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ORIGINAL ARTICLE

PAHs distribution and cultivable PAHs degraders' biodiversity in soils and surface sediments of the impact zone of the Novocherkassk thermal electric power plant (Russia)

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Abstract
The qualitative and quantitative composition of polycyclic aromatic hydrocarbons (PAHs) pollutants at 13 points of the impact zone of the Novocherkassk thermal electric power plant (NchPP) has been determined. This territory has been polluted with emissions from brown coal combustion for several decades. A total of 200 isolates from PAH-degrading bacteria were selected from the soils and surface sediments, heavily contaminated with PAHs. Taxonomic identification of isolates was carried out by sequencing of the 16S rRNA genes and by MALDI-TOF analysis of protein spectra. Soil cultivable PAH-degrading microorganisms were represented by six species of four genera of actinobacteria (*Arthrobacter*, *Rhodococcus*, *Oerskovia* and *Isoptericola*). Surface sediments cultivable PAH degraders were represented by six species of the genus *Pseudomonas*. *R. erythropolis* was the most numerous of soil isolated strains. The studied soils heavily contaminated with PAHs display low biodiversity of cultivable PAH-degrading bacteria and do not have a connection between the quantity of PAHs in the soil samples and taxonomic variety of PAH degraders. Cultivable PAH degraders were represented in the soils exclusively by Gram-positive bacteria, and in the surface sediments only by Gram-negative bacteria.

Keywords Polycyclic aromatic hydrocarbons · Soils · Surface sediments · Microbial communities · Cultivable PAH degraders

Introduction

Polycyclic aromatic hydrocarbons are widespread environmental pollutants because they are disseminated through a wide variety of anthropogenic activities. They are widespread in soil, surface sediments (SS) and water (Samanta et al. 2002), as they get there with gas, liquid and solid waste when fossil fuel, plant residues and organic garbage are burnt, and at oil development and liquid and gas fuel production. PAHs are persistent pollutants toxic for living organisms, and they display mutagenic and carcinogenic properties (Harvey 1991; Alexander 1999). According to the earlier works, the half-life period of low-molecular PAHs in soils is 5.7 years and of high-molecular PAHs—9.1 years (Wild et al. 1991). However, in the recent work of Bandowe et al. (2014) the assessment of concentration and composition of PAHs in a sediment core is carried out over the period of 2600 years.

Bioremediation of soil contamination with PAHs and their derivatives is an important task in connection with the constantly increasing environmental pollution with these substances. As a rule, purely physical and chemical methods of remediation of PAHs contamination are expensive and, furthermore, cause damage to the purified biotopes. Microbial remediation is labile, easily scalable, cost effective and environmentally friendly method of cleaning up such contamination, effective for a wide range of PAHs concentrations (Habe and Omori 2003). Due to the fact that PAHs are hydrophobic and tightly bound with organic matter of soil, the rate of their biodegradation, the quantitative and qualitative composition of PAHs degraders is largely determined by PAHs bioavailability (Crampon et al. 2014).

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Traditionally, hydrocarbon-degrading microbial communities were characterized by isolation of pure cultures or consortia of cultivated microorganisms (Kanaly and Harayama 2010). However, this approach does not allow to assess the real diversity of microorganisms involved in biotransformation and their contribution to hydrocarbon degradation. This limitation can be overcome by culture-independent methods, allowing to estimate the diversity of genetic material (Yang et al. 2015; Festa et al. 2017), ^{13}C-labeled cell lipids (Johnsen et al. 2002) and the variety of intermediate metabolites (Tauler et al. 2016) of microorganisms involved in microbial PAHs biotransformation.

Taking into account the great practical importance of microbial hydrocarbon degraders' cultivation, the combination of the methods listed above is the most acceptable nowadays. Such approaches allow to obtain both individually cultivated microbial PAHs degraders and their consortia and characterize the hydrocarbon-degrading microbial community of the studied soils in a more detailed way.

Microbial degradation is the main process used in biological remediation of chronically contaminated soils (MacNaughton et al. 1999). There is an approach that focuses on maximum restoration of the existing ecosystems back to the original parameters solely by degradation of potential indigenous microbiota (Haritash 2009; Fukuhara et al. 2013). This approach is called natural attenuation. Its disadvantage is that usually hydrocarbon degraders constitute only a small part of the entire soil microbial population (Yu et al. 2005) and restoration of the ecological community can take a considerable time.

Another method, namely bioaugmentation, involves introduction of individual strains or consortia of pre-adapted hydrocarbon-degrading allochthonous microorganisms into contaminated soil (Fantroussi and Agathos 2005). The effectiveness of bioaugmentation usage depends on interaction between autochthonous and allochthonous bacterial populations, and on how suitable natural conditions are for the introduced microorganisms. Furthermore, bioaugmentation changes the composition of native microbiocenosis.

Isolation of autochthonous strains and consortia of PAHs degraders and their further application for bioremediation appears to be the most promising (Shankar et al. 2014). Such microorganisms are adapted to local environmental conditions and do not introduce qualitative changes in the existing microbiocenosis. On the other hand, the share of PAHs-degrading bacteria increases dramatically in microbial population. The speed of PAH degradation is significantly enhanced by biostimulation. It is achieved by nutrients application and optimization of physical and chemical parameters (pH, oxygen concentration and so on) to avoid metabolic limitations (Viñas et al. 2005). The combination of introduction of earlier isolated autochthonic cultures and biostimulation seems to be a good compromise since it does not change the species composition of the developed soil microbiome and activates biodegradation of PAHs. Also the used strains are well adapted for conditions of this soil.

For a deeper understanding of the remediation contribution and effective application of indigenous strains of PAHs-degrader, the cultivable microbial community diversity should be studied in dependence on qualitative and quantitative PAHs contaminants composition. Also, it is necessary to study their spatial spreading in PAHs-polluted biotopes.

The purpose of this study is evaluation of cultivable PAHs-degrading bacteria variety and their spatial distribution in soils and surface sediments of the impact zone of the Novocherkassk thermal electric power plant (NchPP). This NchPP (2258 MW power) has been burning fossil fuel (coal and natural gas) for more than 40 years, and its emissions (gas and dust emissions and ash) are a source of PAHs contamination of the surrounding area (Sazykin et al. 2015). Then, it is possible to use the characterized autochthonic isolates for bioaugmentation of this polluted site.

Materials and methods

Sampling of soils and surface sediments

Sampling of soils and surface sediments was carried out at 13 monitoring sites located in the impact zone of the NchPP in May 2015. Monitoring sites 1, 2, 3, 5, 6, 7, 11, 12 are found 1–3 km to the northeast, southwest, northwest, north and southeast from the electric power station; sites 8, 9, 10 and 8a are located 5, 10, and 15 km to the northwest and 5 km to the west from the electric power plant in accordance with the wind rose controlling the distribution of atmospheric emissions. This zone stretches directly from the contamination source and crosses the residential areas of Novocherkassk city and Krivyanskaya settlement. Fallow land sites were taken as the monitoring sites. Sampling sites scheme is shown in Fig. 1.

