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Bioremediation of river sediment polluted with polychlorinated biphenyls: A laboratory study

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Abstract: Persistent organic pollutants (POPs) are lipophilic, constant and bioaccumulative toxic compounds. In general, they are considered resistant to biological, photolytic, and chemical degradation with polychlorinated biphenyls (PCBs) belonging to these chemicals. PCBs were never produced in Serbia, but they were imported and mainly used in electrical equipment, transformers, and capacitors. Our study aimed to analyse sequential multi-stage aerobic/anaerobic microbial biodegradation of PCBs present in the river sediment from the area known for long-term pollution with these chemicals. The study with an autochthonous natural microbial community (NMC model system) and NMC augmented with allochthonous hydrocarbon-degrading (AHD) microorganisms (isolated from location contaminated with petroleum products) (NMC-AHD model system) was performed in order to estimate the potential of these microorganisms for possible use in future bioremediation treatment of these sites. The laboratory biodegradation study lasted 70 days, after which an overall >33 % reduction in the concentration of total PCBs was observed. This study confirmed the strong potential of the NMC for the reduction of the level of PCBs in the river sediment under alternating multi-stage aerobic/anaerobic conditions.

Keywords: persistent organic pollutants (POPs); remediation; Topčider River; Čukarički Rukavac.

INTRODUCTION

Persistent organic pollutants (POPs) are lipophilic toxic compounds that persist in the environment. They can bioaccumulate through the food chain and cause adverse effects on human health and the environment.[1,2] Production of

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POPs in the last century was deliberate and focused on defence against pests and production of more robust industrial materials. It was not until several decades had passed that they were considered toxic, and now, in many countries, production and usage of these compounds is prohibited.[3,4] Their resistance to photolytic and chemical degradation is based on the exceptional strength of the carbon-chlorine bond, and with a higher degree of substitution of hydrogen with chlorine atoms, greater resistance of the chlorine to degradation occurs.[5] Resistance to degradation and the fact that POPs are generally aromatic structures with stronger connections than those in aliphatic compounds contribute to the low aqueous solubility.[6] However, in spite of their chemical stability, several microorganisms are described in the literature with a potential to biotransform or mineralize the PCBs aerobically or anaerobically.[7] Aerobic oxidation and anaerobic dechlorination are the best known biodegradation pathways of PCBs.[8,9] Pollution with POPs can lead to disorder of the reproductive, immune, endocrine and the nervous system, as well as to carcinogenesis, mutagenesis and teratogenesis.[10] The problem of their high potential for bioaccumulation and toxicity to humans and the environment led to the 2004 adoption of the Stockholm Convention on POPs chemicals. Its primary objective is to protect human health and the environment from POPs. The countries that signed this Convention should determine, prohibit or restrict production, sale, and usage of POPs, and have additional obligation to reduce or eliminate emissions of POPs.[11]

Based on the Convention, POPs are classified into three categories: pesticides, such as aldrin, dieldrin, chlordane, toxaphene, mirex, endrin, heptachlor, hexachlorobenzene (HCB), dichloro-diphenyl-trichloroethane (DDT), *etc.*; industrial chemicals (PCBs); and by-products of industrial processes, and combustion processes as polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzo-*p*-furans (PCDF) and polycyclic aromatic hydrocarbons (PAH). In addition, from 2017, the production and use of the perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOSF) was under restriction and based on the latest revised Convention from 2019, the production and use of perfluorooctanoic acid (PFOA), its salts and PFOA related compounds, together with PFOS, its salts and PFOSF should be eliminated, except for some cases as stated in the Convention.[11–14]

Among POPs, PCBs have a special place. There are ten groups of PCBs, from mono- to decachlorobiphenyl. With an increase in the percentage of substitution, solubility decreases,[15] and these compounds are extremely chemically inert, heat resistant, non-combustible with a high dielectric constant.[16] In Serbia, PCB-based fluids were never produced, but they were imported and used in transformers, capacitors, electric motors with liquid cooling, hydraulic systems, heat transfer systems, electromagnets, fluorescent light fittings, fluid-filled cables, and as additives to pesticides, inks, oils, lubricants, *etc*. A preliminary inventory of PCBs was conducted in the framework of preparation of the first National Implementation Plan of the Stockholm Convention.[17] Besides PCB fluids in electrical equipment, waste consisting of, containing, or contaminated with, PCBs can be found in different physical forms, including: solvents, construction waste, contaminated oils, soil, sediments, sludge, rock and aggregates, tanks, barrels and containers.

