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Experimental Study of Polycyclic Aromatic Hydrocarbons Impact on Conifers

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ABSTRACT

The present work is aimed at modeling artificial aerial treatment of trees with polycyclic aromatic hydrocarbons (PAHs) specific to technogenic emissions, at exploring peculiarities of accumulating these compounds by needles and evaluating their negative impact on plants by the change in the content of photosynthetic pigments and biogenic elements (nitrogen, phosphorus, magnesium, potassium, sodium). Scots pine (*Pinus sylvestris* L.) 8-10-year-old trees were treated with the mixture of 6 PAHs specific to technogenic emissions (naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene). After 2 months of treatment, PAHs accumulation in pine needles (surface content – accumulated by cuticular wax layer, internal content – accumulated by needle internal tissues and total) was determined. The portion of PAHs accumulated in cuticular wax layer was shown to be much smaller (18% of the total sum) than their content in needle internal tissues (82%). Accumulation of individual substances by needles varied significantly: pyrene was "the most accumulated" substance, whose overall content exceeded control values by more than tenfold while fluorene was the "least accumulated" substance with its content exceeded control values less than twice. The needles of test trees demonstrated statistically significant decrease in chlorophyll *a*, *b* levels (by 15-25% of control) and carotenoids (20% of control), increase in total nitrogen content, mainly due to the rise of its non-protein fraction, and also decrease of magnesium and sodium levels.

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INTRODUCTION

Within the framework of investigation of the impact of persistent organic pollutants (POPs) on live organisms, polycyclic aromatic hydrocarbons are an object of specific attention, as individual compounds of this group possess mutagenic and carcinogenic properties, and, according to WHO experts, fall under the category of genotoxic carcinogens [1]. PAHs are widely distributed air pollutants, sources of which can be both anthropogenic (any technological process involving combustion of organic matter, especially burning of fossil fuels at power plants and in motor vehicle engines) and natural (forest fires, volcanic eruptions, natural oil seeps, decay of organic matter) [2]. There is much more scarce evidence concerning PAHs negative influence on plants, with the available data mainly focusing on benzo[a]pyrene [3-5]. The impact of benzo[a]pyrene on plant organism has been mainly studied on cell level. Experiments have demonstrated changes in RNA and protein synthesis activity [6], inhibition of mitotic processes and morphological changes of cells [3], emergence of disturbances in ultrastructure of ribosomes, mitochondria and nucleus [7]. The latest publications argue that other representatives of PAHs also produce negative influence on plant organisms. Thus, there was monitored reduction in total content of green pigments, magnesium and ribulose 1,5-bisphosphate carboxylase in the needles of 2 years old seedlings of *Pinus densiflora* following treatment with high concentrations of phenanthrene and fluoranthene [8]. *In vitro* studies established that addition of a mixture of phenanthrene, pyrene and benzo[a]pyrene to the cultivation medium results in suppression of grain crops growth and fall of their survival [9]. Tests manifested reduction of root biomass accumulation in some cultural plants (radish, lettuce) following anthracene application to the soil [10].

Identification of herbaceous and arboreal plant species for monitoring atmospheric pollution with organic pollutants is another promising area of research [11, 12]. Results of such studies are of great significance for

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defining toxic agents, which are most hazardous for natural environment and humans. Thus, a number of countries has already approved lists of such toxic agents including PAHs, which, along with their derivatives and analogues, account for a large part of the known carcinogenic compounds [13, 14]. In the course of environmental monitoring most researchers take in consideration the list comprising 16 priority (most frequent and toxic) PAHs [15].

Our studies are also related to the monitoring of natural environments pollution with PAHs, as this issue is critical for many regions locating numerous industrial enterprises whose exhaust might contain considerable amounts of PAHs [16]. However, we largely focus our attention on PAHs impact on arboreal plants and forest pollution monitoring. Thus, the possibility of using Scots pine (*Pinus sylvestris* L.) needles as a natural matrix accumulating PAHs of the priority list has been demonstrated [17]. Levels of PAHs accumulation in the needles in the territories subjected to various degrees of technogenic pollution including exhaust of aluminum plants, heat power plants and automobiles have been identified. Strong positive correlations have been found not only between atmospheric air pollution by PAHs and accumulation of these substances in pine needles, but also between PAHs content in tree needles and degree of tree oppression, which suggests presence of phytotoxic effect caused by PAHs [18].