The soils were sampled from studied territory from the depth of 0–20 cm. A sampling of the surface sediments was performed from water bodies located close to four monitoring sites in the impact zone of NchPP. Surface sediments 2 SS, 6 SS, 8 SS, 8a SS were collected in the coastal zone of reservoirs (50–100 cm from the shore). Samples were obtained using the Peterson grab sampler at 5 cm deep. The samples were transferred and kept in clean plastic bags and placed in a cooled container. Sediment samples were stored at − 20 °C before further processing in the laboratories. Physical and chemical properties of the studied soils and sediments samples were analyzed by the commonly used standard method for the Russian Federation (Vorob'eva 2006). The exchangeable bases were determined with 1 M NH_4OAc; the cation exchange capacity (CEC) was analyzed

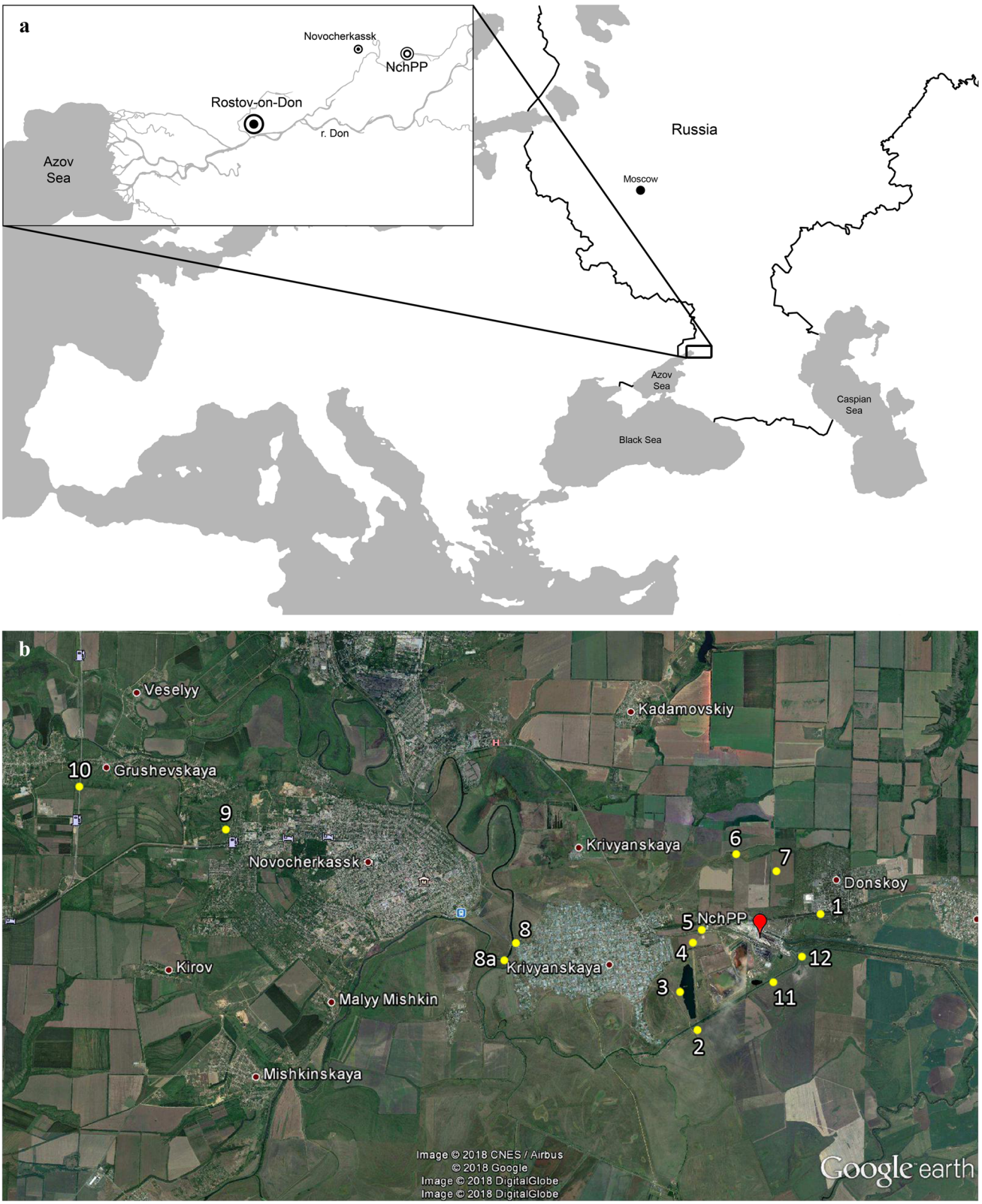

Fig. 1 Study area location and sampling sites: **a** NchPP location on the map of Russia; **b** scheme of sampling sites in the NchPP impact zone

with 1 M NH_4OAc after soil saturation with 1N $BaCl_2$ (pH 6.5); the pH was measured by potentiometry in the supernatant suspension of a soil:water in a ratio of 1:2.5; the $CaCO_3$ was determined by acid neutralization method. The particle-size distribution was determined by the pipette method (with pyrophosphate procedure of soil preparation) (Shein 2009). The organic carbon content in soils and sediments samples was determined by wet combustion with $K_2Cr_2O_7$ and concentrated H_2SO_4 according to the Tyurin method (Vorob'eva 2006), which is very close to the Walkley–Black method (1934). The difference in the results of the organic carbon determination by the Tyurin method and the TOC method is 0.2–0.3% (Gorbov et al. 2018).

Determination of hydrocarbons in soil samples and surface sediments

The new method of subcritical water extraction was used for extraction of PAHs from the soil and surface sediments (Sushkova et al. 2014, 2016). This method includes the following operations. An air-dried soil or surface sediment samples were ground in a porcelain mortar and passed through a 1 mm sieve. One gram of the sample was placed into the extraction cartridge, and 8 ml of double-distilled water was added. Subcritical water extraction of PAHs from soil and surface sediments was carried out in a specially developed extraction cartridge made of stainless steel and equipped with screw-on caps at both ends. The extraction cartridge containing sample and water was placed into an oven connected to a temperature regulator.

Extraction was conducted under optimum conditions (30 min at 250 °C and 60 atm) (Sushkova et al. 2015). After cooling, the content of the cartridge was filtered (Whatman no. 1) through the funnel and washed with 2 ml of double-distilled water. This operation repeated two or three times until the filtrate was clear. The aqueous solution 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 at 40 °C of the water bath. The residue was dissolved in 1 mL acetonitrile by shaking for 30 min.

The content of PAHs in the extracts was quantified by HPLC (Model 1260, Agilent Technologies, USA, 2014) with fluorescence (FL-3000) detection following ISO 13859:2014 requirements (ISO 13859:2014). The excitation wavelength of the FD is 264 nm, and the emission wavelength of the FD is 408 nm. The PAHs peaks on chromatograms were identified by comparing retention time to that of analytical standard samples (Sushkova et al. 2017). The limit of PAHs detection and quantification was determined using standard solutions and calibration curves. A calibration standard was injected after every six samples to correct for drift in retention time within a run.

Solvents and reagents were of 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. The total 16 priority PAHs standards in acetonitrile with concentration 200 μg/cm^3 (Priority pollutant PAHs (in acetonitrile) NIST® SRM® 1647f) was used to prepare total PAHs standard solutions for HPLC analyses. For every target PAH, the individual standard was used as the internal analytical standard for determination: Naphthalene solution 200 μg mL^{-1} (CAS Number 91-20-3, Beilstein Registry Number 7822574, MDL number MFCD00001742, PubChem Substance ID 329798455), Biphenyl solution 2000 μg mL^{-1} (CAS Number 92-52-4; 48161 SUPELCO), Anthracene solution 200 μg mL^{-1} (CAS Number 120-12-7, Empirical Formula (Hill Notation) C14H10, Molecular Weight 178.23, Beilstein Registry Number 1905429, MDL number MFCD00001240, PubChem Substance ID 24872170), Acenaphthene solution 200 μg mL^{-1} (CAS Number 83-32-9, Empirical Formula (Hill Notation) C12H10, Molecular Weight 154.21, Beilstein Registry Number 386081, MDL number MFCD00003807, PubChem Substance ID 24872166EC, Number 200-659-6), Acenaphthylene certified reference material, TraceCERT® (CAS Number 208-96-8, Empirical Formula (Hill Notation) C12H8, Molecular Weight 152.19, Beilstein Registry Number 774092, MDL number MFCD00003806, PubChem Substance ID 329770136EC, Number 205-917-1), Fluorene solution 5000 μg mL^{-1} (CAS Number 86-73-7, Beilstein Registry Number 3562815, MDL number MFCD00001111, PubChem Substance ID 24864952), Phenanthrene analytical standard, for environmental analysis (CAS Number 85-01-8, Empirical Formula (Hill Notation) C14H10, Molecular Weight 178.2, Beilstein Registry Number 1905428, MDL number MFCD00001168, PubChem Substance ID 24872118EC, Number 201-581-5), Benzo[a]anthracene solution certified reference material, 1000 μg mL^{-1} (CAS Number 56-55-3, Empirical Formula (Hill Notation) C18H12, Molecular Weight 228.29, Beilstein Registry Number 1909298, MDL number MFCD00003599, PubChem Substance ID 329755927), Pyrene solution certified reference material, 100 μg mL^{-1} (CAS Number 129-00-0, Empirical Formula (Hill Notation) C16H10, Molecular Weight 202.25, Beilstein Registry Number 1307225, MDL number MFCD00004136, PubChem Substance ID 57652921EC, Number 204-927-3), Fluoranthene 5000 μg mL^{-1} (CAS Number 206-44-0, Beilstein Registry Number 1907918, MDL number MFCD00001184, PubChem Substance ID 24864911), Chrysene analytical standard (CAS Number 218-01-9,

