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Characterization and *in vitro* biological effects of ambient air PM₁₀ from a rural, an industrial and an urban site in Sulaimani City, Iraq

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Abstract

High urban atmospheric pollution is caused by economic and industrial growth, especially in developing countries. The objective of this study was to assess possible relationships between *in vitro* effects on human alveolar epithelial cells of source-related dust types collected at Sulaimani City (Iraq), and to determine their mineralogical and chemical composition. A passive sampler was used to collect dust particles at a rural, an industrial and an urban sampling site during July and August 2014. The samples were size-fractionated by a low-pressure impactor to obtain respirable dust with aerodynamic diameters of less than 10 µm. The dust was mainly composed of quartz and calcite. Chrysotile fibres (white asbestos) were also found at the urban site. Dust from

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AUTHORS' CONTRIBUTIONS

ATA, CM, PKh, VMS, RGm and RGi contributed to the study concept and design. ATA, CM, MGK, PG, NW, RGm, RGi, interpreted the data and were responsible for writing and revising the manuscript. ATA, CM, MGK contributed to laboratory research, data acquisition and interpretation; RGi, RGm, GT, VMS were involved as project supervisors and in manuscript writing and revising.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

the industrial and urban sites triggered cytotoxic and genotoxic effects in the cells, whereas only minor effects were observed for the sample from the rural site.

Keywords

Ambient air PM₁₀; A549 lung cells; toxicity; Sulaimani City; Iraq

1. INTRODUCTION

The rapid economic and industrial growth taking place in developing countries has led to an increase in anthropogenic emissions into the urban atmosphere through traffic congestion, heavy industrial activity and fossil fuel burning for electricity generation and residential heating purposes (Gieré and Querol 2010; Grobéty et al. 2010). Air pollution comprises both gases (e.g. nitrogen oxides, ozone, sulfur dioxide) and particulate matter (PM). PM ranges in size from a few nanometers to several micrometers (Gieré 2016). The PM is generated through both natural processes and human activities, and includes organic and inorganic carbon, metals, metalloids, minerals, and bacteria (Happo et al. 2008; Jalava et al. 2007). Its chemical and physical properties are influenced by the source as well as by aging processes during transport and residence in the atmosphere (Gieré and Querol 2010; Marris et al. 2013). PM can be classified as coarse, respirable fraction (PM₁₀), and fine, alveolar fraction (PM_{2.5}) (Sharma, Jain, and Khan 2007).

In addition to major atmospheric, environmental and ecological impacts (Abdel-Shafy and Mansour 2016), airborne PM may cause adverse health effects, both acute and chronic, because as we breathe millions of solid particles enter the respiratory system (Lewtas 2007). It is well known from epidemiological and toxicological studies that exposure to PM_{2.5} is linked to increased morbidity and mortality due to respiratory and cardiovascular diseases (Englert 2004; Ferk et al. 2016). There is increasing evidence that PM₁₀ may also cause similar health effects (Brunekreef and Forsberg 2005; Kim, Kabir, and Kabir 2015).

PM_{2.5} is dominated by combustion-derived particles, whereas PM₁₀ and coarser fractions contain minerals, non-exhaust traffic-derived particles, including brake, tire and road-surface wear, as well as biological components such as pollen, spores and plant debris (Tian et al. 2017; Sommer et al. 2018; Binková et al. 2003). Not only the size, shape, or composition of particles, but also particle-associated substances, e.g. reactive compounds adsorbed onto their surface, may be involved in causing adverse effects on human health. These compounds include metals, polycyclic aromatic hydrocarbons (PAHs) and endotoxins (Brunekreef and Forsberg 2005; Sharma, Jain, and Khan 2007).

At the cellular level, several mechanisms have been proposed to explain PM-related health effects such as the ability of PM₁₀ to induce intracellular production of reactive oxygen species (ROS) in epithelial cells and macrophages of the respiratory tract (Könczöl et al. 2011). Oxidative stress activates signaling pathways leading to the release of pro-inflammatory mediators or damaging cellular macromolecules and structures (Donaldson et al. 2003; Ghio et al. 2014; Steenhof et al. 2011). PM₁₀ has also been reported to be cytotoxic and may induce apoptosis as a result of upregulation of pro-inflammatory mediators in the

human alveolar cell line A549 (Hetland et al. 2004; van Eeden et al. 2001). PM₁₀ collected in Mexico City induced genotoxic effects in A549 cells, which suggests that DNA damage could be the mechanism by which PM increases the risk of lung cancer in people living in urban areas (Sánchez-Pérez et al. 2009).

Iraq is a good example of a developing country with high concentrations of PM in the urban environment. In Sulaimani City, for example, PM₁₀ often exceeds the daily limit value set by the European Union (EU) for PM₁₀ of 50 µg/m³, and the average concentration of benzo[a]pyrene (B[a]P), a combustion-related carcinogenic PAH in the PM₁₀, has been found to be higher than the EU limit of 1 ng/m³ at standard temperature and pressure of 273 K and 101325 Pa by a factor of three (Ahmed et al. 2015). However, no work has yet been undertaken to investigate the toxicological effects of dust from this area, where anthropogenic sources include combustion processes, especially those of high-sulfur fuels used extensively in gasoline-powered generators, traffic, construction and solid waste incineration, whereas dust storms are the main natural source of airborne PM (Majid 2011).