For the treatment of this material, export from Serbia for incineration was the dominant method of choice. However, bioremediation can be of potential use for the treatment of soil and sediment polluted with low levels of PCBs, having in mind that industrial-scale bioremediation of soil contaminated with petroleum hydrocarbons was previously confirmed.[18] Based on the current knowledge, biodegradation of PCB can be conducted to some extent, using a variety of bacteria and/or fungi. The biodegradation of PCBs can include two main microbial metabolic steps: anaerobic reductive dehalogenation in which the PCB acts as an electron acceptor, and thus, hydrogen replaces chlorine, so the compound becomes less chlorinated,[4,8,15,16] and aerobic decomposition of the biphenyl structure, which is operative only on the PCBs that are less chlorinated (less than five atoms of chlorine).[8,10,16] Complete mineralization of biphenyl and some PCBs was confirmed using microorganisms from the following bacterial genus: *Pseudomonas*, *Alcaligenes*, *Burkholderia*, *Comamonas*, *Sphingomonas*, *Ralstonia*, *Cupriavidus*, *Achromobacter*, *Acidovorax*, *Nocardia* and *Acinetobacter* as Gram-negative strains, and *Rhodococcus*, *Corynebacterium* and *Bacillus* as Gram-positive strains.[3,9]

The aim of this study was to analyse the potential of the autochthonous natural microbial community (NMC model system), and NMC augmented with allochthonous hydrocarbon-degrading (AHD) microorganisms (isolated from location contaminated with petroleum products, NMC-AHD model system) for biodegradation of PCBs present in the river sediments to protect the environment and estimate future treatment of these sites. Sequential multi-stage aerobic/ /anaerobic microbial bioremediation of sediment polluted with PCBs was applied to stimulate both oxidative and reductive processes, respectively. The river sediments were collected in Belgrade, Serbia, from the confluence of Topčider River with Čukarički Rukavac (CR), which is known for long-term pollution with various organic and inorganic pollutants.[19] In the study, a combined multi-stage aerobic/anaerobic process was applied.

EXPERIMENTAL

Sediment sampling

River sediment samples were collected in Belgrade, Serbia, from the confluence of Topčider River with Čukarički Rukavac. Sediments were sampled from four depths in undisturbed conditions: 0–1, 1–3, 3–6 and 6–10 cm.

Basic physicochemical and chemical analysis of the sediment

The content of total petroleum hydrocarbons (TPH) in the sediment was extracted as per method ISO 16703[20] and determined gravimetrically in accordance with DIN EN 14345[21] as previously described.[18] Sediments were analysed for: the content of sand, clay, and silt, moisture, pH, total, organic and inorganic carbon, sulphur and nitrogen using standard methods.[22,23] Sediments were dried and PCBs were extracted using a Soxhlet apparatus according to the modified method 3540C during 24 h with 5 cycles per hour.[24] Extraction was carried out using a 1:1 volumetric mixture of acetone:hexane, and the PCB content in the extracts (after clean-up) was analysed using GC–MS/MS (Bruker, 320MS). All solvents and reagents used were HPLC grade.

Microbiological analysis of the sediment

The number of microorganisms in the river sediments was determined by plating appropriate serial dilutions on agar plates incubated at 28 °C. The media used were: nutrient agar for total chemoorganoheterotrophs (TC); nutrient agar with 0.5 % glucose for total anaerobic chemoorganoheterotrophs (TAC); malt agar for yeast and molds (YM), and; a mineral base medium containing 2 g of standard D2 diesel fuel in 1 L of medium[25] for hydrocarbon degraders (HD).

