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NATURAL ATTENUATION OF AROMATIC HYDROCARBON FROM SANDY SEDIMENT IN BOA VIAGEM BEACH, GUANABARA BAY, RJ, BRAZIL

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ABSTRACT

The aims of this work was to quantify the concentration of petroleum aromatic hydrocarbon in the intertidal zone at Boa Viagem Beach (Guanabara Bay, RJ, Brazil) and to determine, by *ex situ* treatment, the natural attenuation of these substances in the presence of high organic matter content. We determined concentration and degradation of petroleum aromatic hydrocarbon, organic matter, bacterial carbon (CB) and esterase enzyme activity (EST) and electron transport system (ETSA) during 30 days. The results showed that Boa Viagem Beach's sediment, in the beginning of the assays, had high concentration of aromatic hydrocarbon occurring natural attenuation about 99.9%, when it represented 6.57% of the sediment's organic matter. The electron transport system activity was significantly different between the bioassays. It is based on dehydrogenase enzymes that are the major representatives of oxido-reductase reactions. This activity could be used as a biomarker to detect the aromatic hydrocarbon and remediation processes. The natural attenuation *ex situ* occurred in thirty days, and this time is advantageous in the remediation process of aromatic hydrocarbon. These tools can be used in the environmental impact assessment.

RESUMO

O objetivo do trabalho foi quantificar as concentrações de hidrocarbonetos aromáticos de petróleo no sedimento da zona entre-marés da Praia de Boa Viagem (Baía de Guanabara, RJ, Brasil) e determinar, através de tratamento *ex situ*, a atenuação natural destas substâncias em presença de altos teores de matéria orgânica. Ao longo de 30 dias, foram realizadas análises dos hidrocarbonetos de petróleo, da matéria orgânica, quantificação do carbono bacteriano (CB), atividades das enzimas esterases (EST) e do sistema transportador de elétrons (ASTE). Os resultados mostraram que os sedimentos da Praia de Boa Viagem continham, no início dos ensaios, hidrocarbonetos aromáticos em concentrações elevadas; ocorrendo atenuação natural da ordem de 99,9% desses hidrocarbonetos quando estes representavam 6,57% da matéria orgânica dos sedimentos. A atividade do sistema transportador de elétrons foi significativamente diferente entre os bioensaios. Ela está baseada nas enzimas desidrogenases, que são os maiores representantes das reações de óxido-redução. Esta atividade poderia ser usada como biomarcador para detectar os processos de degradação dos hidrocarbonetos aromáticos e remediação. A atenuação natural *ex situ* ocorreu em trinta dias e este tempo é vantajoso no processo de remediação dos hidrocarbonetos aromáticos. Assim estas ferramentas podem ser usadas na recuperação de ambientes impactados.

INTRODUCTION

Guanabara Bay receives daily 400 tons of domestics effluents and garbage, 7 tons of oil, 0,3 tons of heavy metals and 64 tons of organic material generated by the industries. There are also two refineries which are responsible for processing 17% of the national oil and there are more than 2000 commercial ships annually coming to Rio de Janeiro's Port (PDBG, 2000). Ferreira (1995) has estimated that 18 tons.day^{-1} of petroleum hydrocarbons arrive in this bay and 85% of these come from urban garbage.

Boa Viagem Beach is located in Jurujuba Bigth, Niterói and it is moderately degraded (Teixeira *et al.*, 1987). Sediment grain sizes are tiny sand, with anthropogenic matter in bottom sediments. The organic matter content in sediments is around 0,81% (Baptista Neto e Silva, 1996).

Boa Viagem Beach is a suitable system for studying petroleum aromatic hydrocarbon impacts because of sediment structure, high anthropogenic organic matter content, high hydrological action and the proximity of the Guanabara Bay's entrance.

Several studies have shown that the interaction oil-particles are made by physical transport of spilled oil to the sediment (Muschenheim e Lee, 2002). This oil absorption is promoted by organic and inorganic suspended particles with dispersed oil drops (Payne *et al.*, 1989).

The hydrophobic nature of the hydrocarbon made bounds with particles <2 μm on the water surface. These oiled particles can be transported to many marine environment compartments, and can cause a dramatic augmentation of the rate and extent of hydrocarbon impacts (Hargrave e Kranck, 1976).