Empirical Formula (Hill Notation) C18H12, Molecular Weight 228.29, Beilstein Registry Number 1909297, MDL number MFCD00003698, PubChem Substance ID 329757387EC, Number 205-923-4), Benzo[a]pyrene solution certified reference material, 200 µg mL^{-1} (CAS Number 50-32-8; Beilstein Registry Number 1911333; EC Number 200-028-5; MDL number MFCD00003602; PubChem Substance ID 24872109), Benzo(b)fluoranthene solution 200 µg mL^{-1} (CAS Number 205-99-2, Empirical Formula (Hill Notation) C20H12, Molecular Weight 252.31, MDL number MFCD00010582, PubChem Substance ID 24871998, EC Number 205-911-9), Benzo[k]fluoranthene certified reference material, 200 µg mL^{-1} (CAS Number 207-08-9, Empirical Formula (Hill Notation) C20H12, Molecular Weight 252.31, Beilstein Registry Number 1873745, MDL number MFCD00046287, PubChem Substance ID 329747589EC, Number 205-916-6), Benzo[g,h,i]perylene certified reference material, 200 µg mL^{-1} from 861291 SUPELCO PAH Mix 3, certified reference material, in methylene chloride: methanol (1:1) (varied) (CAS Number 191-24-2, Empirical Formula (Hill Notation) C22H12, Molecular Weight 276.33, Beilstein Registry Number 1913029, MDL number MFCD00004135, PubChem Substance ID 24872193EC, Number 200-838-9), Dibenz[a,h]anthracene analytical standard, 200 µg mL^{-1} (CAS Number 53-70-3, Empirical Formula (Hill Notation) C22H14, Molecular Weight 278.35, Beilstein Registry Number 1912416, MDL number MFCD00003708, PubChem Substance ID 24872126EC, Number 200-181-8), purchased from the Sigma-Aldrich (Merch).

The efficiency of target PAHs extraction from soils was determined using a matrix spike (Anonymous 2008). The fresh soil sample as well as air-dried soil sample (1 g) was placed into a round-bottom flask and BaP standard solution in acetonitrile was added to give the target PAHs concentrations of 2, 4, 6, 8, 16 or 32 ng/g. After evaporating the solvent for 30 min under a hood under ambient conditions, the PAHs-spiked soil samples were incubated for 24 h at 4 °C. The samples were then analyzed by the subcritical extraction method described above with consequent HPLC analysis.

The limit of PAHs detection and quantification was 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. PAHs concentrations in soil samples (A, µg kg^{-1}) were calculated as follows:

$$A = kS_{\mathrm{I}} \times C_{\mathrm{st}} \times V/\left(S_{\mathrm{st}} \times m\right) \quad (1)$$

where S_{st} and S_I are the respective areas of target PAHs peaks in chromatograms of standard and sample solutions; C_{st} is the target PAHs concentration in standard solution (µg ml^{-1}); k is the coefficient of target PAHs recovery from a sample; V is the volume of acetonitrile extract used for HPLC (ml); and m is the mass of the sample (g).

Data handling and statistical analyses were conducted using STATISTICA 11.0 and Sigma-Plot 12.5. The value means standard deviation from 3 analytic replications average. For the data obtained, statistically significant levels of difference were found.

Isolation of cultivable strains of hydrocarbon-degrading microorganisms

To isolate the cultures of hydrocarbon-degrading microorganisms, each soil and surface sediment sample was thoroughly homogenized. Then, 10 g of sample was filled with 40 ml basic mineral salt medium (Sazykin et al. 2016), stirred 1 h and left for 5 min for sedimentation of soil particles. After that, 1 mL of supernatant liquid was introduced into a 50 mL Erlenmeyer flask with 15 ml of mineral salt basic medium and 300 µl of hydrocarbons solution (saturated solution of naphthalene and anthracene in the mixture of 50% hexadecane, 25% benzene and 25% cyclohexane). The mixture was cultivated in a shaker incubator Innova 40R (New Brunswick Scientific, USA) at 30 °C, 170 rpm for 1 week. Then, the enrichment culture was plated on Petri dishes with solid mineral agar medium with the addition of 2% hydrocarbon mix, and cultivated at 30 °C for 1 week. Isolates possessing different phenotypic characteristics such as colony morphology, pigmentation, growth properties were selected as hydrocarbon-degrading strains and later maintained on LB medium (Maniatis et al. 1982) with the addition of 2% hydrocarbon mix.

Identification of isolated strains

Sequencing of 16S ribosomal RNA gene of the isolated hydrocarbon degrading bacteria

DNA from all pure cultures was isolated with the method described by Moore et al. (2004). The isolated DNA was used as a matrix for the PCR with standard primers for amplification of 16S rRNA: 27 F—AGAGTTTGATCMTGG CTCAG; 1492 R—CGGTTACCTTGTTACGACTT.

The PCR was carried out on the Bio-Rad Tetrad 2 (Bio-Rad, USA) amplifier\thermocycler as follows: initial DNA denaturation at 94 °C for 5 min, then 35 cycles of DNA melting at 94 °C for 30 s, primer annealing at 53 °C for 30 s, amplification of DNA at 72 °C for 90 s and after that completion of DNA fragments at 72 °C for 10 min. Electrophoresis of the obtained products was conducted in 1% agarose gel in the electric field of 5 V/cm. Sequencing of amplification products was performed with the Sanger method on the ABI 3730 DNA Analyzer (Life Technologies, USA) sequencer.

Sequencing results were processed using the GenBank Blast program (http://blast.ncbi.nlm.nih.gov).

MALDI-TOF mass spectrometry-based identification of hydrocarbon degraders

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was used to generate protein fingerprint signatures from whole bacterial cells. All cultures of microorganisms were plated on LB medium to obtain individual colonies. Isolated colonies were placed on steel MALDI-TOF MS target plates (Bruker Daltonics, Germany), matrix solution was applied on top and, after drying, identification of microorganisms in a mass spectrometer MALDI Biotyper (Bruker Daltonik, Germany) was implemented in the automatic mode. Spectra of the protein profiles were imported into the Biotyper program and identified according to standard settings. All spectra ranging from the mass-to-charge ratio (*m/z*) 2000–20,000, corresponding to the resulting ribosomal protein profiles, were analyzed using the MALDI BioTyper (MBT) compass explorer software v4.1.60 (Bruker Daltonics) and compared to reference spectra of the MALDI BioTyper Reference library (database [DB-5989] MS; Bruker Daltonics) for automatic identification. The whole treatment from processing acquired spectra to identification was performed automatically without user intervention using the integrated pattern matching algorithm of the software (Barbuddhe et al. 2008; Thouvenot et al. 2018).

