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Evaluation of the effects of metals on biodegradation of total petroleum hydrocarbons☆

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ABSTRACT

In the present paper, the efficiency of biodegradation of petroleum hydrocarbons in the presence of metals in mangroves sediments was evaluated. Two models of remediation (intrinsic bioremediation and phytoremediation) were tested. The metals (Al, Fe, Pb, Cr, Cu, Zn and Ni) were determined employing Flame Atomic Absorption Spectrometry (FAAS). The total petroleum hydrocarbons (TPHs) were analyzed using Gas Cromatograph with Flame Iron Detector (GC-FID). The physical–chemical parameters were monitored for a 90-day period at the pilot scale. The results showed that both techniques were effective at degrading the organic compounds in oil, with phytoremediation (*Rizophora mangle*) being the most efficient (87% removal). It was also observed that the biodegradation model of intrinsic bioremediation did not have a direct correlation with the concentrations of metals; however, a positive correlation with some metals (Cu, Zn, Cr, Ni) for the model with phytoremediation was verified with the removal of hydrocarbons, showing efficiency at phytoextraction and phytostimulation. The results suggest that red mangroves, through their rhizosphere mechanisms are a promising plant that can be used for the removal of petroleum hydrocarbons in the presence of metals in mangrove sediments.

1. Introduction

Mangroves are transitional coastal ecosystems that occur between terrestrial and marine environments and are characteristic of tropical and subtropical regions. They are of great ecological and economic importance. In recent years, data ITOPF [1] have shown that the number of spills in mangrove areas has increased, especially in countries where the oil industry has recorded growth. These accidents have the potential to cause environmental and economic effects on a wide variety of natural resources and services in these regions. Studies on the application of remediation techniques in coastal regions are becoming increasingly necessary, given the importance of these ecosystems for ecological balance and because they are targets of major impacts of petrogenic origin [2,3].

Many mangrove areas that are affected by oil spills contain high concentrations of metals that are enhanced by the composition of the spilled oil. Metals at certain concentrations may limit microbial and plant activities that could degrade the organic compounds, causing serious problems for the application of bioremediation, a technique that uses microorganisms to degraded contaminants sediment, and phytoremediation, a technique that uses microorganisms to degraded contaminants in mangrove sediments [4,5].

Some studies have been conducted regarding the individual effects of toxic metals, and biotic and abiotic factors on the degradation of total petroleum hydrocarbons; however, few studies have addressed the effects of all factors convergent on biodegradation in mangrove sediments [6–8]. These sediments are rich in microbial diversity and rely on a set of interactions with the mangrove plant species to maintain conservation, productivity and recovery of this ecosystem when impacted by some type of antropic activity [9].

The decision to correct hydrocarbon contaminated areas should be based on pilot studies through the integrated assessment of the presence of metals along with the main physical, chemical and biological agents that can act positively or negatively on the biodegradation in mangrove sediments. The objective of this study was to evaluate how the presence of metals and other abiotic and biotic factors influence the degradation of total petroleum hydrocarbons in mangrove sediments using two models of remediation: intrinsic bioremediation and phytoremediation with *Rizophora mangle* L. (red mangrove) simulated at the pilot scale.

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2. Materials and methods

2.1. Sediment sampling and analysis

Mangrove sediment samples were collected at 0–30 cm deep, between the cities of Candeias and São Francisco do Conde, Todos os Santos Bay, Bahia, Brazil (a region with many activities in the petroleum industry: extraction, transportation and refining). These sediment samples were sieved through a 4-mm sieve to remove rocks, and the plant material was homogenized immediately after to ensure uniformity. Five sub-samples of sediment were collected, homogenized and freeze-dried for 72 h and sieved through a 2-mm mesh to determine the initial physical and chemical properties of the selected sediment. Soon after, sediment samples were mixed in a 1:10 ratio with oil residue found in the same area, and five samples of the mixture were collected to analyze the new concentrations of TPHs, metals and nutrients (Table 1). In the homogenized sediments the amount of metals (aluminum (Al), iron (Fe), lead (Pb), chromium (Cr), copper (Cu), zinc (Zn) and nickel (Ni)) was determined by Flame Atomic Absorption Spectrometer (FAAS) with Varian model SpectrAA 220 (Mulgrave, Victoria, Australia). The total petroleum hydrocarbons (TPHs) were determined using a Varian CP 3800 (Varian Inc., CA, USA) gas chromatograph equipped with a DB-5 capillary column (15 m length, 0.25 mm ID, 0.25 lm film thickness) and flame ionization detector (FID). Particle-size distribution was determined after the organic matter was removed with 30% H_2O_2 using the Folk and Ward method [10]. Soil organic matter (TOM) was determined using a modified Mebius method [11]. Total N (nitrogen) was determined by the Kjeldahl's digestion, distillation and titration method [12], while available P (phosphorus) was determined by the Olsen extraction method [13]. Measurements were taken for pH and Eh by using a Potentiometer pH/mV HandyLab1 (Schott Glaswerke Mainz). The salinity was measured by the index of refraction using the portable refractometer atoga S/Mill-E, and dissolved oxygen (DO) was measured with a WTW Oximeter OXI 3151 (SCHOTT-GERÄTE).

