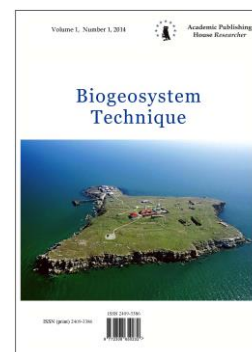


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Benzo(a)pyrene Uptake by Spring Barley Plants in Model Vegetation Experiment

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants. They are formed by incomplete combustion of organic materials (coal, wood, oil and petroleum products). The most dangerous compound among PAHs is benzo(a)pyrene (BaP), as it is a carcinogen and a class 1 mutagen and must be controlled in all natural environments. The aim of this study was to evaluate the accumulation of BaP in soil and plants under the conditions of a model vegetation experiment. The object of the study was spring barley, which was grown under the conditions of a model vegetation experiment, the soil of which was contaminated with various doses of BaP. The soil of the model experiment was incubated under conditions close to natural on the experimental site of the Southern Federal University (Russia, SFU) for 36 months. PAHs were extracted from soil samples using the ecologically clean express method of subcritical water extraction. The concentration of priority PAHs in soil samples was determined, and the concentration of BaP in the vegetative and root parts of barley plants was also determined. During 3 years of the experiment, there was a decrease in the concentration of PAHs in soil samples. The content of BaP in the soil and its accumulation in barley plants decreased during 3 years from the initial contamination.

Keywords: benzo(a)pyrene, PAHs, model experiment, soil contamination, spring barley, soil plant system, accumulation, migration, transformation, toxic effect.

1. Introduction

Long-term studies have made it possible to designate such a dangerous compound as benzo(a)pyrene (BaP), which requires measures for constant monitoring and regulation of its

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content in environmental objects, due to its extreme toxicity. (Tobiszewski, Namieśnik, 2012; Wenzl et al., 2006). Getting to the soil surface as a pollutant, BaP is included in the soil-plant system and migrates along the soil profile. Among the class of polycyclic aromatic hydrocarbons (PAHs), BaP is one of the most dangerous compounds, a carcinogen and a class I mutagen. The background values of the BaP content in ordinary chernozem and plants are 15-20 ng/g, which in turn is justified by the special physicochemical properties of organic substances in the chernozem, as well as by the specific composition of soil microorganisms (Kasimov et al., 2016; Sushkova et al., 2017). Polyaromatic hydrocarbons (PAHs) are usually present in water, air, soil, food and living organisms and persist in the environment for a long time (El-Shahawi et al., 2010). PAHs are relatively inert compounds that are resistant to various degradation processes, and therefore it is usually assumed that they are stable in the environment (Liu et al., 2005). Plants are an integral component of terrestrial ecosystems and are exposed to the entire group of pollutants, and almost 45% of all PAHs released into the environment end up in plants (Eisler, 1987; Huang et al., 2004). The complex of interaction of soils and plants is an important object of environmental pollution control by BaP. Since soils and plants function in close relationship with each other, a whole system arises that links the processes of accumulation and transformation of BaP in soils and plants (Yakovleva et al., 2008). Plants react very quickly to the presence of PAHs, as they influence primary and secondary processes (Tomar et al., 2004). The effect of each PAH can be specific for different plant species and even varieties (Simonich, Hites, 1995). The assimilating organs of plants are a regulatory link in the functioning of the whole organism; they are highly sensitive and stable in response to the action of external factors (Glinushkin, 2008). The carcinogenic effect of BaP consists in the interaction of its metabolites with proteins and nucleic acids, which causes disturbances leading to the transformation of normal cells into tumor cells (Kireeva et al., 2009). The ability of BaP to accumulate in plants and migrate in the soil depends mainly on the sorption properties of the soil matrix, as well as on its physicochemical properties, first of all, its water solubility and the ability to pass into the soil solution (Gennadiev, Tsibart, 2013; Belykh, 2009).

The aim of the study was to evaluate the benzo(a)pyrene uptake by spring barley plants the conditions of a model vegetation experiment.