Toxic effects of the highly lipid soluble aromatic hydrocarbons on organisms are well documented. Information about mutagenicity and carcinogenicity in mamals, due to changes in the integrity of the membranes caused by oils is also available (Sikkema *et al.,* 1995).

The aim of this study was to quantify benzene, toluene, xylene, phenantrene, anthracene, fluorene, benzo[*a*]pyrene, naphthalene and chrysene in the sediments of Boa Viagem Beach and to determine the natural attenuation process *ex situ* in presence of high organic matter content.

METHODS

Study Area

Sediment samples were collected in Boa Viagem Beach (23°40.7' e 23°56.3' S; 43°1.6 e 43°17.4' W) on December 2001, in a maximum depth of 2 cm, in intertidal zone (tide of 0.3) with an extension of 700x30cm^2. This material was weighted in two replicas of 7,5 kg (semi-analytical digital balance, Kern® 440-53, version 1.2) and it was conditioned in aquaria with 50x30x30 cm dimensions. The sediment used in the bioassays A and B was homogenized, mechanically twice a day, with filtrated and sterilized deionized water, to keep the humidity and salinity level of the sediment.

Analytical Procedures

The assays were made during 30 days and measurements were conducted at: 0 (T_0), 1 (T_1), 3 (T_3), 7 (T_7), 11 (T_{11}), 14 (T_{14}), 17 (T_{17}), 24 (T_{24}) e 30 (T_{30}) days.

1) Quantification of aromatic hydrocarbons: benzene, toluene, xylene, phenantrene, anthracene, fluorene, benzo[*a*]pyrene, naphthalene, chrysene. The measurement of hydrocarbons was made by liquid chromatography (HPLC, trend Spectraphysics with ultraviolet and fluorescence detectors, chromatographic column C18 with reversal phase). Methods were performed according to GGDP - PP121006 and standard EPA610. The limits of detection were of 0.01mg.g^{-1} for benzo[*a*]pyrene and

0.1mg.g^{-1} for all others hydrocarbons analyzed. For corrections, reference standards with NIST rastreability were used.

2) Total organic matter (TOM) was determined as the difference between dry weight (60ºC, 24 h) of the sediment and weight of the residue after combustion at 450ºC (2 h) (Crapez *et al.*,2003).
3) CB – bacterial carbon was enumerated by epifluorescent microscopy (Axiosp 1, Zeiss, triple filter Texas Red – DAPI – fluorescein isotiocianate, increasing of 1.000 X) and the fluorochrome fluorescein diacetate (Kepner e Pratt, 1994). Carbon biomass (μg C.cm^{-3}) data was obtained using the method described by Carlucci, *et al.* (1986). The fluorochrome fluorescein diacetate allows to count the viable cells, morphologically differentiated as cocci, rods and spirilum.
4) EST – Esterase enzyme activity was analyzed using the method described by Stubberfield e Shaw (1990). It is based on fluorogenic compounds, which are enzymatically transformed in fluorescent products that can be quantified by absorption on spectrophotometer. These enzymes hydrolyze many polymeric organic matter. The results are in μg fluorescein.h^{-1}.g^{-1} of sediment.
5) ETSA – Electron transport system activity was analyzed using the method described by Houri-Davignon e Relexans (1989), without a surplus of electron donors (Trevors, 1984). It is based on dehydrogenase enzymes that are the major representatives of oxido-reductase reactions. They catalyze the oxidation of substrates producing electrons that can enter into the cells electron transport system (ETSA) and can be quantified by UV-visible absorption on spectrophotometer. The results are in μL O_2.h^{-1}.g^{-1} of sediment.
6) The results were analysed using Tukey Test.

RESULTS

The sediments contained aromatic hydrocarbons such as benzene, toluene, xylene, phenantrene, anthracene, fluorene, benzo[*a*]pyrene, naphthalene and chrysene. They were distributed in patch, as showed by the total concentration, that was 2.30 mg.g^{-1} and 2.53 mg.g^{-1} for bioassays A and B, respectively. The highest concentrations are of benzene, toluene and naphthalene sum 1.83 mg.g^{-1} for bioassay A and 2.02 mg.g^{-1} for bioassay B, that correspond to 80% and of hydrocarbons concentration in the sediments (Tables 1 and 2).

