

Article

Physicochemical Analysis of Water Extracts of Particulate Matter from Polluted Air in the Area of Kraków, Poland

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Abstract: Solubility of transition metal compounds plays a significant role in adverse health effects because that is one of the most important factors of particulate matter bioavailability in the body. In this study, we focus on the chemical analysis of particulate matter (PM) collected at different locations in the area of Kraków, one of the most polluted cities in Poland, and compare them with Standard Reference Material (SRM) 1648a from NIST. The content of four elements (carbon, hydrogen, nitrogen, and sulfur) was determined by elemental analysis, and the ratio between organic and inorganic carbon in PM extracts was confirmed by Total Organic Carbon analysis. Among the most concentrated elements found there are calcium, magnesium, sulfur, silicon, and zinc, whereas copper, iron, and manganese were present in lower concentrations. SEM-EDS analysis showed a similar morphology of the SRM and PM collected in the urban area of Kraków, while PM collected in the industrial area has smaller particles with a smooth surface. The reported analyses are significant for the APARIC project (“Air Pollution versus Autoimmunity: Role of multiphase aqueous Inorganic Chemistry”), which aims to identify the main inorganic components of PM and to understand how they affect the development of immunological diseases.

Keywords: particulate matter; redox-active metal ions; air pollution; reactive oxygen species



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1. Introduction

According to the European Environment Agency, Poland is one of the most polluted countries in Europe. The annual mean PM_{2.5} concentration in 2016 was 24 µg/m³, which is very close to the daily limit set by EU legislation (25 µg/m³) (2018) [1]. The Małopolska voivodship is one of 16 voivodships of Poland. It covers the south-eastern part of the country, the upper and partly middle Vistula river basin, and the upper Warta river basin. The capital of the province is Kraków (50°03'41"N 19°56'18"E). Among the largest emitters of pollutants in this province are steel mills, power plants, and chemical factories near Kraków. An important reason for air pollution in Kraków is its location in the Vistula river basin. On three sides it is surrounded by elevations of the terrain, which limit the movement of air masses. There is often wind silence—about 22% of all days are windless, and for the next 56% of the time, the wind does not exceed 2 m/s. Moreover, due to the specific location of the city, there is a katabatic airflow [2]. Low-stack emissions are a result of burning solid fuels (e.g., low-quality coal) and garbage in central heating boilers. In Kraków, approximately 36% of the total emission of air pollution comes from the municipal and residential sectors [3]. Moreover, it is worth mentioning that a low-stack emission was responsible for 52% of overall PM₁₀ emission sources in Poland and 88% of PM₁₀ was due to individual house heating [4]. In the area of Kraków City, the concentration of PMs is the highest during the winter season and reaches 4 times higher values than during summer. In summer the PM_{2.5} concentration is around 22 µg/m³,

whereas in winter it can reach up to $98 \mu\text{g}/\text{m}^3$; this is 2 times higher than in cities located in central Poland and 3 times higher than in cities located on the coast of the Baltic Sea [5]. The PM concentration and elemental composition depend on the location. Urban and rural air pollution during summer and winter was analyzed, and almost no difference in the PM_{2.5} concentration was observed at different locations, but during the winter season, the variability in urban areas is much higher than for rural areas. According to the report of air quality assessment in the Małopolska voivodship in 2016, the highest PM_{2.5} concentration was monitored in the city center with an annual average of almost $38 \mu\text{g}/\text{m}^3$, whereas in the industrial area close to the steelworks it was lower—around $30 \mu\text{g}/\text{m}^3$ (annual level) [6]. A similar observation was recorded for PM_{2.5} in 2017, during January and February PM_{2.5} concentration was higher than $90 \mu\text{g}/\text{m}^3$ both in urban and rural locations, but this level was exceeded for more days in urban locations [7]. Air pollution in almost whole Europe is directly connected with the same sources: smoke from wood and low-quality fuel burning for home-heating, exhaust fumes from old cars, and industrial emissions. The Balkan area including Skopje, Sofia, Prishtina, and Sarajevo, was ranked as the most polluted area in Europe. Skopje and nearby Tetovo, are predestined to air pollution due to their localization in valleys and surrounding mountains. In this area, the temperature inversion process also occurs, as in Kraków, which makes these cities more susceptible to high air pollution [8]. Sofia is the next city with its geographical location favorable for high levels of air pollution. It is surrounded by the Vitosha massif in the south and the Balkan chain to the north [9]. The best air quality is found in Scandinavian countries. Helsinki has an extremely extensive network of bicycle routes and an excellent public transportation system, which is one of the reasons why the air here is so clean. Moreover, in Finland, a huge part of the energy comes from renewable energy sources. Reykjavik uses natural energy sources, including geothermal energy. Therefore, Iceland does not deal with air pollution problems related to coal combustion. Clearly, air pollution is not only the consequence of human activity but also other, non-anthropogenic contributions.

“Air Pollution versus Autoimmunity: Role of multiphase aqueous Inorganic Chemistry” (APARIC) is an interdisciplinary project supported by the National Science Center in Poland comprising scientists in chemistry, medical biology, and clinical medicine. The main aim of this project is the investigation of environmental pollution, in particular, inorganic PM components, which can aggravate the course of autoimmune diseases, especially influence the immune system response [10]. Properties of particulate matter like chemical composition, particle size, and solubility are crucial for the cellular toxicity mechanism. Inorganic particles can contain elements and ion compounds, which are responsible for their pathogenic properties and reactive oxygen species (ROS) generation activity that affects metabolism and neurotransmitter synthesis. For this reason, it is necessary to investigate how inorganic compounds present in the particulate matter can influence cytotoxicity, and thus—how they can affect human health.

The main goal of this study was to analyze the particulate matter collected in different locations in Kraków in terms of physicochemical properties and to compare them with Standard Reference Material (SRM 1648a) of urban air pollution obtained from the US National Institute of Standard and Technology (NIST). As specified in the Certificate of Analysis for the Standard Reference Material 1648a from NIST [11], this material is a reference for urban particulate matter and is commonly used as a control reference material for many tests. Among others, the NIST sample also contains particulate matter from diesel or general urban dust, so the SRM1648a sample fits the best for our work. The main goal of this project is to account for the influence of inorganic components of particulate matter in Kraków on autoimmune disease development. Our work focused especially on investigating the elemental composition of PM (in particular, PM_{2.5}) and its water extracts. Analysis of the particle size of particulate matter is crucial to understanding its influence on human health. The particle size is one of the most important parameters that control toxicity. PM₁₀, containing particles with an aerodynamic diameter of up to $10 \mu\text{m}$, can enter the respiratory tract and cause bronchitis, asthma, and upper respiratory

tract infections. PM_{2.5} is considerably smaller, with the aerodynamic particle diameter smaller than 2.5 μm . It can penetrate the lower respiratory tract and bloodstream causing cardiovascular infection and diseases. Submicron particles (PM₁ with aerodynamic particle diameter up to 1 μm) penetrate deeper, into the bloodstream, and can cause more harmful effects, for example, heart diseases. Nanoparticles with a diameter <100 nm, referred to as ultrafine particulate matter (UFP) after inhalation are efficiently deposited in all regions of the respiratory tract. With a decrease in particle size, the active surface area of PM increases, showing higher catalytic activity in the generation of reactive oxygen and nitrogen species. As a consequence, a decrease in particle size besides the deeper penetration of the respiratory system should, in general, favor increased toxicity related to catalytic ROS formation. As the size of inhaled PM decreases, their penetration range increases [12]. The particle size distributions in aqueous solutions were measured after different sample preparation times to assess the stability of the PM water extracts. There is not so much information about the inorganic part of particulate matter extracts, their composition of soluble components and size, as well as information about carbon content in such PM. Here we propose a unique approach based on the removal of soot and organic species before extracting water-soluble inorganic components. Therefore, the results of our study can be useful for other scientists who wish to work with PM extracts deprived of organic components, which may be responsible for their own toxicity and reactivity.