2. Research objects

In the experiment, it was used soil and plant samples taken from the top soil layer of 0-20 cm in the virgin area of the Persianovskiy soil nature reserve. The studied soil is Haplic Chernozem with the following characteristics: physical clay content – 52%, silt – 30%, humus – 4.2%, pH_{water} – 7.5, CaCO_3 – 0.4%, CEC – 33.0 cmol (+)/kg. The enzymatic activity of the studied soil was determined: catalase – 4.0 ± 0.3 ml O_2 /g, urease – 5.6 ± 5.0 mg NH_4^+ /10 g, invertase – 1.45 ± 0.09 mg glucose/g. Respiration of soil – 0.11 mg CO_2 /g per day. The initial number of different groups of microorganisms in the soil, assessed by the method of seeding on selective media: heterotrophs – $3.7 \pm 0.5 \times 10^6$ /g; actinomycetes – $4.5 \pm 0.04 \times 10^5$ /g, micromycetes – $3.8 \pm 0.1 \times 10^4$ /g.

Conditions for the vegetation experiment. The studies were carried out under the conditions of a vegetation experiment. The soil was sifted through a sieve with a diameter of 1 mm and placed in 2 kg pots in 4l pots. A solution of BaP in acetonitrile was added to the soil surface based on the creation of a pollutant concentration in the soil of 20, 200, 400, and 800 $\mu\text{g}/\text{kg}$, which corresponds to 1, 10, 20, and 40 MPC. The initial soil and the soil supplemented with pure acetonitrile were used as controls. The experiment was repeated three times. The incubation of the soil in the vessels took place in conditions close to natural conditions, on the territory of a special experimental site of the Southern Federal University for 36 months. To maintain the optimal amount of moisture in the incubation vessels, the soil was watered with distilled water. 1 and 2 years after the start of incubation, the vessels were inoculated with a test culture of spring barley of the "Odessky-100" variety. After 12, 24 and 36 months, before sowing spring barley, a heart sample was taken after stirring it in vessels. In the samples of the initial and incubated soil, the content of BaP was determined at the indicated time by HPLC with preliminary sample preparation of soil samples by subcritical water extraction. Barley plants that were removed from the vessel were cleaned of soil particles, washed with water, dried, weighed, crushed, sieved through a sieve. 1 mm and used the middle sample for analysis. The content of BaP was determined in them by subcritical water extraction.

3. Materials and methods

Soil samples were taken annually and prepared for chemical analysis in accordance with the requirements (GOST, 1986; ISO, 2005). To extract BaP from soil and plant samples, we used the method of extraction with subcritical water (Lekar et al., 2014; Sushkova et al., 2014). Whole barley plants grown in the control background soil were used as a plant sample.

The sequence of the analysis. A weighed portion of a specially prepared soil or plant sample weighing 1 g, sifted through a 1 mm sieve, was placed in a stainless steel extraction cartridge (Sushkova et al., 2014) and hermetically tightened with bolts from both sides. The cartridge was installed in a thermostat and heated to 250°C and a pressure of 100 atm within 30 minutes. After cooling the system, the cartridge was unscrewed, the contents were filtered two or three times until the solution became clear. The resulting aqueous extract was mixed with 5 ml of n-hexane (analytical grade) and placed on a mechanical shaker for 15 minutes. Separation of layers was carried out on a 50 ml separating funnel sequentially in three stages with another portion of hexane. The combined hexane extract was passed through a funnel with anhydrous sodium sulfate into a clean dry round-bottom flask, evaporated on a rotary evaporator at a water bath temperature of 40°C to dry residue. The resulting dry residue was dissolved in 1 ml of acetonitrile with stirring for 30 minutes.

The quantitative determination of BaP in extracts was carried out by high performance liquid chromatography (HPLC) on an AGILENT 1260 instrument. The BaP peak in the chromatogram of the soil extract was identified by comparing the retention time of BaP in the analysis of the extract and the standard sample with simultaneous detection on two detectors. This makes it possible to identify the BaP peak with a sufficiently high degree of reliability and more accurately determine its concentration in the extract. The BaP content in the analyzed samples was calculated using the external standard method. The BaP content in the soil was calculated using the formula:

$$C_n = k \times S_i \times C_{st} \times V / (S_{st} \times m),$$

where C_n is the BaP content in the soil sample ($\mu\text{g}/\text{kg}$); k is the coefficient of extraction of BaP from the sample; S_i and S_{st} are the BaP peak areas of the standard solution and sample; C_{st} is the concentration of a standard BaP solution ($\mu\text{g}/\text{ml}$); V is the volume of the acetonitrile extract (ml); m is the mass of the sample (g).

4. Results and discussion

PAH content in soil and plants of a model vegetation experiment for 3 years

The concentration of priority PAHs in the soil of the model experiment was determined in the first year of the experiment (Figure 1), in the second year (Figure 2) and in the third year (Figure 3).