For these reasons, we chose Sulaimani City for our study, with the objective to investigate the physical, chemical and mineralogical characteristics as well as the toxicological properties of particles deposited and collected at different sites. We investigated their impact on human lung cells by assessing cytotoxicity, genotoxicity, inflammatory potential and ROS formation.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

Sulaimani City, with a population of 1.5 million (2012) is situated in the Kurdistan region of northeastern Iraq (geographic coordinates: 35°33'53" N/ 45°25'58" E; see Fig.S1) at an altitude of 847 m, and covers an area of 114 km². Its climate is semi-arid with very hot, dry summers and cold, wet winters. The city is prone to be windy because of the surrounding mountains (Majid 2011).

Dust was collected in duplicate with open polyethylene boxes (0.8 × 0.5 m; border height: 0.3 m) based on gravitational settling according to (Cao et al. 2011; El-Desoky et al. 2013; Würtz et al. 2005) at the following sites (Fig.S1): rural site (Azmar Mountain; abbreviated as RurSu), industrial site (close to the landfill incineration plant Tanjaro; IndSu), and at Malik Mahmud Street (urban site with moderate to heavy traffic; UrbSu). The samplers were placed 3 m above ground (Table S1). Dust samples were collected continuously from July 1 to August 31, 2014. The samples were then dried for 10 min at temperatures below 60 °C (this may have led to underestimation of volatile organic PAHs, such as naphthalene). The samples were stored in dark glass flasks in a cool and dark place until they were shipped to our laboratories, where they were dry-sieved to particle sizes of < 100 µm. These sieved particles were analyzed by SEM, EDX and XRD. Furthermore, the sieved particles were aerosolized by an aerosol generator (RGB 1000, Palas, Karlsruhe, Germany), and the PM₁₀ fraction was collected by a Berner-type low-pressure impactor (ISAP® B-LPI 27/0.05/2.5/10.0; Ingenieurbüro Schulze, Asendorf, Germany). These PM₁₀ samples were used for the analysis of metals, PAHs, size distribution by transmission electron microscopy

(TEM) and dynamic light scattering (DLS), and toxicological assays, representing the respirable PM.

2.2. Physical, chemical and mineralogical analysis

The sieved samples were characterized by scanning electron microscopy (SEM) (1525 field-emission SEM, LEO Electron Microscope, Thornwood, NY, USA) coupled with energy-dispersive X-ray (EDX) spectroscopy (80 mm² X-MaxN Silicon Drift Detector, Oxford Instruments, UK) at the Institute of Earth and Environmental Sciences, University of Freiburg. Acceleration voltage was 15 kV. Photomicrographs were captured using the secondary electron (SE) signal.

The mineralogical composition of these sieved samples were determined by X-ray diffraction (XRD) (D8 Advance, Bruker, Karlsruhe, Germany) at the University of Freiburg, Germany, in the 2 θ range 2–75°, with a step size of 0.05°, and a counting time of 2 s/step. The raw data were processed with the pattern-matching software BRUKER DIFFRAC.EVA to identify the mineral phases present.

2.2.1. Inductively coupled plasma-mass spectrometry/ inductively coupled plasma-optical emission spectroscopy—The metal and metalloid content of PM₁₀ was determined via inductively coupled plasma-mass spectrometry (ICP-MS) (7500ce, Agilent, Waldbronn, Germany) and inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Arcos, Spectro, Kleve, Germany) at the Medizinisches Labor Bremen, Germany. The concentrations of As, Ba, Be, Cd, Co, Cu, Hg, Mo, Ni, Sb, Pb, Sn, Sr and Zr were determined by ICP-MS, those of Al, Ca, Cr, Fe, K, Mg, Mn, Na, Si, Ti, and Zn by ICP-OES. Prior to ICP analysis, 0.2 g of each PM₁₀ sample was digested with 5 mL aqua regia (3:1 HCl/HNO₃; Sigma Aldrich, Seelze, Germany) under pressure-microwave digestion with a Mars 6 device (CEM, Kamp-Lintfort, Germany). Once digested, the sample volume was adjusted to 20 mL with ultrapure water.

For PAH analysis, the organic fractions of PM₁₀ were extracted according to Piot et al. (2012) and Golly et al. (2015). Briefly, automatic accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA, USA) was used to extract the organic fraction with a solvent mixture of methanol, acetone, and dichloromethane. The extracts were reduced to 1 mL under a gentle stream of N₂, and the PAH separation was achieved by HPLC using a C18 column (EC 250/4.6 Nucleodur®C18 PAHs, 3 μ m, Macherey-Nagel, Düren, Germany) by gradient elution with membrane-degassed methanol-water. PAHs were analyzed using a fluorescence detector (LC 240, Perkin Elmer, Waltham, MA, USA) at variable excitation and emission wavelengths. The method was validated using certified urban dust standard reference material (SRM 1649b, NIST, Gaithersburg, MD, USA).

2.2.2. TEM—To assess mean particle sizes and shapes by TEM, samples of PM₁₀ were placed onto lacey-carbon copper grids. The TEM analysis (Hitachi H600, Tokyo, Japan) was performed at the Poliklinik für Arbeits- und Sozialmedizin der Justus Liebig Universität Gießen, Germany (for details, see Supplemental Material).

2.2.3. DLS analysis—The samples were analysed for their hydrodynamic diameter and their zeta potential in Millipore water (Millipore Elix, Darmstadt, Germany) using a NANO-flex particle size analyser (Microtrac, Meerbusch, Germany) at a concentration of 100 µg/mL. Before characterization, the dispersions were sonicated for 10 min at 37 °C in an ultrasonic water bath (Badelin Electronic, Berlin, Germany).