Bioremediation study

After analysis of the sediments, a composite sample from all four sediments was produced for the bioremediation study, with the mass ratio of sediments from the different depths (0–1, 1–3, 3–6 and 6–10 cm) of 1:2:3:4. Composite sediment and sand were mixed in a 1:1 mass ratio and added to Bushnell–Haas modified medium (chloride-free): magnesium sulphate heptahydrate, 0.2 g L^{-1}; calcium carbonate, 0.2–0.5 %; potassium dihydrogen phosphate, 1.0 g L^{-1}; dipotassium hydrogen phosphate, 1.0 g L^{-1}; ammonium nitrate, 1.0 g L^{-1}; iron sulphate, trace; Tween 80, 0.1 g L^{-1}; pH ~7. Bioremediation of polluted sediment lasted 70 days with alternating anaerobic and aerobic cycles: anaerobic (static, in a CO_2 atmosphere, 28 °C, three weeks (0–21 days)); aerobic (rotary shaker at 200 rpm, 28 °C, three weeks, (21–42 days)); anaerobic two weeks (42–56 days), aerobic two weeks (56–70 days). In parallel, the activity of microorganisms in NMC and NMC-AHD model systems was monitored. In the NMC-AHD model system, bioaugmentation using AHD was conducted at the beginning, 21^{st} and 56^{th} day under sterile conditions. The pH of suspensions in the model systems was measured on days 0, 42 and 70. Abiotic control was sterilized in an autoclave prior to incubation in order to monitor abiotic changes. All analyses were conducted in triplicate and results are given as mean values.

Allochthonous hydrocarbon-degrading microorganisms used for bioaugmentation

Bioaugmentation was carried out by inoculation of AHD biomass (enriched in laboratory conditions) which were previously isolated from sites contaminated with petroleum products: *Pseudomonas* (*sp.* NS009 – GenBank: JF826528.1 and CHNSH-17 –GenBank: JQ292806.1), *Rhodococcus* (*sp.* RNP05 – GenBank: JQ065876.1 and CHP-NR31 – GenBank: JX965395.1) and *Achromobacter* (*sp.* NS014 – GenBank: JF826529.1).[26,27]

Instrumental analysis

The content of PCBs was analysed using GC-MS/MS (Bruker, 320MS). For PCB congener-specific analysis, an HT8-PCB capillary column (60 m, 0.25 mm i.d., Kanto Kagaku, Japan) was used. As standard for PCB determination EC5433 (Cambridge Isotope Laboratories, USA) was used. As an internal standard, ^{13}C-labeled PCB congener mixture MBP-CG (Wellington Laboratories, Ontario, Canada) was used.

RESULTS AND DISCUSSION

Chemical and microbiological properties of sediments

The river sediments were characterized by neutral pH, relatively high content of organic carbon and high content of inorganic carbon (Table I). Silt, particularly fine silt, was the dominant granulometric fraction in all sediments. Among microorganisms determined, the dominant fraction comprised TC microorganisms. The number of TC increased with the depth of the sediment layers, together with HD (Table I). The high counts, determined for all microbial groups examined, suggested that intensive aerobic and anaerobic microbiological processes were occurring in the river sediments, and so these materials would form a suitable matrix for studying possible biotic transformations of organic contaminants, such as PCBs.

TABLE I. Basic chemical and microbiological properties of the river sediments studied; in all standard results: ± deviation for three measurements

Parameter	Sediment layer depth, cm			
	0–1	1–3	3–6	6–10
pH	7.30	7.22	7.23	7.25
Amount of total carbon, %	3.2±0.1	3.9±0.1	4.3±0.1	4.7±0.1
Amount of organic carbon, %	1.8±0.1	2.1±0.1	2.3±0.1	2.6±0.1
Amount of inorganic carbon, %	1.4±0.2	1.8±0.1	2.0±0.1	2.1±0.1
Amount of nitrogen, %	0.32±0.04	0.24±0.04	0.28±0.04	0.22±0.04
Amount of sulfur, %	0.21±0.02	0.19±0.02	0.24±0.02	0.23±0.02
Amount of sand, %	9.7	8.2	8.1	6.3
Amount of silt + clay, %	90.3	91.8	91.9	93.7
Concentration of TPH, g kg^{-1} dw	0.97±0.2	1.12±0.2	0.88±0.2	1.54±0.2
Concentration of PCBs, ng g^{-1} dw	305±10	173±10	220±10	169±10
Number of TC, CFU g^{-1}	2.1×10^6	8.9×10^5	5.1×10^5	6.3×10^4
Number of TAC, CFU g^{-1}	1.2×10^3	5.6×10^3	9.8×10^3	1.2×10^4
Number of YM, CFU g^{-1}	5.4×10^3	5.1×10^3	6.9×10^2	8.4×10^2
Number of HD, CFU g^{-1}	1.1×10^5	5.9×10^5	1.8×10^5	2.3×10^3