2.2. Models for remediation

Over a 90-day period, two models were used to evaluate the remediation of TPH contamination in sediments. One type of remediation used to compare the removal of TPHs was intrinsic bioremediation (natural attenuation monitored), where the degradation by bacteria presented in the mixed sediment was monitored. The other remediation model was phytoremediation using *R. mangle* (red mangrove). This choice was based on pre-tests conducted earlier by our group and other studies that suggested the use of this species for phytoremediation [14–16]. Seedlings of *R. mangle* were collected at low tide, and their height (average of 3 months old) was used to define a standard sampling protocol to maintain comparable results. The density of the bacterial community was characterized to compare the presence of microorganisms with the intrinsic bioremediation method.

Table 1
Some selected physico-chemical properties of the sediments used in the experiment.

Sediment	Uncontaminated	Contaminated
Cu ($\mu g\ g^{-1}$)	13.37	17.86
Zn ($\mu g\ g^{-1}$)	12.66	22.23
Pb ($\mu g\ g-1$)	6.02	18.21
Cr ($\mu g\ g^{-1}$)	7.43	11.47
Ni ($\mu g\ g^{-1}$)	7.49	17.43
Fe ($\mu g\ g^{-1}$)	12,014.88	10,739.67
Al ($\mu g\ g^{-1}$)	7401.69	7925.57
pH	7.51	7.59
Eh	−12	−12.3
T (°C)	26.9	27
Salinity	27	24
DO (mg/L)	4.39	4.53
TPH ($\mu g\ g^{-1}$)	–	33,215.16
TOM (%)	0.63	5.73
TOC (%)	0.37	3.32
TN (%)	0.06	0.36
P (mg/L)	0.90	2.70
C/N	5.74	9.26

2.3. Experimental design

The project design was based on pilot-scale simulation of mangrove dynamics, with the tidal regime in the sediment used for the application of the remediation models. This experiment was conducted in a greenhouse (laboratory deployed to conduct research developed within the network RECUPETRO/UFBA — Cooperative Network Recovery in Areas Contaminated by Petroleum Activities, linked to the Federal University of Bahia) near the mangroves, with environmental conditions very close to the original ecosystem, with an average temperature of 24 °C ± 1. Simulation units were made of glass (50 × 30 × 40 cm). Within each unit of simulation, 6 tubes of glass were added (30 × 10 × 10 cm) and applied to the two models of remediation compared in this study. These tubes of glass were suspended in the unit simulation, allowing the simulation of the tidal regime with water runoff. The tubes of glass were closed at the bottom to prevent loss of chemical residue when watering. All units received the daily tidal regime simulation with an adequate amount of water (approximately 10 L) to maintain the constant humidity of the sediment, as in the mangrove ecosystem. The experiment contained three replicates of each treatment and three samples from each repetition.

2.4. Statistical analysis

Principal components analysis (PCA) was applied to the average concentrations of all the data analyzed in the surface sediments for each type of remediation. PCA was used to determine the primary variables that influence the degradation of TPHs. Other statistical tests that were applied include the K-means, the Kolmogorov–Smirnov test for multiple parameters, the Tukey–Kramer test and the Pearson correlation (Considering extremely positive values between 0.7 and 1.0). All statistical evaluation was performed using the STATISTICA 6.0 and GraphPad software.

