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STUDY OF THE EFFECTIVENESS OF THE NEW METHOD FOR THE RAPID PAHS ANALYSIS IN SOIL

Abstract

The aim of this research was to compare the effectiveness of highly-selective chromatographic PAHs determination method with the new, simpler, non-selective spectrophotometric method, designed for the rapid monitoring of PAHs contamination level in soil. The conducted experiment includes the 2 series of PAHs content determinations, in 4 types of soils, with use of HPLC and spectrophotometric techniques. The obtained results, showed that the developed, PAHs content in soil determination method, can be used for quick and non-specific environmental monitoring of soil. However the effectiveness of this method is dependent on the content of organic matter in the analyzed soil samples.

Key words

PAHs, PAHs determination, Spectrophotometric analysis, HPLC analysis, soil, environmental monitoring.

Introduction

The presence of organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs) in soil, used for crops production, is considered as a big issue in the field of modern agriculture. Due to the toxic nature and mutagenic, cancerogenic and theratogenic activity of some compounds from PAHs group, their occurrence in soil can cause a numerous of problems, such as: induction of economic losses (decreased yield), generation of a threat to human health and impact on the ecological homeostasis (toxic influences to endogenous flora and fauna). The issues associated with risk to humans, results from the fact that, those pollutants can migrate to the plant tissues, causing contamination of food or feed [1,2].

Taking in account the above-mentioned characteristics of pollutants from PAHs group, it can be noted that, conduction of effective monitoring of PAHs contamination levels, in soils used for agricultural purposes, should be a common practice. Unfortunately, for the current moment, the techniques used for determination of PAHs content in soil, are very expensive, labour-intensive and require the use of sophisticated laboratory equipment [3,4]. The most commonly used methods for PAHs analysis in soils are chromatographic techniques, including High Performance Liquid Chromatography (HPLC) and high performance gas chromatography with mass-spectrometry detector (GC-MS) [5]. Both of these methods are characterized as highly accuracy and allow performing a detection of results in the nano and pico-metric scales. However, this type of method requires complex sample preparation, is complicated in execution and generates high procedural costs [1,3,5].

One of the most commonly used PAHs detection techniques is a spectrophotometric method. This technique can be used directly or as part of the chromatographic system. It involves measuring the absorbance of light waves by molecules of presented in studied solution substances. In the case of the compounds from PAHs group, aromatic rings, also called chromophoric systems, absorb light waves. The presence of such structural element in PAHs molecule, cause the absorption of the light spectrum, which leads to the decrease in the electromagnetic energy of wave. The absorbed energy is converted into electron energy, oscillation energy or rotation energy. According to this principle, knowing of a measurable amount of absorbed energy can be calculated in to amount of substance that adsorbs this energy [6,7].

The use of traditional spectrophotometric methods for PAHs determination is associated with some methodological issues. In most cases the presence of a one compound from this group is treated as an indicator. In practice it means that in such situations, in studied material, other substances of similar origin also are present.

Taking into account the above-mentioned fact it can be stated that there is a strong likelihood of interference in data obtained with those types of detections [6,8,9].

The solution to this problem could be the introduction to the process of preparation of samples for analysis of preliminary selective designated analytes separation techniques. This type of methods includes the conventional extraction (for soil solid-liquid extraction) or advanced SPE techniques (solid phase extraction) [10,11,12]. SPE consists of passing a known volume of the test solution (liquid extract) by the material that has the capacity for selective adsorption of the analytes. The most commonly used materials in the PAHs isolation from extracts are silica gel with the modified functional C18 groups. PAHs molecules, present in the solution are retained by the strong hydrophobic gel structure and then they may be washed with an appropriate solvent. This type of procedures has found a wide range of applications in scientific papers and is widely described in publications [11,13].

The aim of this research was to compare the effectiveness of highly selective chromatographic PAHs determination method (HPLC technique) with the new simple, non-selective method, designed for the rapid monitoring of PAHs contamination level in soil.