PCBs in the sediments

The distribution of PCBs in the sediments is given in Fig. 1. The PCBs are water-insoluble chemicals that accumulate in sediments depending on the partition coefficient.[28] The high level of PCBs in the sediment sample is probably due to historical reasons since industrial plant Minel located upstream of Topčider River, was known for the production of transformers and capacitors in the past. The Topčider River water body is small, thus leading to the accumulation of PCBs in sediment. The dominant PCBs in the sediments were pentachlorinated, followed by tetra, tri and hexachlorinated compounds. The highest concentration was determined in the upper layer (0–1 cm), followed by 3 to 6 cm, 1 to 3 cm and 6 to 10 cm layer, respectively. This finding suggests that possible recent contamination also occurred.

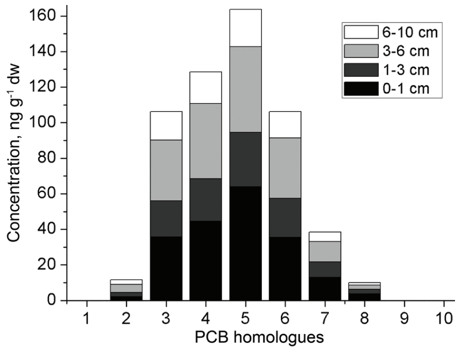

Fig. 1. Distribution of PCBs in the river sediments.

Numbers of microorganisms during the biodegradation study

The number of microorganisms during biodegradation is given in Table II. The number of microorganisms in the NMC model system was reasonably stable, with slight decreases. However, in the NMC-AHD model systems, numbers of TC and HD microorganisms increased by two orders of magnitude by day 42 and then reduced by one order of magnitude by day 70 (Table II).

TABLE II. Numbers of microorganisms (CFU g^{-1}) in the NMC and NMC-AHD model systems on days 42 and 70, and in abiotic control after 70 days

Microorganism	Model					
	Abiotic control		NMC		NMC-AHD	
	Day 0	Day 70	Day 42	Day 70	Day 42	Day 70
TC	7.0×10^5	<1	5.0×10^5	2.5×10^5	4.9×10^7	10^7
TAC	2.1×10^4	<1	1.3×10^5	6.2×10^4	3.9×10^5	2.0×10^5
YM	1.15×10^3	<1	2.5×10^3	4.5×10^3	2.9×10^4	4.0×10^4
HD	1.7×10^5	<1	2.0×10^5	2.3×10^5	2.6×10^7	4.7×10^6

Change in suspension pH during the biodegradation study

The pH values of suspensions in the biodegradation study, measured in the suspensions after stopping the bioremediation process by autoclaving, are shown in Fig. 2. In NMC model systems, the suspension pH decreased from initial pH 7.2 to 6.1 and 6.0 after 42 and 70 days, respectively. In NMC-AHD model systems, the pH decreased from initial 7.2 to 6.0 and 5.8 after 42 and 70 days, respectively. In the abiotic control, sterilized at the beginning of the process, the pH remained the same after 70 days. This change in pH indicated that the activity of microorganisms under aerobic/anaerobic conditions resulted in the decomposition of present hydrocarbons followed by the formation of organic acids as one of the oxidation products during the aerobic step of the study. In addition, in the NMC-AHD model system, the pH decreases slightly more comparing to NMC, which is the result of the microbial activity of AHD used for bioaugmentation of the river sediment.

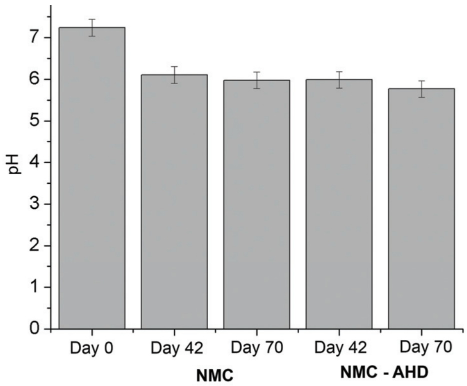

Fig. 2. Change in pH during the bioremediation study in different model systems.