3. Results and discussion

3.1. Initial chemical properties of the sediments

Table 1 presents the physical and chemical analyses of the sediments contaminated and not contaminated by waste oil. The analytical results showed that, except for Fe, all other metals increased after the homogenization of the sediment and residue of oil in the two models, corroborating other studies [8,10]. However, the average values for the metals in the two sediments were below the TEL (Threshold Effect Level) [11], and it was previously found that these concentrations do not influence the biota negatively. The pH, which was between 7.51 and 7.59, of the two sediments was consistent with estuarine waters. These pH data are favorable to the majority of the biodegrading microorganisms in the mangrove sediments and did not compromise the principle bioremediation processes [12]. The temperatures of the two sediments were also very close to those expected for optimal biodegradation, where there is a higher enzyme activity between 25 °C and 38 °C [13]. The initial salt concentrations in the sediment confirmed the brackish characteristics of the medium and selected for particular biodegrading microorganisms but did not adversely affect the application of the remediation techniques [14]. Concentrations of macronutrients (N and P) and organic matter in the sediments were also expected for degradation by the biota in sediments impacted by organic compounds [12]. After homogenization of the sediment and residue oil, the initial concentration of TPHs was approximately 33 $\mu g\ g^{-1}$, which is considered a moderate contamination of the sediment [15].

3.2. Temporal assessment of biodegradation

Over the course of three months, the effectiveness of the two remediation models on the removal of TPHs in the sediment was evaluated. The results showed that during the first days of the experiment, intrinsic bioremediation was more efficient but after day 30 phytoremediation with *R. mangle* was observed which increased degradation of the oil (Fig. 1). This may have occurred because plants need an initial time to adapt to the environment contaminated by organic compounds [16]. After 90 days, the analysis of TPH removal showed that the phytoremediation of contaminants in the sediment was reduced from 33.2 to 4.5 μg g^{-1}, while intrinsic bioremediation resulted in a decrease from 33.2 to 9.2 μg g^{-1}. Therefore, phytoremediation was approximately 17% more efficient at removing TPHs than intrinsic bioremediation. This increased efficiency corroborates other studies in sediments affected by oil organic compounds [17–19].

One must consider that the mangrove plants act in consortium with the microorganisms present in the sediments, magnifying the degradation mechanisms. This fact was proven in this study by the count of bacteria in the two models of remediation (Fig. 2). After the thirtieth day, there was a large decrease in the number of microorganisms using intrinsic bioremediation (1.8×10^6 CFU g^{-1}), whereas in the rhizosphere of *R. mangle*, there was a large increase in bacterial density (24.4×10^6 CFU g^{-1}) when compared to the initial 15 days. This higher concentration of microorganisms in the rhizosphere has been reported by other researchers [20]. In the present study, it is most likely that the red mangroves produced allelopathic compounds, which are similar to organic compounds, and they stimulated the defenses of the microbial communities as a result of environmental stress. Additionally, the production of carbohydrates, organic acids and amino acids could have also stimulated the degradation of organic compounds [21–23].

3.3. Integrated assessment of the analyzed parameters

To evaluate in an integrated manner how metals and other physico-chemical parameters influence the biodegradation of total petroleum hydrocarbons, the Pearson correlation was initially used to indicate the strength and direction of the linear relationship between the variables in both remediation models. Principal Components Analysis (PCA) was also used to obtain a small number of linear combinations of all variables, which makes environmental events understandable, if they were not explained by Pearson correlation.

In intrinsic bioremediation (Table 2), strong positive correlations between metals and TPHs were not observed; however, a strong negative correlation with concentrations of copper (Cu) was observed, which indicates that the more reduced the concentration of organic compounds, the higher the concentration of available copper in the sediment. There was also a strong positive correlation between the concentrations of macronutrients (N, P) and dissolved oxygen (DO) with the concentration of TPHs, indicating that the more nutrients and oxygen available, the greater the degradation of organic compounds in this model. This is most likely because these factors greatly influence the microorganisms in an efficient degradation [12]. In phytoremediation (Table 3), strong positive correlations between the four metals (Cu, Zn, Cr, Ni) and pH and DO were found. Alternatively, strong negative correlations were observed in relation to salinity, TOM and TOC. These types of correlations between metals and the organic compounds derived from petroleum found in sediments with *R. mangle* indicate that the plant, in addition to contributing to the biodegradation of TPHs, is probably performing phytoextraction of the metals in the sediment, which consequently caused a decrease in the concentration of these elements after 90 days, which is similar to the findings of other studies [24,25].