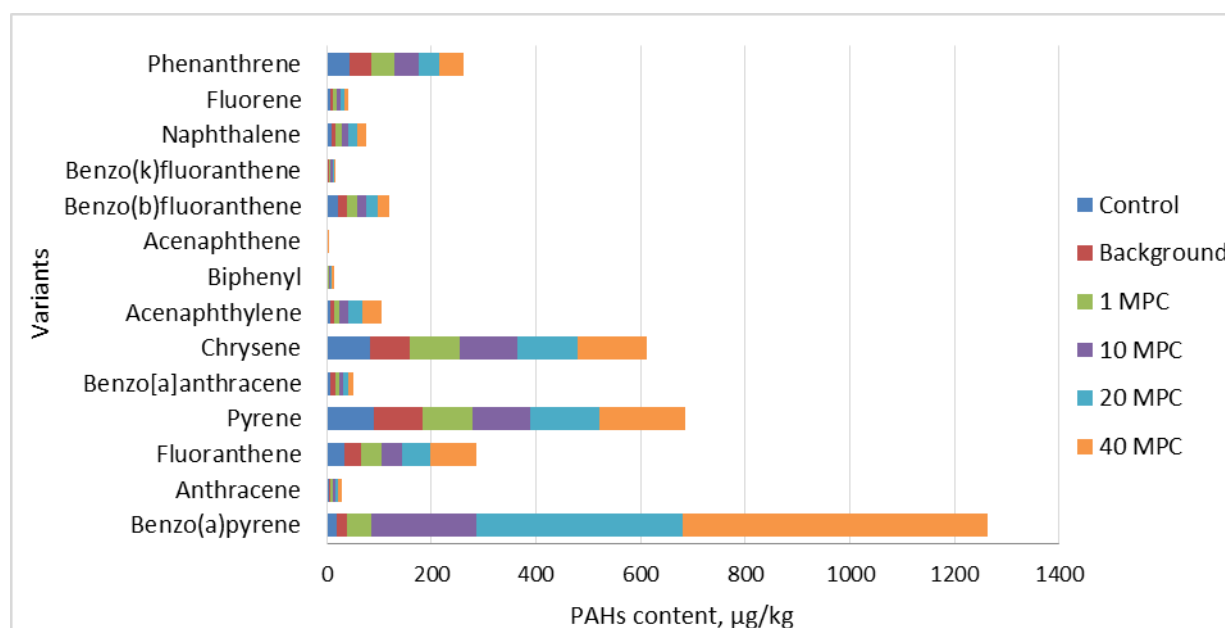


Fig. 1. The content of PAHs in the soil of the model experiment in the 1st year

The control sample and the background sample contained a small amount of such PAHs as phenanthrene, chrysene and pyrene in concentrations equal to 42.4-43.6; 76.3-82.1 and 90.1-92.3 $\mu\text{g}/\text{kg}$, respectively. In the sample with the introduction of 1 MPC BaP, only the concentration of BaP changed, the concentration of the remaining PAHs did not change relative to the control values. With the introduction of 10 MPC, the concentration of BaP in the sample increased, and the concentration of chrysene and pyrene also increased to 109 $\mu\text{g}/\text{kg}$ relative to the control. A similar increase in the concentration of chrysene and pyrene occurred in the samples with the addition of 20 and 40 MPC BaP, but with the introduction of 40 MPC, the concentration of fluoranthene increased up to 82 $\mu\text{g}/\text{kg}$ by 3 times relative to the control values.

