

Elaboration and Approbation of Methods for Benzo[a]pyrene Extraction from Soils for Monitoring of the Ecological State in Technogenic Landscapes

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Abstract: Under consideration is a method for subcritical water extraction of benzo[a]pyrene from soils. The optimum conditions for extraction are described including the soil treatment by subcritical water at 250°C and 100 atm for 30 min. A comparative analysis was made to evaluate the results of benzo[a]pyrene extraction from soils using the subcritical water and organic solvents. The advantages of the subcritical water extraction involve the use of ecologically friendly solvent, a shorter time for the analysis and a higher amount of benzo[a]pyrene extracted from soil (96%).

Key words: Soil · Benzo[a]pyrene · Extraction · Subcritical water · Hexane · Comparative analysis

INTRODUCTION

Benzo[a]pyrene (BaP) as a marker compound contaminates the environment with polycyclic aromatic hydrocarbons (PAHs). This is a very dangerous pollutant, carcinogen and mutagen (Fig. 1).

The BaP content in all the natural objects is under obligatory regulation world-wide according to available legislation [1-4]. For today, some conventional methods of BaP determination in soils based upon a highly efficient liquid chromatography are broadly used for purposes of the environment monitoring [5-14]. However, these methods suffer from common shortcomings: a large volume of organic solvents and a long procedure to prepare samples for their analysis. One of the ways to solve this problem is the use of environment friendly liquid such as CO₂ and the so called subcritical water instead of toxic organic solvents and concentrated acids.

The subcritical water is the water in a liquid state at temperature varying from 100°C to 374°C and pressure higher than that of saturated steams to avoid its transition into the gaseous state [15]. Subcritical water extraction is one of the most recent techniques developed for extracting organic compounds, including pollutants from environmental matrixes and food [16-22].

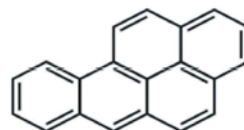


Fig. 1: Structural formula of BaP.

However, some questions have to be investigated in order to use the properties of the subcritical water for extraction from soils and the other environmental objects.

The present study is therefore mainly aimed to develop a new method for BaP extraction from soils using the subcritical water.

From this the Following Objectives Have Been Derived:

- To determine optimum conditions for subcritical water extraction of BaP from soils;
- To compare the obtained results with those of conventional methods based upon the use of organic solvents.

MATERIALS AND METHODS

Subcritical water extraction of BaP was developed and approved using the samples of ordinary carbonate heavy loamy chernozem (virgin, the 0-5 cm topsoil) taken

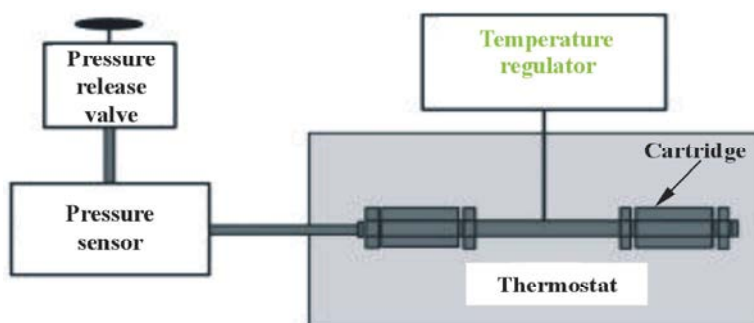


Fig. 2: Scheme of the device used for subcritical water extraction of BaP from solid matrix samples.

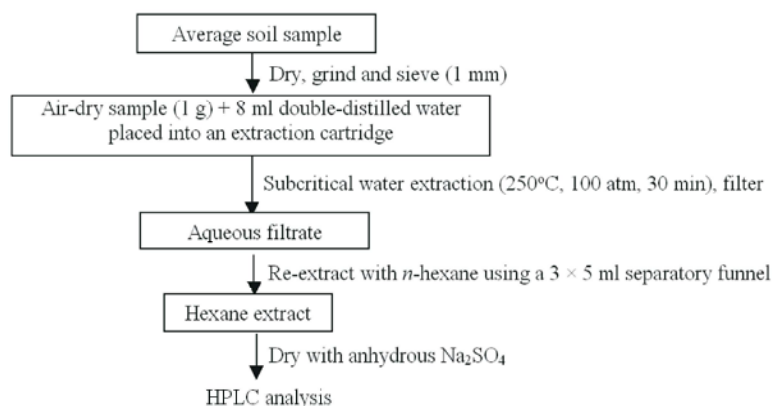


Fig. 3: Scheme of BaP analysis in plant material using subcritical water extraction and HPLC analyses of the extract.

in the Persianovskaya steppe of the Rostov region and located far from possible contamination sources. This soil revealed the following physical and chemical properties: $C_{org} = 3.4\%$; $pH = 7.3$; $ECE = 37.1 \text{ mmol}(+)/100g$; $CaCO_3 = 0.1\%$; the content of physical clay = 53.1% and clay = 32.4% .