At bioassay A, the rate of aromatic hydrocarbons and organic matter biodegradation were 99.9% and 80%, respectively. The rate of petroleum aromatic hydrocarbons in organic matter ranged between 6.57% (T_0) – 0.01% (T_{30}) (Table 2).

At bioassay B, the rate of aromatic hydrocarbons and organic matter biodegradation were 65.6% and 93.3%, respectively. The rate of petroleum aromatic hydrocarbons in organic matter ranged between 2.13% (T_0) – 10.88% ($T_{30,}$ Table 2).

Table 1: Aromatic hydrocarbons in bioassays A and B during T_0 and T_{30} days.

Aromatic Hydrocarbons	A $_{T0}$	A $_{T30}$	B $_{T0}$	B $_{T30}$
	(mg.g^{-1})			
Benzene	1.08	N.D.	1.24	0.64
Toluene	0.46	N.D.	0.49	0.13
Xylene	0.21	N.D.	0.21	0.08
Phenantrene	0.07	N.D.	0.04	0.003
Anthracene	0.04	N.D.	0.10	0.002
Fluorene	0.08	N.D.	0.11	0.01
Benzo[*a*]pyrene	0.03	0.0003	0.02	0.001
Naphthalene	0.29	0.0003	0.29	N.D.
Crysene	0.04	N.D.	0.03	N.D.

Table 2: Hydrocarbons (mg.g^{-1}), organic matter (mg.g^{-1}), PAHs/OM (%),organic matter biodegradation (%) and PAHs biodegradation (%) during T_0 and T_{30} days of experiment.

Bioassays	Hydrocarbons	Organic Matter	PAHs/OM	Biodegradation of OM	Biodegradation of PAHs
	(mg.g^{-1})			(%)	
A_{T0}	2.30	35	6.57	-	-
A_{T30}	0.0006	7	0.01	80.0	99.7
B_{T0}	2.53	119	2.13	-	-
B_{T30}	0.87	8	10.88	93.3	65.6

Bacterial carbon was quantified to rod, cocci and spirilum groups. Bacterial rod carbon started both bioassays with 0.008 μg C.cm^{-3}. At the first day (24 hours), it had 0.035 μg C.cm^{-3}, and decreased 75% until 3 days, and was maintained under 0.005 μg C.cm^{-3} (bioassay A, $p<0.05$). At bioassay B, the biomass decreased significantly during 30 days (Figure 1).

Cocci carbon ranged between 0 – 0.0059 μg C.cm^{-3}, and 0 - 0.00193 μg C.cm^{-3}, at bioassay A and B, respectively ($p>0.05$).

Spirilum carbon ranged between 0 – 0.000058 μg C.cm^{-3}, and 0 - 0.000077 μg C.cm^{-3}, at bioassay A and B, respectively ($p>0.05$).

Esterase enzymes activity ranged between 0.29 – 0.60 μg fluorescein.h^{-1}.g^{-1} of sediment at bioassays A and B ($p>0.05$) (Figures 2 and 3).

Electron transport system ranged between 0.06 – 0.138 μL O_2.h^{-1}.g^{-1} of sediment and 0.06 – 0.113 μL O_2.h^{-1}.g^{-1} of sediment at bioassays A and B, respectively ($p<0.05$) (Figures 2 and 3).

DISCUSSION AND CONCLUSION

Our results of organic matter content showed values to the literature at Boa Viagem Beach (Baptista Neto *et al.,* 2000), excepting biossay B.

