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Seasonal variability of chemical composition and mutagenic effect of organic PM2.5 pollutants collected in the urban area of Wrocław (Poland)

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Abstract. The objective of the study was the assessment of the mutagenicity of chemical pollutants adsorbed on suspended particulate matter with aerodynamic diameter < 2.5 μm (PM2.5) in the four seasons. Samples were collected from the urban agglomeration of Wroclaw, Poland and evaluated for mutagenicity using two *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation with microsomal fraction S9.

The work covered sampling of suspended dusts in four seasons: summer, spring, autumn and winter. The dust samples were collected on glass filters using air aspirator and the organic matter of PM2.5 was extracted using Soxhlet extractor. The levels of polycyclic aromatic hydrocarbon compounds (PAH), nitro-PAH and dinitro-PAH were determined in the extract.

Variable degree of air pollution with mutagenic substances was determined at the selected study site. A greater, negative effect of chemical

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compounds on DNA was determined in dust samples collected in the autumn-winter season in comparison to samples collected in the spring-summer season. In the majority of tests, higher mutagenicity was obtained in analyses conducted on total extracts in comparison to tests conducted in the presence of PAH pollutant fractions. The obtained mutagenic ratio values pointed to the presence of chemical compounds with a character of both promutagens and direct mutagens.

Samples collected in the autumn-winter season were observed to have a higher diversity of organic substances absorbed on PM2.5 dusts. Particular samples differed in the total content and percent contribution of particular PAHs, nitro-PAHs, and other organic compounds. In addition, the identified substances included compounds belonging to different chemical classes: aliphatic compounds, cycloalkanes, mono- and bicycling arenes, polycyclic arenes, compounds containing oxygen, nitrogen, and sulphur.

Key words: particulate matter PM2.5, organic pollutants, PAH, nitro-PAH, dinitro-PAH, mutagenic activities, Wrocław urban area.

1 Introduction

Continuous and systematic changes taking place in the world around us to a lesser or greater extent affect the deterioration of the quality of the environment (water, soil and air). Civilization development causes the introduction of new substances into the environment, both biological and chemical, the presence of which can cause a deterioration in people's quality of life. Air quality is the basic element of good quality of life (Ki-Hyun et al., 2015 ; Wolf-Baca and Piekarska, 2020). The health and ecological effects of atmosphere pollution

has been recognised for years. The problems are particularly serious in the areas of large urban agglomerations, where the observed concentration of pollutants is several hundred times higher than in unpolluted areas. Such high concentration is dangerous, because after entering the organism, the pollutants can function in several different ways: independently, synergistically, or antagonistically (Rückerl et al., 2011; Lim et al., 2014). This concerns pollutants of both natural and anthropogenic origin. Mutagenic pollutants in the air of large cities largely originate from anthropogenic sources (particularly from combustion processes) and constitute a product of chemical reactions occurring in the atmosphere. The atmosphere is currently estimated to contain more than 2000 different chemical, organic and inorganic compounds constituting pollutants (Syafinaz et al. 2018; Wang et al.; 2016).

It has been proved that polycyclic aromatic hydrocarbons (PAHs) can cause carcinogenic and mutagenic effects. An important sources of PAH in air are the processes of incomplete combustion of organic compounds (Abbas et al., 2018; de Kok, Theo MCM, et al., 2006; Landkocz et al. 2017; Alves et al., 2015). The content of PAHs in the air depends on many factors such as volume of dust emitted by industrial plants, methods of heating, intensity of road traffic, applied urban planning solutions facilitating or hindering exchange of air, and meteorological and climatic conditions (Zhang et al. 2017).

Complete chemical analysis of air pollutants impossible due to their heterogenous and complex composition. Moreover, detection and identification of substances based on chemical analysis is costly and requires the application of modern analytical methods. Not all chemical compounds occurring in air have also been identified, and some occur in trace amounts and therefore are outside of the analytical capacity of instrumental methods. Therefore, chemical analysis alone cannot provide the basis for forecasting potential biological effects of pollutants on humans and animals. Therefore, bioindication and biomonitoring methods have been applied to evaluate genotoxic and mutagenic effects on

human health (Shen et al., 2019; Claxton et al., 2004; Crobeddu et al., 2017; Cachon et. al., 2014).

The bacterial *Salmonella* test (Ames test) has been employed to assess the mutagenicity of environmental pollutants that induce DNA damage. The test is based on the verification whether the analysed material causes reverse mutation of special histidine-dependent strains of bacteria *Salmonella typhimurium* LT2. For the purpose of metabolic activation of promutagens, fraction S9 is used in the test, comprising microsomal enzymes isolated from rat liver induced with a mixture of biphenyls. The introduction of the variant with metabolic activation permits transposing results obtained in the bacterial test to mammal organisms (Maron and Ames, 1983).

The *Salmonella* test was applied for detecting the mutagenic effect of organic extractions from suspended dust samples in different cities around the world. For example, research on air pollution conducted in different areas in Poland (IARC monographs, 2017; Piekarska K., 2008).

The current study was concerned to evaluate the air quality in urban sites in Wroclaw, the fourth in terms of population size (approximately 635 thousand residents, approximately 2.1 thousand people/km²) among Polish cities. Wroclaw is an urban-industrial agglomeration where air pollution primarily originates from three sources: so-called low emission, industrial nuisances, and passenger and lorry road transport. The transport, machine, electrotechnical, metal structure, chemical, and food industries play the most considerable role in the economy of the city. The largest point-based sources of pollution emission include a complex of heat and power plants Wrocławski Zespół Elektrociepłowni “Kogeneracja” SA. In spite of a substantial decrease in the emission of pollutants to the air from industrial plants, local exceedance of acceptable values is still recorded. It is particularly due to emission from municipal and household sector: local boiler stations and household furnaces equipped with low emitters, often located in the

central, densely populated areas of the city. An equally important problem in Wroclaw remains the emission of road transport pollutants. It results from the lack of ring roads for transit and city-centre traffic, bad quality of roads, and insufficient rate of modernisation of collective transport in the city (Piekarska, 2008; Bełcik et al., 2018).

The primary objective of this study was to investigate the seasonal variability of the mutagenic effect, by means of a bacterial *Salmonella* test, of primary groups of organic pollutants adsorbed on suspended particulate matter PM2.5 collected in the spring, summer, autumn and winter seasons from urban agglomeration in Wrocław, Poland. According to our knowledge, studies comparing the obtained mutagenic effects for all organic pollutants present in samples of suspended particulate matter with mutagenic effects of primary groups of pollutants such as PAH, nitro-PAH, and dinitro-PAH are hardly available. Such an approach to research may permit correlation of the chemical composition of PM2.5 with the mutagenic effects observed in the *Salmonella* test.

2 Materials and methods

2.1 Sampling suspended dusts

The suspended dust (PM2.5) samples were collected a from the atmospheric air at different sites in Wroclaw, Poland. The study site was located in the vicinity of the city centre, near one of the largest transportation routes (Grunwaldzki Square) in the premises of the campus of the Wroclaw University of Technology (intersection of the Norwida and Wybrzeże Wyspiańskiego Streets), on a roof at a height of the second floor. The sampling took place in the following months; April, July, November and January to represent the four seasons spring, summer, autumn and winter, respectively.

PM 2.5 samples were collected by means of a high-volume air sampler system Staplex® Model with FC-2ETM constant flow controller. The air sampler used meets the requirements of the Environmental Protection Agency's (EPA) PM-10 and PM-2.5 included in the "Federal Reference Method". It permits sampling of large quantities of air and capturing particles from 10 μm to 0.01 μm by means of filters.

The samples were collected on glass fibre anti-hygroscopic filters (Staplex-TFAGF810) with dimensions 20x25 cm, maintaining constant weight in a broad range of humidity fluctuations. The filters were changed every 24 hours. Meteorological conditions during collection of samples were as follows: in April air temperature exceeded multiannual norms, and varied from 2.9˚C to 20˚C (mean 9.5˚C), in July from 10˚C to 35˚C (mean 19.2˚C), in November from -8˚C to 10˚C (mean 2.5˚C), in January from -15˚C to 5˚C (mean -5.4˚C). South-westerly winds were dominant in all months; precipitation was approximate to multiannual norms in July and November; April was very dry (total monthly precipitation did not exceed 1 mm), in July mean precipitation was 97.3 mm (18 days with precipitation, including 5 days with atmospheric storm), in November mean precipitation was 39.2 mm (19 days with precipitation, including 8 days with snowfall), and in January precipitation norms exceeded by 169% (20 days with precipitation, including 6-8 days with snowfall).

2.2 Samples extraction

Samples extraction was performed in accordance with the methodology described by Bełcik et al. (2018)

After collecting the PM 2.5 dust samples, the filters were dried to constant weight (until the absolute difference in dry matter content of two successive weighing did not differ by more than 0.1%). To determine the net mass of particulates collected, the difference in the

dry mass of clean filters and filters with collected dust was calculated. Then, the filters were stored at a temperature of −18 °C until the next step of organic extraction.

Filters together with particulates from individual samplings were combined into one sample, cut into pieces and put into Soxhlet apparatuses and extracted with dichloromethane in the dark for 16 hours plus 15-minute reflux. The extracts were dried in a vacuum evaporator and then further concentrated by purging with nitrogen. The dry extracts obtained were analysed for PAH, nitro-PAH and dinitro-PAH content and also used as the material for the mutagenicity assays (*Salmonella*).

2.3 Analytical methods

Raw extract was fractioned on glass columns filled with silica gel (60, particle diameter 0.063–0.200 nm) dried at a temperature of 150^0C for 16 hours, and then deactivated with distilled water. The column was conditioned with cyclohexane, and then concentrated raw extract was applied on its head.

Three subsequent fractions, after removal of aliphatic hydrocarbons eluted from the column of 20 cm^3 of cyclohexane, were collected in three fractions (ISO 12884, 2000; Zaciera, 2006; Leníček, et al. 2000; Szulejko et. al., 2014).

- Fraction I – PAH, eluted 50 cm^3 of cyclohexane, then 30 cm^3 of 25% dichloromethane solution in cyclohexane.
- Fraction II – nitro-PAH, eluted 20 cm^3 of dichloromethane.
- Fraction III – dinitro-PAH, eluted 10 cm^3 of dichloromethane.

Particular fractions were evaporated in a vacuum evaporator to a volume of 10 cm^3, and then further purged with nitrogen until dry.

PAH content in fraction I was determined by high performance liquid chromatography HPLC (AT 1200 by Agilent Technologies) equipped with fluorescence detector. For this

purpose, the dried residue was diluted with 1 cm^3 of acetonitrile, and then injected into the HPLC.