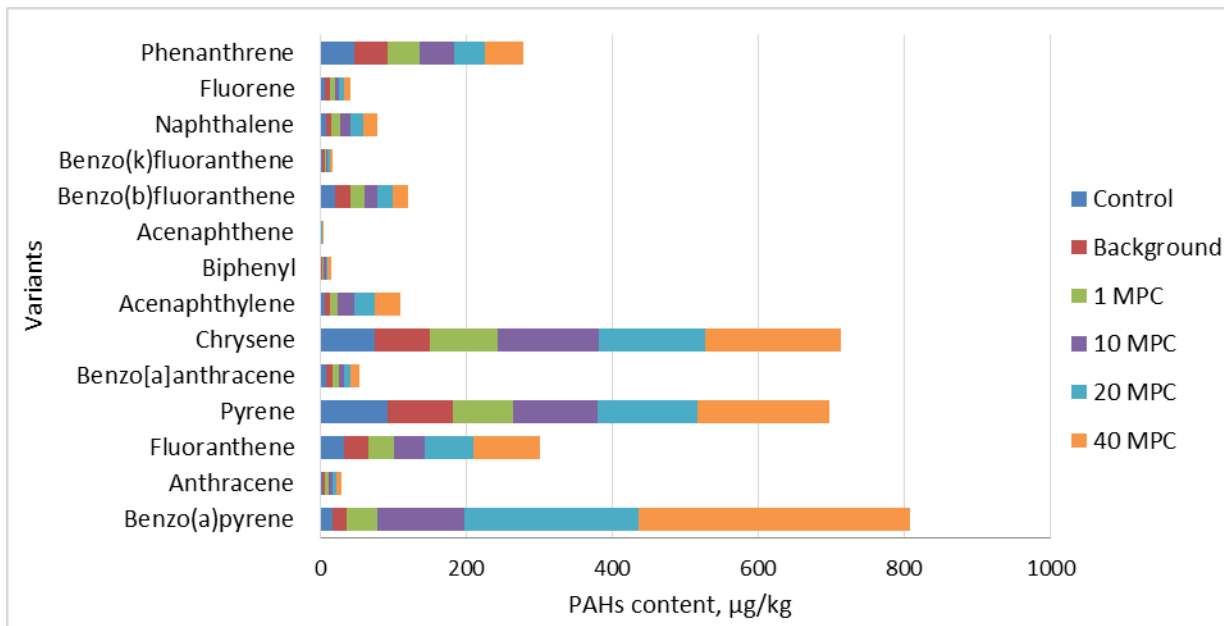


Fig. 2. PAH content in the soil of the model experiment in the 2nd year

In the second year of the experiment, the BaP was observed to decrease in content in relation to the first year of the study. In the sample with 1 MPC BaP, changes in the concentration of Other PAHs are not significant. However, in the samples with the introduction of 10, 20 and 40 MPC, with a decrease in the concentration of BaP, the concentration of chrysene increased by 26.9-40 % and the concentration of pyrene by 3-9 % relative to the first year, as well as in the sample with the introduction of 40 MPC of BaP, the concentration of fluoranthene increased by 6 %. In the control and background samples, no changes in the concentration of PAHs were observed.

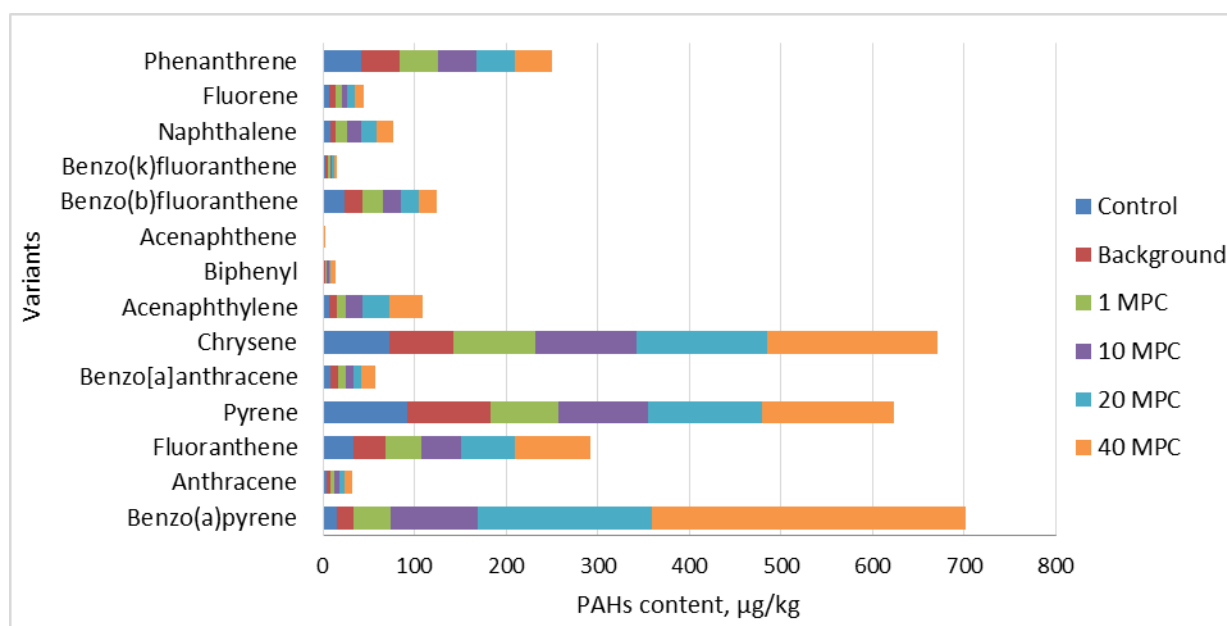


Fig. 3. PAH content in the soil of the model experiment in the 3rd year

In the third year of the experiment, similar to the second year, there was a decrease in the concentration of BaP in the samples, but in all samples there was a decrease in the concentration of the remaining PAHs, approximately to the level of concentration in the first year of the study.

