Evaluation of Polycyclic Aromatic Hydrocarbons Content of Soil from Areas Heavily Industrialised

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Abstract: - This research is part of a broad study to monitor the content of polycyclic aromatic hydrocarbons in the environment and then the chain of traceability, animal fodder, meat and meat preparations. This study aims to identify the possible accumulation of these contaminants in the soil in surrounding areas of the city of Targoviste, which is highly industrialized. Targoviste City area and the upstream have an iron and steel industry, electro technical, petrochemical and chemical. These lands are planted with cereals which are used in animal feed. The effects of the polycyclic aromatic hydrocarbons on the human health, inclusively through the trophy chain and on the environment as a whole are harmful through their concentrations in the ambient air and through their depositions; it should be taken into account the accumulation of these substances in soils and the protection of underground waters. Measurements were made using modern methodology, which allowed detection of even low PAH in the samples analyzed. For this purpose PAHs were dosed by high-performance liquid chromatography (HPLC) of soil samples collected from areas surrounding the city. Soil samples were collected from surface a depth of 5 cm. Fourteen PAHs was determined. Several of them are known to be potential human carcinogens including benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene and benzo[ghi]perylene. PAHs identified in soil samples were benzo (b + k) fluoranten , phenanthrene and pyrene . They have recorded values below accepted European norms in force.

Key-Words: - polycyclic aromatic hydrocarbons, soil, high-performance liquid chromatography, environment, traceability

1 Introduction

Erosion, loss of organic matter, compaction, salinisation, landslides, contamination, are phenomena that lead to land degradation. The European Commission was adopted a Soil Thematic Strategy (COM (2006) 231) and a proposal for a Soil Framework Directive (COM (2006) 232) on 22 September 2006 with the objective to protect soils across the EU. The Strategy and the proposal have been sent to the other European Institutions for the further steps in the decision-making process. [1]

Soil contamination, as result of anthropogenic activities, is a serious health and environmental issue. Based on the principles mentioned in article 175, paragraph (3), Directive 2004/107/CE of The European Parliament and of the Council, the 6th Community Environment Action Programme adopted by the Decision no.1600/2002/CE of the European Parliament and of the Council, establishes the necessity to reduce pollution at levels that can cut to a minimum the noxious effects on the human health, paying particular attention to the sensitive categories of population and to the environment as a whole, to improve the surveillance and the assessment of the air quality, of the polluting agents deposition as well, and to provide information to the public. [2, 3]

The scientific evidence proves that some Polycyclic Aromatic Hydrocarbons (PAH) are genotoxic cancerous agents for the human being and that there is not an identifiable threshold according to which these substances are not a risk for the human health. Their effect on the human health and on the environment is produced by their concentrations in the ambient air and by their deposition.[4, 5, 6, 7]

The Polycyclic Aromatic Hydrocarbons are a group of organic compounds, very stable, composed by at least two fusioned aromatic rings that have

within their composition only carbon and hydrogen atoms. These can penetrate into the aliments during the technological processes. A part of the polycyclic aromatic hydrocarbons can cause cancer and DNA mutations. [8, 9]

Although studies in experimental animals on individual PAHs, mainly on benzo[a]pyrene, have shown various toxicological effects, such as haematological effects, reproductive and developmental toxicity and immunotoxicity, it is the carcinogenic and genotoxic (DNA-damaging) potential of these compounds that has attracted most attention. A number of PAHs have shown carcinogenicity in experimental animals and genotoxicity and mutagenicity in vitro and in vivo. The International Agency for Research into Cancer (IARC) in 1987 concluded that benzo[a]pyrene is a probable human carcinogen. Some other PAHs have also been identified as being carcinogens, with possible genotoxic properties.