The identification result was expressed in assigning a taxonomic identifier of the genus/species and a numerical rating of identification accuracy presented in a logarithmic scale from 0 to 3 to the investigated spectrum. Value 3 corresponds to absolute coincidence; values from 2.300 to 3.000 stand for accurate determination of species; from 2.000 to 2.299—accurate determination of genus and reliable determination of species, from 1.700 to 1.999—reliable identification of genus.

Results and discussion

Soil characteristics

The major part of the territory in the impact zone of the NCHPP is occupied by Haplic Chernozems (sites no 1, 4, 5, 7, 9, 10, 11); in addition, Haplic Chernozems Stagnic (sites 3, 6, 8, 8a, 12) and Calcaric Fluvic Arenosol (site 2) are distinguished within the Tuzlov River floodplain (Table 1).

Haplic Chernozems and Haplic Chernozems (Stagnic) have deep humus horizons (70–100 cm), relatively high levels of organic carbon content (2.2–2.9%) and the CEC (31.2–47.6 cmol (+)/kg) with a high content of exchangeable calcium (76–90% of the sum of exchangeable cations), and content of $CaCO_3$ (0.2–1.0%) with neutral or weakly alkaline pH in water extract (7.3–7.7). By their particle-size distribution, the soils are classified as heavy loamy and light clayey varieties formed on calcareous loess-like deposits

Table 1 Properties of the Novocherkassk thermal electric power plant emissions zone soils and surface sediments

Site no	Soil/surface sediments	Physical clay (particle < 0.01 mm), %	Clay (particle < 0.001 mm), %	Corg, %	pH	$CaCO_3$, %	CEC, -cmol (+)/kg
1	Haplic Chernozems	52.0	27.0	2.5	7.6	0.5	35.0
2	Calcaric Fluvic Arenosol	7.0	3.0	1.8	7.5	0.4	10.9
3	Haplic Chernozems (stagnic)	67.0	37.0	2.7	7.3	0.2	44.8
4	Haplic Chernozems	55.0	29.0	2.7	7.5	0.7	31.2
5	Haplic Chernozems	53.0	27.0	2.5	7.5	1.0	35.7
6	Haplic Chernozems (Stagnic)	55.0	30.0	2.4	7.7	0.8	32.4
7	Haplic Chernozems	51.0	27.0	2.4	7.6	0.7	31.3
8	Haplic Chernozems (Stagnic)	60.0	32.0	2.9	7.4	0.4	47.6
8a	Haplic Chernozems (Stagnic)	60.0	12.0	2.8	7.5	0.5	44.7
9	Haplic Chernozems	52.0	30.0	2.4	7.6	0.6	31.4
10	Haplic Chernozems	53.0	28.0	2.7	7.6	0.5	36.0
11	Haplic Chernozems	33.0	15.0	2.2	7.5	0.6	38.7
12	Haplic Chernozems (Stagnic)	44.0	20.0	2.7	7.4	0.4	42.4
2 SS	Surface sediments	27.7	10.4	1.3	7.3	1.9	35.4
6 SS	Surface sediments	15.0	5.2	0.8	7.0	2.0	24.5
8 SS	Surface sediments	20.5	5.2	1.0	6.9	2.5	31.6
8a SS	Surface sediments	58.4	14.5	1.6	7.7	2.3	50.4

with physical clay content 33–67% and clay content 12–37%. The Calcaric fluvic arenosol is specified by a coarser texture, less thick humus horizon (40–60 cm), lower organic carbon content (up to 1.8%) and CEC (10.6 cmol(+)/kg) with a relatively high content of exchangeable calcium.

Surface sediments 2 SS, 6 SS, 8 SS, 8a SS are characterized by the physical clay content of 27–58% and clay content of 15–38%, organic carbon content of 2.4–2.9%, $CaCO_3$ content of 2.0–2.1%, CEC of 32–50 cmol (+)/kg and alkaline pH (8.1–8.2) (Table 1).

PAHs distribution across the sites

The results of PAHs analysis in soils and surface sediments samples are given in Tables 2 and 3.

The total content of PAHs in different samples ranged from 382.37 to 1604.15 µg/kg for soils and from 634.15 to 1252.14 µg/kg for surface sediments. The maximum concentration of PAHs was detected at the sites located in the direction of the prevailing winds (sites 4, 5, 8a) and the sites closest to the source of pollution (sites 1 and 12). In surface sediment samples, the highest total PAHs concentrations were detected at the sites 6 SS, 8 SS and 8a SS. The lowest PAHs content was registered in soil samples from sites 2, 3, 6 and 7 (382.37–443.96 µg/kg) and surface sediments from site 2 SS (634.15 µg/kg)—according to the wind chart, the export of the emission in the direction of these sites is minimal (Table 2). The results of quantitative determination of PAHs in the studied samples show that the total content of PAHs in them is much lower than in soils of many European cities (Bojakowska et al. 2018) and is slightly lower or slightly higher than the average for rural soils in Europe. Also, it should be noted that the content of benzo[a] pyrene in all samples of soils exceeds the norms (20 µg/kg) established in the Russian Federation (Federal Law No 52-FZ of 1999) 1.5–10 times. For bottom sediment samples, such excess ranges from 4 to 6.5 times. Thus, the pollution level of the NchPP impact zone can be considered average.

To determine the sources of hydrocarbons for the studied soils and surface sediments samples, the diagnostic ratios of various PAHs were used (Yunker et al. 2002; Rocher et al. 2004; Zemo 2009; Hu et al. 2011; Tobiszewski and Namiesnik 2012). For example, the ratio of 2–3 rings low molecular weight (LMW) PAHs and 4-6 ring high-molecular weight (HMW) PAHs characterizes the pyrogenic or petrogenic origin of hydrocarbons (Table 3).

PAHs from petrogenic sources enriched with LMW PAHs can be represented by the ratio LMW PAHs/HMW PAHs > 1. Pyrogenic PAHs are depleted in LMW PAHs and enriched in HMW PAHs, leading to the ratio LMW PAHs/HMW PAHs < 1. Based on this criterion, PAHs from all sites, except site 3, are of pyrogenic origin. Ratio LMW PAHs/HMW PAHs in the sample from site 3 is 1.11, which corresponds to petrogenic pollution. LMW PAHs/HMW PAHs ratio in the samples of surface sediments varies from 0.24 to 0.43, which corresponds to pyrogenic pollution.

PAHs having the ratio of anthracene to anthracene plus phenanthrene An/(An + PhA) < 0.1 are typical for petrogenic sources, while PAHs having the ratio An/(An + PhA) > 0.1 to come mainly from pyrogenic sources. The ratios of Ant/(Ant + Phe) in all the samples of both soils and surface sediments ranged from 0.01 to 0.07, which corresponds to petrogenic pollution by hydrocarbons. It should be noted that some fuels display anomalous ratios of An/(An + PhA) (Yunker et al. 2002). Such anomalous fuels include brown coal, which served as the main fuel for NchPP for several decades. Emissions from the combustion of brown coal result in the ratio An/(An + PhA) < 0.1, which explains the contradiction between LMW PAHs/HMW PAHs and Ant/(Ant + Phe) ratios.

In addition, PAHs sorbed on atmospheric particles had different stability when moving from the source of pollution to the pollutant accumulation site (Behymer and Hites 1985; Kamens et al. 1988). As a result of photolysis, a much greater decrease in the concentrations of anthracene and benzo[a]pyrene occurs in comparison with more stable phenanthrene and benzo[e]pyrene.

The ratio of fluoranthene to fluoranthene plus pyrene (FlA/(FlA + Py)) also characterizes the pyrogenic or petrogenic source of PAHs (Yunker et al. 2002; Havelcová et al. 2014). Ratio FlA/(FlA + Py) < 0.4 indicates typical petrogenic pollution, FlA/(FlA + Py) between 0.4 and 0.5 indicates the oil combustion resulting pollution, and the value of FlA/(FlA + Py) > 0.5 indicates PAHs contamination mainly as the result of combustion of coal and plant biomass. These diagnostic ratios allow to divide all the soil samples into three groups according to the origin/source of PAHs pollution. Sites 1, 2, 3, 7 are contaminated with PAHs of mainly petrogenic origin. Some soil and surface sediment samples are in close proximity to highways and NchPP, and the petrogenic origin of PAH could occur because of pollution with oil products and brown coal which were used as fuel for the power plant.