It is known that reliable evidence of negative impact of PAHs on plants should be based on the results of their direct impact without combination with other polluting agents, which is only possible in the experiment using artificial application of these compounds to plants. Such kind of investigations is difficult due to a number of factors, the most important of which are high hydrophobic properties of many PAHs complicating preparation of water solutions of the required concentration for plant treatment and unacceptability of using solutions on the basis of organic solvents since many of them themselves may be toxic to plants [19]. Due to the above, our first data on negative impact of PAHs were acquired in the experiments with the use of plant cells suspension cultivar [20].

At the next stage we attempted to investigate PAHs influence on the whole plant. Judging by the data provided by a number of authors, PAHs impact on plant organism through soil treatment does not ensure unambiguous result, as transport of PAHs absorbed by roots towards above-ground plant parts with transpiration current is complicated due to PAHs low water solubility [21-23]. PAHs accumulated in plant above-ground parts in polluted territories are deemed to arrive their by air [21, 22, 24, 25].

The present work is aimed at modeling artificial aerial treatment of trees by PAHs present in technogenic emissions, at exploring peculiarities of accumulating these compounds by needles and evaluating their negative impact on plants by the change in the content of photosynthetic pigments and biogenic elements (nitrogen, phosphorus, magnesium, potassium, sodium) in the needles.

MATERIALS AND METHODS

Scots pine (*Pinus sylvestris* L.) which is the prevailing coniferous species in the area was selected as an object of the study due to its high sensitivity to air pollution. The experiments was performed on 8-10 years old Scots pine trees which had been planted on the experimental plot in 2012 and next year during the vegetation period were covered with film chambers (at night time the chambers were opened) and treated with PAHs mixture for 2 months. The mixture consisted of naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene was dissolved in 1% methanol-water solution to significantly increase the compounds solubility without increasing the tested substances impact on plants, as in low concentrations (up to 10% of the volume) methanol in methanol-water solutions is not dangerous to plants [26]. The mixture of substances tested was dispersed with a trigger sprayer inside the chambers. Taking into account chamber volume (1 m^3), the amount of the mixture sprayed inside a chamber at once and concentration of tested substances in the mixture, the PAHs concentrations achieved inside the chambers after spraying the mixture are estimated to be: naphthalene – 40 mcg m^{-3}, acenaphthene – 20 mcg m^{-3}, fluorene – 30 mcg m^{-3}, phenanthrene – 30 mcg m^{-3}, fluoranthene – 30 mcg m^{-3}, pyrene – 30 mcg m^{-3}. Such concentration corresponded to PAHs levels found in the exhaust of electrolysis shops of aluminum plants [14]. Control tree group was not exposed to the PAHs treatment, but was also covered with film chambers.

Upon completion of the experiment quantitative determination of PAHs content in the needles was conducted by chromatography-mass spectrometry with preliminary PAHs ultrasonic-assisted extraction with *n*-hexane [27]. PAHs accumulation on the surface waxy layer and in the internal tissues of the needles were determined separately in order to evaluate the degree of penetration of these substances into assimilative organs. Chlorophyll *a*, *b* and carotenoids content in the needles of tested and control plants was measured by spectrophotometry with ethanol extraction [28, 29]. Content of biogenic elements (nitrogen, phosphorus, potassium, calcium, magnesium, sodium) was determined by atomic absorption and flame photometry methods [30]. All the data acquired were statistically processed using the R computing environment.

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RESULTS AND DISCUSSION

The data acquired by separate evaluation of PAHs levels in waxy layer and in needle internal tissues suggest that airborne PAHs accumulate mainly in internal tissues of pine needles while a significantly smaller portion of them accumulate on needle surface (in waxy layer), as observed in both experimental and control groups of trees. Remarkable is the similarity in PAHs total content distribution between internal and external “layers” of needles both in control and test (treated) trees: a smaller PAHs share is in the waxy layer and a larger one is in the internal organs despite the fact that control and test trees considerably differed in total PAHs content (Fig. 1).