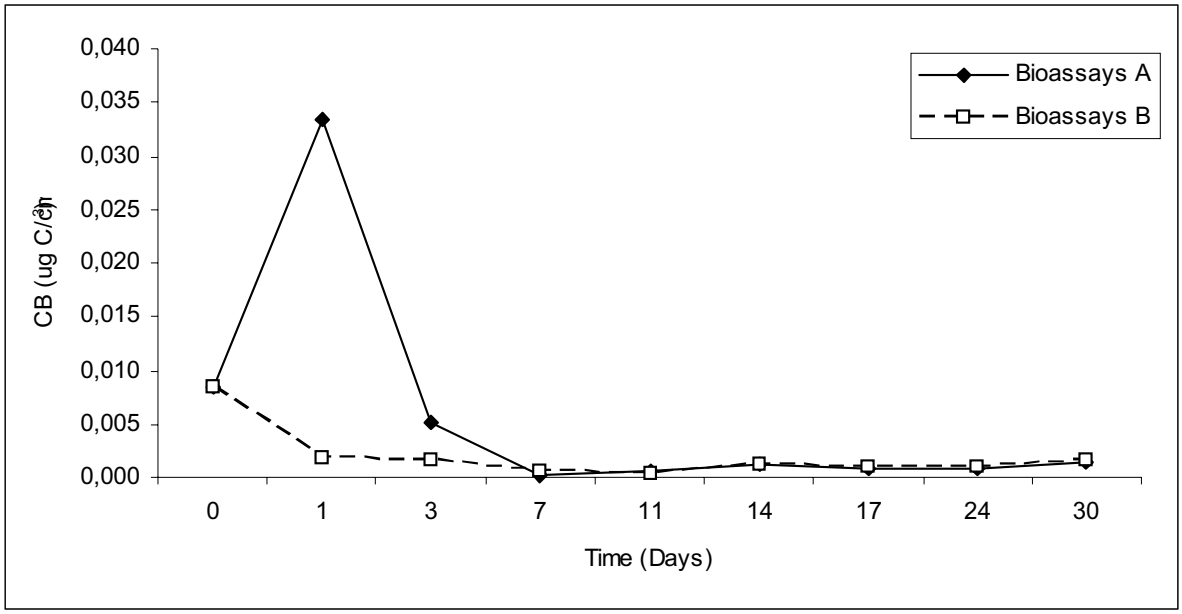

Figure 1: Bacterial rod carbon (CB) (μg C.cm^{-3}) in bioassays A and B, during the 30 days.

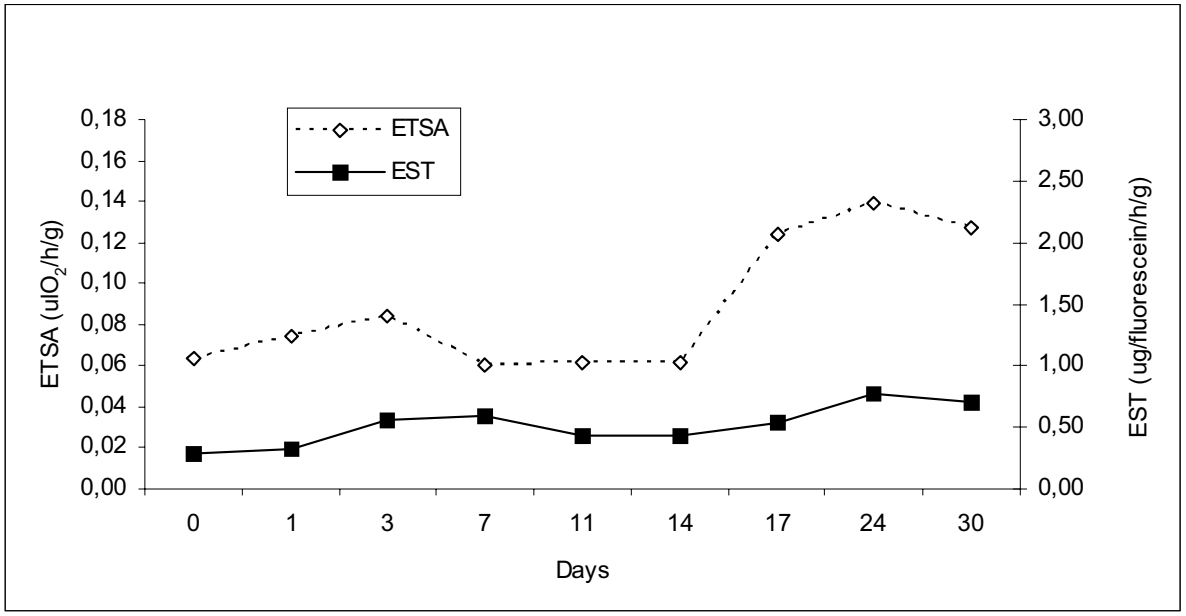

Figure 2: Electron transport system activity (ETSA, ml $O_2.h^{-1}.g^{-1}$) and esterase activity (EST, mg fluorescein.$h^{-1}.g^{-1}$) in bioassay A, during 30 days.