Nitro- and dinitro- PAH (fraction II and III) were determined by gas chromatographer GC-MS (Varian 450) with a mass detector (320 MS) with electron ionisation (EI).The analysis method was validated by Bełcik et al. (2018).

Next, the prepared samples containing the four fractions were used in the biological tests.

2.4 *Salmonella* test

The research employed two test strains of *Salmonella typhimurium*, namely TA98 [TA 1538 his D3052 (pKM101)] and TA100 [TA 1535 His G46 (pKM101)], obtained from K. Sugiyama and M. Yamada from the Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan. Strain TA98 detects mutations causing frameshifts. Before the commencement of the research, each time genotypes of test strains were verified. The presence of mutation in the histidine operon was checked (growth on minimum agar with an addition of L-histidine, and no growth on minimum agar with an addition of biotin), as well as rfa mutation related to the permeability of the cell wall (no growth on nutrient agar around a disc saturated with crystal violet), uvrB mutation involving the removal of the gene related to the DNA repair system (no growth on nutrient agar after irradiation with UV from a distance of 33 cm for 8 seconds), and presence of the pKM101 plasmid (factor R) (growth on nutrient agar with an addition of ampicillin) stimulating the system of erroneous DNA repair and determining resistance of bacteria to ampicillin. Each time the number of spontaneously induced revertants (negative control) and number of revertants induced by diagnostic mutagens (positive control) was also determined. The number of colonies of spontaneous revertants should be within the range specified in the literature, and should be comparable with results obtained in a given

laboratory for a longer period of time (Maron, Ames, 1983; Mortelmans, Zeiger, 2000; Kier et al. 1986; Levya et al., 2020). Control mutagenic compounds should have a chemical structure approximate to that of the analysed compounds. Diagnostic mutagens for analyses conducted without metabolic activation with fraction S9 were: 2,4,7-TNFon (TA98), NQNO (TA100), and for analyses conducted in the presence of fraction S9 2-AF (TA98, TA100).

All the compounds were purchased from Sigma. Table 1 presents the obtained ranges of the number of spontaneous and induced revertants characterising test bacterial strains during the research (OECD, Guideline for the Testing of Chemicals, 1997).

Table 1. Range of the number of spontaneous and induced revertants obtained in the research (Piekarska, 2008).

Strain	**No. of spontaneous revertants**	**Control mutagen (-F)**	**Concentration of control mutagen [µg/ plate] (-F)**	**No. of induced revertants (-F)**	**Control mutagen (+F)**	**Concentration of control mutagen [µg/ plate] (+F)**	**No. of induced revertants (+F)**
TA98	22- 36 (-F) 26-42 (+F)	2,4,7-TNFON	0,2	3242-4552	2-AF	5	1645-2886
TA100	98-179 (-F) 121-194 (+F)	NQNO	0,5	962- 1616	2-AF	5	848- 869

Glass fibre filters to which air samples were collected provided a number of revertants at a level of value of spontaneous reversion characteristic of the strains applied in the study.

The selection of the appropriate concentration of the microsomal fraction S9 in S9-mix applied in the research is very important. The classic procedure, recommended by the authors of the test, stipulates two concentrations of S9 in S9-mix, namely standard (4%, v/v) and high (10%, v/v) (Thybaud et al., 2017). Because different chemical compounds need different optimum S9 concentration for their metabolic conversion, the research applies a broad range of concentrations of the microsomal fraction, from 4% to 30%. The original methodology recommends selection of the concentration of the fraction according to the applied diagnostic mutagen. Because suspended particulate matter extracts contain pollutants with diverse quantitative and qualitative composition, the pilot research involved the determination of optimum concentrations of the microsomal fraction towards the analysed particulate matter extracts (Piekarska, 2008). The research was conducted towards one selected dilution of particulate matter extracts that were chosen in the research with the application of the microsomal fraction concentration of 4% (v/v) in S9 mix. The concentrations caused a mutagenic effect in the test, but still did not cause a toxic effect. Next, tests were performed on the concentrations with the application of 3%, 4%, 5%, 6%, and 9% (v/v) solutions of the fraction. Finally, the following microsomal fraction concentrations were selected for testing: spring and summer sample (TA98-4%, TA100-5%) and autumn and winter samples (TA98-3%, TA100-4%).

Before each test, an appropriate volume of the microsomal fraction was removed in the quantity necessary to perform the planned analyses. Directly before the test, the homogenate was defrosted at room temperature, and microsomal fraction S9 (S9-mix) was immediately prepared. After its preparation, it was kept in ice-water bath. The standard S9-mix mixture should contain: 8mM $MgCl_2$, 33mM KCL, 5mM glucose 6-phosphate, 4mM NADP, 100mM phosphate buffer with pH 7.4, and microsomal fraction in a quantity

depending on its protein content (Maron and Ames, 1983; OECD Guideline for the Testing of Chemicals, 1997).

The analysed sample is considered mutagenic, and therefore potentially cancerogenic, when a linear correlation dose-response (volume of air-number of revertant colonies) occurs with at least double increase in the number of induced revertants in comparison to the number of spontaneous revertants. Obtaining the toxic effect on the dose-response curve fully illustrates the biological effect of pollutants present in the sample depending on its concentration (OECD Guideline for the Testing of Chemicals, 1997).

The mutagenic effect of suspended particulate matter is presented in the form of the mutagenicity ratio (MR), and in the form of mutagenicity (M), i.e. number of revertants induced by 1 m^3 of the analysed air.

The mutagenicity ratio was calculated from the following formula (Trusz-Zdybek et al., 2007):

$$MR = \frac{mean\ numer\ of\ induced\ revertants}{mean\ numer\ of\ spontaneous\ revertants}$$

Samples were considered mutagenic when their mutagenicity ratio MR was ≥ 2.

Seven to thirteen doses of dichloromethane extracts prepared as described above, diluted according to geometric progress with a ratio of 1/2, ranging between 50 and 0.0125 cubic meter-equivalents of air per plate of each sample were tested in triplicate. All organic pollutants present in collected samples (all fraction) were introduced to the tests, as well as pollutants contained in their three fractions: PAH, nitro-PAH, and dinitro-PAH.

2.5 Statistical analysis of results

Statistical analyses were carried out using the STATISTICA software for Windows, v. 7,0 StatSoft. The data were not normally distributed, and the Kruskal-Wallis test (KW), a non-parametric version of the classical one-way analysis of variance (ANOVA), was used to

determine statistical significance. A Spearman correlation analysis was performed to determine the correlation between the chemical composition and biological response. The mean differences and correlations were considered significant when $P < 0.05$ (Bernstein, 1982).

3. RESULTS AND DISCUSSION

3.1 Collection and chemical analysis of dust samples

Data concerning PM2.5 dust samples collected in the spring-summer and autumn winter season in Wroclaw are presented in Table. 2 and Figure. 1.

Table 2. Data concerning collection of PM2.5 dust samples

Type of sample	Number of filters taken for tests	Sampling time [h]	Air volume [m^3]	Weight of dusts [$\mu g/m^3$]	Weight of tar substances [$\mu g/m^3$]
Spring	30	186.59	16 233.33	33.55	3.80
Summer	31	238.76	20 772.12	31.20	9.89
Autumn	30	244.03	21 230.61	66.24	8.78
Winter	31	237.58	20 669.46	88.35	12.30

According to the data, approximately twice more dusts were collected in autumn and winter than in spring and summer at the same study site. Concentrations of the sampled PM2.5

dusts varied from 31.20 μg/m^3 (summer) to 88.35 μg/m^3 (winter). Concentrations of organic compounds adsorbed on suspended dust (tar substances) were in a range from 3.8 μg/m^3 (spring) to 12.30 μg/m^3 (winter).

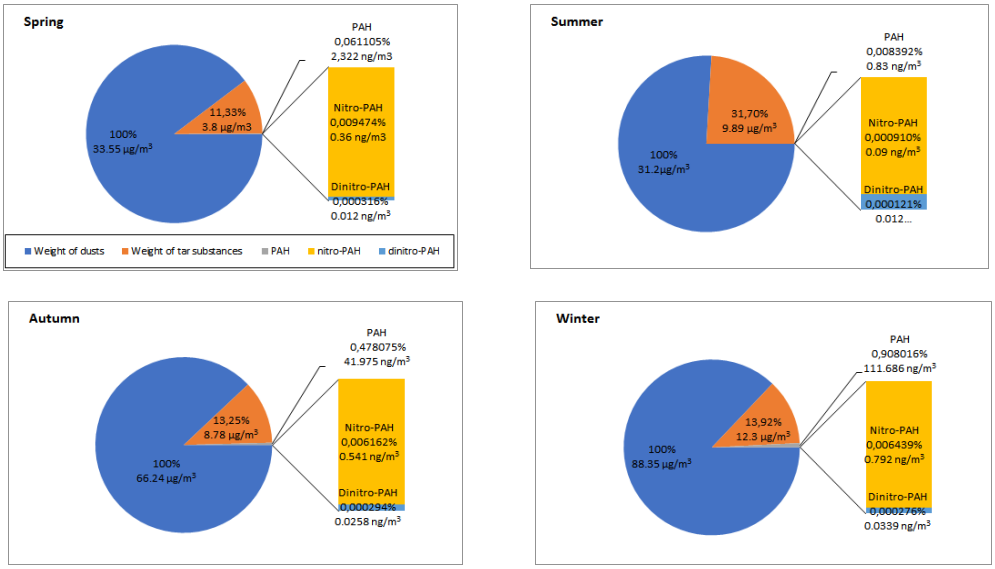

Figure 1 Percent content of the mass of tar substances and particular fractions in the mass of particulate matter together with concentrations.

Concentrations of pollutants emitted to the atmosphere are strongly restricted in provisions of particular countries. The situation is similar in the case of concentration of particulate matter. Norms of concentrations of suspended particles in particular countries are specified in local provisions, although in the case of EU Member States the norms were collectively adopted in the directive of the European Parliament and Council of Europe 2016/2284 of 14 December 2016 on the reduction of national emissions of certain types of atmospheric pollution.

The said directive was implemented into the legislation of the Republic of Poland, and is binding as the Regulation of the Minister of the Environment of 8 June on assessing the levels of substances in the air (Journal of Laws of 2018, item 1119). In the case of

acceptable levels of pollutants of fraction PM2.5, the regulation stipulates exclusively concentrations for the period of averaging results equal to a calendar year. In this case, the acceptable level was stipulated for 25 μg/m^3. The provisions stipulate no possibility of exceeding the acceptable level in the specified averaging.

In spite of the strict requirements concerning the concentration of dust pollutants in atmospheric air in the territory of Poland, norms stipulated in provisions are not always maintained. A major part of cases of exceedance of the acceptable level occurs in the autumn-winter period in urbanised and urban areas. In the Wrocław agglomeration, exceedance of acceptable levels of dust air pollutants have been regularly observed over the recent years in the autumn-winter period.