2.2.4. Endotoxin determination—The endotoxin content of the PM₁₀ samples was determined by the Kinetic Limulus chromogenic Amebocyte Lysate assay (ToxinSensor™ Gel Clot Endotoxin Kit, GenScript, Piscataway, USA). For details, see Supplemental Material.

2.3. Sample preparation for biological assays

PM₁₀ were suspended in Millipore water (stock suspension) with a final concentration of 1 mg/mL; these were vortexed (Vortex mixer, Heidolph Reax Top Heidolph, Schwabach, Germany) for 1 min and sonicated (Ultrasonic water bath Badelin electronic, Berlin, Germany) for 10 min immediately before cell exposure to disperse the particles and reduce particle agglomeration. Next, the cells were exposed to PM₁₀ for incubation at final concentrations of 0.3, 1, 3, 10, 30 and 100 µg/mL at a total volume of 200 µL/well in a 96-well plate (Greiner Bio-One, Kremsmünster, Austria).

Nano-SiO₂ (Sigma Aldrich, Seelze, Germany) and dolomite (Imerys, Axat, France) were used as control substances.

2.3.1. Cultivation of A549 cells and exposure to PM₁₀—The adenocarcinoma human alveolar basal epithelial cell line (A549) was obtained from the American Type Culture Collection (ATCC). The cells were cultivated in DMEM with high glucose with L-glutamine, pyruvate (Life Technologies, Karlsruhe, Germany), supplemented with 10 % (v/v) heat-inactivated Fetal Calf Serum (FCS) (Gold PAA Laboratories GmbH, Linz, Austria) and 100 U/mL penicillin/streptomycin (Invitrogen, Darmstadt, Germany) under humidified conditions (at 37° C, 5 % CO₂ (v/v)). The cells were sub-cultured in 75 cm² flasks (Greiner Bio-One, Kremsmünster, Austria) or the cells were plated in 96-well plates (Greiner Bio-One, Kremsmünster, Austria) at a density of 10,000 cells in 100 µL of medium for 24 h, thus ensuring that the cells reached a confluent monolayer. Next, the particle samples were added to the cell cultures at final concentrations of 0.3, 1, 3, 10, 30 and 100 µg/mL (100 µL/well) in a 96-well plate (Greiner Bio-One, Kremsmünster, Austria). Cells were exposed to particles for 24 h and kept at 37° C, 5 % CO₂ (v/v) in humidified air in an incubator.

2.3.2. Cell viability—The Water Soluble Tetrazolium assay (WST-1, Roche, Mannheim, Germany) was used to measure cell viability in cultured cells. For details, see Supplemental Material.

2.3.3. Detection of ROS

A) Acellular ROS: A Bruker E-SCAN EPR spectrometer (BIO III, Noxygen Science Transfer & Diagnostics, Elzach, Germany) was used to measure hydroxyl-radical (HO•)

generation by PM₁₀ without cells in the Hank's buffered salt solution (HBSS, Gibco, Darmstadt, Germany) and to elucidate possible interactions between the spin probe CAT1-H (1-hydroxy-2, 2, 6, 6-tetramethylpiperidin-4-yl-trimethylammonium chloride • HCl) and the particles. The spin probe (CAT1-H) was obtained from Noxygen Science Transfer & Diagnostics, GmbH, Elzach, Germany.

The amount of ROS generated by the PM₁₀ alone (without cell exposure) was measured after incubation of the PM₁₀ suspensions (100 µg/mL) with the spin probe (200 µM) for 4 h at 37° C, 5 % CO₂. The analysis was done as described below for the intracellular ROS generation.

B) Intracellular ROS in A549 cells: Generation of intracellular ROS in A549 cell culture supernatants after exposure to the investigated PM₁₀ was measured using the spin trap (CAT1-H). For more details, see Supplemental Material.

2.3.4. Detection of cytokine and chemokine release in A549 cells—Release of interleukin-8 (IL-8) by A549 cells after exposure to PM₁₀ for 24 h was determined in the cell culture supernatants and measured in 96-well plates (Greiner Bio-One, Kremsmünster, Austria) according to the manufacturers protocol (Peprotech, Rocky Hill, NJ, USA). The optical densities of the samples were measured at 405 nm and normalized against reference wavelength value at 650 nm using a spectrophotometric Microplate reader (Infinite® 200 PRO Tecan Group Ltd., Männedorf, Switzerland). For calculation of the concentrations, a standard curve with a four-parameter polynomial curve fitting was previously performed.

Additionally, release of further pro-inflammatory cytokines and chemokines, i.e. interleukin 6 (IL-6), interleukin-1β (IL-1β), regulated on activation normal T cell expressed and secreted (RANTES), granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein-1α (MIP-1α), monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), and tumor necrosis factor α (TNF-α) were determined in cell-culture supernatants after 24 h exposure of A549 cells to dolomite, SiO₂ and all three PM₁₀ samples (multiplexed immunoassay based on Luminex technology (IKDT GmbH; Berlin, Germany)).

Lipopolysaccharide (LPS) was used as a positive control to obtain precise information on the cell type-dependent intrinsic sensitization ability.

2.3.5. Measurement of DNA damage in A549 cells by the DNA Alkaline Unwinding Assay (DAUA)—PM₁₀-induced strand breaks in DNA of A549 cells were evaluated by the alkaline unwinding assay (DAUA) using the hydroxyapatite batch procedure (Hartwig and Schlepegrell 1995; Arif et al. 2017) with minor modifications (for details, see Supplemental Material).