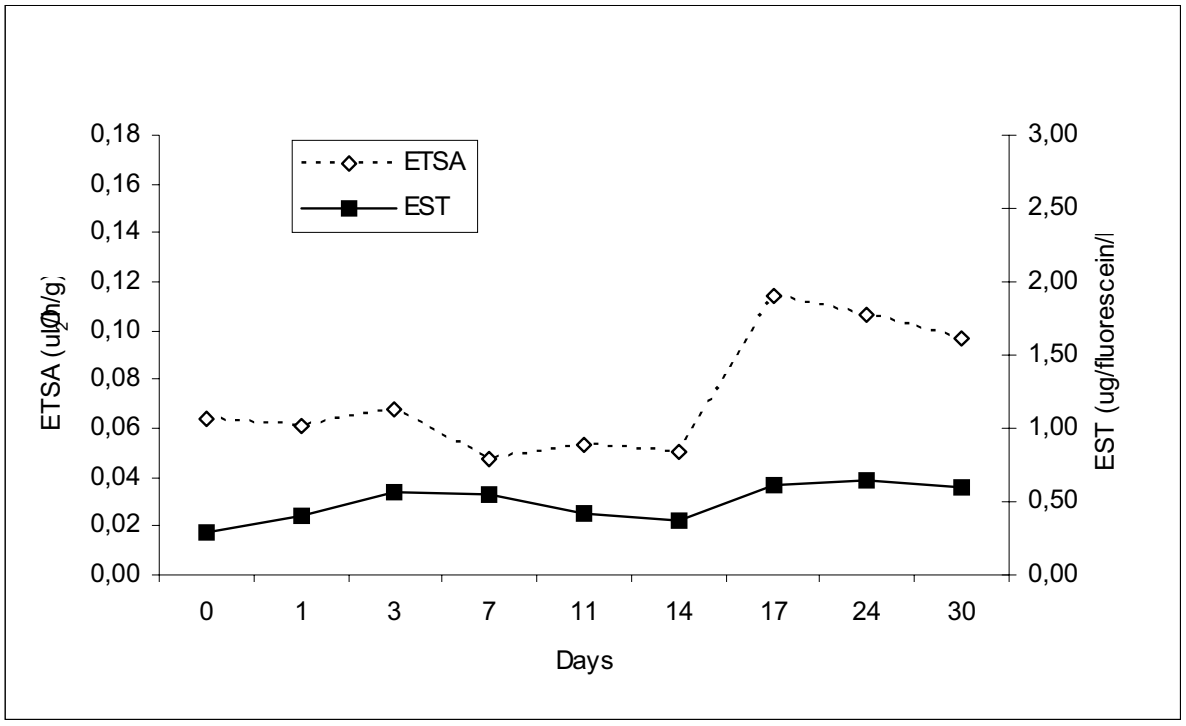

Figure 3: Electron transport system activity (ETSA, l $O_2.h^{-1}.g^{-1}$) and esterase activity (EST, mg fluorescein.$h^{-1}.g^{-1}$) in bioassay B, during 30 days

The samples collected at Boa Viagem Beach showed that organic matter, petroleum aromatic hydrocarbons and bacterial carbon were distributed in patch. Gibson (1984) showed that the hydrocarbon degradation only occurred after the adaptation process of bacterial community in the sediments. This process increase the bacterial biomass. The bioassay B had more organic matter and aromatic hydrocarbons than bioassay A. However the hydrocarbonoclastic bacteria had a more efficient degradation on bioassay A than B, with a degradation of 99.9% of hydrocarbon, overbounding only 0.0003 mg.g^{-1} of

benzo[*a*]pyrene and naphthalene. The rate increase of PAHs/OM in bioassay B showed that the bacterial community degraded organic matter rather than the aromatic hydrocarbons.

Manilal e Alexander (1991), observed a reduction of phenantrene trough mineralization in soils containing high values of organic matter, whereas in soils with small organic matter values this was not seen. They suggested that this reduction was caused by adsorption of phenantrene by the organic matter. Bispo *et al.* (2001) and Crapez *et al.* (1993) showed that high organic matter content did not allow the mineralization by microbial activity of petroleum hydrocarbons to happen, due to adsorption of these hydrocarbons by the organic matter.