The analysed dust extractions were investigated in terms of levels of concentrations, most frequently occurring in atmospheric air, of twelve PAH and eight nitro- and dinitro- PAH. The results are displayed in Figures 2-4.

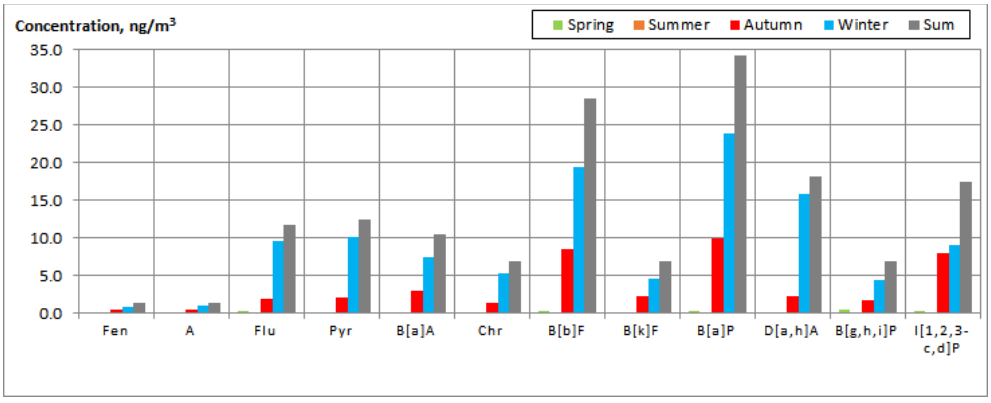

Figure 2. PAH concentration in organic extracts of suspended particulates PM2.5

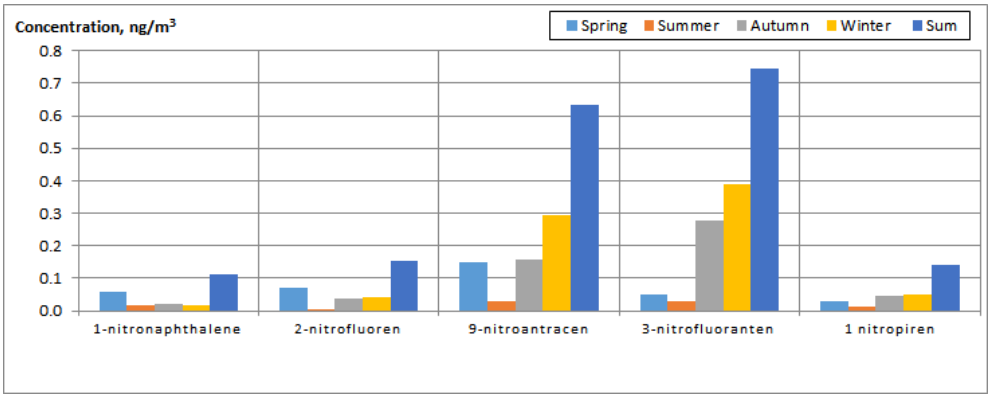

Figure **3. N**itro-PAH concentration in organic extracts of suspended particulates PM2.5

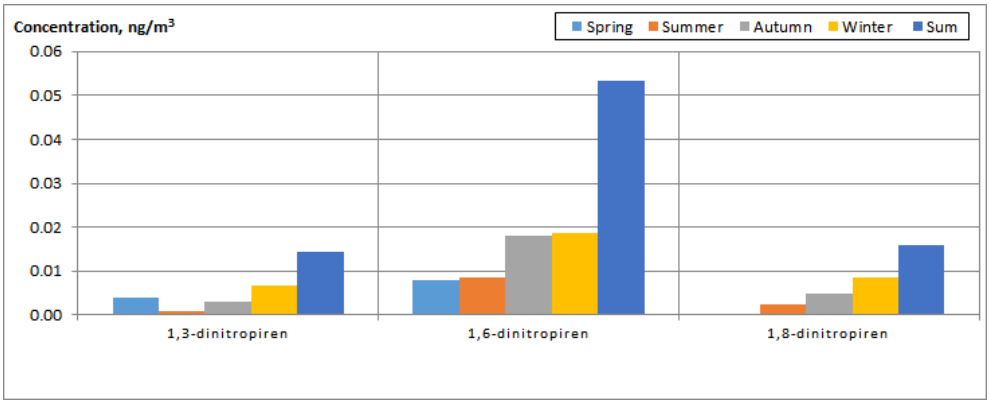

Figure **4.** Dinitro-PAH concentration in organic extracts of suspended particulates PM2.5

Total PAH concentrations detected in the analysed samples varied from 0.830 ng/m^3 (summer) to 111.686 ng/m^3 (winter). Total detected PAH concentrations in the extraction of PM2.5 dusts sampled in summer was therefore 135 times lower in comparison to the extraction of dusts sampled in winter. All particulate matter samples were found to contain benzo[a]pyrene (B[a]P)in concentrations ranging from 0.079 ng/m^3 (summer) to 23.9 (winter) ng/m^3 (Figure 2). The highest concentrations of the PAHs were recorded in the autumn-winter period (16 times more in comparison to the spring-summer season).

Both the total content of PAHs in the researched samples and their profile was consistent with literature reports about other European cities (Iakovidesa, et al.; 2019; Pateraki et al., 2020; Liu et al., 2017).

PAHs contained in the extracts included three (benzo[a]anthracene, benzopyrene, dibenz[a,h]anthracene] classified by IARC (International Agency for Research on Cancer) as belonging to the group of hydrocarbons potentially carcinogenic for people (2A), and three (benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-347 c,d]pyrene) classified to group 2B, potentially cancerogenic for people (Agents classified by the IARC Monographs, 2019). Benzo[a]pyrene is an indicator compound used for the assessment of the carcinogenic properties of other PAHs, a relative carcinogenicity index k=1 was adopted for the compound (Appendix 1). Only benzo[a]anthracene, the presence of which was also detected in the analysed samples, shows a higher strength of carcinogenic properties (k=5). Concentrations of this hydrocarbon were also high in the autumn-winter season, varying from 0.029 ng/m^3 (summer) to 7.5 ng/m^3 (winter).

Statistically significant differences between concentrations of particular PAHs were obtained depending on the season of collected samples. Samples collected in winter showed a higher, statistically significant concentration of particular PAHs in the extract of dusts PM2.5.

Content of nitrated derivatives of PAHs were also determined in the particulate matter extracts (Figure 3). Nitrite PAHs are mutagenic and carcinogenic. Some nitro-PAHs may form during combustion processes. However, most of them are formed in the atmosphere as a result of PAH reactions in the gas phase (Zhuo et.al, 2017). Total concentration of the compounds was in a range from 0.102 ng/m^3 (summer) to 0.8259 ng/m^3 (winter). The highest total concentration of nitrated derivatives of PAHs was recorded in samples collected in winter and autumn. No statistically significant differences were recorded for concentrations of particular nitrated derivatives of PAHs in the analysed extracts of PM2.5

samples in different seasons. The analysed samples were found to contain 2-nitrofluorene, 1-nitropyrene, 1,6-dinitropyrene, and 1,8-dinitropyrene belonging to group 2B (Appendix 1). 1-nitropyrene and 3-nitrofluorantene are characteristic fuel combustion products in a Diesel engine. The compounds are not observed in any other reactions occurring in the gas phase (Bandowe and Meusel, 2017).

The quantitative analysis of particulate matter extracts (Appendix 2) showed the presence of the highest quantity of chemical compounds in PM2.5 samples collected in winter and autumn. Samples collected in the autumn-winter season were observed to have a higher diversity of organic substances absorbed on PM2.5 dusts. The identified substances included compounds belonging to different chemical classes: aliphatic compounds, cycloalkanes, mono- and bicycling arenes, polycyclic arenes, compounds containing oxygen, nitrogen, and sulphur. Particular samples differed in the total content and percent contribution of particular PAHs, nitro-PAHs, and other organic compounds. Coefficients of correlation between masses of dusts and masses of tar substances obtained in the research and PAH and nitro-PAH concentrations presents in Appendix 3. The highest correlation coefficients were obtained between the suspended particulates concentration and concentration of: B[a]P (r=0.833), B[a]A (r=0.857), B[g,h,i]P (r=0.857), and total PAH (r=0.857).

3.2 Bacterial reverse mutation assay (*Salmonella* test)

The *Salmonella* test (Ames test) covered two strains of *Salmonella typhimurium* TA98 and TA100 (for frameshift mutation and base-pair substitution respectively). The tests were performed without and with metabolic activation with microsomal fraction S9. The *Salmonella* mutagenicity results of airborne particles are presented in tables 3-6 and Appendixes 4-7.

In tests conducted on strain TA98 (Table 3, Appendix 4) with participation with all organic pollutants, very high MR values were observed for the sample collected in autumn. Such a situation occurred both in tests conducted with metabolic activation with fraction S9 and without it. A stronger mutagenic response was obtained in tests without the fraction in higher concentrations of the extract, suggesting the presence of a higher amount of pollutants in the sample collected in autumn potentially directly affecting genetic material. The situation was similar for the sample collected in winter. In the case of the sample collected in winter, it is ambiguous what mutagens were the most abundant: those directly affecting genetic material (test without metabolic activity) or those with indirect effect (tests in the presence of fraction S9), because similar MR values were obtained in such tests. The lowest MR values were obtained for the sample collected in summer.

In tests of total extracts with strain TA100 (Table 3, Appendix 4), considerably lower values of the MR coefficient were obtained in comparison to tests conducted with strain TA98. In tests without metabolic activation, the lowest MR values were obtained for the sample collected in spring. In tests with fraction S9, the highest amount of mutagens with direct effect were found in the sample collected in winter.

In the case of the remaining extracts with strain TA98, the highest MR values were obtained for extracts of the sample collected in winter containing PAHs, nitro-PAHs, and dinitro-PAHs (Tables 4-6, Appendixes 5-7),

Results of *Salmonella* tests for particular fractions in the presence of strain TA100 are presented in (Tables 4-6, Appendixes 5-7). Like in the case of tests of the total extract (all), considerably lower values of the MR coefficient were obtained for particular fractions in comparison to tests with strain TA98. Only in a test with metabolic activation on autumn extract of nitro-PAHs, the obtained MR values were higher than those obtained for total extract. In the remaining tests, higher MR values were obtained for total extracts. Results similar to those for nitro-PAHs were obtained for extracts containing dinitro-PAHs

The tested extracts of airborne dust showed mutagenic activity towards test strains *Salmonella typhimurium* TA98 and TA100. In all conducted tests, statistically higher values of the mutagenicity coefficient (MR) were obtained for extracts of air samples collected in the autumn-winter period in comparison to tests with extracts of dust pollutants collected in spring and summer. The same seasonal variability is observed in other countries (Bocchi et.al, 2016, Alves et. al, 2015). In summer, at higher temperatures, part of pollutants transitions to volatile and semi-volatile phase. In the autumn-winter season, low emission and emission by combustion engines using more fuel increases (Claxton, 2007; Bocchi, et. al, 2017; Islama, et. al; 2020). In the conditions of the climatic zone of Wrocław, low emission can still contribute to air pollution in the spring season, as observed in tests of total extract on both test strains with and without activation, and in the case of test of nitro-PAH extract on strain TA98 without metabolic activation.