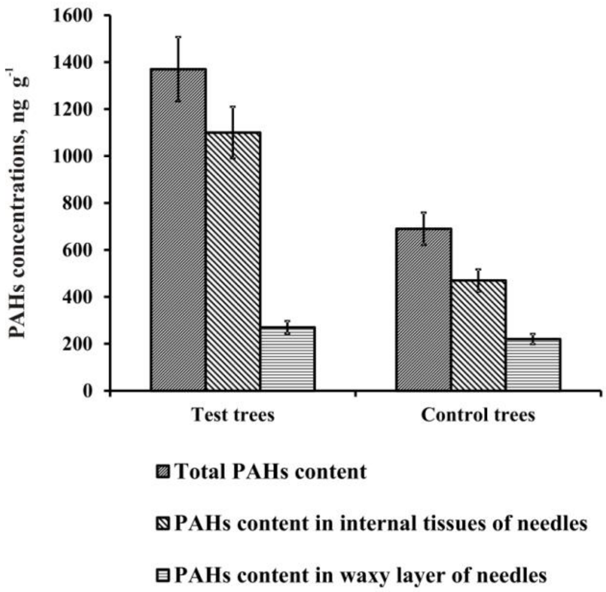

Fig. 1: Polycyclic aromatic hydrocarbons content in the needles of control and test trees: total, accumulated by internal tissues, absorbed by waxy layer.

Quantification of individual PAHs accumulated in needles of test trees demonstrated that pyrene was most accumulated, with its total content exceeding control values 12-fold. Concentration of acenaphthene in the needles of test trees exceeded control values by about 6 times; values of naphthalene, fluorene, phenanthrene and fluoranthene accumulated by needles in the test exceeded their concentrations in control by 1,5-2,5 times (Table 1). Distribution of accumulated individual PAHs between needle waxy layer and its internal tissues was similar to distribution of total PAHs content – a smaller share was found in waxy layer, a larger share – in internal tissues. This regularity is particularly salient, when the data acquired are converted into percentages (Table 2). But there are also exceptions – fluoranthene and pyrene levels in waxy layer of control trees are higher than in needle internal tissues; this may be due to their higher hydrophobic property entailing an initial slightly higher accumulation on the needles at background concentrations.

Table 1: Accumulation of individual polycyclic aromatic hydrocarbons (ng g^{-1}) and their sums in needle internal tissues and on the surface of the needles of control and test pine trees (P = 0,95; δ = 10 %).

PAHs	Control			Test		
	Waxy layer	Internal needles tissue	Total amount	Waxy layer	Internal needles tissue	Total amount
Naphthalene	120 ± 10	240 ± 24	360 ± 36	130 ± 13	450 ± 45	580 ± 60
Acenaphthene	1,1 ± 0,1	14 ± 1	15 ± 2	3,5 ± 0,4	91 ± 9	95 ± 10
Fluorene	2,7 ± 0,3	28 ± 3	31 ± 3	1,6 ± 0,2	49 ± 5	50 ± 5
Phenanthrene	34 ± 3	170 ± 17	200 ± 20	34 ± 3	280 ± 30	310 ± 30
Fluoranthene	14 ± 1	8,3 ± 0,8	22 ± 2	21 ± 2	36 ± 3,6	57 ± 6
Pyrene	10 ± 1	7,7 ± 0,8	18 ± 2	44 ± 4	160 ± 16	200 ± 20
Total PAHs	180 ± 20	470 ± 50	650 ± 65	230 ± 23	1100 ±100	1300 ± 130

Table 2: Polycyclic aromatic hydrocarbons distribution (%) between pine needles waxy layer and internal tissues in control and after artificial treatment of trees (P = 0,95; δ = 10 %).

PAHs	Control		Test	
	Waxy layer	Internal needles tissue	Waxy layer	Internal needles tissue
Naphthalene	33	67	22	78
Acenaphthene	7	93	3	97

Fluorine	9	91	4	96
Phenanthrene	17	83	11	89
Fluoranthene	64	36	37	63
Pyrene	56	44	22	78
Total PAHs	28	72	18	82

Available literature data indicate that PAHs waxy layer-internal tissues partitioning is mainly depends on their phase, with gaseous PAHs capable to penetrate via stomata or diffuse through epicuticular waxes and cuticular membrane and accumulate in needle internal tissues while particle-bound PAHs tend to accumulate on needle surface immersed in epicuticular waxes [31]. The atmospheric partitioning of PAHs between the particulate and the gaseous phases in its turn is determined by PAHs molecular weight: lower weight substances with 2 and 3 rings existing predominately in vapor form, while higher molecular weight PAH with 5 and 6 rings are mainly associated with the particles. Medium molecular weight PAHs with 4 rings can exist in both particle-bound form and vapor phase, depending on the ambient air temperature and vapor pressure and tend to volatilize into the vapor phase with summer increase in air temperature. This explains why in the experiment the investigated PAHs with 2-4 rings mainly accumulated in the internal tissues of the needles.