Solvents and reagents were HPLC grade and included ethanol (96%, analytical grade), *n*-hexane (99%, analytical grade), potassium hydrate (98%, analytical grade), acetonitrile (99.9%, analytical grade), NaOH (97%, analytical grade) and anhydrous Na_2SO_4 (purchased from Aquatest, Rostov on Don, Russia). A BaP standard in acetonitrile (GSO 7515-98; Aquatest) was used to prepare standards for HPLC analyses.

Subcritical water extraction of BaP from soil samples was conducted in a specially developed extraction cartridge made of stainless steel and equipped with screw-on caps at both ends. It was also equipped with a manometer that included a valve for pressure release to maintain an internal pressure of 100 atm. The extraction cartridge containing a sample and water was placed into an oven connected to a temperature regulator. This device is schematically represented on Fig. 2.

The process of BaP analyses in soils based on subcritical water extraction is schematically shown on Fig. 3. It consisted of the following step-by-step operations. An air-dried sample of the soil was ground in a porcelain mortar and passed through a 1 mm sieve. One gram of sample was placed into the extraction cartridge and 8 ml of double-distilled water was added. The extraction cartridge was sealed from both sides with the screw caps. The cartridge was placed into an oven held at 230, 240, 250, 260 or 270°C for 20, 30 or 40 min. Subsequent extractions were conducted under optimum conditions (30 min at 250°C and 100 atm.).

After cooling, the content of the cartridge was filtered (Whatman no. 1) into a conical glass flask and washed with 2 ml of double-distilled water. This operation was repeated two or three times, until the filtrate was clear. The aqueous extract was re-extracted three times with 5 ml of *n*-hexane by shaking for 15 min in a separatory funnel. The hexane extracts were combined and filtered through anhydrous Na_2SO_4 and evaporated to dryness in a pear-shaped flask on a vacuum evaporator in a 40°C water bath. The residue was dissolved in 1 ml of acetonitrile by shaking for 30 min. The BaP concentration in the acetonitrile extract was determined by HPLC.

The efficiency of BaP extraction from soil was determined using a matrix spike [23]. The air-dried vegetation sample (1 g) was placed into a round-bottom flask and BaP standard solution in acetonitrile was added to give BaP concentrations of 2, 4, 6, 8, 16 or 32 nanogram BaP in g soil (ng/g) (0.002, 0.004, 0.008, 0.016, 0.032 mg/kg respectively). After evaporating the solvent for 30 min under a hood under ambient conditions, the BaP-spiked soil samples were incubated for 24 h at 7°C. The samples were then analyzed by the subcritical extraction method described above.

The results of the subcritical water extraction of BaP from soil were compared to those obtained by a standard method in modification permitting to remove limiting lipid macrocomponents from soil by the saponification method for increasing the efficiency of the pollutant extraction [24].

The saponification method has been elaborated with the view of determining the BaP in bottom deposits but it was used and approbated for soils. The given method involves treating the soil samples with a boiling mixture of 1g soil and 100ml of alkali and ethyl alcohol (96%) for 3 hours, further on, the re-extraction of BaP in 15 ml of the hexane solution three times. Subsequent stages in analyzing the obtained extracts are identical to those described for the extraction method using the subcritical water.

BaP in the extracts was quantified by HPLC (Model 2000, Thermo Separation Products, Waltham, MA, USA) with simultaneous ultraviolet (UV-1000) and fluorescence (FL-3000) detection following ISO 13877 requirements [25]. The BaP peak on chromatograms of soil sample extracts was identified by comparing retention time to that of the analytical standard sample using the two detectors. The limit of BaP detection and quantification were determined using standard solutions and calibration curves. A calibration standard was inserted after every six samples to correct for drift in retention time within a run.

BaP concentrations in soil samples (A, nanogram /g (ng/g)) were calculated as follows:

$$A = k S_1 \times C_{st} \times V / (S_{st} \times m) \quad (1)$$

Where S_{st} and S_1 = respective areas of BaP peaks in chromatograms of standard and sample solutions; C_{st} = BaP concentration in standard solution (ng/ml); k = coefficient of BaP recovery from a sample; V = volume of acetonitrile extract used for HPLC (ml); m = mass of the sample (g). Data handling and statistical analyses were conducted using Microsoft EXEL.

RESULTS AND DISCUSSION

The results of the subcritical water extraction of BaP from soil showed that this method allows shortening the time extraction up to 30-40 min instead of 11-48 hours requiring for those obtained by traditional methods (Table 1).

The oxidation of the soil lipid fraction and the maximum transition of BaP into the water solution occur in a hermetic reactor at 230-270°C and pressure of 100 atm for 30-40 min. Under these conditions the water reveals a dielectric permeability equaled to $\epsilon = 32.6-24.3$ at 230-270°C respectively, what is comparable with $\epsilon = 36.2$ at 25°C obtained for acetonitrile as an ideal BaP solvent, thus leading to a complete dissolution of BaP by the subcritical water.