Pollution of sites 4, 6, 8, 8a, 10, 11 and 12 is of mixed origin, possibly with a significant participation of the liquid fuel combustion products. It should be noted that fuel oil is used to serve as the backup fuel for NchPP for decades and currently the main fuel is natural gas. Finally, at sites 5 and 9 the source of PAHs contamination is primarily solid fuel burning (Table 2). Burning plant residues such as straw on neighboring fields and wood at stove heating also contributes to the mixed pollution.

For surface sediments, samples 6 and 8 indicate the pyrogenic source of PAHs pollution. Sample 2 has the petrogenic

Table 2 PAHs concentrations (μg/kg) in soils of the impact zone of the Novocherkassk thermal electric power plant

Site no	Naphthalene	Biphenyl	Anthracene	Acenaphthene	Acenaphthylene	Fluorene	Phenanthrene	Benzanthracene	Pyrene	Fluoranthene	Chrysene
1	26.7 ± 2.2*	4.2 ± 0.8	5.5 ± 2.0	2.4 ± 0.9	9.3 ± 2.1	9.4 ± 1.3	311.7 ± 44.1	35.3 ± 9.8	136.4 ± 11.4	63.2 ± 7.4	59.6 ± 7.2
2	31.1 ± 4.2	4.6 ± 1.6	1.8 ± 0.4	1.0 ± 0.3	10.1 ± 2.4	4.6 ± 0.8	143.7 ± 31.7	10.2 ± 3.4	43.0 ± 8.0	20.8 ± 1.8	45.9 ± 3.6
3	53.7 ± 9.8	11.8 ± 2.1	2.1 ± 1.2	2.6 ± 0.8	10.1 ± 2.7	5.1 ± 1.5	116.0 ± 18.9	11.0 ± 3.8	43.7 ± 7.4	11.1 ± 2.4	23.2 ± 2.9
4	41.4 ± 3.7	16.4 ± 1.7	3.3 ± 0.9	8.7 ± 1.4	25.8 ± 3.8	6.4 ± 1.8	450.2 ± 57.4	65.9 ± 12.1	216.8 ± 39.6	164.2 ± 21.6	96.4 ± 12.3
5	47.2 ± 2.9	8.1 ± 1.1	4.5 ± 1.1	1.1 ± 0.2	18.6 ± 3.1	5.6 ± 2.1	314.9 ± 49.6	48.0 ± 8.4	119.9 ± 10.4	124.9 ± 18.4	79.3 ± 8.4
6	40.8 ± 8.4	3.3 ± 0.6	3.5 ± 1.6	1.0 ± 0.6	10.8 ± 2.4	5.4 ± 2.8	138.5 ± 26.7	7.1 ± 0.9	32.2 ± 8.5	25.5 ± 3.9	47.2 ± 5.2
7	19.5 ± 2.6	11.8 ± 1.7	3.5 ± 2.4	1.7 ± 0.4	9.0 ± 1.7	5.3 ± 3.1	144.1 ± 22.4	9.0 ± 1.7	62.0 ± 6.9	38.6 ± 4.0	38.3 ± 4.3
8	14.5 ± 3.3	18.8 ± 1.4	4.7 ± 1.7	3.3 ± 0.8	20.0 ± 4.6	1.6 ± 0.6	122.9 ± 18.9	37.6 ± 5.7	82.2 ± 10.2	76.7 ± 15.4	62.4 ± 7.2
8a	42.7 ± 6.9	4.5 ± 0.6	10.3 ± 3.8	2.5 ± 1.0	15.6 ± 3.2	9.3 ± 3.1	209.6 ± 15.4	45.7 ± 5.9	106.9 ± 11.4	97.9 ± 20.3	46.3 ± 8.4
9	14.3 ± 5.5	21.0 ± 3.7	1.9 ± 0.5	3.4 ± 1.1	11.5 ± 1.9	7.9 ± 2.4	233.5 ± 19.1	40.5 ± 3.8	46.3 ± 7.8	48.1 ± 6.9	64.6 ± 5.7
10	35.5 ± 6.3	8.3 ± 1.6	7.4 ± 1.2	1.6 ± 0.4	13.5 ± 2.5	4.9 ± 2.1	191.1 ± 15.6	40.7 ± 3.2	105.3 ± 9.7	104.1 ± 11.7	61.5 ± 7.7
11	46.1 ± 4.2	11.9 ± 2.1	2.8 ± 0.7	1.0 ± 0.3	9.1 ± 3.4	1.8 ± 0.7	180.9 ± 21.4	21.6 ± 2.9	62.4 ± 6.4	48.7 ± 5.8	43.9 ± 5.8
12	44.5 ± 7.4	4.9 ± 0.9	10.6 ± 3.1	2.6 ± 0.8	5.6 ± 2.1	9.6 ± 2.1	210.4 ± 25.8	59.4 ± 6.7	118.9 ± 12.6	115.9 ± 12.1	64.3 ± 7.3

Site no	Benzo [a] pyrene	Benzo [b] fluoranthene	Benzo [k] fluoranthene	Benzo [g, h, i] perylene	Dibenz [a, h]-nthracene	∑ PAHs	LMW PAHs/ HMW PAHs	An/(An + PhA)	FLA/ (FLA + PYR)	BaA/ (BaA + Chry)
1	49.4 ± 7.1	56.8 ± 6.7	10.12.8 ±	32.4 ± 4.7	3.3 ± 0.8	815.5 ± 132.0	0.83	0.02	0.32	0.37
2	30.9 ± 3.9	42.7 ± 3.9	3.9 ± 0.7	14.8 ± 3.6	2.6 ± 1.1	411.7 ± 55.4	0.92	0.01	0.33	0.18
3	31.2 ± 5.6	39.2 ± 2.4	1.5 ± 0.4	17.3 ± 4.2	2.7 ± 1.5	382.4 ± 114.7	1.11	0.02	0.20	0.32
4	205.0 ± 12.4	161.0 ± 15.3	44.2 ± 5.6	87.5 ± 12.7	11.0 ± 2.1	1604.2 ± 213.9	0.52	0.01	0.43	0.41
5	113.7 ± 15.7	90.2 ± 12.8	15.5 ± 3.2	30.3 ± 5.8	1.6 ± 0.4	1023.4 ± 172.5	0.64	0.01	0.51	0.38
6	48.1 ± 3.7	32.1 ± 7.4	3.9 ± 1.1	30.6 ± 6.3	3.2 ± 1.0	433.3 ± 88.6	0.88	0.02	0.44	0.13
7	34.0 ± 6.7	44.9 ± 8.6	3.6 ± 1.4	16.6 ± 2.7	2.3 ± 1.3	444.0 ± 131.5	0.78	0.02	0.38	0.19
8	59.0 ± 8.6	105.6 ± 15.7	17.7 ± 2.7	38.8 ± 3.3	2.6 ± 1.1	668.3 ± 168.4	0.39	0.04	0.48	0.38
8a	51.3 ± 5.4	158.3 ± 20.6	23.0 ± 3.5	40.2 ± 5.9	4.1 ± 0.8	868.2 ± 201.7	0.51	0.05	0.48	0.50
9	91.4 ± 10.0	122.9 ± 14.3	14.1 ± 2.4	47.4 ± 6.9	1.5 ± 0.3	770.1 ± 185.3	0.62	0.01	0.51	0.39
10	69.4 ± 8.2	96.6 ± 16.8	29.2 ± 3.1	39.6 ± 4.5	3.5 ± 1.1	812.3 ± 202.1	0.48	0.04	0.50	0.40
11	57.3 ± 7.6	66.9 ± 12.4	17.3 ± 2.2	25.2 ± 6.7	2.2 ± 0.6	599.1 ± 65.7	0.73	0.02	0.44	0.33
12	74.5 ± 8.4	155.1 ± 13.7	31.3 ± 3.0	49.2 ± 11.4	4.9 ± 1.5	961.5 ± 107.4	0.43	0.05	0.49	0.48