The results of photosynthetic pigments quantification show 25% decrease in chlorophyll *a* content, 15 % decrease in chlorophyll *b* content, 18% decrease in total chlorophyll content and 20% decrease in carotenoids level in the needles of test trees after PAHs treatment (Fig. 2).

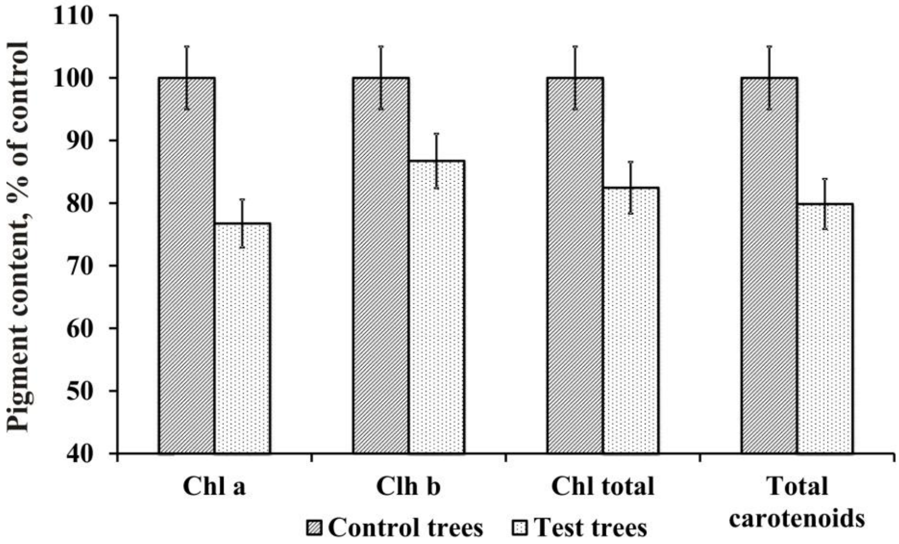

Fig. 2: Changes in the content of photosynthetic pigments in pine needles after artificial treatment with polycyclic aromatic hydrocarbons.

According to the data available, decrease in the content of photosynthetic pigments in plant assimilation organs is a non-specific reaction of plant organism action to unfavorable conditions, including toxic agents impact [32, 33]. Experimentally observed reduction in chlorophylls and carotenoids concentration in needles confirms negative influence of PAHs, which may be accounted for by pigments molecules destruction resulting from the activity of free-radical processes caused by these compounds [8]. Since pigments content characterizes potential photosynthetic ability of a plant, reduction in the efficiency of photosynthetic processes under the impact of PAHs may be assumed.

Biogenic elements analysis shows 26% increase in total nitrogen content and 11% decrease in protein nitrogen content of test trees needles; non-protein nitrogen content increased by 2.4 times as compared to the control group (Fig. 3).

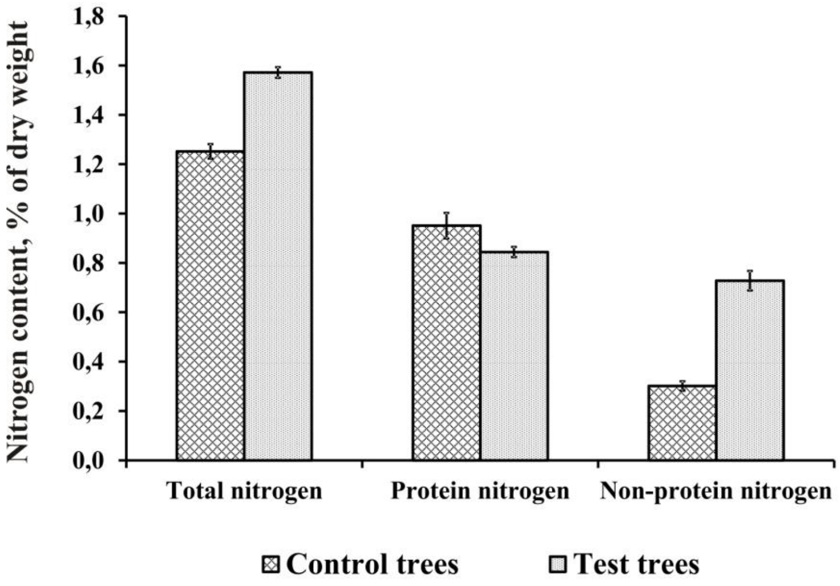

Fig. 3: Changes in the content of nitrogen in pine needles after artificial treatment with polycyclic aromatic hydrocarbons.

