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PHENANTHRENE QUANTIFICATION METHODOLOGY IN BIOREMEDIATION MICROCOSMS

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1. INTRODUCTION

Countless environmental, social and economic damages were caused by oil spills in the world. In 2019, a large amount of crude oil was spilled and spread along 3000 km from the north coast to southeastern Brazil, being considered the largest in the country's history and one of the most extensive recorded worldwide (Lourenço et al., 2020; Magris and Giarrizzo, 2020). Even with the physical removal process, part of the contamination still remains in place, requiring other remediation techniques, as bioremediation, which exploits the genetic diversity and metabolic versatility of microorganisms to transform contaminants into less toxic end-products (Almeida et al., 2021).

Bioremediation studies of contaminated areas by polyaromatic hydrocarbons such as phenanthrene can be previously performed in microcosms, in order to acquire knowledge about the degradation of the interest compound.

The PAH identification and quantification were performed by gas chromatography with flame ionization detection (GC-FID) and mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) with fluorescence detection (FL) and ultraviolet-visible, whose main analytical characteristics are selectivity, accuracy and precision. Sediment samples require an initial extraction step with organic solvents or a mixture of solvents with suitable polarity (USEPA, 1986).

Tests in microcosms use reduced reaction volumes, therefore the analytical methods need to be adapted, so the objective of this study was to adapt a methodology for the identification and quantification of phenanthrene in microcosm samples from mangrove sediment.

2. MATERIAL AND METHODS

The determination of phenanthrene was carried out in a Shimadzu Prominence liquid chromatograph, with a quaternary pump system, mobile phase degassing system, thermostated compartment for analytical column and UV-Vis detector with diode array. Merck's Lichrospher® 100 RP-18 column was used. The mobile phase used was composed of 90% acetonitrile and 10% ultrapure water in an isocratic regime of 1 mL.min^{-1}, injection volume of 20 µL, temperature of the thermostat column compartment of 40 ºC and UV-Vis detection at 274 nm.

Phenanthrene extraction was performed in 1 g of microcosm mangrove sediment, previously spiked with 1, 50 and 150 mg.kg^{-1} of this compound. 1 g of anhydrous sodium sulfate, 5 mL of hexane/acetone (4:1) and 200 µL of a 100 mg.L^{-1} pyrene solution were added as internal standard. The contents were homogenized by vortexing, 30 minutes in ultrasonic bath and centrifuged at 4000 rpm for 5 minutes. The supernatant was collected and this procedure was performed three times, where the extracts were combined and concentrated in a sample concentrator (EZ-2 Elite, Genevac SP Scientific). The final extract was resuspended with 1 mL of methanol, filtered through a 0.45 µm porosity regenerated cellulose filter and injected into the HPLC.

The method was validated according to National Institute of Metrology, Quality and Technology (INMETRO) document DOQ-CGCRE-008. The figures of merit evaluated were selectivity, linearity, limit of quantification (LQ), limit of detection (LD), precision and accuracy (INMETRO, 2020).

3 RESULTS AND DISCUSSION

The validation parameters were obtained after defining the chromatographic conditions of the method. Comparing the analytical standard chromatogram with and without the addition of sediment (Figure 1), it was observed that there was no displacement of the retention time in the complex matrix and loss of peak resolution due to the matrix components. These results, in addition to the spectra obtained in the UV-Vis system with diode array (DAD), allow us to state that the method was selective.

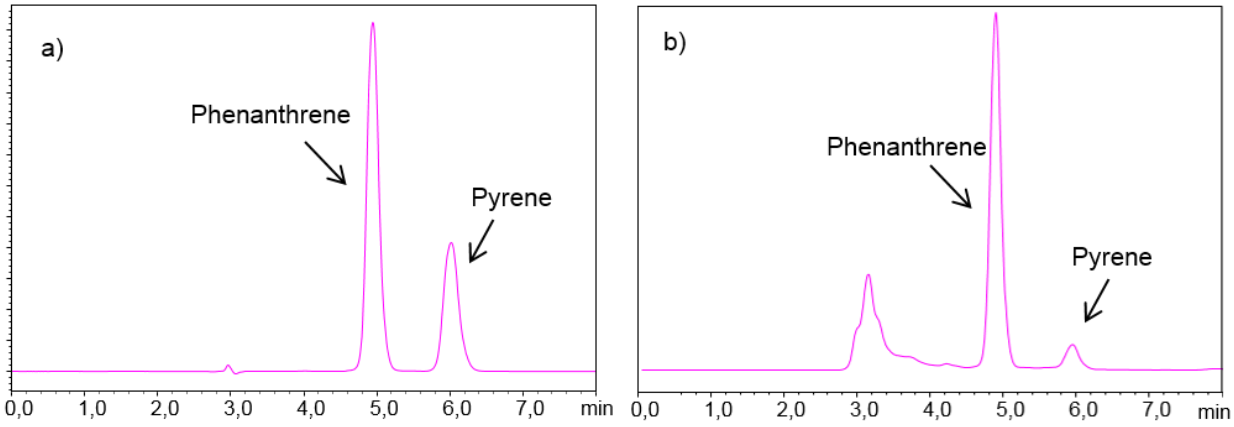

Figure 1. *Chromatogram of the analytical standard without sediment (a) and with sediment (b).*

Linearity was evaluated by the calibration curve. The method showed linearity in the range of 0.5 to 12 mg.L^{-1}, evidenced by the average correlation coefficient (n=3) greater than 0.99. The detection limit (LD) and the limit of quantification (LQ) were 0.034 and 0.105 mg.L^{-1} , respectively.

The precision, expressed by the value of the coefficient of variation (CV), was 2.91%. For INMETRO (2020), CV values of up to 20% are acceptable, so it can be said that the method is accurate for the phenanthrene.

To evaluate the recovery of the method, which expresses the ratio between the value found and the value accepted as a reference, tests will still be necessary in which known concentrations of the analytical standard of phenanthrene are added to the sediment.

4 REFERENCES

LOURENÇO, R.A., COMBI, T., ALEXANDRE, M. da R., SASAKI, S.T., ZANARDO-LAMARDO, E., YOGUI, G.T. 2020. Mysterious oil spill along Brazil's northeast and southeast seaboard (2019–2020): trying to find answers and filling data gaps. Mar. Pollut. Bull., 156: 111219.

MAGRIS, R.A., GIARRIZZO, T. 2020. Mysterious oil spill in the Atlantic Ocean threatens marine biodiversity and local people in Brazil.Mar. Pollut. Bull., 153: 110961.

ALMEIDA, F. F., FREITAS, D., MOTTERAN, F., FERNANDES, B.S., GAVAZZA, S. 2021. Bioremediation of polycyclic aromatic hydrocarbons in contaminated mangroves: Understanding the historical and key parameter profiles. Mar. Pollut. Bull., 169: 112553.

USEPA (1986): Method 8100. Polynuclear aromatic hydrocarbons.

Instituto Nacional De Metrologia, Normalização E Qualidade Industrial (INMETRO). (2020). Orientação sobre validação de métodos de ensaios químicos. Documento orientativo DOQ-CGCRE-008. 35 p.