Material and methods

The experiment was conducted in four stages. In the first stage, the initial calibration, with use of spectrophotometric method, was performed. This procedure includes a series of absorbance measurements, 5 dilutions of the standard solution (0.1; 0.075; 0.05; 0.025; 0.01). The used standard was a RESTEK mixture of 16 PAH's - RESTEK 610 Calibration MIX A. This solution contains a mixture of listed below compounds, dissolved in acetonitrile. The list of PAHs in used standard includes: acenaphthene (1000 µg/ml), acenaphthylene (1000 µg/ml), anthracene (1000 µg/ml), benz(a)anthracene (500 µg/ml), benzo(a)pyrene (500 µg/ml), benzo(b)fluoranthene (500 µg/ml), benzo(k)fluoranthene (500 µg/ml), benzo(g,h,i)perylene (500 µg/ml), chrysene (500 µg/ml), dibenz(a,h)anthracene (500 µg/ml), fluoranthene (500 µg/ml), fluorene (1000 µg/ml), indeno(1,2,3-c,d)pyrene (500 µg/ml), naphthalene (1000 µg/ml), phenanthrene (500 µg/ml), pyrene (500 µg/ml). On the base of obtained results, the linear calibration curve has been prepared and the formula for total PAHs content calculation was developed. The calibration curve is presented on the chart - figure 1.

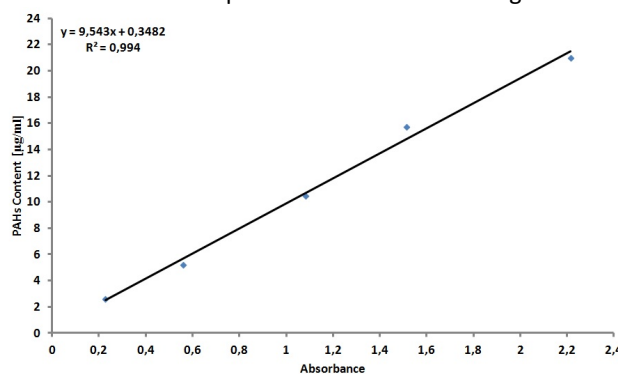


Fig. 1. Spectrophotometric calibration curve

The second step includes the analysis of basic physical and chemical properties of used materials. In the experiment, the four types of soils were used. First soil material was taken from the crop field (cultivation of *Brassica napus*), located in the Silesia region Poland. This material was characterized as loamy sand with low level of organic matter and low pollutants content. Second soil comes from the fallow lands, located near the highway. This material also was characterized as loamy sand with low level of organic matter and low pollutants content. The third soil material was the commercially available, cultivation medium. It consist high level of organic matter and medium level of pollutants content. The last soil was taken from the heavy contaminated industrial site, located in Silesia region of Poland. This material consist a medium level of organic matter and very high level of pollutants content. All collected materials were subjected to following analysis: dry matter; organic matter; pH in water (the active acidity), pH in potassium chloride solution (the exchangeable acidity) - (PN-ISO 10390:1997); CEC (Cation Exchange Capacity) Kappen's method [14,15].

The third stage, was concerned on the analysis of PAHs contamination levels in all above mentioned soil samples. This procedure was performed by two techniques. The first, was selective chromatographic analysis (HPLC

method), while the second, was spectrophotometric analysis. To the PAHs analysis, the air-dry soil samples (samples weights: 10g - soils 1, 2, 4; 1g - soil 3) were used. In order to provide a wide range of statistically correct results all air-dry soil samples were divided into 6 subsamples. To the carefully weighed soil subsamples (3-reps for each subsample) the 30 ml of acetonitrile were introduced. Next they were subjected to the sonification for 30 min. in an ultrasonic bath. The obtained extracts were also been shaken for 24h. After that extracts were purified by centrifugation and filtration over PP membrane filters (0.45 m). The final phase of samples preparation was the concentration of extracts, by using SPE (Solid Phase Extraction) technique. For this purpose the ChromaBond C18 6ml/500mg columns were used. Following the above procedure, 3 ml sample volumes were obtained. Spectrophotometric analysis was performed on HACH DR 6000, and the chromatographic analysis was run on Thermo Scientific SpectraSystem [16,17].