Our previous work demonstrated that reduction in protein nitrogen content along with simultaneous increase in non-protein nitrogen content manifests deterioration of vital state of the trees exposed to unfavorable influences, including technogenic pollution [33]. Increase in trees oppression respectively involves reduction of N protein/N non-protein ratio in the needles due to the increase in the level of non-protein nitrogen and decrease in protein nitrogen content. Test trees needles demonstrated a change in the content of nitrogenous substances and their individual fractions, which proves negative influence on the plants.

Determination of other elements levels in pine needles demonstrated the general tendency towards reduction in their concentrations, although statistically reliable are only the data on reduction in magnesium and sodium concentration (Fig. 4). The level of stress load (PAHs impact) in this test might have been not high enough to change the content of all the elements measured. It may however be ascertained that PAHs do produce a certain negative effect on the level of biogenic elements in test trees needles.

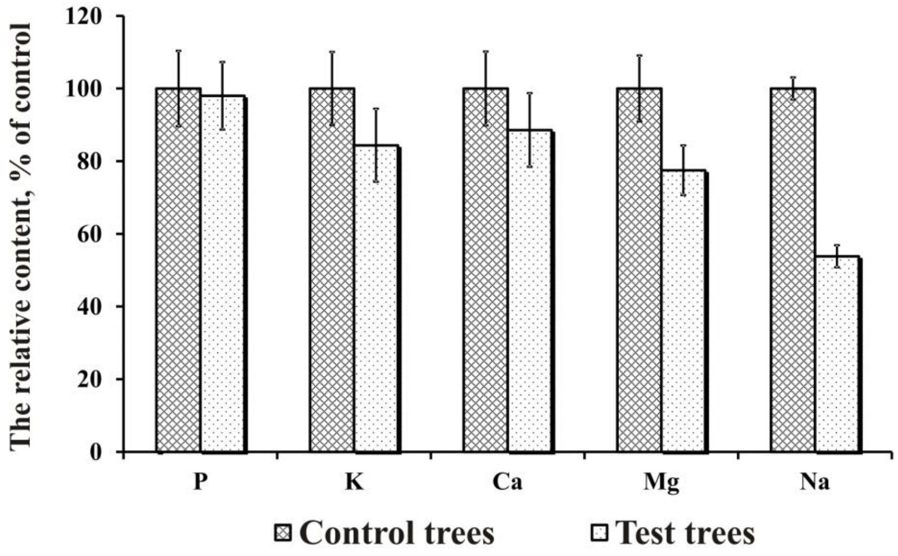

Fig. 4: Changes in the content of phosphorus, potassium, magnesium, calcium, sodium in pine needles following artificial treatment of trees with polycyclic aromatic hydrocarbons.

Conclusion:

The results of the experiments demonstrate the negative impact of PAHs specific for technogenic emissions on Scots pine trees. Significant PAHs accumulation in the needles (total, absorbed by waxy layer, and accumulated in needle internal tissues), changes in the content of photosynthetic pigments and biogenic elements in the needles were registered after 2 months treatment of trees with PAHs mixture comprising substances with the highest water solubility among this group of chemical compounds and prevailing in gaseous phase of technogenic exhaust (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, pyrene). The portion of PAHs absorbed on the needles surface, in waxy layer, was shown to be much smaller (18% of the total sum) than their content found in needle internal tissues (82%). Accumulation of individual substances by the needles varies significantly – fluorene was the least accumulated substance, pyrene – the most accumulated substance, whose overall content (in waxy layer and needles internal tissues) exceeded control values by more than one order. Determination of the content of photosynthetic pigments in test trees needles has demonstrated statistically significant decrease in the level of chlorophylls *a, b* (by 15-25% of control) and carotenoids (20% of control). Changes in the content of biogenic elements in the test tees needles showed in statistically significant increase in total nitrogen content, mainly due to the rise of its non-protein fraction and decrease in magnesium and sodium levels.