2.4. Statistical Analysis

All biological results are expressed as mean ± SD of three experiments, and significance was determined by parametric statistical analysis (ANOVA) followed by Dunnett's post-hoc test. P values < 0.05 were considered significant.

3. RESULTS

3.1. Mass deposition rate of collected dust

Site description and sample collection information are listed in Table S1. The deposition rate was highest at UrbSu, where the value was more than three times higher than that determined at RurSu.

3.2. Characterization of the collected sieved dust

3.2.1. SEM-EDX investigation—SEM images combined with EDX spectroscopy revealed the morphology and qualitative elemental composition of the particles in the sieved dust (< 100 µm in physical diameter) collected from the three sampling sites. The samples were examined to distinguish particles from anthropogenic sources from those produced naturally. All samples, shown at same magnification in Fig. 1, contained mixtures of particles of different sizes and shapes. Sample RurSu contained many sub-micrometer particles and a variety of bigger particles (up to 20 µm in diameter) with distinct angular and rounded shapes and smooth or rough surfaces. In contrast, SEM images of IndSu contained only a few particles < 1µm; particles in the size range 5–10 µm dominated, in some cases forming agglomerates of up to 50 µm across. UrbSu mainly contained relatively large particles, albeit with great morphological variety: Some types of particle displayed angular shapes with smooth surfaces; others were rounded with rough surfaces. Another type consisted of bundles of fibers of up to 60 µm in length. The EDX spectra of these particles documented the presence of Mg and Si as major components (see spectrum shown in Fig. 1), indicating that the fibers were white asbestos (chrysotile, $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$).

3.2.2. XRD—The XRD patterns for the sieved dust (<100 µm), available for two samples only, revealed the presence of abundant quartz, especially in RurSu, with minor amounts of muscovite, calcite and chlorite (for qualitative order of abundance, see Table S2). Minor peaks indicated presence of other phases, but as they did not exceed the minimum intensity of three times the noise level, these phases could not be identified. Small background signals of the XRD patterns indicated that minor amounts amorphous or micro-crystalline substances of unknown chemical composition (inorganic or organic) were present in both samples.

3.2.3. Elemental analysis—The PM_{10} samples contained predominantly Si, Al, K, Na, Mg, Ca and Fe (Table 1). Additionally, some heavy metals known to be toxic were also present in the three PM_{10} samples (e.g. Cu, Zn, Ni, Pb). In RurSu, Ca was the predominant element (nearly 15 wt %), whereas Si was most abundant in IndSu and UrbSu (both ~19 wt %). Iron was abundant in all three samples, especially in IndSu (6.5 wt % Fe). The highest values of K, Mg and Na were found in RurSu (3.7, 2.0 and 0.9 wt %). On the other hand, Al is only abundant in IndSu and UrbSu (~2 wt %), which also contained considerable amounts of Ti (0.5 and 0.9 wt %, respectively). The compositional similarity between IndSu and UrbSu, and the distinction from RurSu, are also reflected in the trace elements concentrations, e.g. those of As, Ba, Cd, Co, Cu, Ni, Pb, Sn, Sr, and Zn. Worth of note is that, except for Co, Cu and Ni, RurSu was richer in these elements than IndSu and UrbSu. Especially noteworthy are the very high Cu concentrations in IndSu and UrbSu (> 1 wt%).

3.2.4. Polycyclic aromatic hydrocarbons (PAHs)—The PAH concentrations in PM₁₀ from the three sampling sites in Sulaimani City are shown in Table 2. The total PAH concentration (PAHs) was nearly three times higher for IndSu than for RurSu and UrbSu, which had similar concentrations. With a few exceptions, such as triphenylene (Tri) in UrbSu and naphthalene (Nap) in RurSu, the IndSu sample showed the highest concentration of individual PAH compounds. In particular, mutagenic and carcinogenic B[a]P was found at a concentration of 6.4 µg/kg in the IndSu sample. The PAH profiles of the PM₁₀ from the three sampling sites, calculated as relative concentrations, are presented in Fig.S2.

3.2.5. Hydrodynamic particle size characterization—The size distribution of all three PM₁₀ samples was bimodal (Fig.S3). RurSu showed a first maximum at 0.2 µm, holding about 30 % of the particle mass, and a second peak at 0.8 µm. The first maximum for IndSu, accounting for more than 90 % of the particles mass, was at 0.5 µm, whereas the second (and minor) peak was located at 2 µm. UrbSu showed a first maximum at 0.3 µm, holding almost 85 % of the particles by weight, a second peak at 1.2 µm, and a third, minor peak, at 2 µm.

Zeta potential measurements were performed to analyze the surface charge of the studied PM₁₀ samples. All three samples showed a negative particle surface charge measured in distilled water (Table S3).

3.2.6. Transmission electron microscopy (TEM)—PM₁₀ from RurSu was composed of elongated, acicular sub-micrometer particles forming agglomerates of ~1 µm across (Table S3). Also present were sub-micrometer-sized cubic shaped particles, presumably (secondarily formed) halite crystals (Fig. 2A). PM₁₀ from IndSu contained aggregates of rounded and elongated, as well as angular particles of sub-micrometer size (Fig. 2B). In addition to sub-micrometer particles, PM₁₀ from UrbSu contained fibers with diameters of ~ 50 nm and lengths of several µm (Fig. 2C). Such fibers were only observed in this sample, and they might have been single fibers of chrysotile asbestos, as previously observed in SEM images of the corresponding sieved dust sample (Fig. 1). As expected, TEM images of nano-SiO₂ revealed tiny spheres of uniform size (~40 nm in diameter), building chains and forming aggregates of about 600 nm across (Fig. 2D). Dolomite showed irregular particles ranging from several nanometers to about one micrometer in size (Fig. 2E).