The concentration of BaP in the soil of the model experiment was determined for three years (Figure 4).

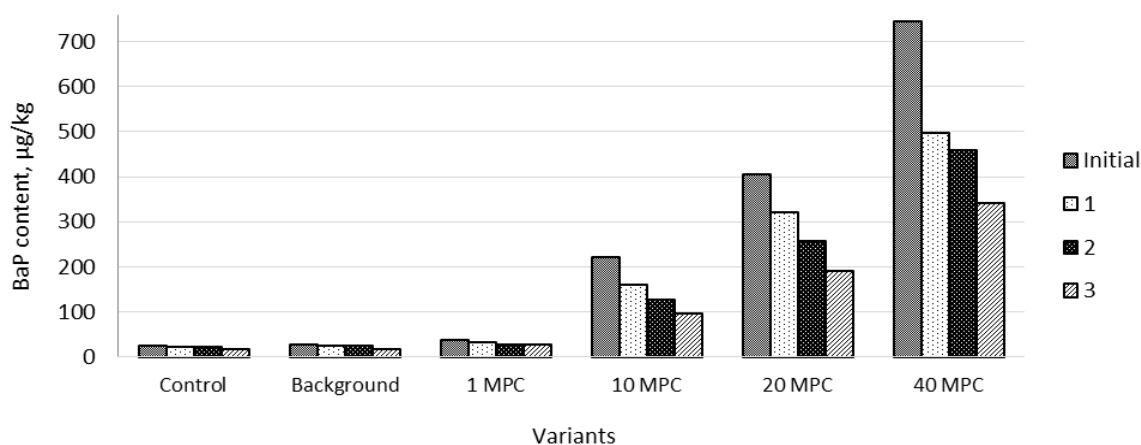


Fig. 4. Concentration of BaP in the soil of the model experiment for three years

In the initial soil in the control sample and with the addition of acetonitrile (background), the concentration of BaP was at the level of 24.3 and 27.2 µg/kg, respectively. With the introduction of 1 MPC BaP, the concentration increased to 45.4 µg/kg. In samples with 10, 20, and 40 MPC BaP, the concentration of the pollutant increased to 221, 406, and 743.4 µg/kg, respectively.

In the first year of the experiment, the concentration of BaP slightly decreased in the control and background samples to 21 µg/kg in the control and to 25 in the background. Also, a slight decrease in the concentration of BaP occurred in the sample with the addition of 1 MPC BaP up to 31 µg/kg. More significant reductions occurred in samples with 10, 20 and 40 MPC BaP to values equal to 159, 321, and 497 µg/kg, respectively.

In the second and third years, there was a gradual decrease in the concentration of BaP. During the second year of the experiment, a significant decrease in the concentration of BaP occurred in the samples with the introduction of 10, 20, and 40 MPCs of BaP to values of 127, 256,

and 458 $\mu\text{g}/\text{kg}$, respectively, and in the third year, the concentration of BaP in these samples decreased to 95, 190, and 342 $\mu\text{g}/\text{kg}$, respectively. In the second year of the experiment, no significant decrease in the concentration of BaP was found in the control and background samples, and in the third year, the values decreased to 15 $\mu\text{g}/\text{kg}$ in the control and 17 in the background samples. In the sample with the introduction of 1 MPC BaP, the concentration of BaP in the second year decreased to 27 and in the third year did not change and does not differ significantly.

We obtained data on the concentration of BaP in the roots (Figure 5) and vegetative part of the plants (Figure 6) for three years of the experiment. In the vegetative part, the concentration of BaP was lower than in the root part. The increase in the accumulation of BaP occurred in proportion to the increase in its concentration in the soil of the model experiment. The minimum accumulation of the pollutant in plant parts was observed in the control sample and reached values equal to 0.8 $\mu\text{g}/\text{kg}$ in the roots and 0.3 $\mu\text{g}/\text{kg}$ in the vegetative part, and the maximum in the sample with the introduction of 40 MPC BaP in the first year of the experiment and reached 23.4 $\mu\text{g}/\text{kg}$ in the root and 12.4 $\mu\text{g}/\text{kg}$ in the vegetative.