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REFERENCES

WHO, 2003. Polynuclear aromatic hydrocarbons in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. Retrieved from http://www.who.int/water_sanitation_health/dwq/chemicals/polyaromahydrocarbons.pdf.

Lee, B.-K., 2010. Sources, Distribution and Toxicity of Polyaromatic Hydrocarbons (PAHs) in Particulate Matter. In: V. Villanyi (Ed.), *Air Pollution*. Retrieved from http://dx.doi.org/10.5772/10045.

Krestovskaya, T.V., E.I. Slepyan and I.A. Kalinina, 1979. Effects of benzo(a)pyrene on *Allium cepa*. In: E.I. Slepyan (Ed.), *Blastomogens and plants* (pp. 38–42). Nauka, Leningrad (in Russian).

Forrest V., T. Cody, J. Caruso and D.I. Warshawsky, 1989. Influence of the carcinogenic pollutant benzo(a)pyrene on plant development: fern gametophytes. Chemico-biological Interactions, 72(3): 295-307.

Kummerova M., L. Slovak and I. Holoubek, 1995. Phytotoxicity studies of benzo[a]pyrene with *Lactuca sativa*. Toxicological & Environmental Chemistry, 51: 197-203.

Durmishidze, S.V. and D.I. Dzhokhadze, 1979. The effect of benzene and benz(a)pyrene on the RNA and protein synthesizing activity of isolated cell nuclei and chloroplasts. In S.V. Durmishidze (Ed.), *Metabolism of biosphere chemical pollutants in plants* (pp. 138–150). Metsniereba, Tbilisi (in Russian).

Buadze, O.A., S.V. Durmishidze, M.D. Kakhaya and G.V. Zaalishvili, 1979. Effects of different concentrations of benzidine, benzo(a)anthracene and 3,4-benzo(a)pyrene on ultrastructure of maize root cells. In S.V. Durmishidze (Ed.), *Metabolism of biosphere chemical pollutants in plants* (pp. 179–191). Metsniereba, Tbilisi (in Russian).

Oguntimehin, I., N. Nakatani and H. Sakugawa, 2008. Phytotoxicities of fluoranthene and phenanthrene deposited on needle surfaces of the evergreen conifer, Japanese red pine (*Pinus densiflora* Sieb. et Zucc.). Environmental Pollution, 154(2): 264-271.

Reynoso-Cuevas, L., M.E. Gallegos-Martinez, F. Cruz-Sosa and M. Gutierrez-Rojas, 2008. *In vitro* evaluation of germination and growth of five plant species on medium supplemented with hydrocarbons associated with contaminated soils. Bioresource Technology, 99(16): 6379-6385.

Wieczorek, J.K. and Z.J. Wieczorek, 2007. Phytotoxicity and accumulation of anthracene applied to the foliage and sandy substrate in lettuce and radish plants. Ecotoxicology and Environmental Safety, 66(3): 369-377.

Sojinu, O.S., O.O. Sonibare, O. Ekundayo and E.Y. Zeng, 2010. Biomonitoring potentials of polycyclic aromatic hydrocarbons (PAHs) by higher plants from an oil exploration site, Nigeria. Journal of Hazardous Materials, 184(1–3): 759-764.

Ratola, N., J.M. Amigo, M.S.N. Oliveira, R. Araujo, J.A. Silva and A. Alves, 2011. Differences between *Pinus pinea* and *Pinus pinaster* as bioindicators of polycyclic aromatic hydrocarbons. Environmental and Experimental Botany, 72: 339-347.

Nisbet, I.C.T., M.A. Schneiderman, N.J. Karch and D.M. Siegel, 1984. Review and evaluation of the evidence for cancer associated with air pollution. EPA Report No EPA-450/5-83-006R. Retrieved from http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000NJ6V.txt.

Suzdorf, A.R., S.V. Morozov, L.I. Kuzubova, N.N. Anshits and A.G. Anshits, 1994. Polycyclic aromatic hydrocarbons in the environment: sources, profiles and conversion routes. Chemistry for sustainable development, 2: 449-473.

Keith L.H. and W.A. Telliard, 1979. ES&T special report – priority pollutants: I-a perspective view. Environmental Science & Technology, 13: 416-423.