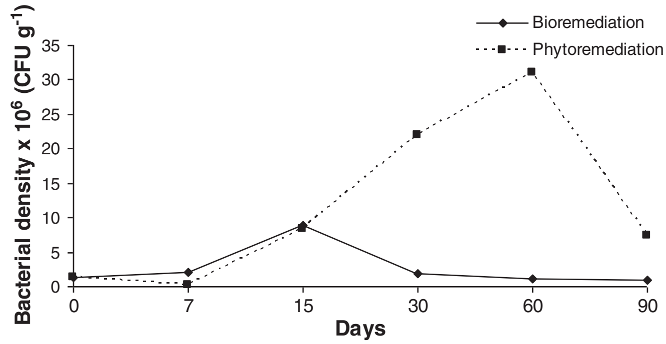

Fig. 2. Comparison of total bacteria over a 90-dayperiod for two models of remediation (n = 3).

PCA from the intrinsic bioremediation method indicated that the two main factors can explain 79.74% of the considered analytical data variation, with the first factor alone explaining 55.76% of the data (Fig. 3). The values of TPHs, P (Phosphorus), TN (Total Nitrogen), Sal (Salinity), C/N (Carbon/Nitrogen ratio), Pb, Cu and Zn are well represented in the graph. Strong positive correlation was observed between TPHs with pH and P, suggesting that these factors converged for biodegradation. However, it was evident that the degradation of TPHs was independent of the variables Al, Ni, Cu, Zn, Fe and Pb, confirming the results of the Pearson correlation that showed that, in this model of remediation, the metals did not influence the process [26,27]. Finally, a negative correlation was found between the variables DO (Dissolved Oxygen), TOC (Total Organic Carbon), TOM (Total Organic Matter), Sal, and C/N with the TPHs, which must have occurred because the degradation of organic compounds in sediments with indigenous microorganisms was directly linked to the consumption of oxygen, organic matter and nutrients [12].

For the model of phytoremediation, the two main factors explained 81.02% of the variation in the analyzed data, and factor 1 explained 55.66% of the data (Fig. 4). In this case, the values of TPHs, TN, P, TOM, Cu, Ni, Pb, Fe, Cr and pH were well represented in the graph. A strong positive correlation was found between Cu and Ni with TPHs,

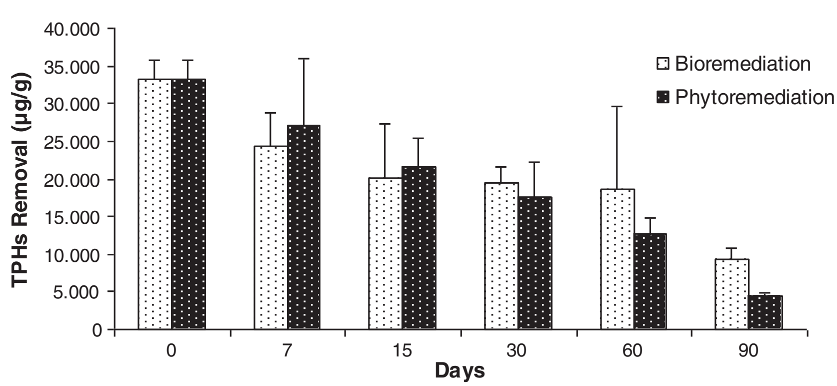

Fig. 1. TPH removal (μg g^{-1}) in the mangrove sediment as a function of time for the two models (n = 3).