3.3. Assessment of biological effects of PM₁₀ samples on A549 human lung cells

3.3.1. Endotoxin determination—Endotoxin was detectable by LAL in all sieved dust samples (<100 µm), as well as in the dolomite control substance. However, at about 0.2 EU/mL, the endotoxin content of the 1 mg/mL stock solutions was relatively low (data presented in Fig.S4).

3.3.2. Cell viability in A549 cells—Significant cytotoxic responses were observed in A549 cells after 24 h exposure to the two control substances nano-SiO₂ and dolomite at concentrations from 10 – 100 µg/mL, and to PM₁₀ samples at concentrations of 100 µg/mL.

Of the studied PM₁₀, the most pronounced effect was observed for the UrbSu sample, where the effect was already noticeable at 30 µg/mL (see Fig. 3).

3.3.3. Hydroxyl-radical production (acellular & intracellular ROS generation)

A) Acellular ROS production: None of the PM₁₀ samples alone induced noticeable ROS production in an acellular environment at concentrations of 100 µg/mL after 4 h exposure. Similarly, no effects were observed for the control substances nano-SiO₂ and dolomite (data presented in Fig.S5).

B) Intracellular ROS production: Exposure of A549 cells to our PM₁₀ samples for 4 h induced slight, albeit non-significant, concentration-dependent ROS production in the cell culture supernatants for all three investigated samples. By contrast, ROS production was significant for both nano-SiO₂ and dolomite, starting at concentrations of 10 µg/mL and 30 µg/mL, respectively (Fig.S6). The positive control (PC) menadione (100 mmol/L) induced strongest (and significant) ROS production in A549 cells.

3.3.4. Pro-inflammatory cytokine/chemokine release—Release of IL-8 by A549 cells in culture supernatants was detectable in a dose-dependent manner after exposure to the investigated PM₁₀ samples (Fig. 4). The particles significantly induced IL-8 at similar levels and concentrations of between 1 – 100 µg/mL. In addition, release of various chemokines (MIP-1α, MCP-1, RANTES) and various cytokines (VEGF, IL-6, TNF-α, IL-1β, GM-CSF) was triggered by all the PM₁₀ samples as well as dolomite at a concentration of 30 µg/mL; also by nano-SiO₂ at a concentration of 10 µg/mL (see Fig.S7). Of our PM₁₀ samples, UrbSu induced higher releases of IL-1β and VEGF, whereas the RurSu sample induced the highest releases of MCP-1, VEGF and RANTES. All three samples released similar amounts of GM-CSF, MIP-1α, and TNF-α. For several cytokines, concentration-dependent induction was also observed by unspecific stimulation with the positive control LPS.

3.3.5. DNA damage in A549 cells—Fractions of ds-DNA in A549 cells after 24 h exposure to PM₁₀ samples as well as to nano-SiO₂ and dolomite are shown in (Fig. 5). Nano-SiO₂ (at 10, 30 and 100 µg/mL) and dolomite (at 30 and 100 µg/mL) significantly induced DNA damage. PM₁₀ from UrbSu induced DNA damage at concentrations of 30 and 100 µg/mL, PM₁₀ from IndSu at a concentration of 100 µg/mL, whereas no effects were observed for the PM₁₀ from RurSu, even at the highest concentration of 100 µg/mL.

4. DISCUSSION

There is increasing interest in the association between adverse health effects and exposure to various types of dust suspended in ambient air. Especially respirable airborne PM is of concern for inhalation, and may increase the risk of human morbidity and mortality (Morman 2013; Miller, Shaw, and Langrish 2012). To study PM pollution in ambient air at different locations in Sulaimani City, we collected dust deposited at three different sites in and around the city. Mineralogical and physical characterization of the sieved samples was performed by SEM and EDX. For metal, metalloid and PAH contents, size-distribution, and toxicological investigations we focused on the PM₁₀ fraction, because this is a probabilistic

cutoff diameter for particle aerodynamic sizes that reach deeper lung areas (Wilson and Suh 1997).

Our results demonstrate that of the three sites, UrbSu had the maximum average dust deposition rate during the two summer months (July to August) when collection took place. SEM and XRD investigations showed that the particles in the three samples differed in shape and composition. The dust samples contained a high percentage of quartz and calcite, which is consistent with another study of airborne mineral dust and aerosols in Iraq (Engelbrecht and Jayanty 2013). The abundant presence of quartz in RurSu and UrbSu can be explained by the re-suspension of road particles (Grobéty et al. 2010). Calcite is widely used as a soil conditioner for agricultural needs and is also found in natural dust in this area (Khanaqa and Karim 2015); this could explain both the high calcite content and the high concentration of Ca (~15 wt%) in PM₁₀ from the RurSu site. A study comparing the mineralogical and chemical composition of dust samples with the surroundings of Sulaimani city showed that local geological formations and soils around the city strongly influence the properties of the dust particles investigated (Majid 2011). In particular, these data suggest that soil from wind erosion or re-suspension is an important natural contributor to local air pollution. Another source of dust are regional dust storms, which, originating in the Arabian Desert, carry silt and clay, including muscovite and chlorite, to Sulaimani city. These dust storms are especially common between mid-June and mid-September, i.e. during our sampling period (Sissakian, Al-Ansari, and Knutsson 2013). The two-month sampling period allowed to average all these effects. Consequently, the collected dusts are representative for the sampling period.