2. Materials and Methods

2.1. Materials

SRM 1648a was supplied by NIST. Methanol 99.9% was purchased from POCh, phosphate-buffered saline, PBS (pH = 7.4) from Merck. The PBS buffer was used to mimic the intracellular environment.

2.2. Particulate Matter Sampling and Extraction

Location of all PM collectors are presented in Figure 1.

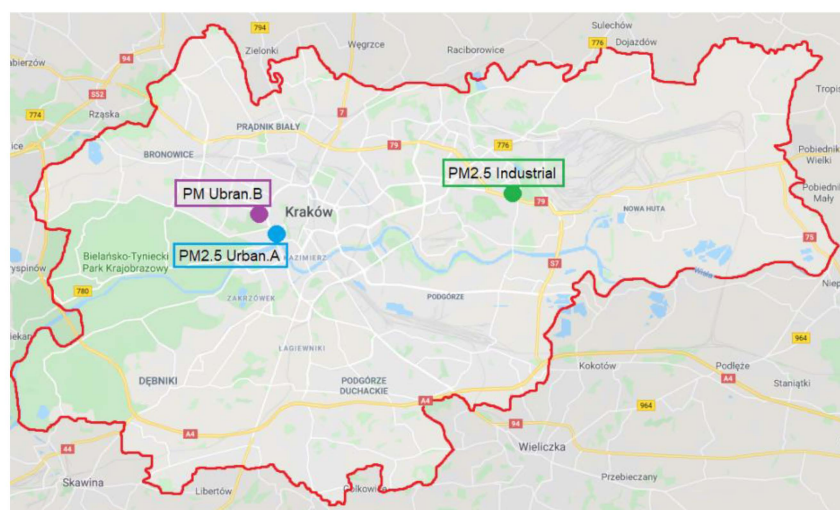


Figure 1. Localization of PM collectors in Kraków.

PM_{2.5} Urban.A and PM_{2.5} Industrial samples were collected on quartz fiber filters (diameter of 47 mm, pore size 2.2 μm) according to procedures of the Voivodship Inspectorate for Environmental Protection (VIEP) in Kraków. The air quality assessment in Poland was carried out based on relevant legal acts that define the air monitoring system, and the scope and method of testing. The relevant regulations can be found on the website <http://monitoring.krakow.pios.gov.pl/ocena-jakosci-powietrza> (accessed on 27 April 2021). The PM_{2.5} Urban. A collector was located in the city center close to busy roads and traffic, while the industrial area (sample PM_{2.5} Industrial) is a suburb of Kraków in the

neighborhood of a power plant and steelworks. We built our own homemade system for PM collection, which is a modified version of conventional systems and allows for much larger samples to be collected (sample name Urban.B). It was located in the city center, 200 m from the collector of urban PM_{2.5} of VIEP. A specially designed plexiglass cassette with 16 holes was made for 16 polytetrafluoroethylene (PTFE) filters with a diameter of 47 mm and pore size of 2.5 µm. All filters were held with metal rings in fixed positions in the cassette. This cassette was connected tightly to a pump which sucked the air from outside (ca. 30 m³/h) through the filters and allowed the collection of particulate matter on the filters. Filters were exchanged every week. All filters with particulate matter were extracted with methanol and sonicated for 1 min (PTFE filters) or 15 min (quartz filters) in a water-bath sonicator (SONIC-5, Polsonic, 620 W). Extraction of PM from quartz filters was more difficult, which resulted in a longer sonication time (15 min). For Teflon filters, 1 min of sonication was sufficient for the extraction process. Each of the filters were sonicated 2 times to assure total extraction of collected particulate matter. Subsequently, the PM was dried overnight at 60 °C (Pol-Eko-Aparatura Sp. J.). Finally, altogether ca. 50 mg of dry PM_{2.5} Urban.A and dry PM_{2.5} Industrial powders were collected. Due to a high airflow the amount of PM Urban.B collected during 3 months was much higher, around 400 mg.

2.3. Preparation of Kraków PM Extracts

Appropriate amounts of dried powder of the particulate matter samples were suspended in water and phosphate-buffered saline (pH 7.4) to reach the concentration of 1 g/L. Suspensions were incubated on a shaker for a maximum of 7 days at room temperature. Extracts were prepared in 3 ways: the first sample—only incubation, the second sample—incubation with additional centrifugation (1350 rpm, 10 min or 30 min), and the third sample—incubation, filtration (syringe filter with 0.22 µm pores), and centrifugation. Extracts prepared in these ways were analyzed for elemental composition (Inductively Coupled Plasma—Optical Emission Spectrometer (Plasm 40, Perkin Elmer, Waltham, MA, USA) and particle size (Dynamic Light Scattering measurements, Malvern Zetasizer ZS, Malvern, UK). The three procedures for PM extract preparation were used to investigate in detail the particles that actually occur in our body after inhalation. In general, PM in the air is present in the solid state, but during rain, fog, or high humidity, we deal with a totally different system. Many components of PM are soluble under such conditions. We inhale them as the high humidity is common for seasons with the highest air pollution (autumn and winter). After entering our body, PM components are also soluble in body fluids. Limiting the particle size reflects particle size reduction after crossing the barriers in our organisms.

2.4. Analysis of the Carbon, Nitrogen, Hydrogen, Sulfur Elemental Content and Removal of Carbon Compounds

The removal of components containing carbon was performed as described in our earlier study [13]. Low-temperature plasma enables the conversion of organic contaminants into volatile products, leaving the inorganic components in their basic forms. PM samples were treated with a low-temperature oxygen plasma for 2 h in the case of NIST [13] and 5, 10, and 15 h for Urban.C PM at the maximum power of the device (100 W) and referred to as NIST SRM/2 h and Urban.C/5 h, Urban.C/10 h and Urban.C/15 h, respectively. For PM collected in Kraków, plasma treatment was much longer (up to 15 h) compared to NIST SRM 1648a (2 h treatment). This was due to fact that the carbon content in PM from Kraków was 3 times higher than in the SRM 1648a sample. Elemental analysis was used to monitor the contents of carbon, hydrogen, nitrogen, and sulfur (Elementar, Vario Micro Cube, Okehampton, UK), and a total organic carbon analyzer (Shimadzu, Kioto, Japan, TOC-V series) was used to determine the organic and inorganic carbon content.

2.5. Morphology Characterization and Elemental Analysis of Kraków PM

PM samples collected at different locations in Kraków were analyzed with a Scanning Electron Microscope TESCAN (Brno, Czech Republic) Vega 3 LMU (LaB₆ cathode)

equipped with the EDS spectrometer (Oxford Instruments, X-act, SDD 10 mm², Oxford, UK). EDS spectra were collected for several areas of PM samples to check the sample inhomogeneity. Results were compared with those collected for the Standard Reference Material, NIST 1648a.