Table 2
Pearson correlation in intrinsic bioremediation.

	pH	Eh	T	Sal	DO	Cu	Zn	Pb	Cr	Ni	Fe	Al	TOM	TOC	TN	P	TPH
pH	1																
Eh	–0.92	1															
T	–0.23	0.18	1														
Sal	–0.80	0.58	0.31	1													
DO	–0.31	–0.07	0.10	0.74	1												
Cu	–0.21	–0.09	0.04	0.73	0.90	1											
Zn	–0.04	0.03	0.93	0.27	0.09	0.17	1										
Pb	–0.39	0.28	0.76	0.71	0.46	0.57	0.85	1									
Cr	–0.17	0.23	0.88	0.32	–0.02	0.11	0.97	0.86	1								
Ni	–0.01	0.08	0.64	0.29	0.03	0.30	0.87	0.84	0.92	1							
Fe	–0.46	0.32	0.79	0.65	0.50	0.39	0.72	0.84	0.70	0.54	1						
Al	0.48	–0.34	0.43	–0.30	–0.22	–0.14	0.59	0.33	0.57	0.60	0.40	1					
TOM	–0,47	0.22	0.05	0.85	0.86	0.89	0.13	0.60	0.14	0.27	0.57	–0.05	1				
TOC	0.50	–0.23	–0.71	–0.82	–0.73	–0.68	–0.67	–0.87	–0.60	–0.50	–0.82	0.00	–0.65	1			
TN	0.67	–0.38	–0.50	–0.91	–0.83	–0.68	–0.39	–0.72	–0.35	–0.23	–0.83	0.13	–0.79	0.92	1		
P	–0.52	0.24	0.43	0.91	0.86	0.87	0.46	0.83	0.44	0.46	0.76	–0.02	0.90	–0.91	–0.93	1	
TPH	0.52	–0.16	–0.24	–0.85	0.91	–0.83	–0.18	–0.54	–0.09	–0.07	–0.49	0.44	–0.74	0.84	0.87	0.86	1

suggesting that the phytostimulation of the microorganisms by plants to degrade the organic compounds was also removing the two metals from the sediment. However, it was evident that the degradation of TPH in this model was independent of the variables Al, Cr, Sal, C/N, TOM and TOC, suggesting that the plants have provided allelopathic compounds for microorganisms to degrade. The PCA analysis in this

Table 3
Pearson correlation in intrinsic phytoremediation.

	pH	Eh	T	Sal	DO	Cu	Zn	Pb	Cr	Ni	Fe	Al	TOM	TOC	TN	P	TPH
pH	1.00																
Eh	–0.15	1.00															
T	–0.51	–0.16	1.00														
Sal	–0.86	0.31	0.37	1.00													
DO	–0.55	–0.53	0.50	0.19	1.00												
Cu	0.87	0.08	–0.76	–0.65	–0.55	1.00											
Zn	0.76	0.16	–0.06	–0.48	–0.71	0.55	1.00										
Pb	–0.05	–0.60	0.37	0.11	0.02	–0.40	0.11	1.00									
Cr	0.78	–0.04	–0.77	–0.79	–0.54	0.71	0.36	–0.10	1.00								
Ni	0.82	0.19	–0.78	–0.75	–0.68	0.81	0.50	–0.28	0.96	1.00							
Fe	–0.09	–0.72	0.21	0.08	0.14	–0.37	–0.10	0.96	–0.01	–0.25	1.00						
Al	0.57	–0.42	–0.82	–0.49	–0.21	0.67	0.01	0.07	0.71	0.60	0.26	1.00					
TOM	–0.86	0.29	0.51	0.76	0.59	–0.62	–0.61	–0.33	–0.87	–0.80	–0.33	–0.65	1.00				
TOC	–0.86	0.29	0.51	0.76	0.59	–0.62	–0.61	–0.33	–0.87	–0.80	–0.33	–0.65	1.00	1.00			
TN	0.24	0.87	–0.47	0.04	–0.65	0.54	0.36	–0.72	0.19	0.45	–0.80	–0.06	0.04	0.04	1.00		
P	0.03	0.78	–0.16	0.10	–0.22	0.34	0.17	–0.88	–0.13	0.14	–0.96	–0.32	0.40	0.40	0.87	1.00	
TPH	0.85	0.31	–0.48	–0.76	0.72	0.74	0.75	–0.30	0.78	0.90	–0.38	0.25	–0.72	–0.72	0.52	0.30	1.00