The HPLC analysis was based on the existing methodologies [3,5,11]. The used procedure was based on gradient elution technique, with use of a mixture of water, methanol and acetonitrile as mobile phase. The analytes separation was carried out in the reversed phase, on the Restek Pinnacle II PAH HPLC column, at a temperature of 30°C. Detection of results was carried out by using a UV-VIS detector at 254 nm wavelength. Spectrophotometric analysis was based on the absorbance measurements also at 254 nm wavelength [16,18].

The final stage of the study includes the comparison of the results of total PAHs content, obtained in both types of conducted analysis. It consisted of calculations of the Pearson correlation coefficients and the graphical presentation of the level of similarity between them.

Results and discussion

The results, obtained during the execution of physical and chemical properties analysis, of all studied materials, are presented in Table 1. Based on those information's, it can be observed that the first two types of soils 1 and 2, have high dry matter content and low organic matter content. The pH of these soils is in range of 7 and the cation exchange capacity (CEC) is relatively low. In the case of soil 3, it can be seen that this material had the lowest content of dry matter and the highest content of organic matter. The pH of this material is slightly acidic and the cation exchange capacity is twice higher than in the previously discussed soils. The last type of soil 4 was characterized as material with approximately high dry matter content and a relatively high content of organic matter. The pH of the material is acidic and cation exchange capacity is placed among the highest values of all tested materials.

Table 1. The physical and chemical properties of used materials

Parameter	Soil 1	Soil 2	Soil 3	Soil 4
Dry matter [%]	92,4	93,2	48,7	88,8
Organic matter [%]	4,9	4,2	88,9	45,7
pH (in H ₂ O)	6,99	7,15	6,42	5,99
pH (in KCL)	7,15	7,57	6,58	6,15
CEC [cmol(+)/kg]	16,3	14,5	32,3	44,8

The next section of charts contains the set of results obtained from the both spectrophotometric and chromatographic analysis. Figure 2 presents the results obtained from the determination of the sum of PAHs in soil 1 and 2. Figure 3 contains a set of data obtained from the soil 3 and 4 analyses.

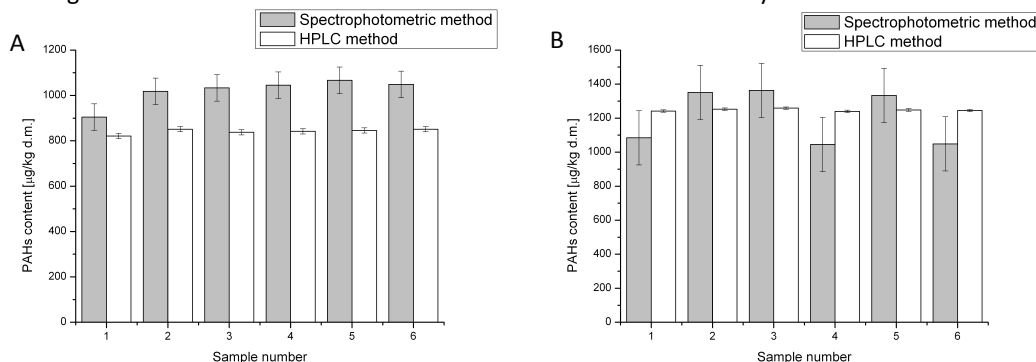


Fig. 2. The results of PAHs determinations conducted with both spectrophotometric and HPLC methods. Chart A applies to soil 1 and chart B applies to soil 2.