The SEM-EDX and TEM data revealed presence of chrysotile in the UrbSu dust, but this mineral could not be identified by XRD. The two main XRD peaks of chrysotile were at almost the same position as two significant peaks of chlorite, and, we cannot exclude that we falsely identified chlorite rather than chrysotile, or that the chrysotile signal was overlain by the chlorite signal. Both minerals are phyllosilicates (sheet silicates) with similar structures and therefore have similar XRD signals. Another issue is the difficulty of detection by XRD of fibrous materials in general, because of their preferred orientation in the sample, which gives incomplete XRD patterns.

From the presence of chrysotile in the PM₁₀ from UrbSu, a site with a high traffic volume, we conclude that the mineral may have originated from friction pads being used for automotive brakes, as shown by Saito (1991). This conclusion is supported by the fact that asbestos is not known to occur naturally in the study area. Asbestos fibers are associated with adverse health effects when inhaled by humans and animals; they are, for example, causally linked to the development of lung cancer, malignant mesothelioma and pleural pulmonary fibrosis (Mossman et al. 2007). Asbestos released from brake wear into the ambient air of cities requires a unique strategy for removal (Saito 1991). Therefore, we recommend investigating and monitoring in depth the occurrence of asbestos in dust from Sulaimani City (and other cities).

Because PM is a complex mixture of particles from different origins, its composition often varies greatly depending on emission sources and particle size (Gualtieri et al. 2009;

Mirowsky et al. 2013). The results of our elemental analysis showed that the PM₁₀ samples from IndSu and UrbSu were chemically quite similar, except for Fe, the concentration of which was much higher in IndSu. PM₁₀ from RurSu was distinct in its composition, especially in regard to its content of Si, Ca and Al: the concentration of Si and Al was significantly lower, whereas that of Ca was significantly higher. This probably reflects a high calcite input from farming activities in the rural area, where limestone is prominent (Khanaqa and Karim 2015). The concentrations of Cu, Fe, Ni and Ti were high in PM₁₀ from IndSu and UrbSu, whereas they were low in PM₁₀ from RurSu, where levels of Ba, Mn, Pb, Sb and Zn were elevated compared with the other two samples. The relatively high heavy metal content in IndSu and UrbSu could be related to anthropogenic sources like traffic, industry and combustion. This hypothesis correlates with the larger number of small particles contained in these two samples. The metals Cr, Cu, Fe and Mn in PM are reported to come from engine and break wear, whereas metals like Ni, Cr, Cd and Al derive from municipal waste burning outdoors, alloys in automobiles as well as from burning coal (Srithawirat, Latif, and Sulaiman 2016). Zinc in urban environments may be released as corrosion products from galvanized steel road equipment, such as crash barriers, road signs and lamp posts, or may be released by tire wear (Sommer et al. 2018; Gunawardana et al. 2012).

PAHs in the environment originate from both natural and anthropogenic sources, but especially from combustion of organic materials (e.g. fossil fuels, wood or straw), motor vehicle emissions, waste incineration, cigarette smoke and many industrial activities (Abdel-Shafy and Mansour 2016; Tapanainen et al. 2012). Most of the PAHs with low vapor pressure in the air can be adsorbed onto particles (for discussion, see (Garra et al. 2015)), and they are typically products of incomplete combustion and are defined as soot precursors (Eberle, Gerlinger, and Aigner 2017), which explains why they are found in PM. The concentration of PAHs in road dust particles increases with decreasing particle size, because smaller particles have larger surface areas relative to their mass available for deposition of PAHs emitted from vehicular exhaust (Dong and Lee 2009). Our study is the first report on the presence of PAHs in deposited dust samples collected in and around Sulaimani City. PAHs were detected at relatively high concentrations in all three PM₁₀ samples, but were considerably higher in the sample from IndSu. High concentrations (up to 6.44 ng/g) of the mutagenic and carcinogenic compound B[a]P were found in all PM₁₀ samples. These PAH values are in accordance with those generally found in road dust samples taken from industrial and urban areas in other cities (Ha et al. 2012; Besombes et al. 2001; Sharma, Jain, and Khan 2007; Dong and Lee 2009). It has been reported that concentrations of PAHs in ambient air in rural areas resulting from combustion of biomass fuels (e.g. crop residues and brushwood) are higher globally in developing countries than from combustion of solid fuel in urban areas (Li et al. 2015). This might explain why the value obtained for B[a]P concentration is higher at the rural site (3.26 ng/g) than at the urban site (2.97 ng/g). Furthermore, evaluation of the PAH profiles showed that Phe, Fla, and Pyr were the most abundant PAH compounds at all three sampling sites in Sulaimani City. Fla and Pyr can be produced during many types of combustion process typically taking place in urban areas (Schauer et al. 1996; Müller, Hawker, and Connell 1998). The ratio (B[a]P)/(B[ghi]P)

pointed to production from traffic emissions, whereas (B[a]A) and Chr corresponded to gasoline emissions (Abbas et al. 2018).

All the dust samples investigated here contained low amounts of endotoxins, in agreement with previous reports indicating that endotoxins can be present in fine and ultrafine samples (De Vizcaya-Ruiz et al. 2006; Steenhof et al. 2011). However, the results of our study demonstrate that the toxic response of these endotoxins was low and had no influence on human lung-cell toxicity.