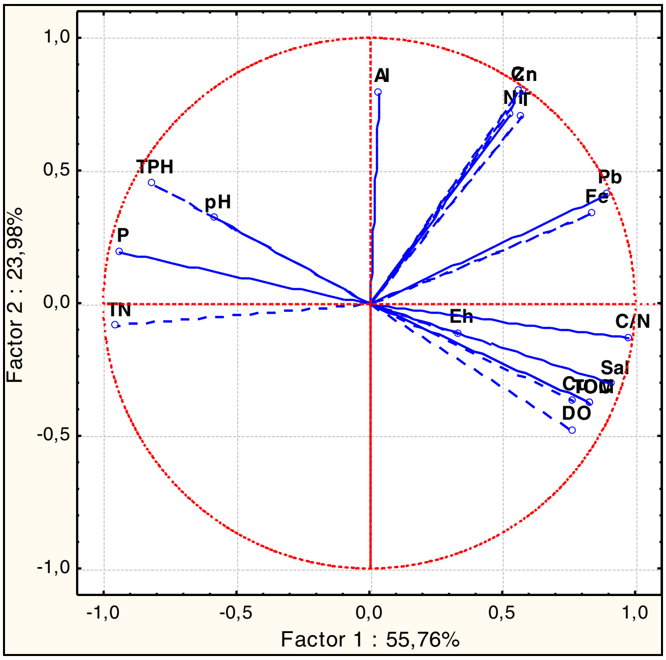

Fig. 3. PCA in intrinsic bioremediation (n = 3).

model did not show a strong negative correlation between some variables and TPHs, although there is a moderate negative correlation between DO and TPHs with T, suggesting that the further degradation of the compounds occurred with higher temperatures and increased dissolved oxygen. The fact that a higher oxygen concentration occurred over time can be explained by the presence of the roots of *R. mangle* that may help to increase the oxygen, providing a greater degradation of TPHs [22,23].

4. Conclusions

This study showed that the use of *R. mangle* in phytoremediation was more efficient than intrinsic bioremediation for the biodegradation of organic compounds derived from petroleum in the sediments of mangroves, at the pilot scale. It was observed that metals do not show a positive correlation in the biodegradation model of intrinsic bioremediation, and Cu may have been a negative factor, causing lower efficiency of the model. In phytoremediation, the analyzed metals (Cu, Zn, Cr, Ni) had a direct and positive influence on the degradation of hydrocarbons, confirming the important role of red mangrove on the mobilization and removal of contaminants while being a fundamental factor for the growth of microorganisms in the sediment. The relevance of the interaction between the plants and the microorganisms in the model when evaluated using phytoremediation was apparent. The biodegradation of hydrocarbons in the presence of metals was affected by the presence of the plant and caused a decrease in the concentration of toxic metals in the sediment. However, it must be evaluated if similar results would be found if the concentrations of metals and hydrocarbons were larger and more diverse. For the present study, *R. mangle* was classified as a promising species for the remediation of mangrove sediments impacted by moderate concentrations of TPHs and toxic metals.

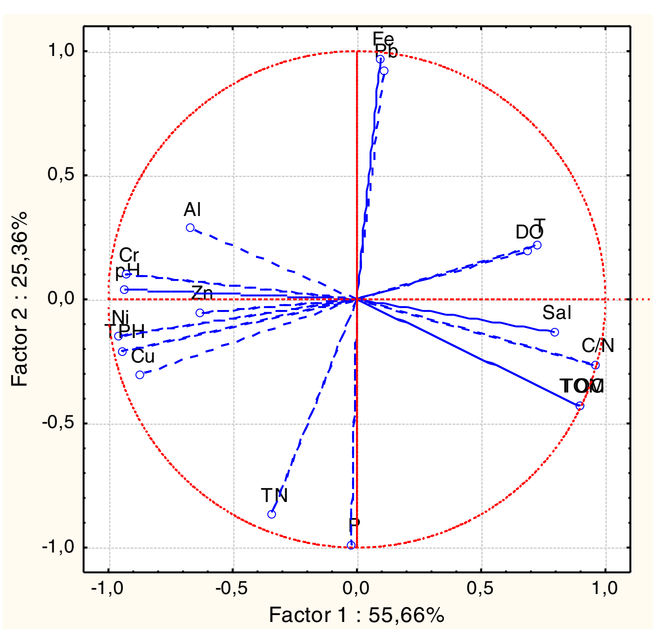

Fig. 4. PCA in phytoremediation (n = 3).

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