Strain TA100, detecting mutagenic substances causing base-pair substitution, proved the least sensitive to the analysed pollutants. The highest number of positive results for strain TA100 was obtained by introducing pollutants collected in autumn and winter to tests. In this case, statistically higher MR values were obtained in tests with metabolic activation (prevalence of promutagens), both for total extracts and PAH and nitro-PAH extracts. In the case of tests with the application of strain TA98, detecting chemical compounds causing frameshift mutation, in the majority of cases higher MR values were obtained in tests with total dust extracts. Like in tests with strain TA100, the lowest number of positive results was obtained in tests with dust extracts collected in summer and spring, particularly for extracts of PAHs and nitro-PAHs. For samples collected in autumn and winter, very high values of the MR coefficient were obtained (particularly for dusts collected in autumn), statistically higher than those obtained for strain TA100.

Based on the above, the analysed extracts contained both pollutants with potential indirect (promutagens) or direct effect on genetic material. Indirect effect of pollutants on genetic

material is related to the presence of unsubstituted PAHs, and direct effect to the occurrence of nitro-, amino-, and oxy-PAHs. The obtained results confirmed the presence of high amounts of direct mutagens in the analysed extracts. The majority of papers regarding the issue shows that the mutagenic effect of airborne dusts is largely determined by moderately polar and highly polar classes of compounds (Crobeddu, et.al, 2017, De Kok T.M.C.M, et. al.,2006, Erdinger et. al., 2005, Lemos et. al, 2016). More polar fractions usually contain higher concentrations of nitro-PAHs than unsubstituted PAHs. Chemical compounds with such effect are nitro- and amino-PAHs, polar aromatic compounds, heterocyclic compounds, and phenols. They are produced in the process of combustion of fuels and as a result of reactions of organic pollutants occurring in the atmosphere. They are present among others in extracts of diesel exhaust particles (e.g. nitrofluorene, nitroanthracene, nitrofluoranthene, nitrobenzo[a] pirene). The majority of these products, however, are produced in the atmosphere as a result of reactions of PAHs in the gas phase. The transformation of PAHs into nitro-PAHs is an irreversible reaction. PAHs contained in the gas phase react with hydroxyl and nitrate radicals occurring in the air. It results in production of many different derivative compounds, among others nitro-PAHs. Although nitro-PAHs occur in lower concentrations in comparison to unsubstituted aromatic hydrocarbons, they show high stability in the solid phase and higher mutagenicity (2x105 times) and carcinogenicity (10 times) of some of them in comparison to PAHs. Nitro-PAHs should be therefore considered in risk assessment regarding air pollutants. Their low concentrations in this environment, however, pose analytical problems (Kawanaka et. al 2008; Künzli et. al, 2000, Landkocz et. al, 2017; Thybaud et. al., 2017).

Table 3. Dependency of MR ± S.D. on concentrations of extracts of all organic dust pollutants in the analysed air samples (All) determined by means of the *Salmonella* test.

Concentration m^3/cm^3		50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.097	0.049	0.025	0.013
TA98 -S9mix	Spring	9.47±0.3	6.75±0.2	5.37±0.2	5.12±0.1	3.91±0.2	3.80±0.1	3.31±0.1	2.26±0.1	2.10±0.2	1.44±0.1	1.32±0.1	-	-
	Summer	4.48±0.4	3.17±0.2	2.17±0.1	1.87±0.1	1.43±0.1	1.23±0.1	1.13±0.1	1.00±0.1	0.98±0.1	0.91±0.2	-	-	-
	Autumn	20.98±0.7	18.87±0.6	14.02±0.5	10.71±0.4	8.56±0.3	6.60±0.4	5.56±0.3	4.50±0.3	4.31±0.3	3.90±0.2	2.24±0.2	1.57±0.1	1.11±0.1
	Winter	11.31±0.6	9.13±0.4	8.32±0.3	5.51±0.2	4.12±0.2	2.70±0.2	2.13±0.2	1.52±0.1	1.32±0.1	0.96±0.1	-	-	-
TA98 +S9mix	Spring	4.30±0.3	2.11±0.1	1.53±0.2	1.27±0.1	1.20±0.2	0.99±0.1	0.97±0.1	0.98±0.2	1.00±0.1	1.04±0.1	1.02±0.1	-	-
	Summer	3.67±0.3	1.98±0.2	1.26±0.2	1.22±0.1	1.14±0.2	0.95±0.1	1.00±0.1	1.09±0.1	1.14±0.1	0.86±0.2	-	-	-
	Autumn	15.6±0.3	12.78±0.3	9.87±0.2	9.10±0.2	6.84±0.1	6.04±0.3	5.23±0.2	4.44±0.4	3.99±0.3	2.01±0.2	1.84±0.1	1.23±0.1	1.17±0.1
	Winter	11.4±0.5	8.91±0.4	8.62±0.5	5.65±0.3	4.41±0.3	2.73±0.2	2.10±0.2	1.25±0.1	1.43±0.2	1.12±0.2	-	-	-
TA100 -S9mix	Spring	3.96±0.4	2.74±0.2	2.14±0.2	1.93±0.1	1.16±0.1	1.03±0.2	1.01±0.1	1.00±0.1	0.99±0.2	0.89±0.1	1.01±0.1	-	-
	Summer	1.68±0.2	1.40±0.2	1.38±0.2	1.16±0.1	1.14±0.1	0.93±0.1	0.82±0.2	0.84±0.1	0.92±0.2	0.99±0.2	-	-	-
	Autumn	2.28±0.2	1.98±0.2	1.95±0.1	1.66±0.1	1.65±0.2	1.45±0.1	1.3±0.1	1.17±0.1	1.16±0.2	0.91±0.2	-	-	-
	Winter	1.95±0.2	1.75±0.2	1.56±0.2	1.51±0.1	1.47±0.1	1.02±0.1	0.93±0.1	0.95±0.1	1.02±0.1	0.99±0.1	-	-	-
TA100 +S9mix	Spring	3.07±0.3	2.5±0.3	1.46±0.2	1.27±0.1	1.06±0.2	1.04±0.1	1.04±0.2	0.94±0.1	1.01±0.1	1.00±0.1	1.00±0.2	-	-
	Summer	2.12±0.1	1.78±0.2	1.6±0.2	1.43±0.1	1.37±0.1	1.18±0.1	1.12±0.1	1.05±0.2	1.04±0.1	1.02±0.2	-	-	-
	Autumn	3.00±0.3	2.60±0.3	2.29±0.2	2.12±0.2	2.02±0.2	1.42±0.1	1.4±0.1	1.21±0.2	1.17±0.1	1.1±0.1	-	-	-
	Winter	3.63±0.2	3.56±0.3	3.31±0.3	2.87±0.2	2.47±0.2	1.81±0.3	1.45±0.2	1.16±0.2	1.04±0.1	0.94±0.1	-	-	-

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Table 4. Dependency of MR ± S.D. on concentration of hydrocarbons extracted from the analysed dust samples (PAH) determined by means of the *Salmonella* test.

Concentration m^3/cm^3		**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**	**0.78**	**0.39**	**0.195**	**0.097**
TA98-S9mix	**Spring**	1.43±0.3	1.27±02	1.07±0.2	1.05±0.1	1.05±0.1	0.96±0.1	0.94±0.1	0.92±0.2	0.90±0.2	0.83±0.1
	Summer	1.66±0.2	1.62±0.2	1.51±0.1	1.47±0.1	1.38±0.1	1.23±0.1	1.12±0.1	-	-	-
	Autumn	3.14±0.4	2.92±0.2	2.71±0.2	2.34±0.2	1.94±0.1	1.54±0.1	1.42±0.1	1.23±0.2	1.09±0.1	-
	Winter	4.88±0.5	3.57±0.4	2.62±0.4	1.84±0.2	1.77±0.2	1.54±0.1	1.24±0.1	1.09±0.1	-	-
TA98+S9mix	**Spring**	1.62±0.2	1.51±0.2	1.44±0.3	1.38±0.1	1.36±0.2	1.34±0.1	1.19±0.1	1.05±0.2	0.83±0.3	0.67±0.2
	Summer	1.44±0.1	1.31±0.2	1.30±0.1	1.29±0.2	1.27±0.3	1.23±0.1	1.13±0.1	-	-	-
	Autumn	3.88±0.2	3.68±0.2	2.46±0.2	2.10±0.1	1.63±0.2	1.23±0.1	1.18±0.1	1.09±0.1	-	-
	Winter	4.14±0.3	3.53±0.3	3.20±0.2	2.27±0.1	1.52±0.2	1.32±0.2	1.21±0.2	1.15±0.1	-	-
TA100-S9mix	**Spring**	1.34±0.1	1.26±0.1	1.12±0.2	1.12±0.1	0.88±0.2	0.95±0.1	1.00±0.1	-	-	-
	Summer	1.86±0.2	1.82±0.1	1.72±0.1	1.58±0.2	1.32±0.2	1.21±0.3	1.18±0.1	-	-	-
	Autumn	1.78±0.2	1.59±0.2	1.44±0.1	1.34±0.1	1.20±0.1	1.10±0.1	1.09±0.1	-	-	-
	Winter	2.06±0.2	1.90±0.2	1.85±0.1	1.63±0.1	1.54±0.1	1.26±0.1	1.12±0.1	-	-	-
TA100+S9mix	**Spring**	1.41±0.1	1.40±0.1	1.23±0.1	1.11±0.2	1.1±0.2	1.03±0.1	1.01±0.1	-	-	-
	Summer	2.08±0.2	2.04±0.2	1.69±0.2	1.59±0.1	1.43±0.2	1.15±0.1	1.02±0.1	-	-	-
	Autumn	1.62±0.2	1.55±0.1	1.21±0.1	1.13±0.1	1.05±0.1	1.04±0.1	0.95±0.1	-	-	-
	Winter	3.06±0.4	2.44±0.3	1.55±0.1	1.16±0.1	1.15±0.1	1.09±0.2	1.00±0.1	-	-	-

Table 5. Dependency of MR ± S.D. on concentration of nitro-PAHs extracted from the analysed dust samples determined by means of the *Salmonella* test.