2.6. Determination of Elements Present in Water Extracts

The NIST SRM 1648a powder and NIST SRM 1648a after 2 h of plasma treatment were suspended in distilled water (in concentration 1 g/L). After a few minutes of sonication, samples were shaken for 24, 72, and 168 h. Different incubation times were applied to assess whether it had any influence on the elemental composition of the PM extracts. After incubation, each suspension was centrifuged and filtrated through a syringe PTFE filter with 0.22 µm pores. Water extracts of Standard Reference Materials, as well as Kraków PM extracts, were analyzed by Inductively Coupled Plasma—Optical Emission Spectrometry (Plasm 40, Perkin Elmer).

2.7. Effect of Sonication Time, Filtration and Centrifugation on Particle Size Distribution (SRM/2 h and SRM 1648a)

The NIST SRM 1648a and NIST SRM/2 h samples were suspended in distilled water (in concentration 1 g/L). After a few minutes of sonication, samples were shaken for 72 h, centrifuged for 10, 30, or 40 min, and 2 mL of each suspension was collected. Then, one part of the samples were filtrated through a syringe PTFE filter with 0.2 µm pores. Samples prepared in this way were analyzed by the Dynamic Light Scattering technique (Malvern Zetasizer ZS, Malvern, UK).

3. Results

3.1. Analysis of the Carbon, Nitrogen, Hydrogen, Sulfur Elemental Content and Removal of Carbon Compounds

The content of carbon, hydrogen, nitrogen, and sulfur was measured by elemental analysis and summarized in Figure 2.

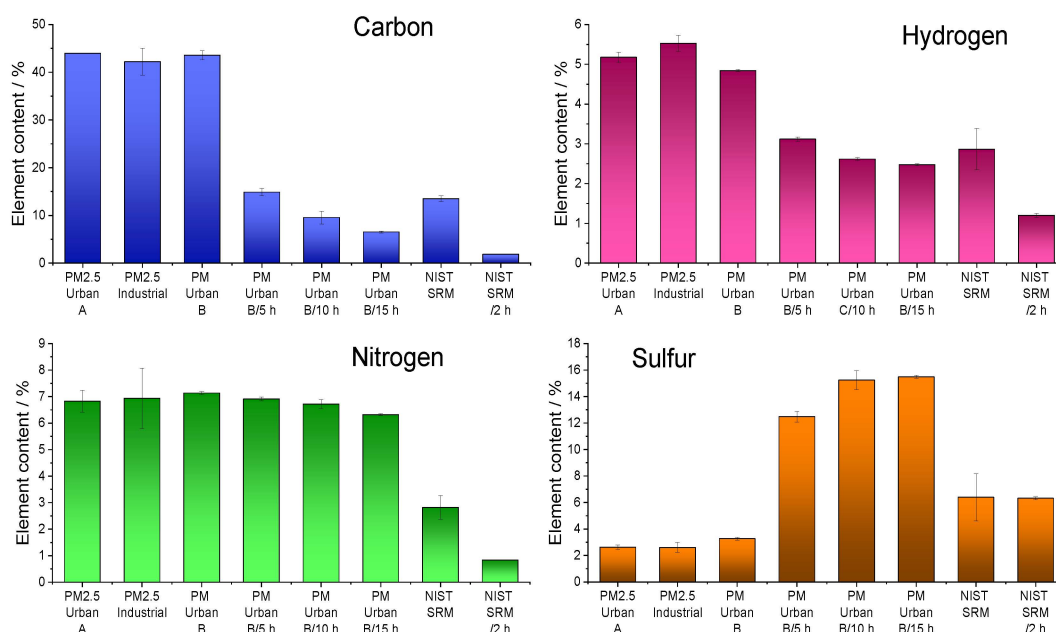


Figure 2. Comparison of the C, H, N, and S elemental content in NIST SRM 1648a, particulate matter collected in Kraków, and plasma-treated samples.

In comparison to our earlier data [13] PMs collected in Kraków are much richer in organics than standard reference urban particulate matter NIST SRM 1648a. The carbon

content in NIST SRM 1648a is certified as 12.7% [11] and we obtained a similar value of 13.5%. For all PM samples collected in Kraków (Urban.A, Urban.B, Industrial), samples contain more than 40% of carbon. Black carbon is one possible form of carbon in air pollution and it is produced during the incomplete combustion of fossil fuels (e.g., soot). Organic carbon which can be formed in the atmosphere as a result of chemical and photochemical reactions is another component of PM [14]. Four main factors affect the organic carbon content in PM: biomass combustion, traffic, secondary organic aerosol, and long-range transport. In Poland, people heat their homes often using coal, or low-quality fuels, which contribute to the production of PM rich in soot. During winter, emissions of PM are 4–5 times higher than those measured during summer [6,7]. A wide range of different polycyclic aromatic hydrocarbons (PAHs) can be found in the particulate matter collected in Kraków. Among them, fluoranthene, pyrene, benzo[α]anthracene, and chrysene are the most concentrated components [15]. Extremely high concentrations of organics in Kraków air pollution can be because the samples were collected mostly during the winter season, and the propensity of aggregation of organics from air pollution to total suspended particles increases with decreasing temperature [16]. Removal of the organic content from PM Urban.B was performed in the same way as for NIST 1648a, i.e., using the low-temperature oxygen plasma. For Urban.B, 2 h of plasma treatment were not sufficient to obtain the carbon-free fraction of air pollution, and even after 15 h of plasma treatment (Urban.B/15 h) the remaining carbon content (52%) was higher than in the NIST SRM/2 h sample.

Furthermore, the nitrogen content is higher in Kraków PM (around 7.4%) than in NIST SRM (2.8%). From the decreasing content of nitrogen upon plasma treatment (mainly in the case of NIST SRM 1648a), it can be concluded that a certain amount of nitrogen may be due to the presence of PAHs. The most concentrated nitro-PAH in Kraków air is 2-nitrofluoranthene, the same as in NIST SRM 1648a, but in fifty times higher concentration, viz. 12.9 compared to 0.2 mg/kg, respectively [15]. The sulfur content is two times lower than in NIST SRM 1648a. The main source of this element in PM is ascribed to the combustion of fossil fuels and some minerals (e.g., gypsum) [13].

3.2. Analysis of Organic and Inorganic Carbon Content

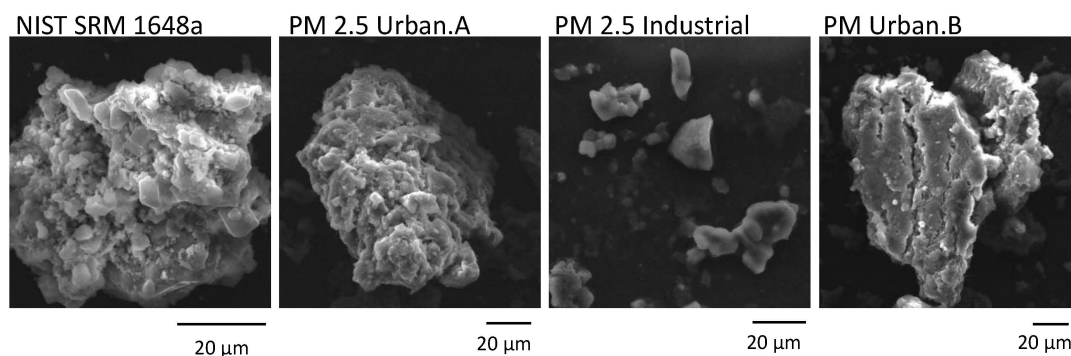
The carbon content was also examined using the Total Organic Carbon Analyzer, which enabled us to distinguish between organic and inorganic forms of carbon. The obtained results are summarized in Table 1. In the first procedure, only 3 days of incubation were applied. In the case of inorganic carbon (IC), the extracted content was higher in PBS than in the water in every sample. This can confirm the presence of less water-soluble, better PBS-soluble species of IC in the PM. Moreover, the content of IC in all investigated samples was very low, which means low carbonate content. For total organic carbon (TOC) this relation is not discernible, since carbon compounds seem to be similarly soluble in both solvents. After 10 min of centrifugation of incubated suspensions of PM, the general content of carbon decreased a few times. Probably, centrifugation causes the separation of larger particles that are richer in carbon compounds and only soluble carbon compounds remain in the solvent. Following centrifugation, a trend with higher solubility of carbon compounds in PBS was observed, mainly for IC, whereas in aqueous solution the amount of IC was almost zero. For TOC we observed a higher content of organic carbon compounds in PBS; only for the reference material (both before and after plasma treatment) the amount of TOC is higher in water. Additional filtration during the preparation of extracts did not have a significant impact on the carbon content, since all values were comparable. Consequently, it can be concluded that the content of carbon from carbon-containing compounds soluble in water/PBS can be estimated just after incubation and short centrifugation, while filtration does not affect the TOC/IC content. PBS seems to be a better solvent for organic carbon-containing compounds present in Kraków particulate matter.