The general characteristics common to the class are high melting and boiling points, low vapour pressure, and very low water solubility which tend to decrease with increasing molecular mass. PAHs are soluble in many organic solvents and are therefore lipophilic (soluble in fat). At the temperature of 18-20 Degrees Celsius the PAH are solid, without color or white or light yellow-greenish colored, providing high points of melting and boiling. PAH are used as intermediate agents in the processes of obtaining different plastic materials, coloring agents and dyes, pesticides. [10] They penetrate into the environment because of the incomplete decrepitations of the organic combustibles during industrial processes or because of other human activities. One of the major contaminants of soil is polycyclic aromatic hydrocarbons (PAHs). [11, 12]

The main pollutants from traffic are, besides the waste originated during and after the life cycle of vehicles and in accidents, mainly CO_2, CO, NO_x, N_2O, SO_2, O_3, Pb, Cd, Ni, Cr, platinum metals, volatile organic substances, CH_4, 1,3-butadiene, benzene, toluene, xylenes, phenols, aldehydes, ketones, tar, but also persistent organic pollutants in the form of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, dibenzo-p-dioxins, dibenzofurans, etc. [13,14, 36]

PAHs are characterized by high hydrophobicity, resistance to natural degradations and carcinogenic properties. They are largely rendered from incomplete combustion of hydrocarbon-containing fuels. The main sources of these human activities include open fires, engine exhaust emissions, manufactured gas plants by-products and domestic heating systems (United States Environmental Protection Agency 2008). Apart from coal mining activities, contamination sources are most often prevalent within populated areas such as urban zones where there are concentrated transport systems, large industrial bases and dense housing [15]

In heavily contaminated soils were initiated researches on the biodegradation of PAHs, in which bacteria or fungi have been used. PAH-contaminated soil, to focus on harnessing the biobacteria and fungi to create a cooperative environment for PAH degradation, and to further investigate the array of PAHs that can be lost during the composting process by either leaching or volatilization [16, 17]

Many analytical methods have been developed, optimized and applied for the determination of PAHs in marine sediments [18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30]

These methods provide for both high performance liquid chromatographic (HPLC) and gas chromatographic (GC) approaches for the determination of PAHs. [31] The gas chromatographic procedure does not adequately resolve the following four pairs of compounds: Anthracene and phenanthrene; chrysene and benzo(a)anthracene; benzo(b)fluoranthene and benzo(k)fluoranthene; and dibenzo(a,h) anthracene and indeno (1,2,3-cd)pyrene. Unless the purpose for the analysis can be served by reporting the sum of an unresolved pair, the liquid chromatographic approach must be used for these compounds. The liquid chromatographic method does resolve all 14 of the PAHs listed. [32,33]

In the current study were analyzed by HPLC, soil samples from different areas, located in the vicinity of roads and some industrial companies, potentially polluting. [31, 34] These soils are planted with grain and other fodder from farms and feed in their own households. Farm animals are slaughtered and the meat is used for direct consumption or production of meat products. A good traceability requires accurate information about a critical point, locating the exact stages in which PAHs can accumulate over the meat supply chain. [35]

2 Problem Formulation

Most of the PAHs in Targoviste area are introduced into soil from atmospheric deposition due to exhaust gases supported by the presence of PAHs in the soil of regions remote from any industrial activities, such as those in the Doicesti, Fieni. Pollution in this region is caused by processing coal,

oil, natural gas, iron production, steel, power and heating in residences, fire. Soils, surface waters and precipitation may be contaminated with PAHs in the atmosphere, due to oil spills and emissions from cars in traffic. Animal feed may be contaminated by depositing particles in the air or by developing them in a contaminated soil.

2.1 Compounds determination

In this study were analyzed 14 PAHs recognized as high priority pollutants by the U.S. Environmental Protection Agency (U. S. EPA, 1993). These compounds were selected because of their highest toxicity potential, but also of their mobility and transmission to the human.

The polycyclic aromatic hydrocarbons determined were presented in the Table 1.

Table 1
Polycyclic Aromatic Hydrocarbons evaluated in the soil samples

Nomenclature	Formula	Molecular weight
Naphthalene	$C_{10}H_8$	128,173
Phenantrene	$C_{14}H_{10}$	178.233
Fluoranthene	C_6H_{12}	202.255
Fluorene	$C_{13}H_{10}$	166.223
Anthracene	$C_{14}H_{10}$	178.23
Acenaphthene	$C_{12}H_{10}$	154.21
Chrysene	$C_{18}H_{12}$	228.28
Benzo [a]anthracene	$C_{18}H_{12}$	228.2928
Benzo [e] pyrene	$C_{20}H_{12}$	252.3148
Benzo[g,h,i] perylene	$C_{22}H_{12}$	276.3368
Benzo [k] fluroanthene	$C_{20}H_{12}$	252.3148
Benzo [b] fluroanthene	$C_{20}H_{12}$	252.3148
Indeno [1,2,3] cd pyrene	$C_{22}H_{12}$	276.3368
Pyrene	C_6H_{10}	202.255

2.2 Samples

Soil samples were taken from three different places of Targoviste and surrounding areas. The mainly pollutant sources from Dambovita County are presented in Fig.1.