Marinayte, I.I., A.G. Gorshkov, E.N. Taranenko, E.V. Chipanina and T.V. Khodzher, 2013. Distribution of polycyclic aromatic hydrocarbons in natural objects over the territory of scattering the emissions from the Irkutsk aluminum plant (Shelekhov city, the Irkutsk Region). Chemistry for Sustainable Development, 21: 135-146. Retrieved from http://sibran.ru/upload/iblock/b36/b3646fb5e3c85c6cf87039404b73f5ab.pdf.

Gorshkov, A.G., T.A. Mikhailova, N.S. Berezhnaya and A.L. Vereshchagin, 2006. Pine Needles as a Biomonitor for Estimation of the Regional-Scale Distribution of Organic Pollutants. Doklady Earth Science, 408(4): 599-601.

Gorshkov, A.G., T.A. Mikhailova, N.S. Berezhnaya and A.L. Vereshchagin, 2008. Needle of Scotch Pine (*Pinus sylvestris* L.) as a Bioindicator for Atmospheric Pollution with Polycyclic Aromatic Hydrocarbons. Chemistry for Sustainable Development, 16: 155-162.

Ugrekhelidze, D. Sh. and S.V. Durmishidze, 1984. Penetration and detoxification of organic xenobiotics in plants. Metsniereba, Tbilisi (in Russian).

Mikhailova, T.A., V.N. Shmakov and E.N. Taranenko, 2013. Evaluation of polycyclic aromatic hydrocarbons toxicity for plants. The Bulletin of Irkutsk State University, Series “Biology, Ecology”, 2: 27-33 (in Russian).

Briggs, G.G., R.H. Bromilow and A.A. Evans, 1982. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. Pesticide Science, 3(5): 495-504.

Paterson, S., D. Mackay, D. Tam and W.Y. Shiu, 1990. Uptake of organic chemicals by plants: A review of processes, correlations and models. Chemosphere, 21: 297-331.

Wild, S.R. and K.C. Jones, 1992. Polynuclear aromatic hydrocarbon uptake by carrots grown in sludge-amended soil. Journal of Environmental Quality, 21(2): 217-225.

Hellström, A., 2004. Uptake of organic pollutants in plants. Department of Environmental Assessment Swedish University of Agricultural Sciences, Uppsala, Sweden. Retrieved from http://info1.ma.slu.se/IMA/Publikationer/internserie/2004-01.pdf.

Collins, C., M. Fryer and A. Grosso, 2006. Plant uptake of non-ionic organic chemicals. Environmental Science & Technology, 40(1): 45-52.

Cossins, E.A. and S.K. Sinha, 1965. The utilization of carbon-1 compounds by plants: II. The formation and metabolism of formate by higher plant tissues. Canadian Journal of Biochemistry, 43(6): 685-698.

Gorshkov, A.G., 2008. Determination of polycyclic aromatic hydrocarbons in the needles of a Scotch pine (*Pinus Sylvestris* L.), as biomonitor of atmospheric pollution. Journal of Analytical Chemistry, 63(8): 805-811. Retrieved from http://dx.doi.org/10.1134/S1061934808080169.

Wettstein, D., 1957. Chlorophyll-letale und der submikroskopische formwechsel der plastiden. Experimental Cell Research, 12(3): 427-506.

Wintermans, J.F.G.M. and A. DeMots, 1965. Spectrophotometric characteristics of chlorophylls *a* and *b* and their phenophytins in ethanol. Biochimica et Biophysica Acta, 109(2): 448-453.

Proydakova, O.A., V.D. Tsyhansky, L.N. Matveeva, T.S. Gormashev and V.K. Haltueva, 1986. Physico-chemical methods for the determination of macro- and micronutrients in the environment. In: E.V. Pinneker (Ed.), *Geochemistry of technogenesis* (pp. 124–130). Nauka, Novosibirsk (in Russian).

Lehndorff, E. and L. Schwark, 2004. Biomonitoring of air quality in the Cologne Conurbation using pine needles as a passive sampler – Part II: Polycyclic aromatic hydrocarbons (PAH). Atmospheric Environment, 38: 3793-3808.

Nikolaevskiy, V.S., 1999. Ecological assessment of environmental pollution and terrestrial ecosystems state by phytoindication methods. MSFU Publ, Moscow (in Russian).

Mikhailova, T.A., N.S. Berezhnaya, L.V. Rusakova and L.S. Yan'kova, 2000. Pigment complex state parameters of Scots pine oppressed by air industrial emissions. Siberian Ecological Journal, 6: 693-697.