I and II were 0.9 ± 1.1 (median: 0.2 %), and 7.0 ± 9.0 (median: 4.0 %), respectively. Analogous to the clays, no clear dependence of xylene degradation on the N/C-ratio was obtained. However, a dependence of the specific abundance of XOBC on the P-content for the sands in group II is evident (Fig. 4c).

On the basis of the established probability that xylenes and phosphorus are accumulated mainly in different fractions of sands, *i.e.*, xylenes are preferentially associated with the constituents of the coarse and the fine fraction (Fig. 5a), whereas phosphorus is almost entirely associated with the constituents of the middle grain fraction of sands (Fig. 5b), a specific mechanism of phosphorus availability in the sands is proposed. The main characteristic of this mechanism being the dependence of P-availability on the desorption kinetics from the substrates and transport processes, either of orthophosphate ions located on the constituents of the MFS or of xylene located on the constituents of the CFS and FFS.

CONCLUSION

1. In a phosphorus-poor alluvial aquifer consisting of clays and sands, which had been polluted with xylenes, the association patterns of xylene and of total phosphorus with the sediment constituents depend on the mineralogical and granulometric composition of the sediments, the N/C-ratio of the organic matter and the redox-conditions. These association patterns are, to a certain degree, different in the clay- and the sandy-environments.

2. In the clays, depending on the intensity of the sulfate-reducing conditions, two association patterns for xylene and a single one for phosphorus were established. In the clays with unstable reducing conditions, URC, (depths < 3.3 m, FeS content 0.12 ± 0.09 μmol/g), xylene (71 – 347 μg/kg) is associated, directly and/or indirectly, with the micas, illite, montmorillonite, the accessory minerals, and the OM. Phosphorus is associated with illite, Mg-species and OM, and, most probably, forms complex associations, such as those proposed by Sigg *et al.*[33] and Boers and DeBles,[11] *e.g.*, Mineral–OM–Mg-phosphate and Mineral–Mg-phosphate. In the clays with rather stable reducing conditions, SRC, (depths > 4.4 m; FeS content 10.6 ± 3.9 μmol/g), xylene (46 – 9772 μg/kg) is associated with illite, OM, quartz and the micas, whereas phosphorus shows the same association patterns as in the URC-clays.

3. The main proof for the occurence of xylene degradation in the clays is the presence of bacteria which oxidize xylenes as the single carbon source. The average percentual share of these bacteria was, for the URC-clays 6.7 ± 8.8 %, and for the SRC-clays 0.9 ± 0.7 %.

4. As a consequence of the strong binding of P in the clays, the bacterial degradation of xylene depends on the concentration of phosphorus, but no clear dependence on the N/C-ratio of the OM was obtained.

5. In the sands, due to the facilitated circulation of water, xylene and phosphorus were relatively widely distributed between the sediment constituents. Xylene (26 – 17783 μg/kg) is almost entirely sorbed on the constituents of the coarse grain (1.000 – 0.600 mm) and fine grain fractions (0.100 – 0.050 mm). The principal individual substrate of xylene appears to be the OM, because the xylene *vs.* sand fractions correlation depends strongly on the N/C-ratio of the OM. Phosphorus is al-

most entirely associated with the middle grain fraction (0.400 – 0.200 mm). Agglomerated clay minerals, OM, quartz, Mg- and Ca-species being the most probable substrates of P, the association pattern of which, like that in the clays, consists of a system Solid phase with ionisable functional groups – Me-ion – phosphorus, is likely. The separated locations of xylene and of phosphorus, in the sands imposes a specific mechanism of P-availability characterized by its dependence on desorption and transport processes.

6. Xylene degradation in the sandy sediments with a higher N/C-ratio depended on the P-content. A proof for the occurrence of xylene degradation in the sands is the percentual share of bacteria which oxidize xylenes as the single C-source (0.03 – 19.9 %; median: 1.6 %).

И З В О Д

АСОЦИЈАЦИЈЕ КСИЛЕН–СУПСТРАТ ОДНОСНО ФОСФОР–СУПСТРАТ У АЛУВИЈАЛНОМ АКВИФЕРУ И ЊИХОВ УТИЦАЈ НА *in situ* ДЕГРАДАЦИЈУ КСИЛЕНА

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Испитивани су глинени и песковити седименти Макишког поља, загађени ксиленом (26–17800 μg/kg), с циљем да се утврди утицај асоцијација ксилен–супстрат, односно фосфор–супстрат на *in situ* деградацију ксилена. Седименти Макишког поља (алувијални седименти млађег Квартара), имају релативно низак садржај фосфора. Анализирањем корелација ксилена и фосфора са 32 састојка седимената утврђено је да асоцијације ксилена и фосфора зависе од минералошког и гранулометријског састава седимента, структурних карактеристика органске супстанце и редокс услова средине. Фосфор и ксилен у песковитим и глиненим седиментима граде различите типове асоцијација, што се одражава и на деградацију ксилена. Знатан удео бактерија које оксидују ксилен као једини извор угљеника представља поуздан показатељ да се у седиментима одвија деградација ксилена.

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