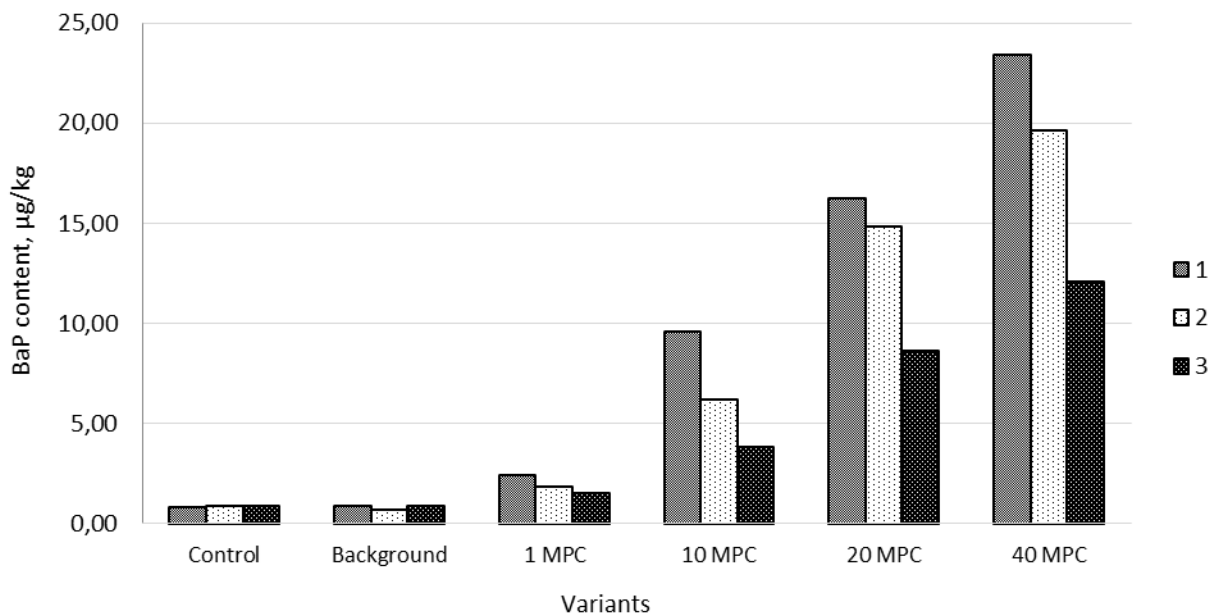


Fig. 5. Concentration of BaP in barley roots for three years

In the first year of the experiment, 0.8 and 0.9 $\mu\text{g}/\text{kg}$ BaP were found in the plant roots in the control sample and the sample with the addition of acetonitrile. When 1 MPC BaP was introduced into the soil, 2.4 $\mu\text{g}/\text{kg}$ BaP accumulated in the roots, which is higher than the control values. With an increase in the dose of introduced BaP to 10, 20 and 40 MPC, BaP accumulated in plant roots in values equal to 9.6, 16.2, and 23.4 $\mu\text{g}/\text{kg}$, these values are 12.20 and 29 times higher than the values obtained in the control sample.

In the second and third years of the experiment, the accumulation of BaP by the roots decreased. Thus, in the sample with the introduction of 1 MPC BaP in the second year, the accumulation in the roots decreased to 1.8 $\mu\text{g}/\text{kg}$ of BaP, and in the third to 1.5 $\mu\text{g}/\text{kg}$, which is comparable to the control. In samples with doses of BaP introduced at 10.20 and 40 MPC, the accumulation of BaP by plant roots decreased and reached values in the second year: 6.2; 14.8 and 19.6 $\mu\text{g}/\text{kg}$, and in the third year, values were obtained equal to 3.8; 8.6 and 12.1 $\mu\text{g}/\text{kg}$, respectively.