The following notations were used:
- SA - soil sample taken from a depth of 5 cm from the industrialized area of Targoviste;
- S1 - soil sample taken from a depth of 5 cm, at a distance of 2 km from the industrialized area of Targoviste
- S2 - soil sample taken from a depth of 5 cm, at a distance of 7 km from the industrialized area of Targoviste.

The weight of the soil samples was about 200g. The sampling was performed in glass vessels, observing the norms for taking, preservation and transport of the soil samples

Fig.1 The pollution sources in Targoviste city and surrounding area

2.3 Method and reagents

The principle of determining the polycyclic aromatic hydrocarbons in soil was based on liquid chromatography technique, according to SR EN

ISO17993: 2004. High performance liquid chromatograph (HPLC) is an analytical system complete with column supplies, high pressure syringes, detectors, and compatible strip-chart recorder. A data system is recommended for measuring peak areas and retention times. High performance liquid chromatography (HPLC) is an evolution of a more ancient, classical column chromatography, which serve primarily the preparative isolation of natural compounds. Scheme of work for a HPLC: solvent pump→injector → column→detector→recorder.

Pump is considered one of the most important components of HPLC as allow a constant flow of eluent through the entire system: injector, column, detector, greatly increasing the speed of separation. By placing pumps are working at higher pressures (200atm), development of efficient stationary phase, more small-scale (recently stationary phase consisting of spherical granules with diameters of 2-5μm), in columns all short (3-10cm) resulted in initial improvement method.

The method is based on extraction of PAHs in soil with organic solvents (acetone and petroleum ether) followed by HPLC separation and detection by UV-VIS (MWD).

Extraction is done sequentially with acetone and petroleum ether, which is performed after purification of the organic extract by passing the sorbent SPE cartridge especially for PAH. Finally it performs the extract concentration analysis using Rota evaporator and chromatographic analysis.

The determinations of PAH compounds were performed with an Agilent Technologies HPLC chromatograph type 1100, equipped with quaternary pump, auto sampler, column thermostat and UV-VIS detector.

The column used for separation of PAHs was Lichrospher DAP 250x3cm RP C18, 5 μm, with guard column and precolumn. The mobile phase: acetonitrile - water gradient from 60:40 to 100% acetonitrile was used.

Acetonitrile was by HPLC quality, distilled in glass. Characterization of silica gel: 100/200 mesh, desiccant, Davison, Grade-923 or equivalent. Before use, activate for at least 16 hours at 130°C in a shallow glass tray, loosely covered with foil.

3 Problem Solution

The European Commission issued a Recommendation (2005/108/EC), by all EU Member States, which provides that making a random monitoring of these PAHs in food. It aims to provide information on sources of environmental contamination by PAHs of the food.

The references values, in Romania, for the chemical elements from soil are mentioned by the Order 756/1997 of the Ministry of Waters, Forests and Environmental Protection for the approval of the Regulation referring to the evaluation of the environmental pollution . Normal limits for PAHs in soil ranged between 0.02-0.5 mg/kg dry substance.

Limits of detection (LOD) for HPLC separation and detection by UV-VIS (MWD) are 0.01 mg/kg dry substance. Using GC-MS, LOD is 10 ng/g [16].