Concentration m^3/cm^3		50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.097
TA98-S9mix	**Spring**	2.46±0.2	1.56±0.1	1.16±0.1	1.14±0.1	1.05±0.2	1.01±02	0.99±0.2	0.99±0.1	0.96±0.1	0.96±0.1
	Summer	1.82±0.2	1.74±0.1	1.32±0.2	1.30±0.2	1.29±0.1	1.80±0.1	1.07±0.1	-	-	-
	Autumn	4.79±0.4	3.63±0.2	2.89±0.3	2.69±0.1	2.07±0.1	1.56±0.1	1.14±0.2	1.07±0.2	1.00±0.1	-
	Winter	8.33±0.5	4.64±0.4	3.31±0.1	2.64±0.2	2.08±0.2	1.17±0.1	1.08±0.1	1.00±0.1	-	-
TA98+S9mix	**Spring**	1.83±0.1	1.43±0.1	1.41±0.2	1.24±0.2	1.21±0.1	1.21±0.1	1.17±0.2	1.05±0.2	0.99±0.1	0.94±0.2
	Summer	1.83±0.1	1.43±0.1	1.41±0.2	1.24±0.2	1.21±0.2	1.21±0.2	1.17±0.1	1.05±0.1	0.99±0.1	0.94±0.2
	Autumn	5.79±0.6	4.54±0.4	3.96±0.4	2.98±0.2	2.84±0.3	2.00±0.3	1.83±0.2	1.32±0.2	-	-
	Winter	11.26±0.5	6.83±0.3	3.43±0.3	2.12±0.2	1.42±0.2	1.21±0.1	1.02±0.1	1.01±0.1	-	-
TA100-S9mix	**Spring**	1.26±0.1	1.21±0.1	1.14±0.2	1.14±0.1	1.13±0.3	1.06±0.2	1.04±0.1	-	-	-
	Summer	2.04±0.2	1.96±0.1	1.82±0.1	1.76±0.1	1.54±0.1	1.36±0.2	1.19±0.2	-	-	-
	Autumn	2.26±0.3	2.55±0.3	1.31±0.1	1.20±0.2	1.15±0.1	1.12±0.2	0.97±0.1	-	-	-
	Winter	3.55±0.2	2.02±0.2	1.47±0.1	1.38±0.1	1.19±0.2	1.11±0.1	1.06±0.1	-	-	-
TA100+S9mix	**Spring**	1.35±0.2	1.27±0.1	1.16±0.1	1.15±0.2	1.04±0.2	1.00±0.1	0.98±0.1	-	-	-
	Summer	1.51±0.1	1.49±0.1	1.47±0.2	1.39±0.2	1.35±0.3	1.23±0.1	1.19±0.1	-	-	-
	Autumn	5.41±0.4	3.18±0.2	3.1±0.2	2.72±0.1	1.53±0.1	1.48±0.1	1.32±0.1	-	-	-
	Winter	6.93±0.6	3.37±0.2	2.16±0.3	1.75±0.1	1.16±0.1	1.01±0.2	1.02±0.1	-	-	-

Table 6. Dependency of MR ± S.D. on concentration of dinitro-PAHs extracted from the analysed dust samples determined by means of the *Salmonella* test.

Concentration m^3/cm^3		50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.097
TA98-S9mix	Spring	1.25±0.1	1.05±0.2	1.01±0.2	1.01±0.1	1.01±0.1	1.01±0.1	0.99±0.2	0.90±0.1	0.89±0.1	0.77±0.2
	Summer	2.00±0.2	1.74±0.2	1.43±0.1	1.19±0.1	1.13±0.1	1.11±0.2	0.98±0.2	-	-	-
	Autumn	2.98±0.2	1.82±0.2	1.18±0.1	1.02±0.1	0.99±0.1	1.05±0.1	1.00±0.1	1.00±0.1	1.01±0.2	-
	Winter	5.04±0.3	3.86±0.3	2.00±0.1	1.64±0.2	1.24±0.2	1.00±0.1	1.02±0.1	0.99±0.1	-	-
TA98+S9mix	Spring	1.54±0.1	1.36±0.2	1.26±0.2	1.19±0.2	1.04±0.1	1.03±01	1.00±0.1	0.99±0.1	0.92±0.1	0.83±0.1
	Summer	1.42±0.2	1.39±0.2	1.36±0.2	1.23±0.1	1.22±0.2	1.05±0.1	1.05±0.1	-	-	-
	Autumn	2.97±0.2	2.09±0.2	1.51±0.1	1.49±0.1	1.27±0.1	1.12±0.1	1.09±0.1	0.99±0.1	-	-
	Winter	4.26±0.3	2.77±0.2	2.17±0.2	1.87±0.1	1.13±0.1	1.02±0.1	0.98±0.1	1.01±0.1	-	-
TA100-S9mix	Spring	1.17±0.1	1.20±0.1	1.15±0.2	1.12±0.2	1.00±0.1	0.99±0.2	1.01±0.1	-	-	-
	Summer	2.34±0.1	2.09±0.2	1.89±0.2	1.53±0.1	1.25±0.1	1.1±0.14	1.06±0.1	-	-	-
	Autumn	2.02±0.1	1.89±0.2	1.87±0.3	1.46±0.2	1.38±0.2	1.17±0.1	1.02±0.1	-	-	-
	Winter	2.73±0.2	1.98±0.1	1.76±0.2	1.35±0.1	1.25v0.1	1.12±0.1	0.99±0.1	-	-	-
TA100+S9mix	Spring	1.34±0.1	1.08±0.2	1.00±0.1	0.85±0.2	0.75±0.1	0.84±0.1	0.98±0.1	-	-	-
	Summer	1.95±0.1	1.83±0.1	1.75±0.1	1.47±0.1	1.46±0.2	1.28±0.2	1.17±0.1	-	-	-
	Autumn	5±0.4	3.61±0.3	2.98±0.3	2.23±0.2	1.55±0.1	1.23±0.1	1.19±0.1	-	-	-
	Winter	2.91±0.2	2.09±0.2	1.99±0.1	1.79±0.1	1.35v0.1	1.14±0.1	1.05±0.1	-	-	-

3 Conclusions

Due to the complex chemical composition of dust air pollutants, the detection and identification of particular compounds, and therefore determination of substances responsible for the mutagenicity of extracts of air samples is extremely difficult. Despite the applied analytical methods, we are not able to detect all pollutants contained in collected samples. Moreover, we cannot determine the concentration of many of them. Such complex mixtures of substances are subject to different interactions, potentially leading to an increase or decrease in their biological activity. The resultant activity is the effect of the synergy or antagonism of particular substances occurring in the mixture. Therefore, the assessment of mutagenicity of all pollutants present in the analysed samples is more useful than chemical analysis of particular compounds. On the other hand, the determination of the effect of the primary groups of organic pollutants present in airborne dust such as: PAHs, nitro-PAHs, and dinitro-PAHs on genetic material, and comparison of the obtained results with the activity of all pollutants present in the tested extracts may permit correlating the chemical composition of PM2.5 with the observed mutagenic effects in the bacterial *Salmonella* test. Due to high exposure of people to environmental factors, analyses of mutagenicity of dust air pollutants should be introduced to environmental monitoring, developing a screening system able to detect different mutations on the molecular level, providing a potential basis for the estimation of the risk of cancer morbidity. Air quality monitoring should consider the assessment of mutagenicity of organic pollutants by means of the *Salmonella* test, performed with at least two test strains of *Salmonella typhimurium* TA98 and TA100.

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Appendix 1. Carcinogenicity of the analysed PAHs and nitro-PAHs (Rogula-Kozłowska, 2014)

Compound	Short	Number of CAS[a]	Carcinogenicity [b]	relative carcinogenic coefficients, k[c]
Phenanthrene	Fen	85-01-8	3	0.001
Anthracene	A	120-12-7	3	0.01
fluoranthene	Flu	86-73-7	3	0.001
Pyrene	Pyr	129-00-0	3	0.001
Benzo[a]anthracene	B[a]A	56-55-3	2A	0.1
chrysene	Chr	218-01-9	3	0.01
Benzo[b]fluoranthene	B[b]F	205-99-2	2B	0.1
Benzo[k]fluoranthene	B[k]F	207-08-9	2B	0.1
Benzo[a]pyrene	B[a]P	50-32-8	2A	1
Dibenzo[a,h]anthracene	D[a,h]A	53-70-3	2A	5
Benzo[g,h,i]perylene	B[g,h,i]P	191-24-2	3	0.01
Indeno[1,2,3-c,d]pyrene	I[1,2,3-c,d]P	193-39-5	2B	0.1
1-nitronaphthalene	-	86-57-7	3	-
2-nitrofluoren	-	607-57-8	2B	-
9-nitroantracen	-	602-60-8	3	-
3-nitrofluoranten	-	892-21-7	3	-
1 nitropiren	-	5522-43-0	2B	-
1,3-dinitropiren	-	75321-20-9	3	-
1,6-dinitropiren	-	42397-64-8	2B	-
1,8-dinitropiren	-	42397-65-9	2B	-

a Chemical Abstract Service registry number,

b According to IARC: Group 2A- potential carcinogen, Group 2B- potential carcinogen, Group 3- not classified as carcinogenic for people, relative carcinogenic coefficients (according to Nisbet and LaGoy) for particular PAHs towards B[a]P [**13d**,13e].

Appendix 2. Compounds identified in extracts of suspended particulates PM2.5.