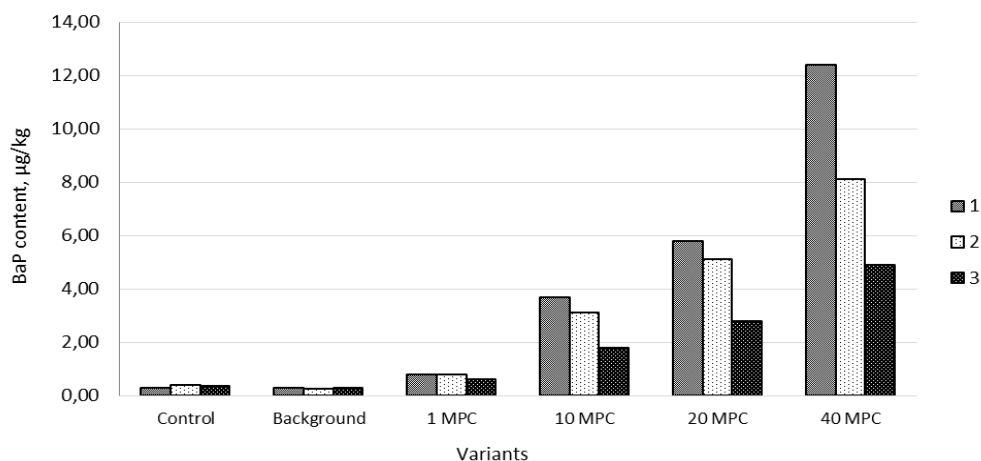


Fig. 6. Concentration of BaP in the vegetative part of barley for three years

In the first year of the experiment in the vegetative part of the plants in the control sample and with the addition of acetonitrile, the BaP content was 0.3 and 0.28 µg/kg, respectively. The accumulation of BaP in the vegetative part in the sample with the addition of 1 MPC BaP to 0.8 µg/kg slightly increased from the control. However, in the samples with the introduction of increased doses of BaP equal to 10, 20 and 40 MPC, the accumulation in the vegetative part of the plant reached values equal to 3.7; 5.8 and 12.4, which are 12, 19 and 41 times higher than the control values, respectively.

Thus, in the second and third years of the experiment, the values of BaP accumulation in the vegetative part of plants decreased in the samples with the addition of 10, 20, and 40 MPCs for BaP. In the second year, the values dropped to 3.1; 5.1 and 8.1 µg/kg, and in the third year up to 1.8; 2.8 and 4.9 µg/kg, respectively. In the control sample, in the sample with the addition of acetonitrile, and in the sample with the addition of 1 MPC of BaP, no significant differences were found in the accumulation of BaP in the vegetative part of the plants.

5. Conclusion

For the first time, the features of accumulation, migration and transformation of benzo(a)pyrene (BaP) in the soil-plant system were studied using original methods of analysis under the conditions of a unique model experiment with ordinary carbonate chernozem contaminated with BaP. This model can be used to predict the situation in different regions prone to contamination with such hazardous organic pollutants as PAHs, as well as to assess the likelihood of PAH accumulation in plants and their involvement in the food chain.

The concentration of priority PAHs in the soil of the model experiment was determined. During the three years of the experiment, the total concentration of PAHs in the soil in the control and with the addition of acetonitrile (background) was in the range of 382-327 and 379-322 µg/kg, respectively. In the sample with the introduction of 1 MPC BaP, the concentration of PAHs was 445-363 µg/kg, and with the introduction of large doses of PAHs, such as 10, 20 and 40 MPC, the concentration of PAHs was within the limits of 666-476, 920-658 and 1234-920 µg/kg, respectively.

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References

- Belykh, 2009 – Belykh, L.I. (2009). Raspredeleniye politsiklicheskikh aromaticseskikh uglevodorodov v sisteme pochva rasteniye [Distribution of polycyclic aromatic hydrocarbons in the soil-plant system]. *Pochvovedeniye*. 9: 1083-1089. [in Russian]
- Eisler, 1987 – Eisler R. (1987). Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates: a synoptic review. *Fish and Wildlife Service, US Department of the Interior*. 11.