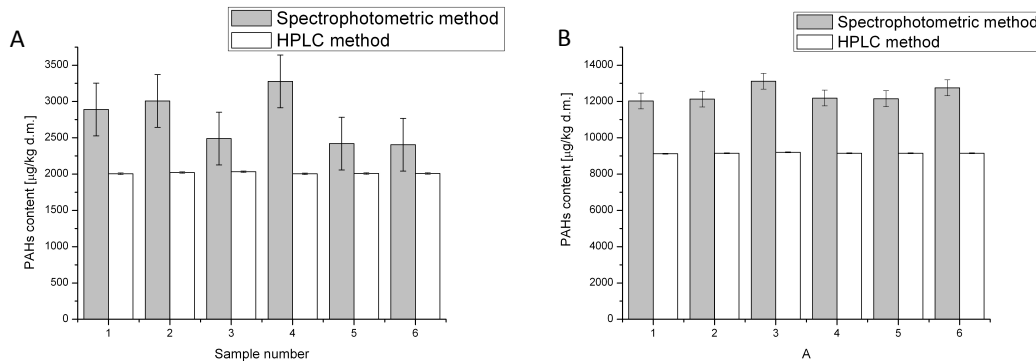


Fig. 3. The results of PAHs determinations conducted with both spectrophotometric and HPLC methods. Chart A applies to soil 3 and chart B applies to soil 4.

On the basis of presented above results, it can be seen that in all studied cases the PAHs contents, obtained by using spectrophotometric method, were higher than the values obtained with use of HPLC technique. This phenomenon can be caused by the non-specific nature of spectrophotometric analysis. Techniques based on the chromatography enable to separate designated groups of compounds from other substances (background), which may be expressed by seemingly reduced analytes content. However such situations does not mean, that HPLC methods are less effective. Selective isolation of only the desired substances, and precise detection of data, makes chromatographic methods much more reliable then spectrophotometric techniques. This fact is supported by the observed in obtained results standard deviation values. In case of the results obtained during spectrophotometric analysis, the values of this parameter were significantly higher than values obtained from HPLC analysis.

The results also show that the soil characterized by increased content of organic matter (soil 3), causes significantly greater measurement errors, during the spectrophotometric analysis. A similar situation also applies to the results obtained from the highly contaminated industrial soils (soil 4).

The presence of large amounts of soil organic matter is associated with the intensive background influences on the absorbance measurement. The compounds with the most important impact on this issue are phytohormones, natural dyes, pesticides and oil origin pollutants (compounds that do not belong to the group of PAHs). Due to the fact, that the detection methods, based only on the spectrophotometry, are not specific, the presence of mentioned above compounds within the soil matrix, can contribute a significantly high level of disturbance in the effectiveness and reliability of this type of methods.

The last set of results concerns the statistical comparison of both PAHs determination methods. The results of this step of research are presented at 4 charts. Figure 4 applies to the soils 1 and 2 while figure 5 to soils 3 and 4. These charts illustrate the degree of linear relationship, between the results of spectrophotometric analysis and HPLC analysis. In the table 2 the standard deviations and calculated correlation coefficients for both studied method are presented.

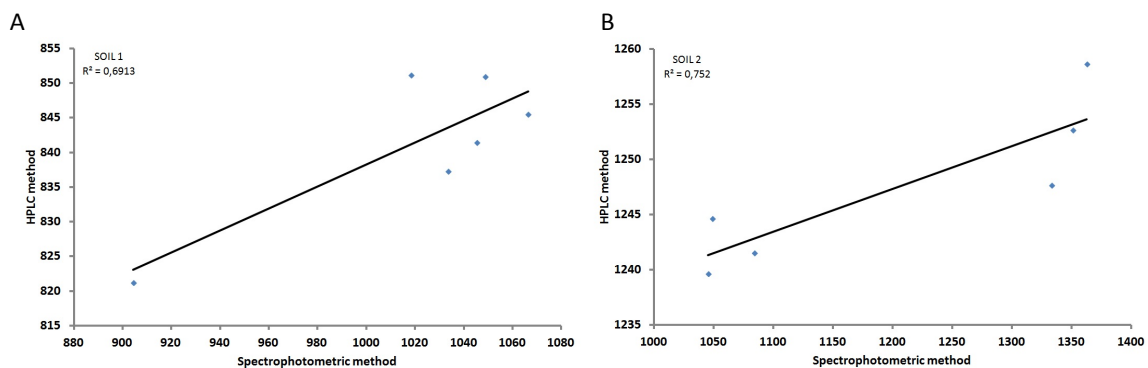


Fig. 4. Statistical comparison of both PAHs determination methods. Chart A applies to soil 1 and chart B applies to soil 2.