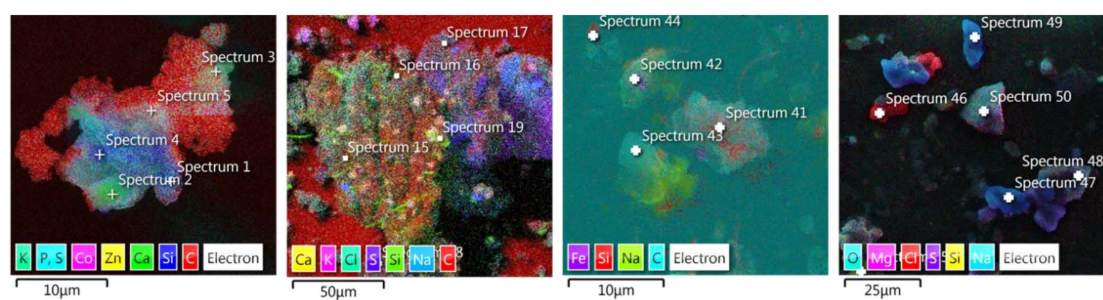
Table 1. Inorganic carbon (IC) and total organic carbon (TOC) content of prepared extracts.

| Sample | Solvent | Content (in %) | | | | | |
|------------------|------------------|--|-----------|---|-----------|---|-----------|
| | | Sample Preparation: 3 Days of Incubation | | Sample Preparation: 3 Days of Incubation, 10 Min Centrifugation | | Sample Preparation: 3 Days of Incubation, Filtration, 10 Min Centrifugation | |
| | | TOC | IC | TOC | IC | TOC | IC |
| NIST SRM 1648a | H ₂ O | 21.6 ± 0.5 | 0.1 ± 0.1 | 4.9 ± 0.3 | - | 3.1 ± 0.1 | 0.1 ± 0.1 |
| | PBS | 19 ± 3 | 1.5 ± 0.1 | 3.6 ± 0.1 | 0.5 ± 0.1 | 4.1 ± 0.3 | 0.4 ± 0.1 |
| NIST SRM/2 h | H ₂ O | 4.0 ± 0.2 | 0.2 ± 0.1 | 2.3 ± 0.3 | - | 3.9 ± 0.2 | - |
| | PBS | 3.4 ± 0.4 | 0.5 ± 0.1 | 1.2 ± 0.2 | 0.4 ± 0.1 | 1.6 ± 0.1 | 0.3 ± 0.1 |
| PM2.5 Urban.A | H ₂ O | 31 ± 2 | 0.2 ± 0.1 | 14.4 ± 0.3 | 0.1 ± 0.1 | 11.0 ± 0.7 | 0.1 ± 0.1 |
| | PBS | 45 ± 4 | 1.3 ± 0.1 | 16.9 ± 0.2 | 0.6 ± 0.1 | 16.5 ± 0.2 | 0.5 ± 0.1 |
| PM2.5 Industrial | H ₂ O | 47.4 ± 0.2 | 0.2 ± 0.1 | 7.4 ± 0.2 | 0.1 ± 0.1 | 11.9 ± 0.1 | - |
| | PBS | 38 ± 3 | 1.2 ± 0.1 | 10.0 ± 0.4 | 0.8 ± 0.1 | 10.3 ± 0.5 | 0.5 ± 0.1 |
| PM Urban.B | H ₂ O | 48 ± 5 | 0.2 ± 0.1 | 10.4 ± 0.6 | 0.1 ± 0.1 | 9.5 ± 0.3 | 0.1 ± 0.1 |
| | PBS | 46 ± 3 | 1.0 ± 0.1 | 14 ± 1 | 0.5 ± 0.1 | 11 ± 1 | 0.5 ± 0.1 |
| PM Urban.B/10 h | H ₂ O | 19 ± 2 | 0.1 ± 0.1 | 9.2 ± 0.1 | 0.3 ± 0.1 | 11.1 ± 0.4 | 0.1 ± 0.1 |
| | PBS | 25 ± 3 | 0.7 ± 0.1 | 11.1 ± 0.4 | 0.7 ± 0.1 | 12.8 ± 0.2 | 0.8 ± 0.1 |

3.3. Analysis of Morphology of Particulate Matter from Different Kraków Locations

Scanning electron microscopy images of PM from Kraków do not show significant differences in morphology. In all samples, relatively large aggregates of particles of different shapes and sizes, from nano- to even a few micrometers, were observed. Samples collected in the urban areas (Urban.A, Urban.B) are characterized by particles similar in size and surface morphology to the reference material, while PM2.5 Industrial consists of smaller aggregates, up to 20 µm, showing a smooth surface (Figure 3). Elemental analysis was performed by Energy Dispersive Spectroscopy (Figure 4). The mass content of elements was determined for selected sites and is also presented in Figure 4. Carbon is the most common element; the content of this element in the PM2.5 Industrial is comparable with NIST SRM 1648a, whereas the content of this element in the PM2.5 Urban.A is higher. This may be associated with increased exhaust gas emissions because our collectors of urban PM samples are located in the center of the city and is one of the most crowded streets in Kraków. Interestingly, EDS measurements revealed nitrogen only for the NIST SRM 1648a sample, but it can be explained by selectively determining the content of elements in one point of the samples. All PM2.5 samples contain comparable amounts of sulfur and silicon, whereas iron was found in larger quantities for PM2.5 Urban.A. This might be related to the abrasion of brake pads. The amount of copper in all samples is negligible, but this result may originate from local analysis of elements in one particle, while the total content of elements may be different. Samples collected in the urban area contain small amounts of titanium, which is not present in the PM2.5 from the industrial area. The content of Na and Cl in the PM from Kraków can be explained by the fact that the PM was collected mainly during the winter period when roads are salted.