Kohl e Rice (1998) showed different adsorption of organic matter types to organic contaminants. The chemical properties of the mineral fraction in marine sediments play an important role in the adsorption of organic polar/ ionic/hydrophobic molecules to sediment particles, making its surface highly lipophilic with a low kinetic of desorption (Gustafsson *et al.*, 1997; Stoffyn-Egly e Lee, 2002).

Several models were proposed to study the adsorption of organic contaminants to the particles of soot, lignine and humic and fulvic acids. It was verified that benzene, naphthalene and pyridine can form hydrogen bounds with fulvic acids, coal and soot (Kubicki e Apitz, 1999). The mineral-oil aggregate is formed as a result of a natural process of oil removal in coastal environment (Bragg e Owens, 1994; Bragg e Yang, 1995).

The interaction between low viscosity oil with quartz particles and kaolinite can contribute to form a drop type (μm) oil-mineral aggregate (Omotoso *et al.*, 2002). Since quartz is the main component of the sediment of Boa Viagem Beach, this is one of the strongest factors to form the oil-mineral aggregate, the adsorption of hydrocarbons from the sediment by the quartz particles, when it becomes unavailable to biodegradation.

The hydrocarbons benzene, toluene and xylene are volatile but are retained in the sediments of Boa Viagem Beach. This adsorption can be explained by physical and chemical phenomena, such as adsorption or encapsulation (Lee *et al.*, 2002).

Aerobic rod shaped was the main adapted hydrocarbonoclastic bacteria found in bioassays and showed no significantly increase in biomass during the experiment. The cellular death that occurred during the first 24 hours allowed a acclimatation of the bacteria and the growth of a specific biomass that degraded the aromatic hydrocarbon, this phenomena was a little stronger in bioassay A. After 1 (bioassay B) and 3 (bioassay A) days all bacterial biomass decreased to a minimum and remained so through the rest of the experiment. These results, with values 0.008 μg $C.cm^{-3}$, are higher than the ones previously found at Boa Viagem Beach, with only 0.001μg $C.cm^{-3}$ (Crapez *et al.*, 2001).

At the same time the esterase enzymes activity showed an increase during the bioassays. Bispo *et al.* (2001) and Crapez *et al.* (2001) found maximum of 0.17 and 0.360 μg fluorescein.$h^{-1}.g^{-1}$ also at Boa Viagem Beach. These enzymes are not inhibited in the presence of petroleum hydrocarbons and metals and produce carbon substrates that can be used as an energy source by bacteria (Crapez *et al.,* 2003).

Electron transport system activity is based on dehydrogenase enzymes, and this activity also showed significant changes during the experiment ($p<0.05$). There is an initial increase, then a decrease which could be interpretaded as an acclimatation and finally a more significantt increase in this activity in bioassay A.

Bispo *et al.* (2001) found maximum values of 0.110 μl $O_2.h^{-1}.g^{-1}$ during the summer time and Crapez *et al.* (2000) found 7.48 l $O_2.h^{-1}.g^{-1}$ both at Boa Viagem Beach. These index are directly related with the biomass (Crapez *et al.*, 2001).

Analyzing all the results obtained in this study we could describe this phenomena in two stages. Firstly, the biopolymers of the organic matter were degraded, because these sources of carbon and energy are easily assimilated by bacteria in the environment. Moreover, the consumption of the organic matter exposes the encapsulated aromatic hydrocarbons, and the hydrocarbon-degrading bacteria increase their own biomass (bioassay A). The results showed that up to 6.57% of the ratio aromatic hydrocarbons/ organic matter, bacteria are capable of utilizing both compounds as a source of carbon. When the ratio reaches 10.88%, the bacteria degrade the organic matter preferably, and the oxidation process of the petroleum will be compromised, because there is reduction of dehydrogenase activity. Irha *et al.* (2003) also suggest that dehydrogenase activity is inhibited by these substances. This phenomenon was also observed in this study in bioassays A and B, between 7 and 14 days. After that, it increased only in bioassay A, with the simultaneous oxidation of the organic matter and petroleum hydrocarbons. This activity could be used as a biomarker to detect the aromatic hydrocarbon and remediation processes. The natural attenuation *ex situ* occurred in thirty days, and this time is advantageous in the remediation process of aromatic hydrocarbon. These tools can be used in the environmental impact assessment. These tools can be used in the environmental impact assessment.

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(Footnotes)

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