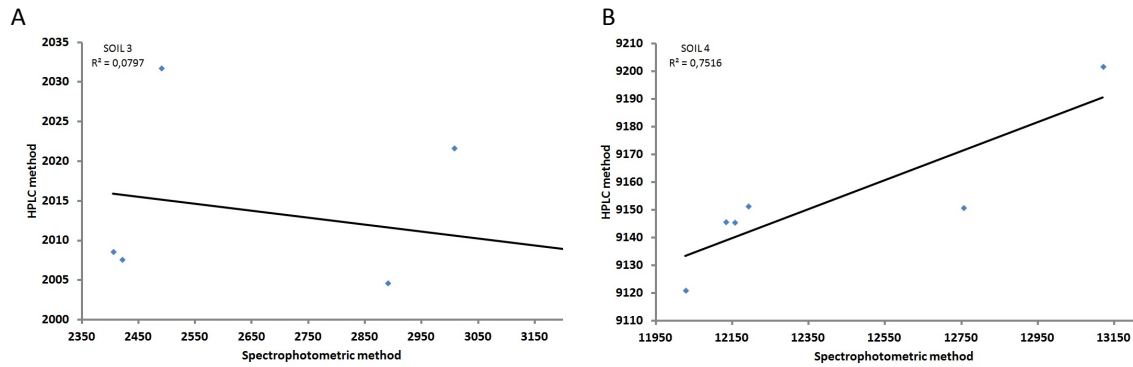


Fig. 5. Statistical comparison of both PAHs determination methods. Chart A applies to soil 3 and chart B applies to soil 4.

Table 2. Correlation coefficients and standard deviations of the studied methods

	Correlation coefficient	± HPLC [%]	± Spectrophotometric [%]
Soil 1	0,83	4,18	12,65
Soil 2	0,87	5,14	11,37
Soil 3	-0,28	9,35	25,03
Soil 4	0,87	7,45	31,64

During the results discussion the following thesis was assumed: the results obtained with the use of chromatographic method are reliable and provide a reference for the results obtained with the use of spectrophotometric technique. A statistical comparison of both those methods shows that in the case of soils 1, 2 and 4, a high degree of correlation was observed, values > 0.8 . In case of soil 3, the correlation coefficient was lowest and reaches only a value < 0.3 . Comparing the precision of studied spectrophotometric method with methods presented by other authors, it should be noted that the developed technique has greater measurements errors. For example the method used by Juliane Hollender et. al. showed standard deviations in range 2-10 %, depending on the analyzed compounds. Similar variations can be observed at Oleszczuk et. al., Smol et. al. and Sun et. al. [19,20,21]. Presented in current paper, spectrophotometric technique, depending on the type of soil, has the standard deviations in the range of 11.37% to even 31.64%, in the case of highly contaminated soil (sample 4). In result, such large discrepancy of obtained data, disqualify the presented method from most research work, however this technique was designed only for estimating purposes and they scope of potential applications is quite different. Due to the fact, that spectrophotometric method is much more affordable (cheaper test equipment) and the time of analytical procedure is much shorter (spectrophotometric method 2-5 min/sample; chromatographic methods - up to 60 min/sample) this type of technique might find some applications in the fields of implementation of a preliminary assessment of soil contamination, during cyclical monitoring of PAHs content in soils or during the performance of preliminary tests, that do not require precise determination of the strict composition in studied medium.

Summary and conclusions

On the basis of described above results, the following conclusions can be made:

- The developed PAHs in soil determination method can be used for quick and non-specific environmental monitoring of soil.
- The effectiveness of new spectrophotometric method is dependent on the organic matter content in the analysed soil samples. High levels of organic matter have negative impact on the reliability of obtained results. The spectrophotometric method is affected by higher measurement errors than chromatographic techniques. The measurement errors increase proportionally with the increase in environmental contamination of analysed soils.
- The developed method can be used for quick and non-specific environmental monitoring of soil.

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