Sample	**Identified compound**	Number of CAS
Spring	Myristic (tetradecanoic acid)	544-63-8
	Myristic acid isopropyl ester	110-27-0
	1-hexadecanol	36653-82-4
	Palmitic acid isopropyl ester	142-91-6
	Octadecanic acid	57-11-4
	1-octadecanol	112-92-5
	hexacosane	630-01-3
	palmitic acid	57-10-3
	eicosan $CH_3(CH_2)18CH_3$	112-95-8
	oleic acid	112-80-1
	hexatriacontane $CH_3(CH_2)34CH_3$	630-06-8
	cyclohexanol	108-93-0
	cyclohexanone	108-94-1
Summer	cyclohexanol	108-93-0
	cyclohexanone	108-94-1
	eicosane	112-95-8
	1,2-epoksycycloheksan	286-20-4
	isobutyl phthalate	84-69-5

	5-hexenal	764-59-0
	palmitic acid	57-10-3
	4,4,5-trimethyl-2-hexene	55702-61-9
	nondekan	629-92-5
Autumn	cyclohexanol	108-93-0
	7-cykloheksylotridekan	13151-92-3
	Cyclopenta [c, d] pyrene	
	docosane $CH_3(CH_2)_{20}CH_3$	629-97-0
	4,4-dimetylo-2-penten	690-08-4
	2,7-dimetylofenantren	1576-69-8
	1,3-dimetylopiren	64401-21-4
	eikozan	112-95-8
	2-etylodibenzotiofen	89816-98-8
	3-etylodibenzotiofen	89817-03-8
	2-fenylonaftalen	35465-71-5
	heksadekan	544-76-3
	heksatriakontan $CH_3(CH_2)_{34}CH_3$	630-06-8
	heptadecane	629-78-7
	1 metyloantracen	610-48-0
	1 metylochryzen	3351-28-8
	5-metylochryzen	3697-24-3
	4-metylofenantren	832-64-4
	1 metylopiren	2381-21-7
	nondekan	629-92-5

	octadecane	593-45-3
	pentadecane	629-62-9
	perylene	198-55-0
	tetracosane $CH_3(CH_2)_{22}CH_3$	646-31-1
	2,6,10,14-tetramethyl hexadecane	638-36-8
	2,6,10,15-tetramethyl heptadecane	54833-48-6
	2,6,10,14-tetramethylpentadecane	1921-70-6
	tricosane $CH_3(CH_2)_{21}CH_3$	638-67-5
Winter	Naphthalene anhydride	81-84-5
	7H-Benz [de] anthracen-7-one	82-05-3
	Benzo (e) pyrene	192-97-2
	Benzo (j) fluoranthene	205-82-3
	Bicyclohexyl	92-51-3
	cyclohexanol	108-93-0
	cyclohexanone	108-94-1
	cyklotetradekan	295-17-0
	4,4-dimethyl-2-pentene	690-08-4
	2,6-Di-tert-butyl-p-Cresol	128-37-0
	diphenylacetylene	501-65-5
	eicosane	112-95-8
	1,2-epoxy cyclohexane	286-20-4
	1,4-epoksycycloheksan	279-49-2
	2-etylodibenzotiofen	89816-98-8
	3-etylodibenzotiofen	89817-03-8

	ethyl phthalate	84-66-2
	isobutyl phthalate	84-69-5
	9-fluorenone	486-25-9
	heptadecane	629-78-7
	3-hydroxy-cyclohexanone	823-19-8
	2-hydroksycyklopentadekanon	4727-18-8
	palmitic acid	57-10-3
	5-metylochryzen	3697-24-3
	1 metylopiren	2381-21-7
	2-metylopiren	3442-78-2
	4-metylopiren	3353-12-6
	octadecane	593-45-3
	1-octadecene	112-88-9
	pentadecane	629-62-9
	pentanal	110-62-3
	tetradecane	629-59-4
	1,1,2,3-tetrametylocyklopropan	74752-93-5
	tridecane	629-50-5

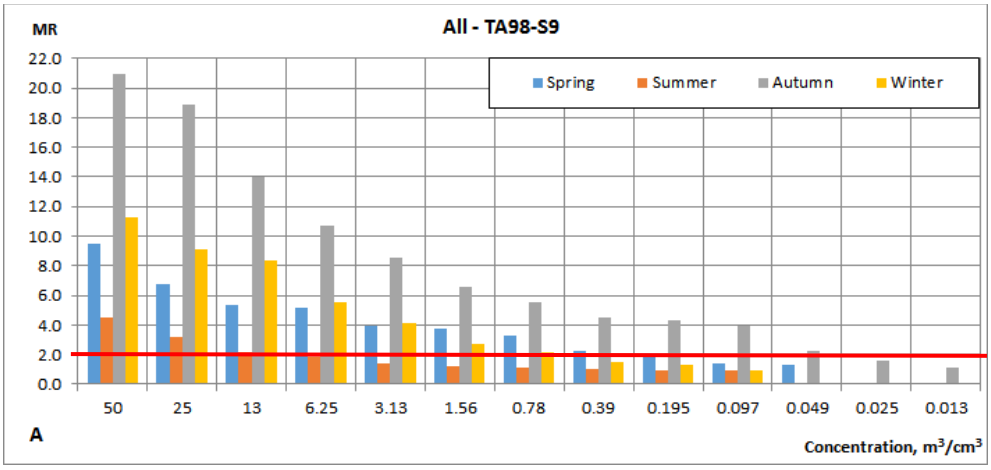
Appendix 3. Correlations between the concentration of dusts [μg/m^3] and tar substances [μg/m^3] in the analysed samples and the content of selected PAHs [ng/m^3] and nitro-PAHs [ng/m^3].

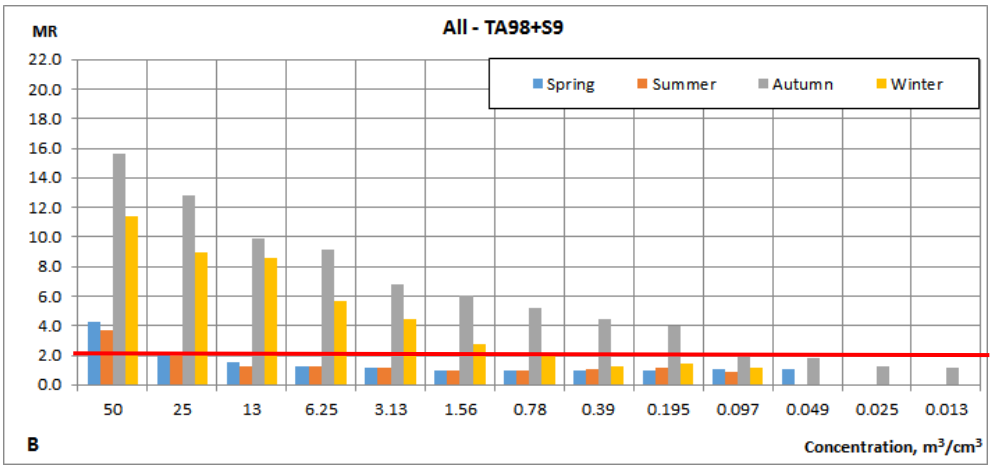
Componud	**Concentration of dusts**	**Tar substances**
B[a]P	0.833 (0.0102)	0.548 (0.16)

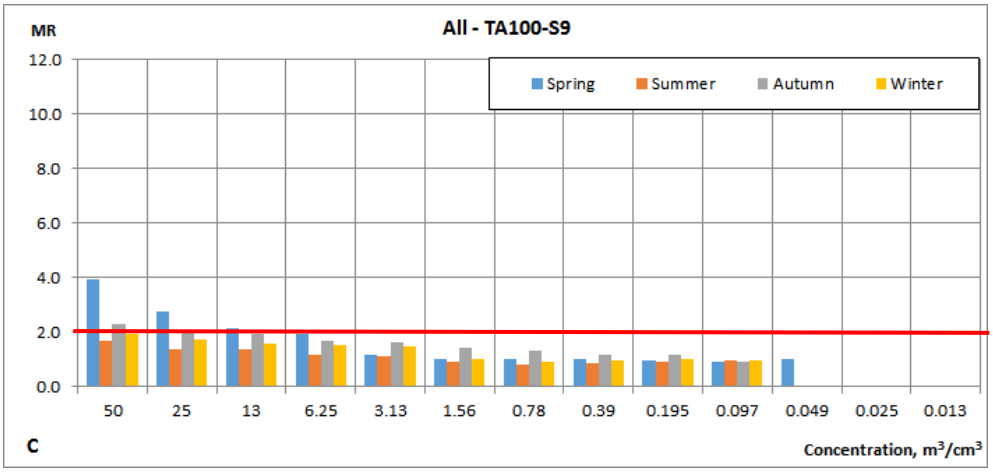
B[a]A	0.857 (0.0065)	0.690 (0.058)
D[a,h]A	0.786 (0.0208)	0.762 (0.028)
B[g,h,i]P	0.857 (0.0065)	0.714 (0.0465)
2-nitrofluoren	-0.048 (0.9108)	-0.262 (0.531)
Sum WWA	0.857 (0.0065)	0.69 (0.058)
Sum nitro-WWA	0.381 (0.3518)	0.238 (0.57)

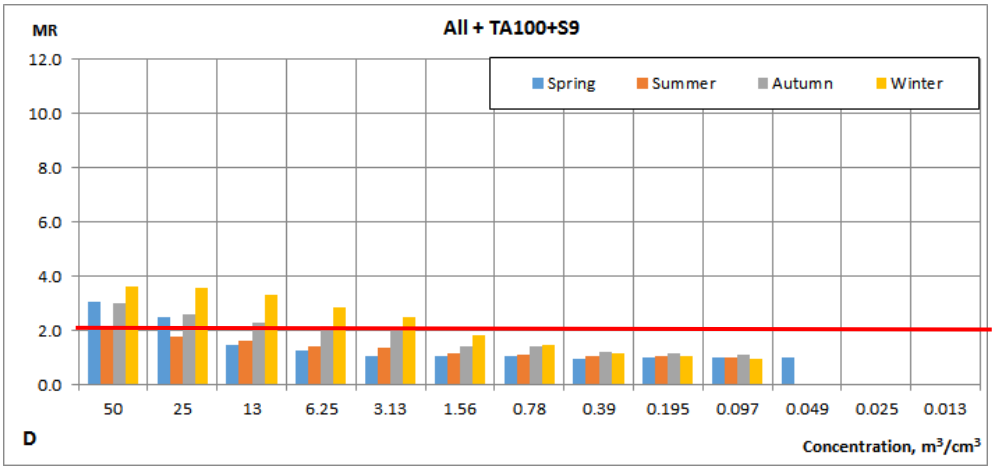
Spearman rank-order correlation coefficient (level p)

Appendix 4. Dependency of MR on concentrations of extracts of all organic dust pollutants in the analysed air samples (All) determined by means of the *Salmonella* test.