**Figure 3.** Comparison of PM collected in different locations in Kraków.



| | NIST SRM 1648a | | | PM Urban.B | | | PM2.5 Urban.A | | | PM2.5 Industrial | | |
|----|----------------|------|------|------------|------|------|---------------|------|------|------------------|------|------|
| | S1 | S3 | S5 | S15 | S17 | S19 | S41 | S42 | S43 | S45 | S47 | S48 |
| C | 19.5 | 23.1 | 39.0 | 59.0 | 68.9 | 28.4 | 36.7 | 41.9 | 45.6 | 31.9 | 4.9 | 39.0 |
| N | 1.9 | 0.9 | 9.9 | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| O | 4.7 | 3.4 | 15.7 | BD | BD | 6.4 | BD | 12.0 | BD | 14.2 | 1.0 | BD |
| Na | 0.02 | BD | 0.3 | 0.2 | 1.3 | BD | 0.1 | 1.1 | 0.2 | 0.7 | 0.3 | 0.35 |
| Mg | 0.03 | BD | 0.05 | BD | BD | BD | BD | 0.07 | BD | BD | BD | BD |
| Al | 0.6 | 0.02 | 0.3 | BD | 0.1 | BD | BD | 0.06 | 0.01 | BD | BD | 0.05 |
| Si | 1.3 | 0.01 | 0.6 | 0.4 | 0.2 | BD | 1.4 | 0.3 | 0.1 | 6.6 | BD | 0.2 |
| S | 0.2 | 0.01 | 0.6 | 0.4 | 1.7 | BD | 0.1 | 0.6 | 0.2 | 0.4 | 0.9 | 0.6 |
| Cl | 0.02 | BD | 0.4 | 0.4 | 0.8 | BD | 0.1 | 0.2 | 0.02 | 0.8 | 0.02 | 0.4 |
| Ca | 0.1 | BD | 0.1 | 0.04 | 0.03 | BD | BD | 0.05 | BD | 0.04 | BD | 0.09 |
| Ti | 0.04 | BD | 0.03 | 0.04 | 0.06 | 0.09 | BD | 0.08 | BD | BD | BD | BD |
| Fe | 0.3 | BD | 0.2 | 0.3 | 0.4 | BD | 0.4 | 5.3 | BD | 0.03 | BD | 0.1 |
| Cu | BD | BD | 0.04 | 0.07 | BD | BD | BD | 0.1 | BD | 0.03 | BD | 0.1 |
| Zn | BD | BD | BD | 0.04 | 0.3 | BD | 0.03 | 0.2 | BD | 0.1 | BD | 0.09 |
| K | BD | BD | BD | 0.06 | 0.2 | BD | 0.04 | 0.07 | 0.02 | 0.2 | 0.03 | 0.1 |

Figure 4. Energy dispersive spectroscopy mapping of PM samples and contents of selected elements (in wt%) of Kraków PM samples determined by EDS analysis. BD—below the detection limit.

3.4. Determination of Elements Present in Water and PBS Extracts from Kraków and NIST SRM Particulate Matter

One of the aims of PM incubation in water was to determine elements in the water-soluble components of PM and to investigate whether longer incubation times affect the element concentration in water. Principally, the same elements were found for both NIST SRM 1648a before and after plasma treatment, but in extracts of NIST SRM/2 h higher amounts of different elements were found (Figure 5). Sulfur is present in the highest amount among water-soluble elements analyzed both for NIST SRM and NIST SRM/2 h water fractions with concentrations of 160 and 200 mg/L, respectively. Silicon occurs in the NIST SRM 1648a in large amounts (12.8%), whereas the ICP-OES analysis shows that a negligible concentration of this element is present in both extracts. This indicates that silicon is present in the form of insoluble compounds. The concentration of calcium is the second highest and is five times lower than the sulfur concentration. The third element with the highest concentration is phosphorus with an average concentration of 7 mg/L in both extracts.

Among the most interesting elements from the standpoint of bioinorganic redox chemistry, manganese is one with the highest concentration and its concentration in extracts is two times higher for plasma-treated PM samples. A similar situation occurs for copper, but compared to manganese, its concentration in the extracts is two times lower. The iron concentration is higher in the PM extract before oxygen plasma treatment. More interesting, the concentration of iron in NIST SRM 1648a is the highest among all metals (of around 3.9%), but in extracts, the amount of iron is much lower, viz. 0.05 mg/L, which can indicate that iron also exists mainly in an insoluble form. The last element of interest is nickel, whose content in the water extracts of both PM is comparable. Only in the case of elements with high concentrations, the time of PM incubation has a small influence on the number

of elements in the water extracts, viz. the concentration of these elements in the extract increases slightly with longer incubation time.

Elements of Kraków PM extracts were examined in two solvents: water and PBS. Elements that are included in the composition of phosphate-buffered saline buffer were not considered. Calcium, magnesium, sulfur, silicon, and zinc are among the elements with the highest concentration, whereas sulfur is the most concentrated element in all extracts, similar to extracts of the standardized PM where this value is two times smaller (Figure 6). Interestingly, a high concentration of sulfur was observed for PM Urban.C/10 h, a plasma-treated sample of PM with 4 times higher sulfur concentration than in the sample before plasma treatment. This concentration was also confirmed in elemental analysis where the content of this element was 4 times higher. This also means that nearly all sulfur-containing compounds are soluble in water.