*The value means average from three replications and standard deviation is given after ± symbol

Table 3 PAHs concentrations (μg/kg) in surface sediments of the impact zone of the Novocherkassk thermal electric power plant

Site no	Naphthalene	Biphenyl	Anthracene	Acenaphthene	Acenaphthylene	Fluorene	Phenanthrene	Benzanthracene	Pyrene	Fluoranthene	Chrysene
2 SS	25.1 ± 1.7*	0.9 ± 0.4	1.1 ± 0.5	0.5 ± 0.2	8.2 ± 1.1	2.7 ± 0.8	109.0 ± 12.7	7.3 ± 2.4	42.5 ± 3.7	12.3 ± 8.7	118.6 ± 11.4
6 SS	72.1 ± 3.4	24.2 ± 3.8	5.1 ± 2.1	2.1 ± 0.7	17.9 ± 0.8	5.7 ± 1.2	138.5 ± 14.6	46.4 ± 2.2	98.4 ± 8.5	155.7 ± 16.4	235.9 ± 18.5
8 SS	41.0 ± 2.6	3.9 ± 1.4	10.0 ± 3.0	2.0 ± 0.7	5.5 ± 0.9	7.9 ± 2.4	206.3 ± 15.4	67.2 ± 1.8	155.7 ± 10.0	160.7 ± 15.3	156.4 ± 16.7
8a SS	51.8 ± 8.5	3.8 ± 0.8	8.6 ± 3.6	1.5 ± 0.5	8.6 ± 1.1	6.0 ± 2.3	197.3 ± 17.9	63.4 ± 1.7	135.9 ± 12.1	129.8 ± 12.3	178.7 ± 18.4

Site no	Benzo [a] pyrene	Benzo [b] fluoranthene	Benzo [k] fluoranthene	Benzo [g, h, i] perylene	Dibenz [a, h]-nthracene	∑ PAHs	LMW PAHs/ HMW PAHs	An/(An + PhA)	FLA/ (FLA + PYR)	BaA/ (BaA + Chry)
2 SS	113.5 ± 9.7	122.6 ± 8.3	31.9 ± 2.4	36.1 ± 4.0	1.8 ± 0.6	634.1 ± 27.4	0.30	0.01	0.22	0.06
6 SS	132.4 ± 12.4	221.7 ± 11.2	28.7 ± 3.6	59.4 ± 6.1	7.9 ± 1.1	1252.1 ± 107.3	0.27	0.04	0.61	0.16
8 SS	91.1 ± 10.6	201.2 ± 9.4	33.9 ± 2.9	49.6 ± 5.2	3.2 ± 1.0	1195.3 ± 98.6	0.30	0.05	0.51	0.30
8a SS	83.9 ± 7.4	197.2 ± 10.1	35.9 ± 3.1	56.4 ± 3.4	6.3 ± 0.9	1165.1 ± 75.4	0.31	0.04	0.49	0.26

*The value means average from three replications and standard deviation is given after ± symbol

nature of contamination, and sample 8a is contaminated with the products of oil hydrocarbon combustion.

Wang and Fingas (2003) found that benzo[a]anthracene/(benzo[a]anthracene + chrysene) (BaA/(BaA + Chry)) can also serve as an indicator of pyrogenic or petrogenic PAHs source. Ratio BaA/(BaA + Chry) < 0.2 corresponds to a petrogenic source of PAHs, the value of 0.2 < BaA/(BaA + Chry) < 0.35 is an oil products combustion indicator, and BaA/(BaA + Chry) > 0.35 correlates to the origin of PAHs from coal and plant biomass combustion. Soil samples demonstrate mainly pyrogenic PAHs origin (sites 1, 4, 5, 8, 8a, 9, 10, 12). Soils of sites 2, 6, 7 are petrogenously polluted, and PAHs of two sites (3, 11) have origin from the oil products combustion. Surface sediments of sites 6 and 8 are pyrogenically contaminated. Sample 2 has a petrogenic contamination source, and sample 8a has a mixed type of PAHs contamination.

Thus, at the investigated sites the types of PAHs contamination are mainly of pyrogenic or mixed origin with a marked axis of pyrogenic contamination moving along the direction of the prevailing winds toward the points 4, 5, 8, 8a, and 9. The main source of PAH pollutants in this area is the NchPP.

Biodiversity of cultivable PAHs-degrading bacterial strains

A total of 200 isolates from hydrocarbons supplemented mineral agar plates were selected and kept for further study. Taxonomic identification of isolates was carried out by sequencing of the 16S rRNA genes and by MALDI-TOF analysis of protein spectra. The 16S RNA gene sequences of isolated strains of PAHs-degrading bacteria are deposited in GenBank (GenBank accession numbers MH718753-MH718790).

The obtained sequences were analyzed using the GenBank Blast program, and nucleotide collection database was used; the number of matching sequences was not less than 96%. Mass spectra of proteins were analyzed using the Biotyper program; the values of rating accuracy of identification were in the range of 1.998–2.455 (for most isolates, the value exceeded the 2.300 threshold). Identification of isolates by means of two different methods led to the identical results presented in Table 4.

As a result of identification, all isolates were assigned to the five genera and 12 species. Representatives of the genera *Rhodococcus* (*R. erythropolis*), *Isoptericola* (*I. variabilis*), *Oerskovia* (*O. turbata*), and *Arthrobacter* (3 species—*A. aurescens*, *A. polychromogenes* and *A. sulfonivorans*) were isolated from soil samples. From surface sediments, six species of *Pseudomonas* genus (*P. koreensis*, *P. brassicacearum*, *P. chlororaphis*, *P. kilonensis*, *P. stutzeri*, *P. putida*) were obtained. *R. erythropolis* was the

Table 4 Species of PAHs-degrading bacteria isolated from soils and surface sediments in the impact zone of the Novocherkassk thermal electric power plant

Site no.	Species of bacteria isolated from soils	Species of bacteria isolated from surface sediments
1	*Rhodococcus erythropolis*	
2	*Arthrobacter aurescens*	
2 SS		*Pseudomonas koreensis*
3	*Rhodococcus erythropolis*	
4	*Isoptericola variabilis*	
5	*Rhodococcus erythropolis*	
6	*Oerskovia turbata* *Rhodococcus erythropolis*	
6 SS		*Pseudomonas brassicacearum* *Pseudomonas chlororaphis* *Pseudomonas kilonensis*
7	*Rhodococcus erythropolis*	
8	*Rhodococcus erythropolis*	
8 SS		*Pseudomonas stutzeri*
8a	*Arthrobacter aurescens* *Arthrobacter polychromogenes* *Arthrobacter sulfonivorans*	
8a SS		*Pseudomonas putida*
9	*Rhodococcus erythropolis* *Oerskovia turbata*	
10	*Rhodococcus erythropolis*	
11	*Rhodococcus erythropolis*	
12	*Rhodococcus erythropolis*	

most numerous according to the number of isolated strains and was found in the vast majority of soil samples (10 of 13). In two out of the three soil samples in which there was no *R. erythropolis,* representatives of the *Arthrobacter* genus (samples 2 and 8a) were found and in the third (sample 4) *Isoptericola variabilis* representatives were detected. Representatives of these three genera of bacteria were not present in any of the samples simultaneously. In soil samples No. 6 and 9 *R. erythropolis* and *O. turbata* were identified at the same time.

Higher quantity of PAHs degraders' bacterial species was observed only in the direction of the prevailing winds from NchPP, that is, in the main direction of distribution of the gas–dust emissions of the power plant. The connection between the quantitative content of PAHs in the soil samples and species quantity of PAHs degraders was not observed.