PCB concentrations during the biodegradation study

The amount of PCBs and level of substitution through the bioremediation process was analysed using GC–MS/MS and the results are given in Fig. 3. At the beginning of the bioremediation, the total amount of PCBs was 287.5 ng g^{-1} (day 0). In the NMC model system, the concentration of PCBs was 276.8 and 198.5 ng g^{-1} after 42 days and 70 days, respectively. Therefore, significant (>30 %) decrease in PCBs concentration occurred after 70 days.

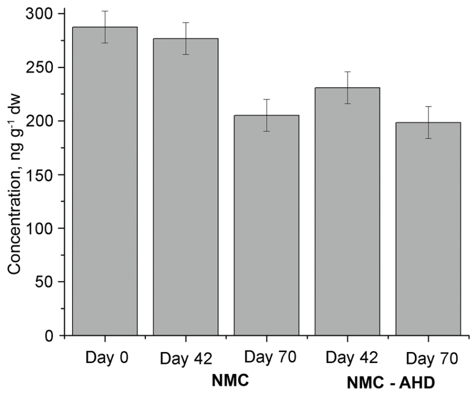

Fig. 3. Change in PCB concentration during the bioremediation study in different model systems.

In the NMC-AHD model systems, a faster decrease in the concentration of PCB in the first 42 days was determined (231.0 ng g^{-1}). After 70 days, the concentration was 195.2 ng g^{-1}. These reductions were 20 and 32 %, respectively, after 42 and 70 days. A slight decline in PCB levels in the abiotic control was also observed, suggesting that sorption processes occurred. Surprisingly, the concentration of PCBs in the NMC and NMC-AHD model systems after 70 days was almost the same, suggesting that NMC, already present in the sediment, has important biodegradation potential for reducing PCBs and carry a major part of the bioremediation potential. Some previous studies also confirmed that PCBs contaminated soils can be the source of PCBs and biphenyl degrading microorganisms.[29] However, it should be emphasized that in our study, bioaugmentation (a process of adding allochthonous hydrocarbon-degrading microorganisms to supplement the autochthonous population) proved to be very useful because it leads to accelerated bioremediation.

Changes in the PCB congener and PCB homologue patterns during the biodegradation study

Changes in the PCB congener pattern during the biodegradation study are given in Fig. 4. The sediment sample contained from mono to octachlorinated biphenyls, wherein the dominant were pentachlorinated congeners (control suspension). A change in congener concentrations and profiles was observed during the bioremediation study. The level of higher substituted congeners was reduced and increases in the level of trichlorinated biphenyls were noticed.

These changes were noticed after the applied anaerobic/aerobic alteration cycles, suggesting that reductive dehalogenation occurred and that the increase in the concentration of trichlorinated biphenyls resulted from dehalogenation of higher congeners. This is supported by the previous studies in which tetra-, penta-, hexa- and hepta-chlorobiphenyls were produced during the dechlorinated process of deca- and hepta-chlorobiphenyls.[30–32] In the study of Song *et al.*[33] the rise of tetra-, penta-, hexa- and hepta-chlorobiphenyls was attributed to the loss of deca- and hepta-chlorobiphenyls. It should also be emphasized that the degree of chlorination and position of chlorine atoms on biphenyl rings may influence the biodegradability of different PCB congeners.

To follow the change in the homologue pattern of the PCBs during the bioremediation study, dihalogenated and trihalogenated homologues were given special attention. Table III lists the analysed homologues, while Figs. 5 and 6 depicts differences in the homologue patterns during bioremediation in NMC-AHD model systems.

The concentration of different PCB congener homologues was different in NMC-AHD model systems after 70 days compared to day 0. PCB12 was completely degraded while the concentrations of PCB-8, PCB-11 and PCB-15 were reduced by 60, 50 and 20 %, respectively. As for trihalogenated PCBs, PCB-18 and PCBs 19, 31 and 33 were the most susceptible to biodegradation, while PCB-28 and PCB-37 were reduced by less than 10 % by the biodegradetion processes that occurred. In abiotic control after 70 days, significant changes were not noticed.