- El-Shahawi et al., 2010 – El-Shahawi, M. S., Hamza, A., Bashammakh, A. S., Al-Saggaf, W. T. (2010). An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Talanta*. 80(5): 1587-1597.
- Gennadiev, Tsibart, 2013 – Gennadiev, A.N., Tsibart, A.S. (2013). Faktory i osobennosti nakopleniya pirogennykh politsiklicheskikh aromatcheskikh uglevodorodov v pochvakh zapovednykh i antropogenno-izmenennykh territoriy [Factors and features of the accumulation of pyrogenic polycyclic aromatic hydrocarbons in the soils of protected and anthropogenically altered territories]. *Pochvovedeniye*. 1: 32-32. [in Russian]
- Glinushkin, 2008 – Glinushkin, A.P. (2008). Agriculture plant protection from pathogens in oil-gas discharge environment. *Oil-Extraction Industry*. 11: 119-120. [in Russian]
- GOST, 1986 – GOST 26423-85. Pochvy. Opredeleeniye pH vodnoy vytyazhki po metodu TSINAO [Soils. Determination of the pH of the aqueous extract by the CINAO method]. M.: Izd-vo Standartov, 1986. [in Russian]
- Huang et al., 2004 – Huang, X.D., El-Alawi, Y., Penrose, D.M., Glick, B.R., Greenberg, B.M. (2004). A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. *Environmental pollution*. 130(3): 465-476.
- ISO, 2005 – ISO 13877-2005. Soil Quality – Determination of Polynuclear Aromatic Hydrocarbons – Method Using High-performance Liquid Chromatography, 2005.
- Kasimov et al., 2016 – Kasimov, N.S., Vlasov, D.V., Kosheleva, N.E., Nikiforova, E.M. (2016). Geokhimiya landshaftov Vostochnoy Moskvy [Geochemistry of landscapes of Eastern Moscow]. M.: Izd-vo APR. P. 276. [in Russian]
- Kireeva et al., 2009 – Kireeva, N.A., Novoselova, E.I., Erokhina, N.I., Grigoriadi, A.S. (2009). Nakopleniye benz(a)pirena v sisteme «Pochva rasteniye» pri zagryaznenii neft'yu i vnesenii aktivnogo ilya [Accumulation of benzo (a) pyrene in the "Soil plant" system during oil pollution and the introduction of activated sludge]. *Vestnik Orenburgskogo gosudarstvennogo universiteta*. 6. [in Russian]
- Lekar et al., 2014 – Lekar, A.V., Borisenko, S.N., Vetrova, E.V., Sushkova, S.N., Borisenko, N.I. (2014). Extraction of quercetin from *Polygonum hydropiper L.* by subcritical water. *American Journal of Agricultural and Biological Sciences*. 9(1): 1.
- Liu et al., 2005 – Liu, X., Zhang, G., Jones, K. C., Li, X., Peng, X., Qi, S. (2005). Compositional fractionation of polycyclic aromatic hydrocarbons (PAHs) in mosses (*Hypnum plumaeformae* WILS.) from the northern slope of Nanling Mountains, South China. *Atmospheric Environment*. 39(30): 5490-5499.
- Simonich, Hites, 1995 – Simonich S.L., Hites R.A. (1995). Organic pollutant accumulation in vegetation. *Environmental Science & Technology*. 29(12): 2905-2914.
- Sushkova et al., 2014 – Sushkova, S.N., Vasilyeva, G.K., Minkina, T.M., Mandzhieva, S.S., Tjurina, I.G., Kolesnikov, S.I., Kizilkaya R., Askin, T. (2014). New method for benzo[a]pyrene analysis in plant material using subcritical water extraction. *Journal of Geochemical Exploration*. 144: 267-272.
- Sushkova et al., 2017 – Sushkova, S.N., Minkina, T.M., Mandzhieva, S.S., Tyurina, I.G., Vasilyeva, G.K., Kizilkaya, R. (2017). Monitoring sodержaniya benz(a)pirena v pochvakh pod vliyaniem mnogoletnego tekhnogennogo zagryazneniya [Monitoring of benzo (a) pyrene content in soils under the influence of long-term technogenic pollution]. *Pochvovedeniye*. 1: 105-116. [in Russian]
- Tobiszewski, Namieśnik, 2012 – Tobiszewski, M., Namieśnik, J. (2012). PAH diagnostic ratios for the identification of pollution emission sources. *Environmental Pollution*. 162: 110-119.
- Tomar, Jajoo, 2014 – Tomar, R. S., Jajoo, A. (2014). Fluoranthene, a polycyclic aromatic hydrocarbon, inhibits light as well as dark reactions of photosynthesis in wheat (*Triticum aestivum*). *Ecotoxicology and Environmental Safety*. 109: 110-115.
- Wenzl et al., 2006 – Wenzl, T., Simon, R., Anklam, E., Kleiner, J. (2006). Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union. *TrAC Trends in Analytical Chemistry*. 25(7): 716-725.
- Yakovleva et al., 2008 – Yakovleva, E.V., Beznosikov, V.A., Kondratenok, B.M., Gabov, D.N., Vasilevich, M.I. (2008). Bioakkumulyatsiya politsiklicheskikh aromatcheskikh uglevodorodov v sisteme pochva-rasteniye [Bioaccumulation of polycyclic aromatic hydrocarbons in the soil-plant system]. *Agrohimiya*. (9): 66-74. [in Russian]