In three of the four sampling points of surface sediments, only one PAHs degraders' species is isolated: site 2—*P. koreensis*, site 8—*P. stutzeri* and site 8a—*P. putida*. The maximum diversity of PAHs degraders is found in the site 6 sediments—*P. brassicacearum*, *P. chlororaphis* and *P. kilonensis*.

Research carried out during the last decade showed that soil contamination with total petroleum hydrocarbons (TPH) not only helps to increase the number, but also stimulates hydrocarbon-degrading bacteria, and promotes augmentation of bacterial biodiversity, microbial activity and function in carbon metabolism (Benedek et al. 2013; Liao et al. 2015). Influence of PAHs pollution on soil microbial community is dramatically different from the effect of TPH. A significant negative correlation was observed between PAHs concentration and diversity of bacterial species, total bacteria and fungi counts and enzyme activities in polluted soils (Pérez-Leblic et al. 2012; Benedek et al. 2013). It is likely that different influence of TPH and combustion PAHs on biodiversity of microbic community is connected not only with their different sorption, but also with more expressed toxicity of PAHs and smaller quantity of the microorganisms capable of their utilization.

In this study of PAHs-polluted soil and surface sediment microbial communities, similar results were obtained. In the surface sediments, cultivable PAHs degraders were represented by only six species of the only genus of Gram-negative bacteria (*Pseudomonas*). Cultivable soil microbial community was also represented by six species of actinobacteria of four genera (*Arthrobacter*, *Rhodococcus*, *Isoptericola* and *Oerskovia*). Low biodiversity is likely to be associated with significant contamination of the investigated soil by PAHs. Interestingly, in the soils, cultivable PAHs degraders were represented exclusively

by Gram-positive microorganisms and in the surface sediments only by Gram-negative microorganisms.

Actinobacteria are recognized as the dominant group both in soils contaminated with PAHs (Benedek et al. 2013) and soil metagenome from soils with mixed contamination by PAHs and heavy metals (Kuppusamy et al. 2016). At the same time in these works, the dominance of *Pseudomonas* in contaminated soils was pointed out, although in our study representatives of this genus are present in the contaminated surface sediments, but not in soils. Such genera of hydrocarbon-degrading microorganisms as *Sphingomonas*, *Mycobacterium*, *Nocardia*, *Stenotrophomonas*, *Burkholderia*, or *Achromobacter*, mentioned in the papers above and Johnsen et al. (2002); Chikere et al. (2017), are not detected in the studied polluted soils by us.

The presence of such active PAH degraders as *Rhodococcus erythropolis, Pseudomonas stutzeri* and *Pseudomonas putida* in the soils and surface sediments of the impact zone of the NchPP indicates chronic hydrocarbon contamination and, on the other hand, a likely significant bioremediation potential of the area. For the purpose of sustainable agricultural use of the territories adjacent to NchPP, it is rational to carry out stimulation of autochthonous microbiocenosis. It would also be necessary to introduce the isolated cultures of autochthonous PAH degraders into the most polluted sites.

Conclusion

The toxic emissions from the NchPP were one of the main factors of environmental pollution and PAHs accumulation in soils and sediments of the studied region. Soil properties and additional pollution sources were also the important factor for PAHs accumulation in the studied region. Summarizing the obtained data, it is necessary to point out that soils and surface sediments are moderately polluted with PAHs mainly of pyrogenic origin, display low biodiversity of cultivable PAHs-degrading bacteria and have considerable variations of cultivable strains. Cultivable PAH degraders from the soil samples are presented exclusively by Gram-positive actinobacteria of four genera (*Arthrobacter*, *Rhodococcus*, *Isoptericola* and *Oerskovia*) with a strong *Rhodococcus erythropolis* dominance. Cultivable PAH degraders, isolated from surface sediments, differed dramatically and included six species of Gram-negative bacteria of the *Pseudomonas* genus.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Alexander M (1999) Biodegradation and bioremediation. Academic Press Inc, San Diego, p 453

Anonymous (2008) Procedure of measurements benz(a)pyrene content in soils, sediments and sludges by highly effective liquid chromatography method. Certificate 27–08. Russian State Standard Publishing House, Moscow, p 56 (in Russian)

Bandowe BAM, Srinivasanb P, Seelge M, Sirocko F, Wilcke WA (2014) 2600-year record of past polycyclic aromatic hydrocarbons (PAHs) deposition at Holzmaar (Eifel, Germany). Palaeogeogr Palaeoclimatol Palaeoecol 401:111–121. https://doi.org/10.1016/j.palaeo.2014.02.021

Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, Chakraborty T, Hain T (2008) Rapid identification and typing of *Listeria* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 74(17):5402–5407. https://doi.org/10.1128/AEM.02689-07

Behymer TD, Hites RA (1985) Photolysis of polycyclic aromatic hydrocarbons adsorbed on simulated atmospheric particulates. Environ Sci Technol 19:1004–1006

Benedek T, Vajna B, Táncsics A, Márialigeti K, Lányi S, Máthé I (2013) Remarkable impact of PAHs and TPHs on the richness and diversity of bacterial species in surface soils exposed to long-term hydrocarbon pollution. World J Microbiol Biotechnol 29:1989–2002. https://doi.org/10.1007/s11274-013-1362-9

Bojakowska I, Tomassi-Morawiec H, Markowski W (2018) PAHs and DDTs in soil and sediment of inland water bodies of Warsaw city and its surroundings. J Geochem Explor 187:57–71

Chikere CB, Azubuike CC, Fubara EM (2017) Shift in microbial group during remediation by enhanced natural attenuation (RENA) of a crude oil-impacted soil: a case study of Ikarama Community, Bayelsa, Nigeria. 3 Biotech 7(2):152. https://doi.org/10.1007/s13205-017-0782-x

Crampon M, Bureau F, Akpa-Vinceslas M, Bodilis J, Machour N, Le Derf F, Portet-Koltalo F (2014) Correlations between PAH bioavailability, degrading bacteria, and soil characteristics during PAH biodegradation in five diffusely contaminated dissimilar soils. Environ Sci Pollut Res Int 21(13):8133–8145. https://doi.org/10.1007/s11356-014-2799-6

Fantroussi SE, Agathos SN (2005) Is bioaugmentation a feasible strategy for pollut-ant removal and site remediation? Curr Opin Microbiol 8(3):268–275

Federal Law No 52-FZ of 1999 On the sanitary and epidemiological well-being of the population. Federal Service for Supervision of Consumer Rights Protection and Human Well-Being. Accepted by The State Parliament on March 12, 1999, Approved by Federation Council on March 17, 1999. Moscow. 1999

Festa S, Coppotelli BM, Madueño L, Loviso CL, Macchi M, NemeTauil RM, Valacco MP, Morelli IS (2017) Assigning ecological roles to the populations belonging to a phenanthrene-degrading bacterial consortium using omic approaches. PLoS One 12(9):e0184505. https://doi.org/10.1371/journal.pone.0184505

Fukuhara Y, Horii S, Matsuno T, Matsumiya Y, Mukai M, Kubo M (2013) Distribution of hydrocarbon-degrading bacteria in the soil environment and their contribution to bioremediation. Appl Biochem Biotechnol 170:329–339

Gorbov SN, Bezuglova OS, Tischenko SA, Gorovtsov AV (2018) Organic matter and elemental composition of humic acids in soils of urban areas: the case of Rostov agglomeration. Geography. https://doi.org/10.1007/978-3-319-70557-6_10

Habe H, Omori T (2003) Genetic of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. Biosci Biotechnol Biochem 67:225–243

Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatichydrocarbons (PAH): a review. J Hazard Mater 169:1–15

Harvey RG (1991) Polycyclic aromatic hydrocarbons: chemistry and carcinogenicity. Cambridge University Press, Cambridge

Havelcová M, Melegy A, Rapant S (2014) Geochemical distribution of polycyclic aromatic hydrocarbons in soils and sediments of El-Tabbin, Egypt. Chemosphere 95:63–74. https://doi.org/10.1016/j.chemosphere.2013.08.018