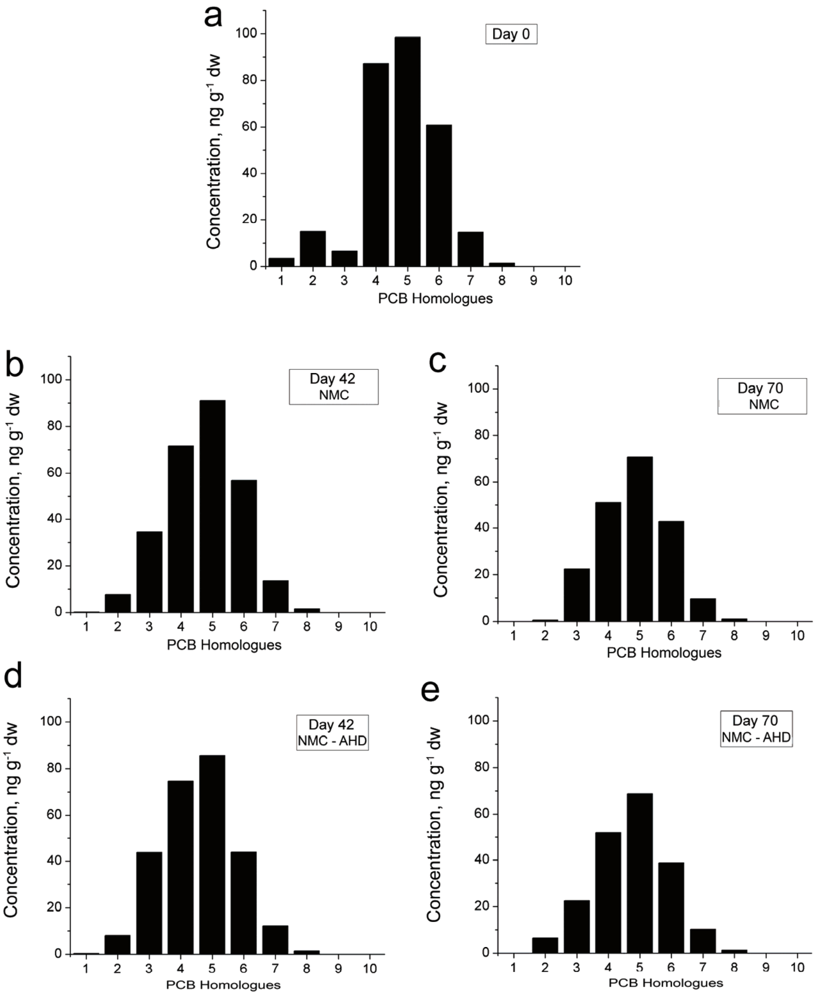

Fig. 4. Change in PCB congener patterns obtained during the bioremediation study in different model systems: day 0 (a), day 42 NMC (b), day 70 NMC (c), day 42 NMC-AHD (d) and day 70 NMC-AHD (e).

TABLE III. List of dihalogenated and trihalogenated PCB homologues analysed

PCB 2 Cl	IUPAC	PCB 3 Cl	IUPAC
–	–	2, 2’, 6	19
2, 6	10	2, 2’, 5	18
2, 2’	4	2, 4’, 5	31
2, 5	9	2, 4, 4’	28
2, 4’	8	2’, 3, 4	33
3, 3’	11	3, 4, 5	38
3, 4	12	3, 3’, 4	35
4, 4’	15	3, 4, 4’	37

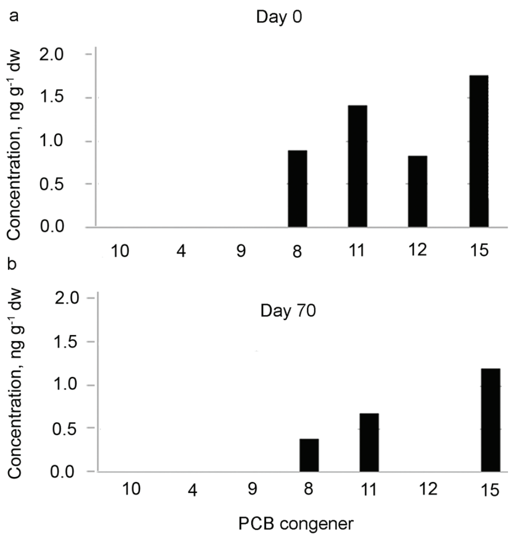

Fig. 5. Changes in PCB homologue patterns of dihalogenated PCBs observed during the bioremediation study in NMC-AHD model system: day 0 (a) and day 70 (b).