The limit of quantitation (LOQ) is an approximate concentration required to yield a positive result at the lowest standard. (Table 2)

Table 2

Carcinogenic PAHs in soil and their limits of quantification

Contaminant	LOQ, ppb	Normal values, mg/kg dry substance
Naphthalene	31250	<0.02
Phenantrene	420	<0.05
Fluoranthene	428	<0.02
Fluorene	2138	<0.02
Anthracene	128	<0.05
Acenaphthene	>62500	<0.02
Chrysene	4.3	<0.02
Benzo [a]anthracene	3.0	<0.02
Benzo [e] pyrene	10	<0.02
Benzo[g,h,i] perylene	>625	<0.02
Benzo [k] fluroanthene	3.9	<0.02
Benzo [b] fluroanthene	3.9	<0.02
Indeno [1,2,3] cd pyrene	12.7	<0.5
Pyrene	1456	<0.02

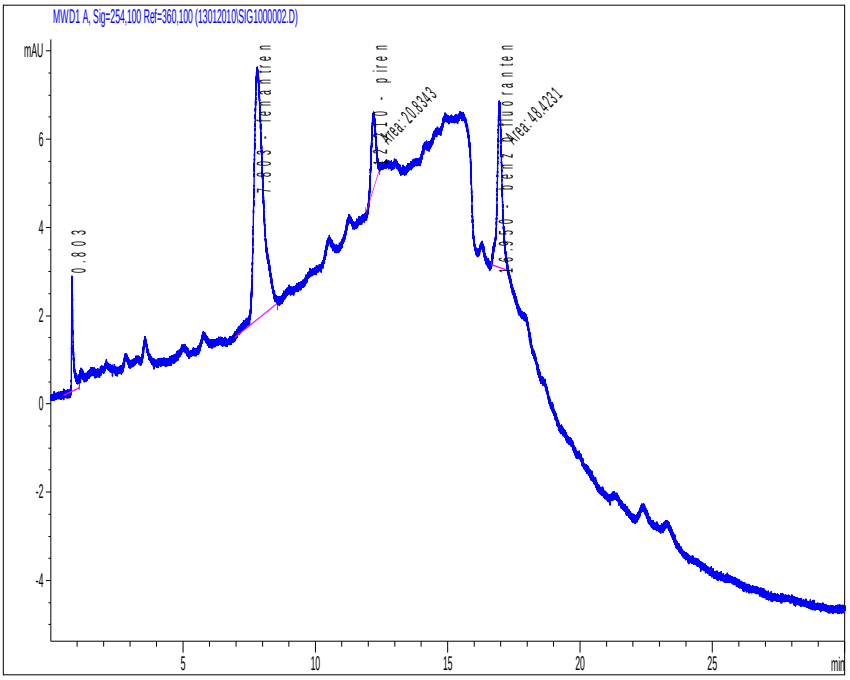

Fig.2 The HPLC chromatogram obtained for the sample SA

Total PAHs normal content should not exceed 0.1mg/kg dry substance, sensitive and alert thresholds are maximum 7.5 mg / kg and the less sensitive value is 25mg/kg.

In Fig.2 is presented the chromatogram obtained through the analysis of the soil sample taken at a depth of 5 cm from the area of Targoviste. Note that after analyzing the results of soil sample (SA) shown the highest concentration of PAHs. Values above the detection limit were found for benzo (b + k) fluoranten (0,022 mg/kg dry substance), phenanthrene (0.043 mg/kg d. s.) and pyrene (0.015 mg/kg d. s.). From all of them only benzo (b + k) fluoranten is shown to be carcinogenic, but the limit allowed it isn't exceeded. The alert threshold sensitivity for pyrene and anthracene is 5 mg / kg, the value by 10mg/kg representing the less sensitive. For benzofluoranten the alert threshold sensitivity is 2 mg / kg and 5mg/kg less sensitive. Other PAHs monitored were below the limit of detection.