Based upon the obtained results the optimum conditions of the BaP extraction from soil have been determined: the soil is treating by subcritical water at 250°C and 100 atm of pressure for 30 min.

When comparing the chromatograms of soil extracts after subcritical water extraction, it was possible to specify advantages of this extraction method (Fig. 4). In chromatograms of the extract obtained by the saponification method within the BaP retention time

Table 1: BaP concentration in ordinary chernozem depending on temperature and time extraction using subcritical water, ng/g (n=9).

Temperature of extraction, °C	Time of extraction, min		
	30	35	40
230	7.6(0.1)*	6.3(0.3)	5.8(0.2)
240	9.4(0.2)	8.5(0.5)	7.6(0.5)
250	11.9(0.2)	11.1(0.6)	10.4(0.5)
260	8.2(0.2)	7.9(0.5)	7.2(0.4)
270	5.4(0.1)	4.7(0.2)	3.9(0.1)

* The value means average from 9 replications and standard deviation is given in brakes

Table 2: BaP extraction degree from ordinary chernozem using the extraction methods of saponification and subcritical water (n=9).

Initial BaP in sample (ng/g)		BaP content by method of extraction (ng/g)		Extraction efficiency of the method (% of initial)	
Background	Spike	Subcritical water	Saponification	Subcritical water	Saponification
11.9±0.7	2.0	10.3(1.1)*	13.4(0.9)	74.1	96.1
	8.0	14.7(1.4)	19.2(1.4)	73.8	96.4
	16.0	20.5(2.9)	27.1(2.5)	73.4	97.0
	32.0	32.4(4.1)	42.2(3.8)	73.8	96.2
	64.0	56.3(3.9)	72.7(4.4)	74.2	95.9

* The value means average from 9 replications and standard deviation is given in brakes.

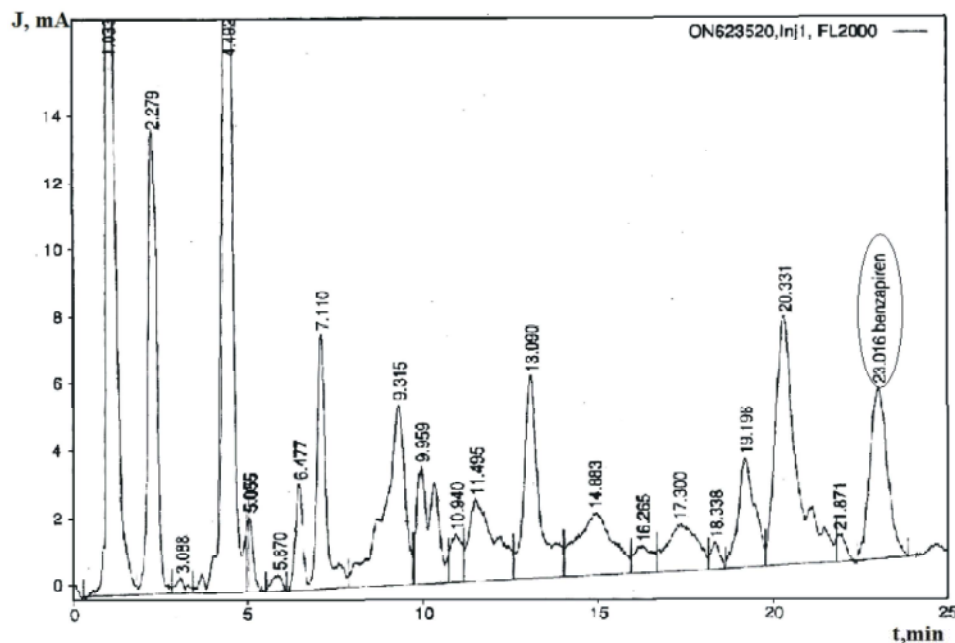


Fig. 4: Chromatogram of extracts from a representative soil sample prepared by subcritical water extraction.

there are additional picks limiting the extraction, whereas the chromatograms of the subcritical water extraction reveal an individual pick permitting to evaluate more precisely the pollutant concentration.

The experimental results with definite BaP concentration in soil showed that the subcritical water extraction allows extracting 96% of pollutant from soil (Table 2), exceeding the results of saponification method by 20%.

A comparative analysis was made to evaluate the results of BaP extraction from soil using the subcritical water and saponification method. For this purpose the soil samples were taken in monitoring sites located in a different distance from Novocherkask hydroelectric station. The above methods demonstrated the similar regularities in increasing the BaP content in soils located near the emission source (Table 2). But the BaP content extracted by subcritical water from samples of

contaminated soils varied in the range of 9-58 nanogram BaP/g, what is higher by 29-35% as compared to that of hexane extraction.

CONCLUSIONS

Thus, a method of BaP determination in soils has been approved as based upon the subcritical water extraction under the optimum conditions. The efficiency of the given method involves the use of subcritical water as an environment friendly solvent, a shorter time for analysis and a higher content of BaP extraction (to 96%) as compared to that extracted by organic solvents.

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