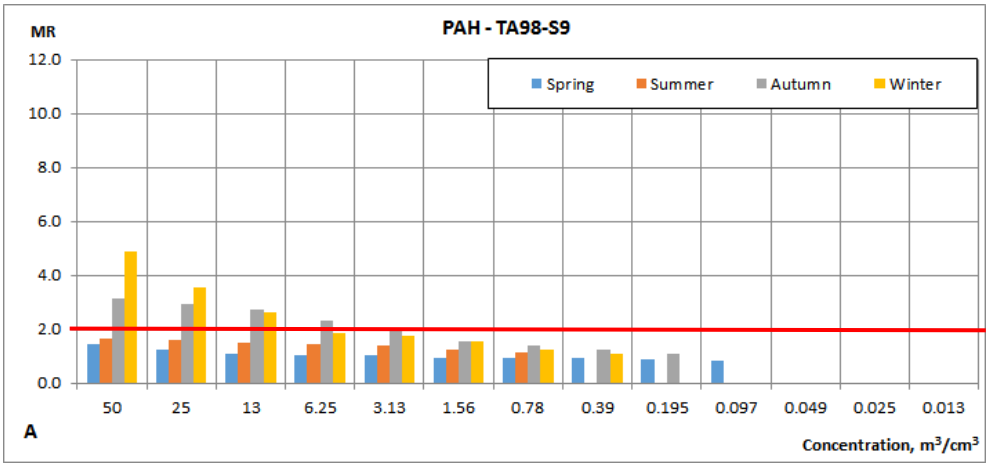


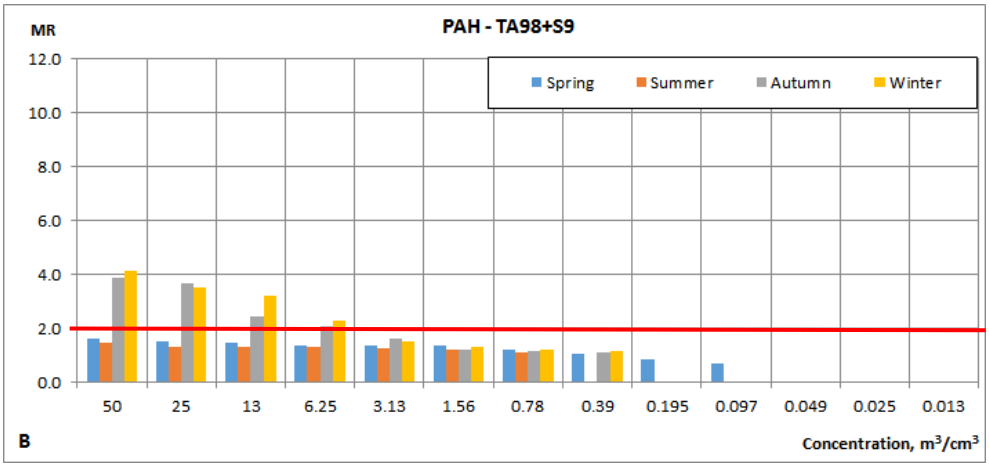


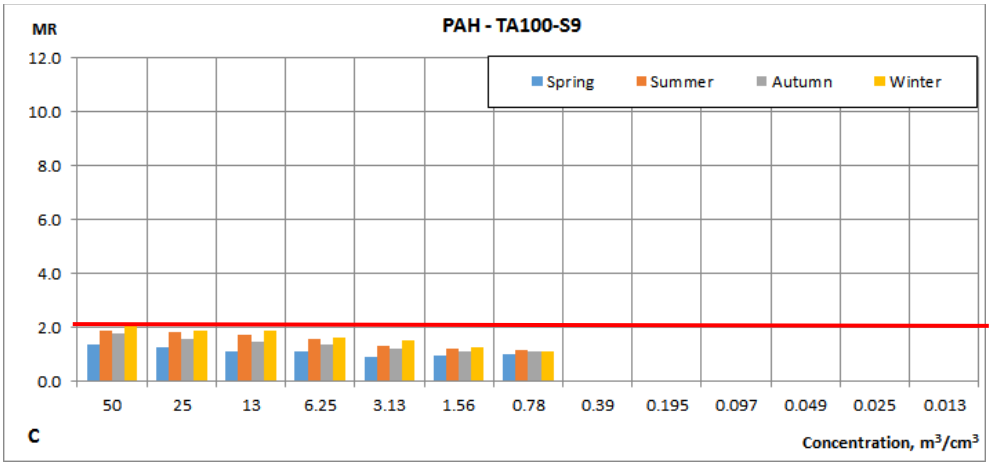


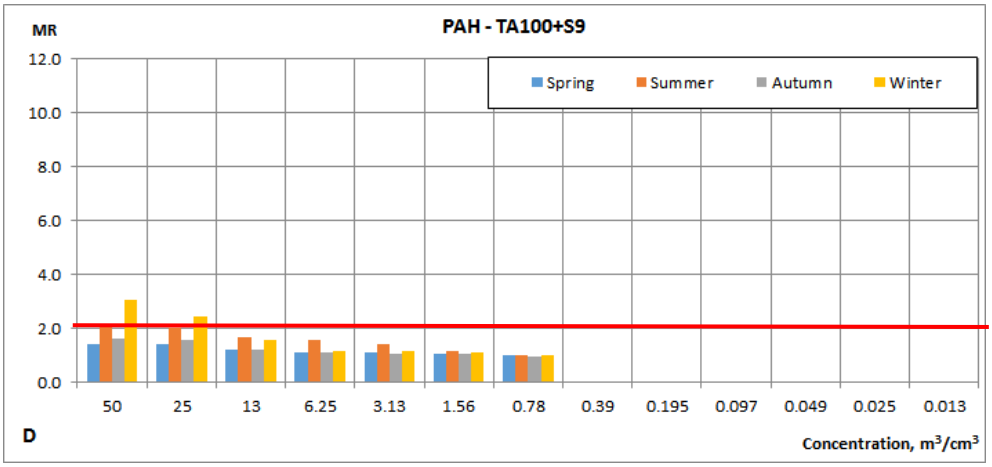


Appendix 5. Dependency of MR on concentration of hydrocarbons extracted from the analysed dust samples (PAH) determined by means of the *Salmonella* test.

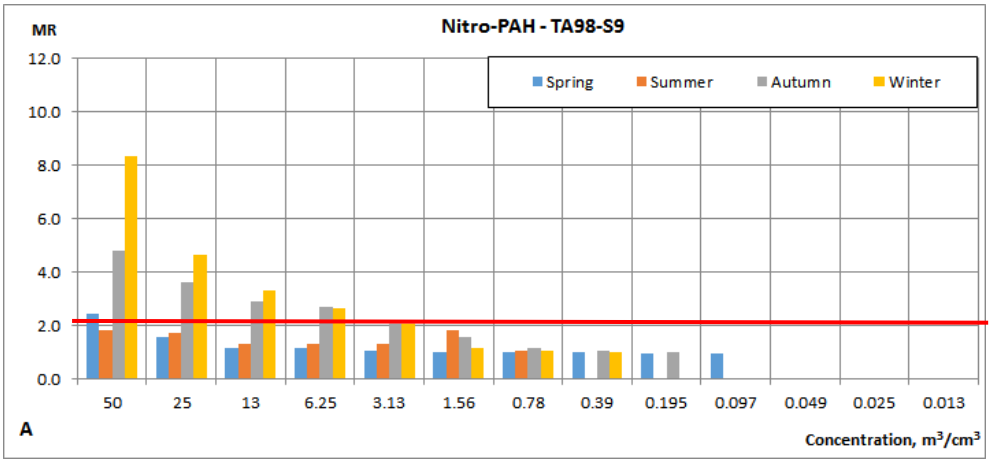

PAH - TA98-S9
MR
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Spring
Summer
Autumn
Winter
50
25
13
6.25
3.13
1.56
0.78
0.39
0.195
0.097
0.049
0.025
0.013
A
Concentration, m³/cm³

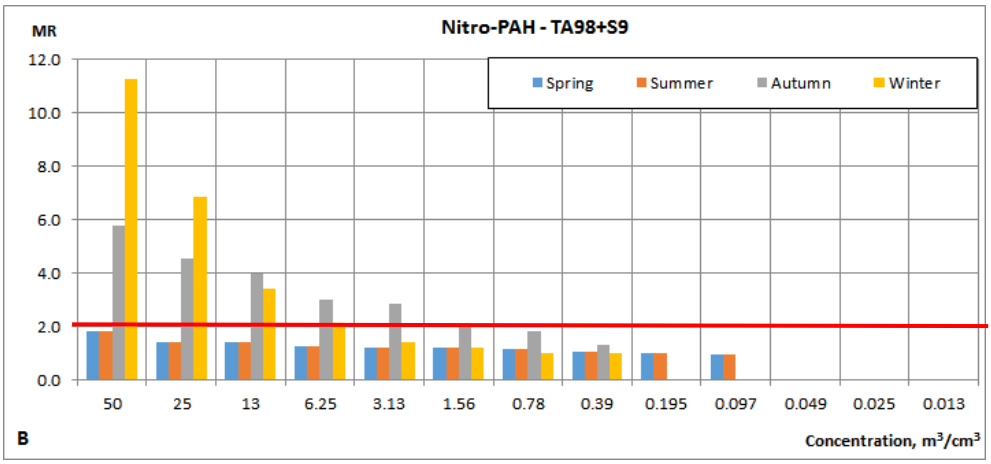

PAH - TA98+S9
MR
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Spring
Summer
Autumn
Winter
50
25
13
6.25
3.13
1.56
0.78
0.39
0.195
0.097
0.049
0.025
0.013
B
Concentration, m³/cm³

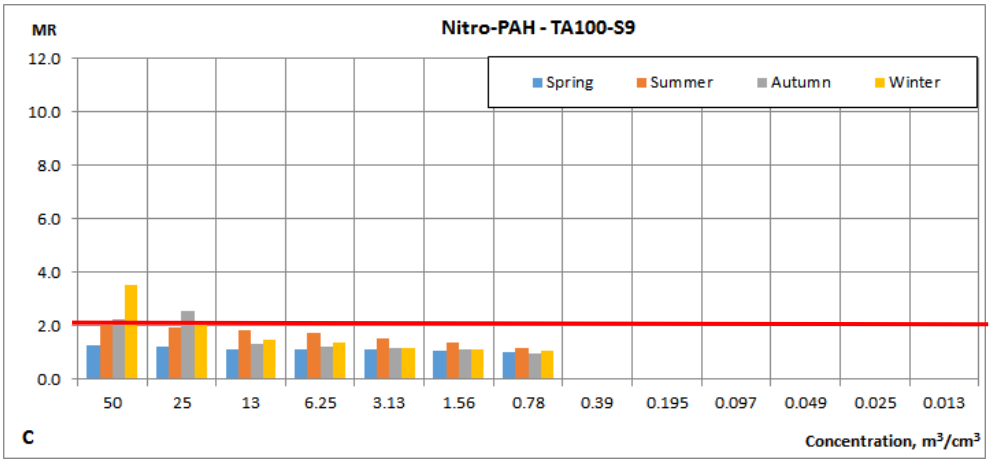

PAH - TA100-S9
MR
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Spring
Summer
Autumn
Winter
50
25
13
6.25
3.13
1.56
0.78
0.39
0.195
0.097
0.049
0.025
0.013
C
Concentration, m³/cm³

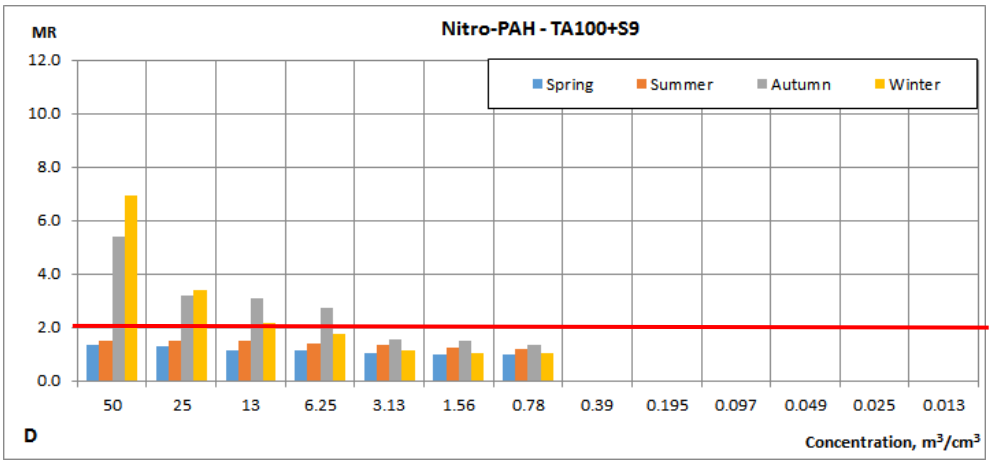


Appendix 6. Dependency of MR on concentration of nitro-PAHs extracted from the analysed dust samples determined by means of the *Salmonella* test.