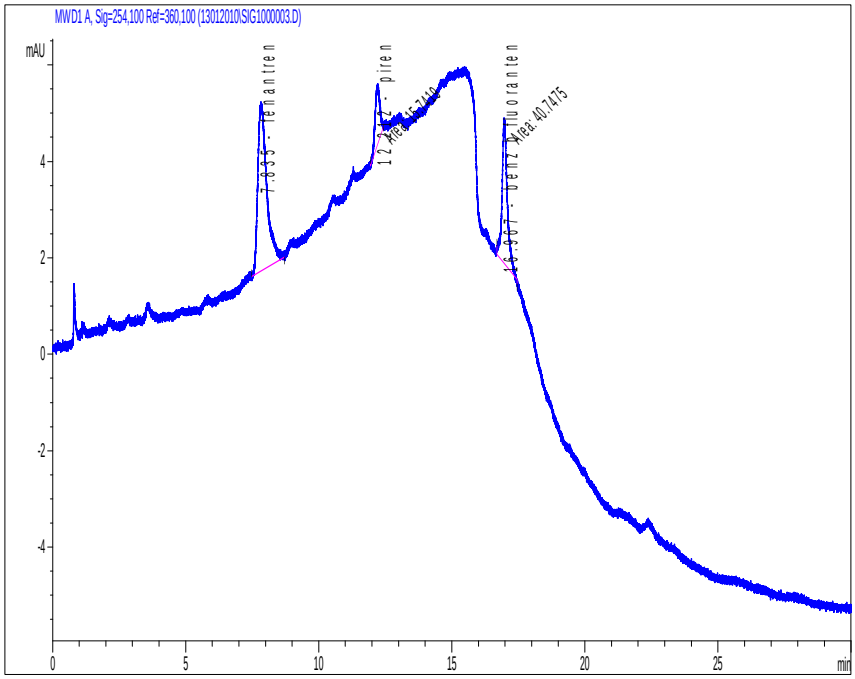

Fig.3 The HPLC chromatogram obtained for the sample S2

Soil sample taken from a depth of 5 cm, to 7 km distance from the industrialized area, was less contaminated with PAHs than the city sample. The resulting chromatogram for this sample is shown in Fig.3.

The same contaminants were found here, but in smaller quantities: benzo (b + k) fluoranten (0,013mg/kg dry substance), phenanthrene (0.025 mg/kg d. s.) and pyrene (0.01 mg/kg d. s.).

Total content of PAHs to the limits of intervention are sensitive to 15mg/kg and threshold for intervention less sensitive 150mg/kg. Sensitive intervention limits for pyrene is 10 mg / kg and 100mg/kg is less sensitive. For benzfluoranten and phenanthrene alert threshold sensitivity is 5 mg/ kg, 50mg/kg and less sensitive.

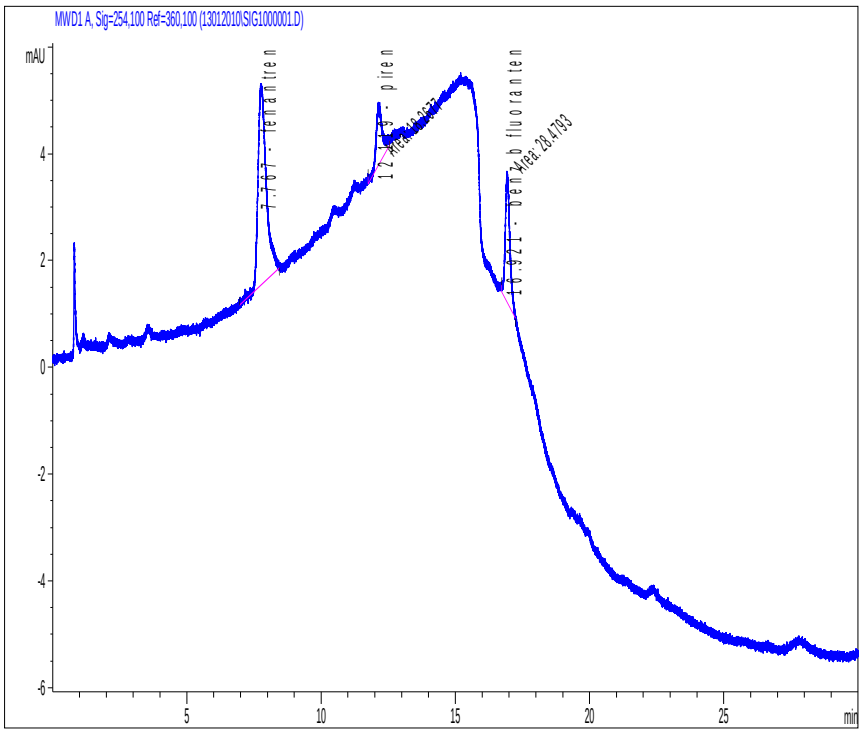

Fig.4 The HPLC chromatogram obtained for the sample S1

In the soil sample S1 taken from a depth of 5 cm to 2 km from the industrialized, naphthalene, phenanthrene, chrysene, benzo (a) anthracene, benzperilen, benzo [e] pyrene, benzo [g, h, i] perylene were found below LOD. The chromatogram is represented in Fig.4, from this one resulting that the content of benzo (b + k) fluoranten was 0.010 mg/kg d. s., phenanthrene 0.025 mg/kg d.s. and pyrene 0.01 mg/kg d. s.