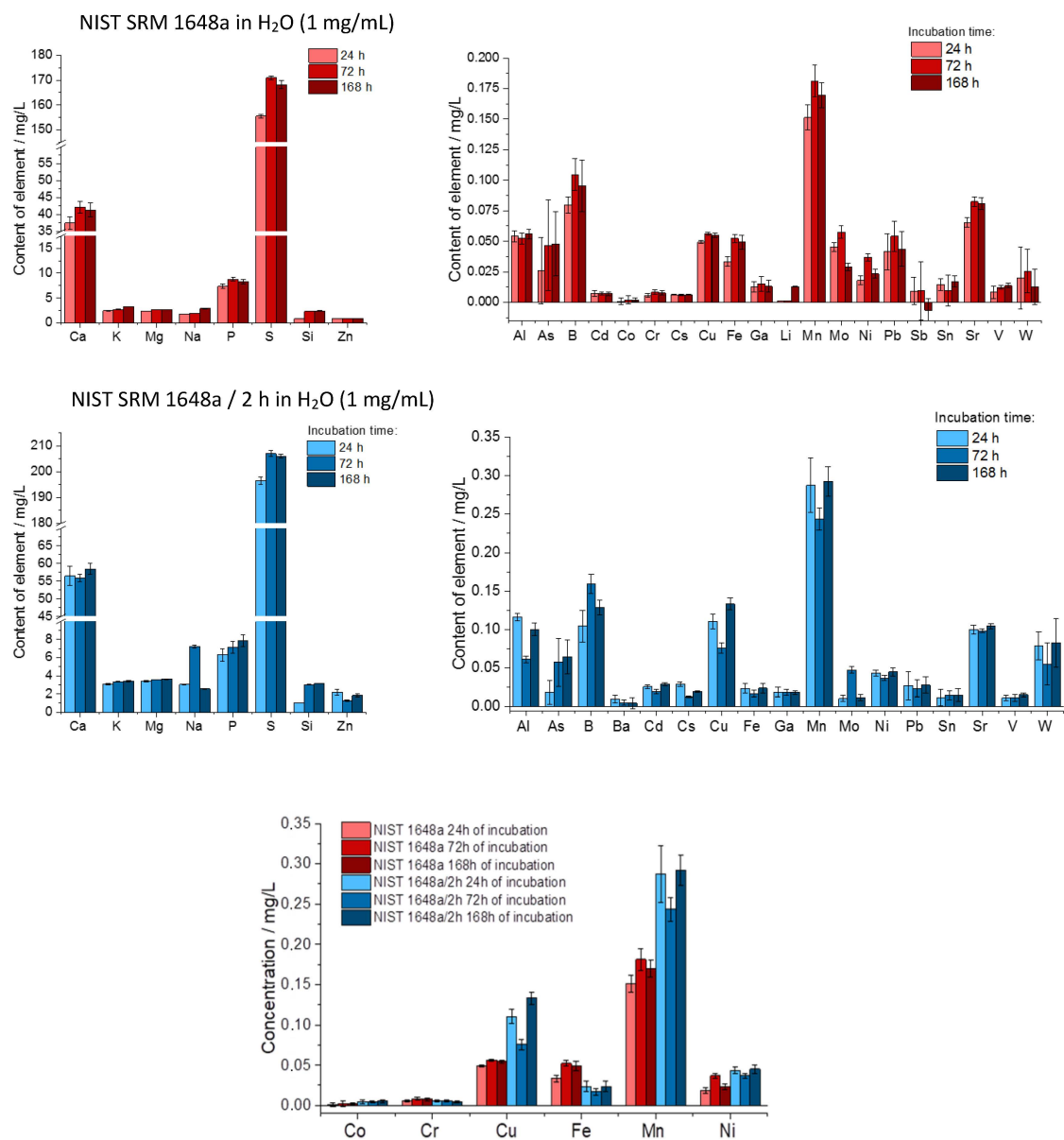


Figure 5. Concentrations of elements in water extracts of NIST SRM 1648a and plasma-treated NIST SRM 1648a samples.

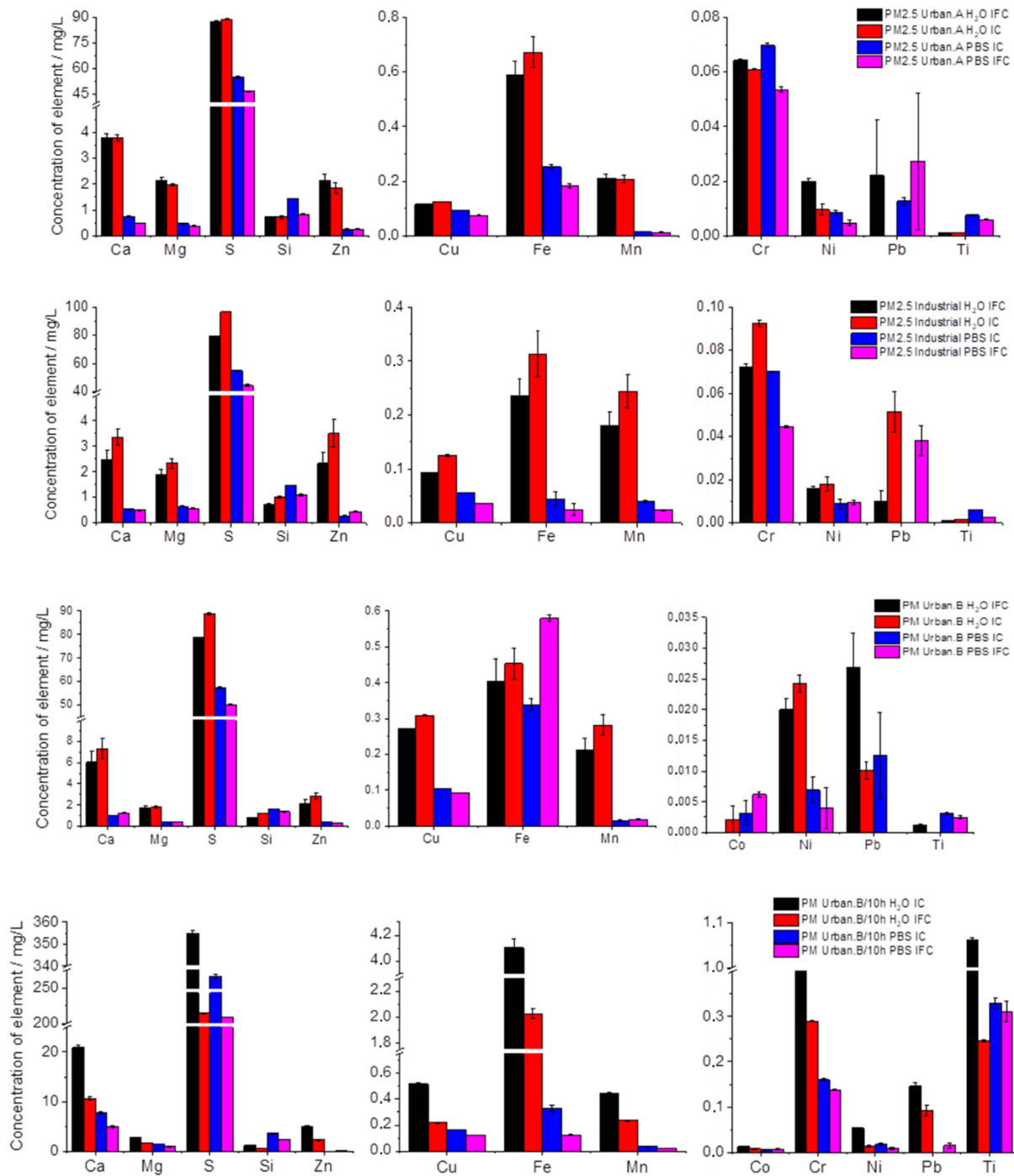


Figure 6. Selected elements for water and PBS soluble compounds from Kraków particulate matter (I—incubation, F—filtration, C—centrifugation).

Sulfates are a common group of compounds present in particulate matter, and the most abundant are calcium and sodium sulfates [17]. Large amounts of sulfates are generated by sulfur dioxide oxidation, which is produced by the combustion of fossil fuels. The next one is calcium, its concentration in Kraków PM extracts is a few times lower than in extracts of NIST SRM 1648a. Magnesium, silicon, zinc are present in Kraków samples in comparable amounts, similar to the standard reference sample. Elements with moderate concentration are copper, iron, and manganese, which are interesting from the standpoint of their redox activity. The iron content is the highest among these three elements and

its concentration is 10 times higher in Kraków PM sample extracts than for NIST SRM 1648a extracts, especially for PM collected near crowded streets [13]. In plasma-treated PM Urban.B/10 h, iron is present even in a higher concentration, up to 4 mg/L, which makes this element important to study due to its redox activity as in the Fenton reaction. It was reported that the solubility of iron is around 10–30% [18], thus it has to exist in a form of insoluble or slightly soluble compounds, like oxides [19], and it is not attached to highly soluble ions like sulfates and nitrates [20]. According to our studies, iron is quite well soluble in water after 10 h of plasma treatment. Copper and manganese are present in a similar concentration as in the NIST SRM 1648a and NIST SRM/2 h extracts; only for PM Urban.B/10 h this concentration is around 2 times higher. The quantity of elements that form the last group with the lowest concentrations is similar in all Kraków samples, as well as in the standardized PM sample. Noteworthy is that after plasma treatment, the concentration of elements like iron, copper, chromium, titanium, and lead is higher than in PM before the treatment. Increased concentration of these elements means increased availability of these elements in the collected PM, which means that plasma sawing somehow releases the bulk of transition metals. In the case of chromium, the concentration of this element after treatment is 1 mg/L, whereas in the original PM sample this concentration was below the detection level. It was hypothesized that a high soot content reduces the dissolution of chromium [20,21]. There is a strong dependence of the concentrations of elements on the type of solvent. Several papers describing water-soluble elements from particulate matter and their concentrations show that the content of soluble components strongly depends on localization. PM_{2.5} from Bologna contains zinc and cadmium among the most soluble elements, while aluminum compounds showed the least solubility [22]. Similar results were obtained by Jiang—the highest concentration among water-soluble cations was observed for zinc, calcium, and cadmium. Aluminum was found in the smallest amount, similarly to chromium [23]. Chromium was also found among the least water-soluble metal compounds in PM_{2.5} collected near Katowice in Poland [24]. However, not too many authors consider the solubility in PBS buffer, which imitates body fluids. Almost all soluble elements are present at higher concentrations in water extracts than in PBS, e.g., sulfur in all Kraków PM extracts is two times, and zinc five times more concentrated in water extracts than in PBS buffer. It is crucial to recognize the prevalent elements in extracts to understand and account for their role in further APARIC studies.