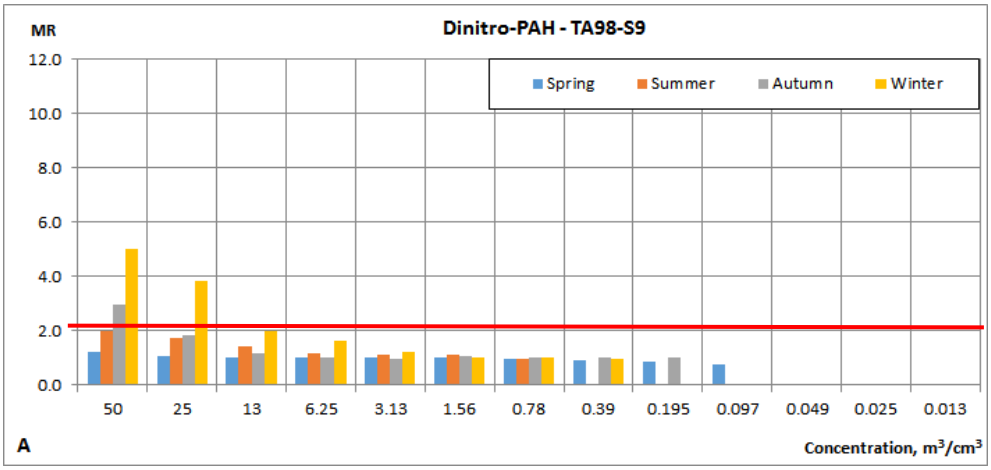


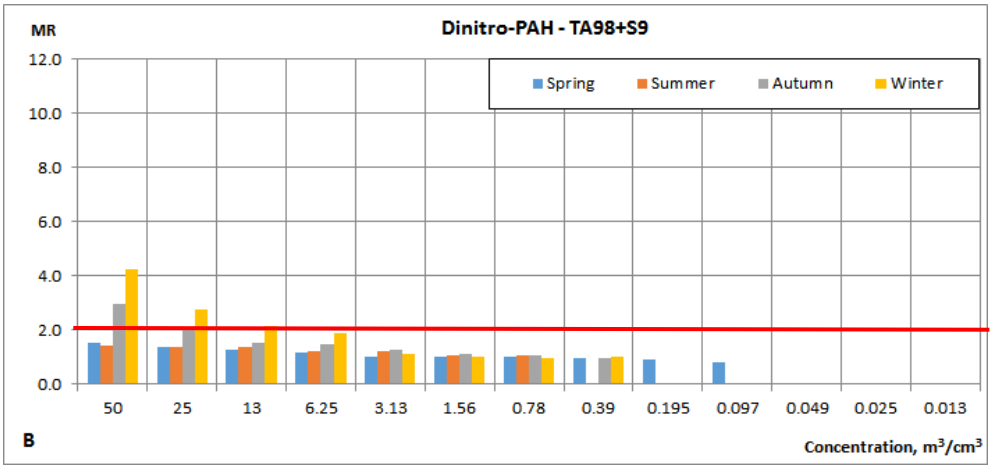


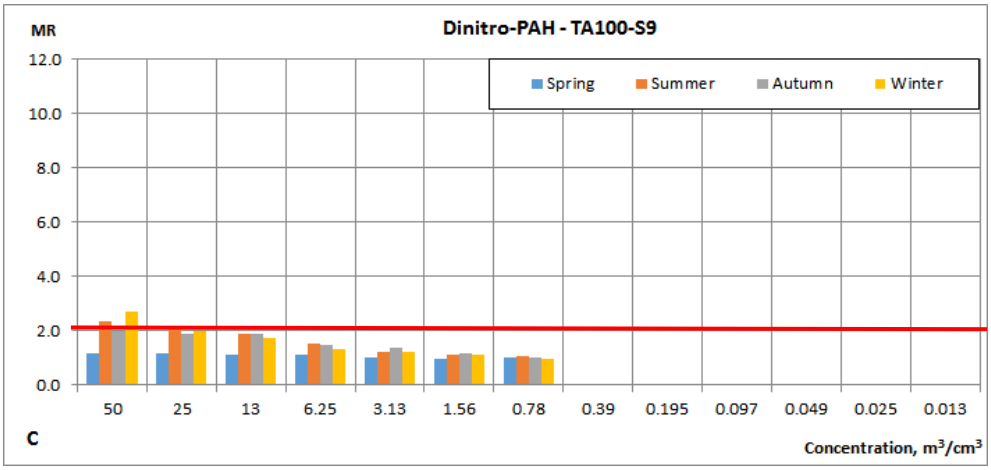


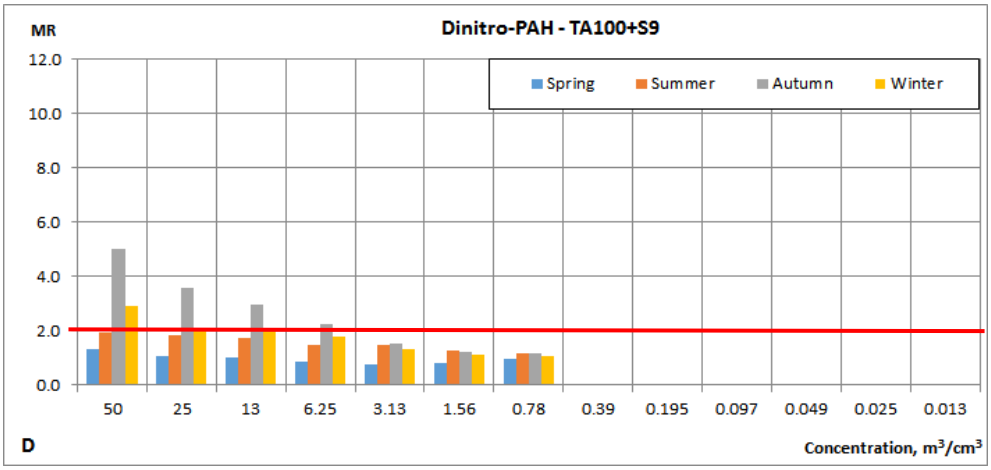


Appendix 7. Dependency of MR on concentration of dinitro-PAHs extracted from the analysed dust samples determined by means of the *Salmonella* test.


MR
Dinitro-PAH - TA98-S9
Spring
Summer
Autumn
Winter
12.0
10.0
8.0
6.0
4.0
2.0
0.0
50
25
13
6.25
3.13
1.56
0.78
0.39
0.195
0.097
0.049
0.025
0.013
A
Concentration, m³/cm³


MR
Dinitro-PAH - TA98+S9
Spring
Summer
Autumn
Winter
12.0
10.0
8.0
6.0
4.0
2.0
0.0
50
25
13
6.25
3.13
1.56
0.78
0.39
0.195
0.097
0.049
0.025
0.013
B
Concentration, m³/cm³

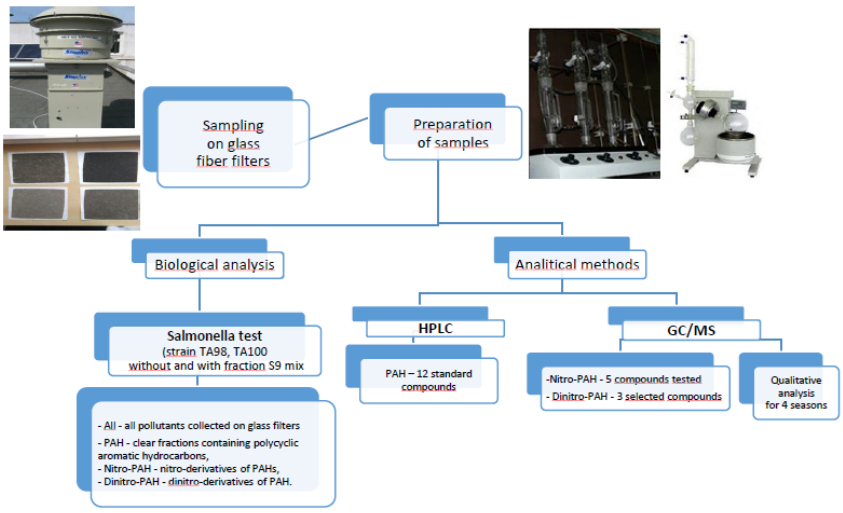

MR
Dinitro-PAH - TA100-S9
Spring
Summer
Autumn
Winter
12.0
10.0
8.0
6.0
4.0
2.0
0.0
50
25
13
6.25
3.13
1.56
0.78
0.39
0.195
0.097
0.049
0.025
0.013
C
Concentration, m³/cm³


MR
Dinitro-PAH - TA100+S9
Spring
Summer
Autumn
Winter
12.0
10.0
8.0
6.0
4.0
2.0
0.0
50
25
13
6.25
3.13
1.56
0.78
0.39
0.195
0.097
0.049
0.025
0.013
D
Concentration, m3/cm3

Declaration of interests

☒ The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Graphical abstract



Highlights

- Particulate matter PM2.5 was sampled at one study site in the centre of Wroclaw in four seasons, followed by its extraction, fractioning, and chromatographic analysis.
- The seasonal variability of the mutagenic effect was analysed by means of Salmonella test for all organic pollutants present in the collected samples of particulate matter and their fractions: PAH, nitro-PAH, and dinitro-PAH.
- A variable degree of air pollution with mutagenic substances was determined at the selected study site, depending on the analysed fraction of pollutants and sampling season.