Hu NJ, Shi XF, Huang P, Liu JH (2011) Polycyclic aromatic hydrocarbons in surface sediments of Laizhou Bay, Bohai Sea, China. Environ Earth Sci 63:121–133. https://doi.org/10.1007/s12665-010-0675-3

ISO 13859:2014. Soil quality — Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)

Johnsen AR, Winding A, Karlson U, Roslev P (2002) Linking of microorganisms to phenanthrene metabolism in soil by analysis of 13C-labeled cell lipids. Appl Environ Microbiol 68(12):6106–6113. https://doi.org/10.1128/aem.68.12.6106-6113.2002

Kamens RM, Guo Z, Fulcher JN, Bell DA (1988) The influence of humidity, sunlight, and temperature on the daytime decay of polyaromatic hydrocarbons on atmospheric soot particles. Environ Sci Technol 22:103–108

Kanaly RA, Harayama S (2010) Advances in the field of high-molecular weight polycyclic aromatic hydrocarbon biodegradation by bacteria. Microb Biotechnol 3:136–164. https://doi.org/10.1111/j.1751-7915.2009.00130.x

Kuppusamy S, Thavamani P, Megharaj M, Venkateswarlu K, Lee YB, Naidu R (2016) Pyrosequencing analysis of bacterial diversity in soils contaminated long-term with PAHs and heavy metals: implications to bioremediation. J Hazard Mater 317:169–179

Liao J, Wang J, Jiang D, Wang MC, Huang Y (2015) Long-term oil contamination causes similar changes in microbial communities of two distinct soils. Appl Microbiol Biotechnol 99:10299–10310. https://doi.org/10.1007/s00253-015-6880-y

MacNaughton SJ, Stephen JR, Venosa AD, Davis GA, Chang YJ, White DC (1999) Microbial population changes during bioremediation of an experimental oilspill. Appl Environ Microbiol 65:3566–3574

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, New York

Moore E, Arnscheidt A, Kruger A, Strompl C, Mau M (2004) Simplified protocols for the preparation of genomic DNA from bacterial cultures. In: Antoon DL, van Elsas JD, de Bruijn FJ (eds) Molecular microbial ecology manual edition, suppl 4, chapter 1.6.1, 2nd edn. Kluwer Academic Publishers, London

Pérez-Leblic MI, Turmero A, Hernández M, Hernández AJ, Pastor J, Ball AS, Rodríguez J, Arias ME (2012) Influence of xenobiotic contaminants on landfill soil microbial activity and diversity. J Environ Manag 95:S285–S290. https://doi.org/10.1016/j.jenvman.2010.07.017

Rocher V, Azimi S, Moilleron R, Chebbo G (2004) Hydrocarbons and heavy metals in the different sewer deposits in the 'Le Marais' catchment (Paris, France): stocks, distributions and origins. Sci Total Environ 323:107–122

Samanta SK, Singh OV, Jain RK (2002) Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends Biotechnol 20:243–248

Sazykin IS, Sazykina MA, Khammami MI, Kostina NV, Khmelevtsova LE, Trubnik RG (2015) Distribution of polycyclic aromatic hydrocarbons in surface sediments of lower reaches of the Don River (Russia) and their ecotoxicologic assessment by bacterial lux-biosensors. Environ Monit Assess 187:277. https://doi.org/10.1007/s10661-015-4406-9

Sazykin IS, Sazykina MA, Khmelevtsova LE, Khammami MI, Karchava ShK Zhuravleva MV, Kudeevskaya EM (2016) Expression of SOD and production of reactive oxygen species in *Acinetobacter calcoaceticus* caused by hydrocarbons oxidation. Ann Microbiol 66(3):1039–1045. https://doi.org/10.1007/s13213-015-1188-9

Shankar S, Kansrajh C, Dinesh MG, Satyan RS, Kiruthika S, Tharanipriya A (2014) Application of indigenous microbial consortia in bioremediation of oil-contaminated soils. Int J Environ Sci Technol 11:367–376

Shein EV (2009) The partiche-size distribution in soils. Problems of the methods of study, interpretation of the results, and classification. Eur Soil Sci 42(3):284–291

Sushkova S, Vasilyeva G, Minkina T, Mandzhieva S, Tjurina I, Kolesnikov S, Kizilkaya R, Askin T (2014) New method for benzo[a]pyrene analysis in plant material using subcritical water extraction. J Geochem Explor 144(Part B):267–272. https://doi.org/10.1016/j.gexplo.2014.02.01

Sushkova S, Minkina T, Mandzhieva S, Borisenko N, Vasilyeva G, Kızılkaya R, Aşkın T (2015) Approbation of express-method for benzo[a]pyrene extraction from soils in the technogenic emission zone territories. Eurasian J Soil Sci 4(1):15–21. https://doi.org/10.18393/ejss.48158

Sushkova SN, Minkina TM, Mandzhieva S, Vasilyeva G, Borisenko N, Turina I, Bolotova O, Varduni T, Kızılkaya R (2016) New alternative method of benzo[a]pyrene extraction from soils and its approbation in soil under technogenic pressure. J Soils Sediments 16(4):1323–1329. https://doi.org/10.1007/s11368-015-1104-8

Sushkova S, Minkina T, Turina I, Mandzhieva S, Bauer T, Kizilkay R, Zamulina I (2017) Monitoring of benzo[a]pyrene content in soils under the effect of long-term technogenic poluttion. J Geochem Explor 174:100–106. https://doi.org/10.1016/j.gexplo.2016.02.009

Tauler M, Vila J, Nieto JM, Grifoll M (2016) Key high molecular weight PAH-degrading bacteria in a soil consortium enriched using a sand-in-liquid microcosm system. Appl Microbiol Biotechnol 100:3321–3336. https://doi.org/10.1007/s00253-015-7195-8

Thouvenot P, Vales G, Bracq-Dieye H, Tessaud-Rita N, Maury MM, Moura A, Lecuit M, Leclercq A (2018) MALDI-TOF mass spectrometry-based identification of *Listeria* species in surveillance: a prospective study. J Microbiol Methods 144:29–32. https://doi.org/10.1016/j.mimet.2017.10.009

Tobiszewski M, Namiesnik J (2012) PAH diagnostic ratios for the identification of pollution emission sources. Environ Pollut 162:110–119

Viñas M, Sabaté J, Espuny MJ, Solanas AM (2005) Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediationof heavily creosote-contaminated soil. Appl Environ Microbiol 71:7008–7018

Vorob'eva LA (ed) (2006) Theory and practice of the chemical analysis of soils. GEOS, Moscow

Walkley A, Black IA (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37:29–37

Wang Z, Fingas MF (2003) Development of oil hydrocarbon fingerprinting and identification techniques. Mar Pollut Bul 27:423–452

Wild SR, Obbard JP, Munn CI, Berrow ML, Jones KC (1991) The long-term persistence of polynuclear aromatic hydrocarbons (PAHs) in an agricultural soil amended with metal-contaminated sewage sludges. Sci Total Environ 101(3):235–253

Yang Y, Wang J, Liao J, Xie S, Huang Y (2015) Abundance and diversity of soil petroleum hydrocarbon-degrading microbial communities in oil exploring areas. Appl Microbiol Biotechnol 99:1935–1946. https://doi.org/10.1007/s00253-014-6074-z

Yu SH, Ke L, Wong YS, Tam NFY (2005) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by a consortium enrichment from mangrove sediments. Environ Int 32:149–154

Yunker MB, Macdonald RW, Vingarzan R, Mitchell RH, Goyette D, Sylvestre S (2002) PAHs in the Fraser River Basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org Geochem 33:489–515

Zemo DA (2009) Use of parent polycyclic aromatic hydrocarbons (PAH) proportions to attribute PAH sources in sediments: a case study from the Pacific Northwest. Environ Forensics 10:229–239