In this study, a series of toxicological assays was performed with the PM₁₀ samples to evaluate their cytotoxic, pro-inflammatory and genotoxic effects on A549 cells *in vitro*. We did not observe any significant changes in ROS production in lung cells exposed to PM₁₀ from the three sampling sites. However, PM-induced ROS production in human cells has been demonstrated in several other *in vitro* studies (Könczöl et al. 2013; Hamad et al. 2016; Sehlstedt et al. 2010). This may be due to the use of the CAT1-H spin probe reagent, which exclusively detects hydroxyl-radical (HO•) generating activities. Hamad et al. (2016) reported strong correlations between chemical species (organics and metals) and ROS production using DCFH-DA (2',7'-dichloro-dihydro-fluorescein diacetate) for ROS measurement in a rat macrophage cell line exposed to PM_{2.5} collected in Baghdad, Iraq (Pearson correlation analysis; r-Pearson of > 0.5 or <0.7).

All three samples of PM₁₀ from Sulaimani City as well as the control substances nano-SiO₂ and dolomite induced significant cytotoxic and inflammatory effects in human A549 lung cells. These results are consistent with several earlier studies of PM in urban air (Rönkkö et al. 2018; Jalava et al. 2012). However, as has been shown here, biological responses exerted by dust are strongly dependent on the specific chemical and mineralogical composition, and hence depend on proximity to industrial activity. Exposure to silica particles, which are known to induce cytotoxicity and to exert pro-inflammatory activity (Kaewamatawong et al. 2005), were found to be a major component of the investigated dust samples.

We found significant genotoxic effects in lung cells exposed to PM₁₀ from UrbSu and, to a smaller extent, from IndSu. No genotoxic effect was observed for PM₁₀ from RurSu. This is in line with results found for PM₁₀ and extracted organic compounds collected in industrial, urban and rural areas in Flanders, Belgium (Brits, Schoeters, and Verschaeve 2004). The genotoxic potency of the particles from UrbSu and IndSu in our study might be explained by their PAH content and their relatively high content of Al, Fe, Ni, Cu, Co and Si compared with the RurSu sample.

Our study and two former investigations (Ahmed et al. 2015; Majid 2011) demonstrate that the air in Sulaimani City is highly polluted with PAH-containing PM, and that these pollutants may be associated with adverse health effects. For example, long-term exposure to ambient air pollution is associated with mortality from cardiopulmonary diseases (Brunekreef and Forsberg 2005; Ahmad Al. and Talib 2011). Moreover, assessment of the results of Brits, Schoeters, and Verschaeve (2004) showed that exposure to inhalable airborne particles of urban origin can cause genotoxic effects and potentially lead to cancer (see also (Buschini et al. 2001; Zhao et al. 2002)). We can further show that PM₁₀, at least

that from the traffic-polluted urban site in Sulaimani City, has the potency to induce DNA damage and immunological modulation *in vitro* and might therefore have implications for cancer and allergy development.

Therefore, mitigation strategies designed to reduce air pollution, especially emissions of PM and other air pollutants associated with increasing motor vehicle operation must be urgently developed. Moreover, continuous monitoring of PM levels and composition at different urban sites is recommended. With these activities in place, improvement of air quality in Sulaimani City is possible.

5. CONCLUSIONS

Mineralogical, physical and chemical characterization revealed that PM₁₀ collected in the city of Sulaimani consists of complex mixtures of inorganic and organic compounds derived from natural and anthropogenic sources. Heavy metals (e.g. Cu, Fe), silica and PAHs were present at relatively high levels in PM₁₀ from an urban (UrbSu) and an industrial (IndSu) sampling site, and at lower levels in PM₁₀ from a rural sampling site (RurSu). Currently, it is not possible to determine which one of these compounds in PM₁₀ is responsible for the cytotoxic, pro-inflammatory and genotoxic responses we observed in human lung cells upon exposure to these particles.

The results of this and former studies suggest that the increase in the burden of air pollution in the last decades in Sulaimani City associated with unhampered economic and industrial growth has made air quality a crucial health problem, probably increasing the risk of death from cardiopulmonary diseases and lung cancer, especially when people are exposed to high concentrations over long periods. To improve public health, and for a better and more comprehensive understanding of the association between compounds in PM₁₀ and their toxicological effects, further studies on a wide range of sampling sites and sources of pollution should be initiated in Sulaimani City. Furthermore, action or strategies on air pollution reduction, especially PM and other air pollutants (gases) from different types of combustion processes are essential. Only with these measures can a permanent and sustainable program for the improvement in the quality of air in Sulaimani City be developed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Secondary electron (SE) images of the sieved dust samples (RurSu; IndSu; UrbSu) showing physical size range and shape of particles on representative sample areas; the energy-dispersive X-ray (EDX) spectrum (below, right) displays the qualitative elemental composition of the fibrous agglomerate found in the SE image of UrbSu (white arrow). The gold peak is due to the coating carbon peak results from substrate.

Figure 2. Transmission Electron Microscopy (TEM) images of the PM10 samples and the control substances. (A) RurSu; (B) IndSu; (C) UrbSu; (D) nano-SiO₂; and (E) dolomite. Fibrous particle in UrbSu (C) is an example of presumed asbestos. Bright spots in UrbSu and dolomite are pores from the Micropore[®] substrate material.

Figure 3.