From the comparative analysis of the level of the PAHs determined for each sample (Fig.5), results that the soil sampled from the industrialized area (SA) had the highest concentration of these pollutants. As the distance from the city increase, the soils are less contaminated with PAHs.

A parallel analysis between the PAH content of the samples and the maximum normal values (according the Order 756/1997 is shown in Fig. 6-8.

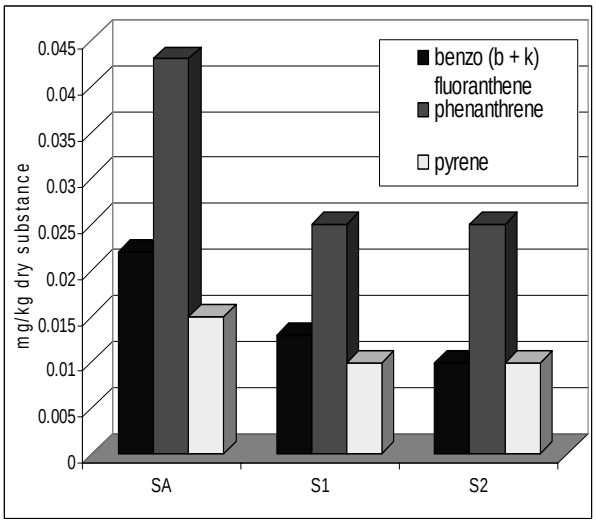

Fig. 5 PAHs comparative values between the analyzed samples

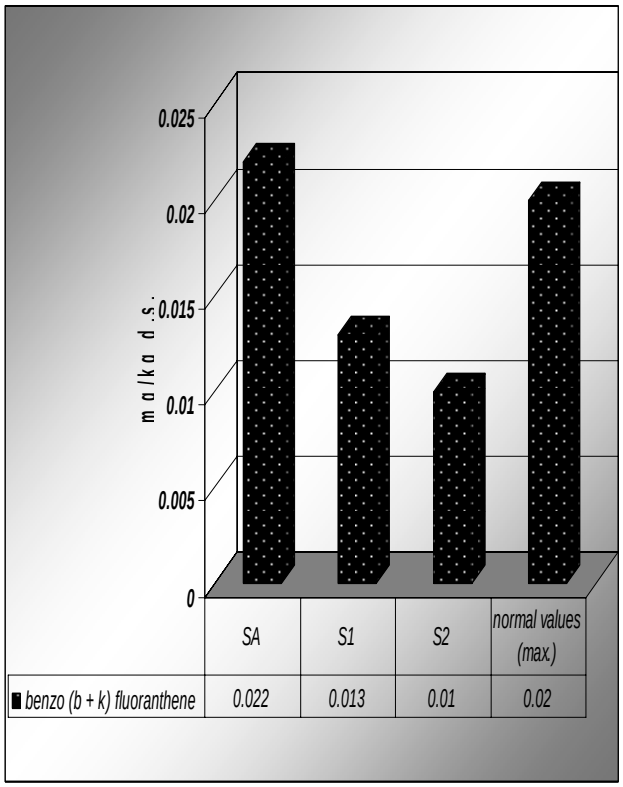

Fig. 6. The benzo (b+k) fluoranthene content of the analyzed samples in comparison with the normal values