3.5. Effect of Sonication Time, Centrifugation and Filtration on Particle Size Distribution (NIST SRM/2 h and NIST SRM 1648a)

For plasma-treated NIST SRM 1648a, there is a large difference in particle size distribution for samples centrifuged for 10 and 30 min (Figure 7). After a short centrifugation time, particles in the water extract of NIST SRM/2 h are homogeneous in size during 3 subsequent measurements, and the mean particle size from these measurements is ca. 850 nm. Longer centrifugation causes a fluctuation in size, which means a low instability. In our experiments, even after filtration through filters with 0.22 µm pores, particles with a larger diameter than the pores were observed in the extracts. In the case of 10 min of centrifugation and filtration, there is almost no change in the size distribution of the particles. The mean diameter from 3 measurements is 0.8 µm and particles are smaller than before filtration but larger than the filter pores. This may point to the aggregation of smaller particles, but they do not sediment and the size distribution remains similar during the measurements. In the case of NIST SRM 1648a, the size distribution dependencies are very similar to results obtained for plasma-treated samples (Figure 7). Shorter centrifugation times are better because the particle size distribution in these experiments is homogeneous and remains almost intact during the experiment (mean diameter is 0.74 µm). Longer centrifugation time causes extension of the size range from 0.3 to even 2 µm with a mean diameter of 0.9 µm. Particles are 0.2 µm larger than after shorter centrifugation times. Moreover, filtration after 10 min centrifugation does not affect the size distribution.

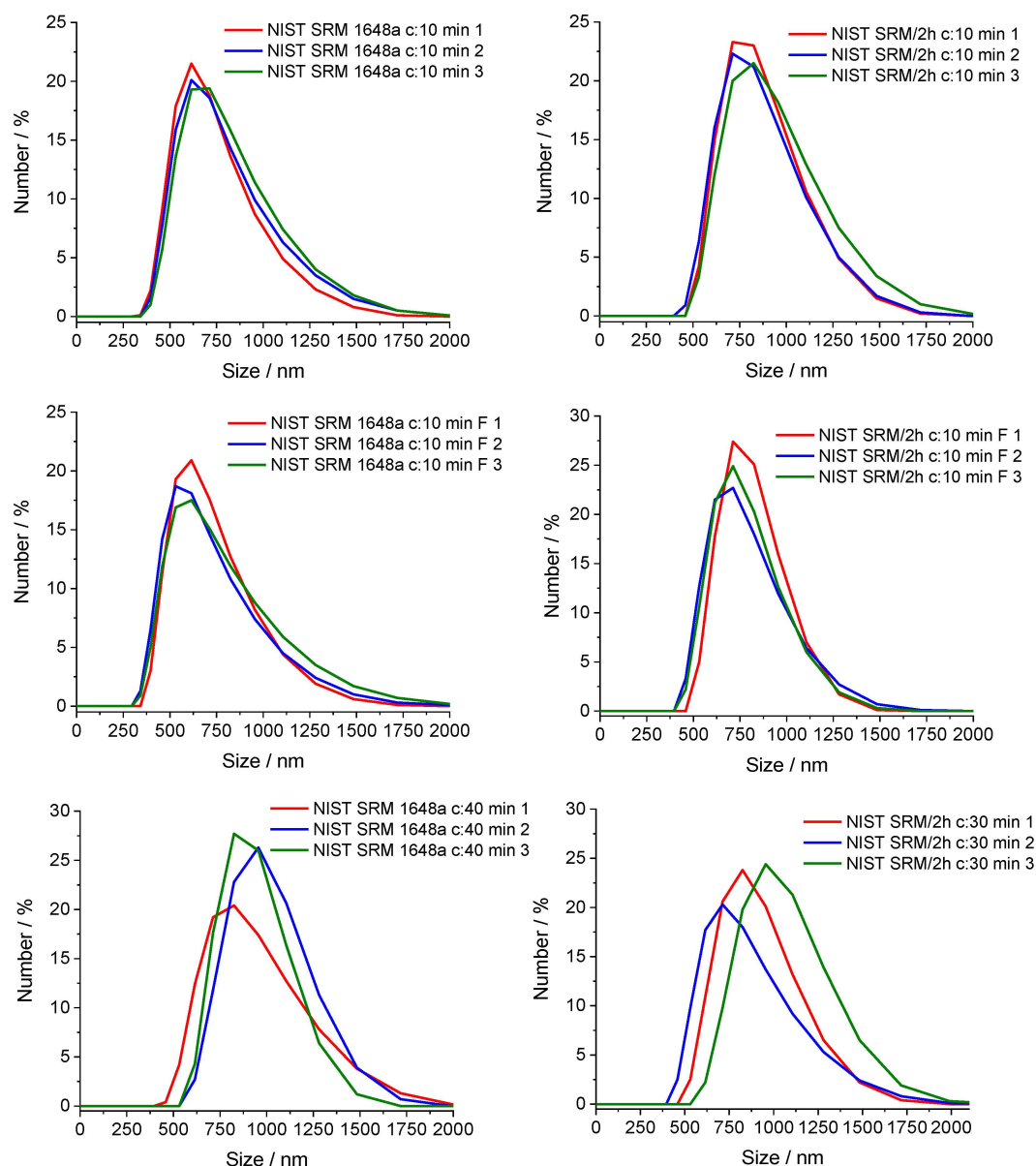


Figure 7. The particle size of NIST SRM 1648a and SRM/2 h prepared in different methods (C10, C30, C40—centrifugation time in minutes, F—filtration). Each sample has been analyzed in triplicate.

3.6. The Influence of the Extract Preparation Procedure on Particle Size Distribution (Kraków PM Samples)

Kraków PM extracts were prepared from PM Urban.B in two solvents, viz. water and PBS, and in different ways: incubation, incubation with centrifugation, incubation with centrifugation, and additional filtration. For all analyzed samples we observed the general trend that the particle size is the largest immediately after incubation and the smallest after the 3 step protocol for extract preparation (Figure 8). Extracts prepared by 3 days of incubation contain particles in two size ranges: smaller (0.1–0.5 μm) and larger (0.5–1.5 μm). Our purpose was to decrease the size to obtain particles smaller than 1 μm by using filtration and centrifugation. 10 min of centrifugation seems to be enough to remove particles from the larger size range and the obtained particles are between 50 and 300 nm. This size is satisfactory for animal tests using the nebulizer device. The samples after incubation, filtration, and centrifugation, contain even smaller particles, from 10 to

150 nm, but this step is not essential since the decrease in size is not spectacular. Particles after incubation and centrifugation are of an ideal size for biological studies. Considering the results for different solvents (water, PBS), the differences are not discernible and the general size ranges are similar in both cases. Furthermore, there were no major differences between the two PM samples collected in Kraków. This may be because these PM samples were collected at the same location using different sampling procedures that did not affect the size.