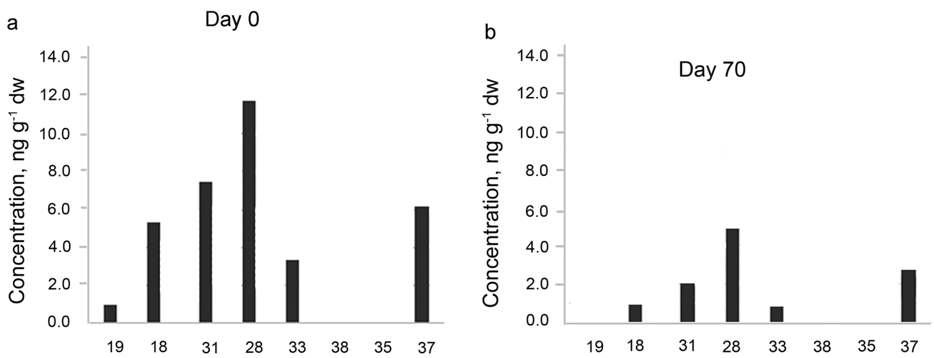

Fig. 6. Changes in PCB homologue patterns of trihalogenated PCBs observed during the bioremediation study in NMC-AHD model system: day 0 (a) and day 70 (b).

CONCLUSION

After 70 days in the both model systems, one with the autochthonous natural microbial community (NMC consortium) and the second with NMC augmented with AHD microorganisms (*Pseudomonas* (*sp.* NS009 – GenBank: JF826528.1 and CHNSH-17 –GenBank: JQ292806.1), *Rhodococcus* (*sp.* RNP05 – GenBank: JQ065876.1 and CHP-NR31 – GenBank: JX965395.1) and *Achromobacter* (*sp.* NS014 – GenBank: JF826529.1) about a 33 % reduction in the concentration of PCBs was observed. Tests have confirmed that NMC consortia can reduce the level of PCBs in the contaminated river sediment under laboratory conditions and that presence of AHD microorganisms facilitates this degradation. The results indicate the strong bioremediation potential of NMC present at the contaminated site if alternating anaerobic/aerobic cycles are used for the treatment of sediments or soils contaminated with PCB compounds. Changes in the level of dominant congeners, *i.e.*, a reduction of the substituted higher fraction and an increase of the substituted lower fraction, during the course of the biodegradation corresponded to the occurrence of anaerobic reductive dehalogenation.

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И З В О Д

БИОДЕГРАДАЦИЈА ПОЛИХЛОРОВАНИХ БИФЕНИЛА У РЕЧНОМ СЕДИМЕНТУ: ЛАБОРАТОРИЈСКА СТУДИЈА

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Дуготрајне органске загађујуће супстанце (POPs) су липофилна, постојана и биоакумулативна токсична једињења. Уопштено говорећи, сматрају се отпорним на биолошку, фотолитичку и хемијску деградацију, а полихлоровани бифенили (PCBs) спадају у групу ових хемикалија. У Србији се PCBs никада нису производили, али су се увозили и углавном користили у електроопреми, трансформаторима и кондензаторима. Циљ нашег истраживања био је да се анализира секвенцијална вишестепена аеробна/анаеробна микробиолошка разградња PCBs присутних у речном седименту са подручја познатог по дуготрајном загађењу овим једињењима. Проучавање активности конзорцијума аутохтоних природно присутних микроорганизама (NMC конзорцијум) и NMC суплементисаних конзорцијумом алохтоних угљоводоник деградирајућих (HD) микроорганизама (изолованих са локација контаминираних нафтним дериватима) (NMC-HD конзорцијум) је изведено како би се проценила способност ових микроорганизама за потренцијалну употребу у будућим биоремедијационим третманима оваквих локалитета. Лабораторијска студија биоразградње је трајала 70 дана, након чега је уочено смањење концентрације укупних PCBs за >33 %. Ово истраживање је потврдило снажан потенцијал аутохтоних микроорганизама за смањење нивоа PCBs у речном седименту у наизменичним вишестепеним аеробним/анаеробним условима.

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