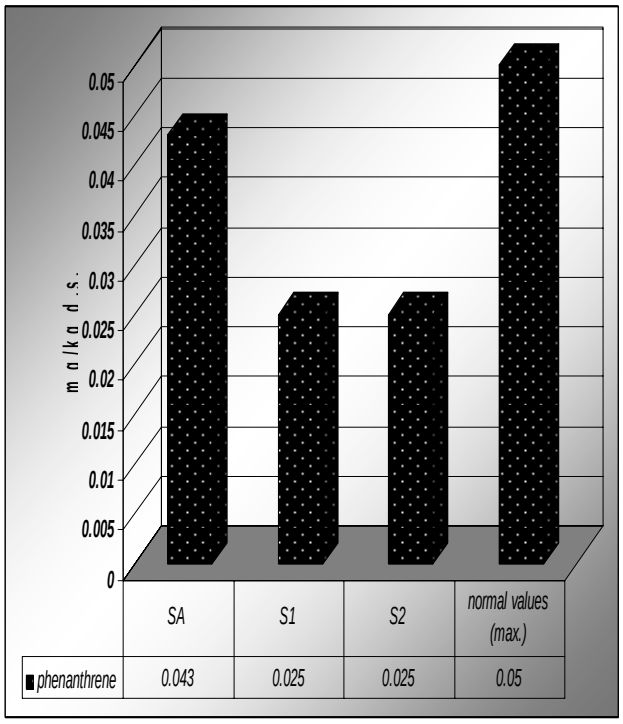

Fig. 7. The phenanthrene content of the analyzed samples in comparison with the normal values

The benzo (b+k) fluoranthene content was exceeded the normal value only in the case of the soil sampled from the city area with 10%.

However, the alert threshold (sensitive type of use) is about 90 times highest than the value obtained in the case of the sample SA, respectively by 200 times highest comparatively with the content of the soil sampled from 7km distance to the city.
The data are also too smaller than the intervention threshold (sensitive type of use) in all the experimental variants.

The phenanthrene content of the sample SA do not exceeded the maximum value stated by the national regulations, but was very close to it (Fig. 7). In the same time, the equal values obtained in the case of the soil sampled outside of Targoviste area were represented only 50% from the normal maximum values.

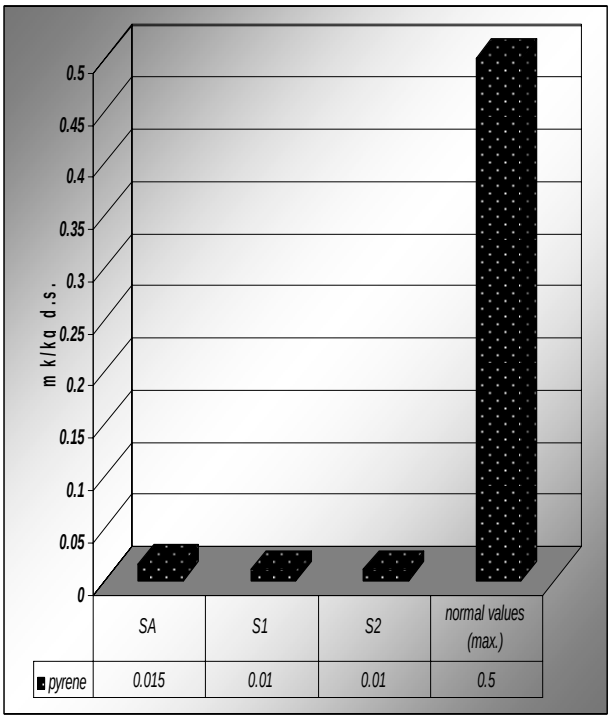

Fig.8 The pyrene content of the analyzed samples in comparison with the normal values

If for the benzo (b+k) fluoranthene and the phenanthrene the results obtained through the HPLC technique were close by the normal maximum values, the pyrene content of all the samples were lesser with 33.33 and 50 times than the normal maximum value.

4 Conclusion

Regarding the content of PAHs in soil from areas considered, we conclude that despite these contaminants, they do not reach the limits laid down in European standards.

PAHs are found in surface soils, indicating pollution of the atmosphere. In rural areas, where the emissions from cars are smaller, but there is thermal processing of coal, which is likely to pollute the environment, level of PAHs is lower.

The least contaminated area is located away from major companies and road congestion. In the context of future research, the chain of traceability, it can be stated as cereals and fodder grown in these areas can be used as feed for animals to be slaughtered for human consumption in various forms.

But because PAHs are accumulated during the trophic chain, with final destination the human body, have been included in the priority substances of the Soil Framework Directive.

The distributions of PAHs in the environment and potentially human health risks have become the focus of much attention. but more frequent monitoring is required. Overall, EFSA reached a similar conclusion to JECFA, although they considered that high consumers of PAH contaminated food could be at some risk. Environmental pollution with PAHs research will continue to measure these contaminants in the flesh, blood and milk of lactating animals, as proposed tracing study.

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