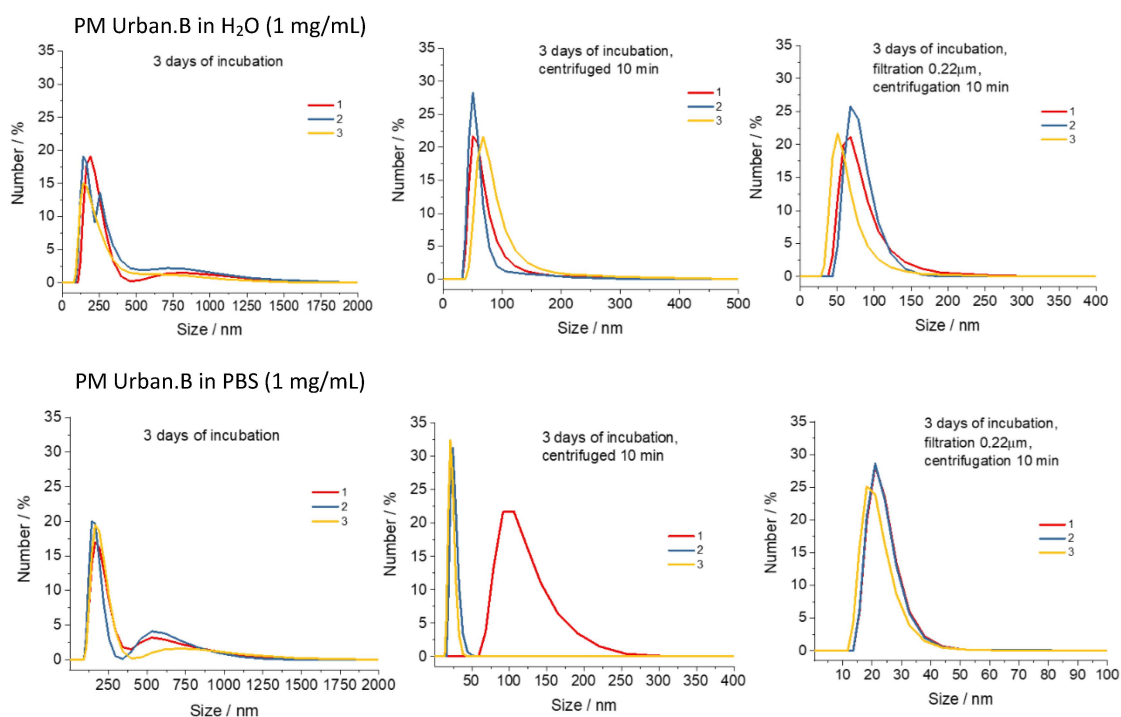


Figure 8. The particle size of Kraków particulate matter in water and PBS after preparation using different procedures. Each sample has been analyzed in triplicate.

4. Discussion

Air pollution is currently one of the most significant environmental problems in many countries. The problem of air pollution is an enormous challenge for the City of Kraków, and the consequences for people in the form of deterioration of health and numerous allergies. That is the reason why the APARIC project wants to face this problem, by investigating how air pollution affects the development of immunological diseases. PM in the air is present in the solid state, but during rain, fog, or high humidity, we deal with a totally different system. Many components of PM are soluble yielding more ions and soluble elements. We inhale them together with moisture, in particular during seasons with the highest air pollution and humidity (autumn and winter). After entering our bodies, PM is also dissolved and/or suspended in body fluids, so in fact, soluble components of particles are very important.

PM collected in Kraków is richer in organics than the employed standard NIST SRM 1648a. Samples from all locations in the City of Kraków contain more than 40% of carbon, which can suggest an increased amount of polycyclic aromatic hydrocarbons. The content of nitrogen is two times higher than in NIST SRM 1648a, which is connected with the presence of nitro substituted organic compounds in the PM. As seen from the data presented in Figure 2, the carbon content decreases upon plasma treatment of PM Urban.B samples, pointing to an efficient removal of soot and organic compounds. This conclusion is consistent with the drop in hydrogen content. A nearly constant level of nitrogen suggests an almost equal content of this element in organic and inorganic components. Only

the sulfur content increases upon plasma treatment, which can be interpreted as evidence for inorganic sulfur, which is more available for analysis when organic components are removed. SEM-EDS analysis showed a similar morphology for standard and PM collected in the city center, while PM collected in the industrial area has smaller particles with a smooth surface. Extracts of PM were prepared in two solvents in three different ways to optimize the protocol which enables to obtain particles with a size under 1 μm . From ICP-OES analysis we can perceive that the same elements come from soluble compounds and suspended nanoparticles in water both for NIST SRM 1648a before and after plasma treatment, but in extracts, from NIST SRM/2 h a higher content of different elements can be found. Among the elements with the highest concentrations in Kraków PM extracts are calcium, magnesium, sulfur, silicon, and zinc, whereas moderate concentrations were found for copper, iron, and manganese. In plasma-treated PM Urban.C, the concentration of these elements is higher. This is because during treatment with plasma a large part of the sample is removed, viz. organic compounds, constituting a large part of the sample that is converted to CO_2 and H_2O . The sample after combustion in the plasma is poorer in the organic fraction, and thus richer in inorganic compounds. For the experiments with extracts, we took the same amount of samples to obtain suspensions with the same concentration. Therefore, the samples after the plasma treatment contain an increased amount of soluble elements such as iron or copper. There is a strong dependence between the concentration of elements and the selected solvent: all soluble elements are present in PBS extracts at a higher concentration than in water. During NIST SRM 1648a and NIST SRM/2 h extract preparation, it is sufficient to centrifuge samples for 10 min. For all analyzed Kraków PM extract samples, we observed the general trend that the particle size is the largest immediately after incubation and the smallest after the 3 step protocol for extract preparation (in the range from 50 to 300 nm) suitable for biological experiments.

5. Conclusions

The APARIC project was initiated with the idea to recognize relationships between transition metal-containing particulate matter and the course of autoimmune diseases. Results presented in this study are crucial for the health of people in the City of Kraków and are important for them to be aware of the threat to their health resulting from air pollution. All presented results show the importance of the air pollution problem in Kraków. The first is the high content of organic carbon compounds. Next is the concentration of important elements like iron and copper which are redox-active and catalytically active metal ions that can affect ROS generation. Redox chemistry also plays a crucial role in targeting the redox balance in inflammatory skin conditions. Finally, the particle size of PM is extremely important.

In this work, we focused mainly on PM_{2.5}. It is worth mentioning that the mean concentration in air of this PM fraction in the spring of 2015 was around $27 \pm 19 \mu\text{g}/\text{m}^3$, while the annual concentration for PM_{2.5} was $25 \mu\text{g}/\text{m}^3$ and PM₁ concentration was $19 \pm 14 \mu\text{g}/\text{m}^3$ [10]. The main conclusion from our study is that PM in Kraków contains much more organics than the standard reference material of particulate matter, which is responsible for many diseases. Carbon cannot be removed totally from PM collected in Kraków by using oxygen plasma. PM is constituted rather of agglomerates of smaller particles after collection on filters, and the average grain size is around 50–150 μm , while after suspending in water/PBS the average size is 1 μm . Extracts of PM contain much smaller particles up to 200 nm which are appropriate for cell tests. Among the most concentrated elements are calcium, magnesium, sulfur, silicon, and zinc, whereas copper, iron, and manganese are present in lower concentrations in all sample extracts. This report may be very useful for many scientists working with PM since in the literature there is still a lack of information about particulate matter extract preparation, their composition of soluble components and size.

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review and editing: W.M., R.v.E., G.S.; funding acquisition: R.v.E., G.S.; supervision: W.M. All authors have read and agreed to the published version of the manuscript.

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