Cell viability shown as percent viable cells (untreated control =100%) in human A549 cells. determined by the WST-1 assay in cells following 24 h exposure to different concentrations (0.3, 1, 3, 10, 30, 100 µg/mL) of PM10 samples from RurSu, IndSu, and UrbSu, as well as nano-SiO2 and dolomite. Each bar represents the data of the mean ($\pm$ SD) of three independent experiments performed in triplicate. Asterisks indicate statistically significant reductions of cell viability as * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ determined by one-way ANOVA followed by Dunnett's post hoc test between exposed groups and control [Ctr.].

Figure 4.

Release of IL-8 from cell culture supernatants of A549 cells after exposure to the control substances (nano-SiO₂ and dolomite) and the PM₁₀ from RurSu, IndSu, and UrbSu for 24 h. at concentrations ranging from 0.3 to 100 µg/mL. Each bar represents the data of the mean ($\pm$ SD) of three independent experiments performed in triplicate. Asterisks indicate statistical significant IL-8 release (* p <0.05, ** p <0.01, and *** p <0.001) determined by one-way ANOVA followed by Dunnett's post hoc test between exposed groups and control [Ctr.].

Figure 5.

Decrease in double-stranded DNA in A549 cells by alkaline unwinding after 24 h exposure to the PM10 samples from RurSu, IndSu and UrbSu as well as the control substances nano-SiO2 and dolomite at various concentrations (0.3, 1, 3, 10, 30, 100 μg/mL). Positive control: ethyl methane sulfonate (EMS). The data represent the mean (±SD) of at least three independent experiments performed in triplicate. Asterisks indicate statistical significance as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ determined by one-way ANOVA followed by Dunnett's post hoc test between exposed groups and control [Ctr.].

Table 1.

Elemental concentrations in PM₁₀ samples (in mg/kg)^a collected at various sampling sites of Sulaimani City as determined by ICP-MS or ICP-OES

Major Elements	RurSu		IndSu		UrbSu		Analytical method
	mg/kg	wt%	mg/kg	wt%	mg/kg	wt%	
Al	3565	0.4	20347	2.0	17841	1.8	ICP-OES
Ca	145038	15	30882	3.1	31862	3.2	ICP-OES
Fe	14084	1.4	64653	6.5	36637	3.7	ICP-OES
K	37308	3.7	10057	1.0	11758	1.2	ICP-OES
Mg	19579	2.0	4988	0.5	10145	1.0	ICP-OES
Mn	4651	0.5	1150	0.1	1049	0.1	ICP-OES
Na	8965	0.9	5683	0.6	5202	0.5	ICP-OES
Si	57325	5.7	187588	19	188964	19	ICP-OES
Ti	739	0.1	4566	0.5	8742	0.9	ICP-OES

Trace Elements	RurSu	IndSu	UrbSu	Analytical method
	mg/kg	mg/kg	mg/kg	
As	22	10	8	ICP-MS
Ba	1595	296	357	ICP-MS
Be	1.25	1.87	1.69	ICP-MS
Cd	9.8	0.6	0.9	ICP-MS
Co	9.8	18.0	20.5	ICP-MS
Cr	104	109	243	ICP-OES
Cu	438	10380	17196	ICP-MS
Mo	3.7	0.9	2.4	ICP-MS
Ni	73	111	243	ICP-MS
Pb	155	18	61	ICP-MS
Sb	3.0	1.0	2.4	ICP-MS
Sn	26.8	3.3	5.2	ICP-MS
Sr	563	183	179	ICP-MS
Zn	1137	126	454	ICP-OES
Zr	33	31	25	ICP-MS

^aMajor elements of each sample are also expressed in wt %

Table 2.PAH content in PM₁₀ at RurSu, IndSu, and UrbSu

Aromatic Rings	PAHs compounds	RurSu	IndSu	UrbSu
		concentrations (µg/kg)		
2	Naphthalene (Nap)	15.9	9.56	1.79
3	Acenaphthene (Ace)	3.32	36.60	1.40
3	Fluorene (Flu)	4.90	3.30	6.04
3	Phenanthrene (Phe)	55.0	82.05	41.4
3	Anthracene (Ant)	1.36	2.8	1.36
3	Retene (Ret)	11.08	49.97	23.32
4	Fluoranthene (Fla)	34.85	97.32	44.17
4	Pyrene (Pyr)	31.90	206.73	39.04
4	Triphenylene (Tri)	3.10	1.22	8.40
4	Benzo[a]anthracene (B[a]A)	11.71	25.33	14.96
4	Chrysene (Chr)	11.17	6.55	15.44
5	Benzo(e)pyrene (B[e]P)	15.56	30.37	21.08
5	Benzo[b]fluoranthene (B[b]F)	15.89	30.61	12.49
5	Benzo[k]fluoranthene (B[k]F)	2.37	1.98	1.92
5	Benzo[a]pyrene (B[a]P) ^a	3.26	6.44	2.97
6	Benzo[ghi]perylene (B[g,h,i]P)	9.22	45.3	6.04
6	Dibenzo[a,h]anthracene (DB[a,h]Ant)	0.43	1.14	0.39
6	Indeno[1,2,3-c,d]pyrene (IndP)	4.87	4.72	3.81
7	Coronene (Cor)	5.86	32.74	5.31
Sum of PAHs concentrations (PAHs) ^b		242	675	251

^a Mutagenic or carcinogenic according to International Agency for Research on Cancer (IARC)^b Sum of 19 PAHs measured in the study