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## Article

# Chemical Composition of PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> Collected in Southern Lebanon and Assessment of Their Toxicity in BEAS-2B Cells

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**Abstract:** Fine particles (PM<sub>2.5</sub>) have generally been reported as the major contributor to the adverse health effects of air pollution. Lebanon is characterized by a high density of transport, the production of electricity by generators, and a problem of uncontrolled incineration of household waste. For the purpose of this paper, the physico-chemical properties of fine (PM<sub>2.5-0.3</sub>) and quasi-ultrafine (PM<sub>0.3</sub>) particulate matter sampled in Southern Lebanon, were studied. Then, an evaluation and comparison of the toxicity of the different extracted fractions from PM (i.e., native PM<sub>2.5-0.3</sub> vs. organic extractable matter fraction (OEM<sub>2.5-0.3</sub>), and non-extractable matter fraction (NEM<sub>2.5-0.3</sub>)) was performed. Also, an examination of the toxicity of PM<sub>0.3</sub> was conducted indirectly through the evaluation of the OEM<sub>0.3</sub> harmfulness. The physico-chemical analysis showed that PM<sub>0.3</sub> was much more concentrated than PM<sub>2.5-0.3</sub> in organic compounds such as polycyclic aromatic hydrocarbons (PAHs) (28-fold) and their nitrated (N-PAHs, 14-fold) and oxygenated (O-PAHs, 10-fold) derivatives. Normal human bronchial epithelial cells (BEAS-2B) were exposed to PM<sub>2.5-0.3</sub>, its derived fractions (i.e., OEM<sub>2.5-0.3</sub> and NEM<sub>2.5-0.3</sub>), and OEM<sub>0.3</sub> before evaluating the global cytotoxicity, metabolic activation of organic compounds, genotoxicity, and inflammatory response. Different responses were observed depending on the considered fraction of particles. The global cytotoxicity showed a pronounced response related to ATP and LDH activities after exposure to the quasi-ultrafine organic extractable matter fraction (OEM<sub>0.3</sub>). There was no significant induction of the AhR cell-signaling pathway by NEM<sub>2.5-0.3</sub>. Despite the apparent difference in the kinetics of induction of the toxicological endpoints under study, OEM<sub>0.3</sub> provoked a higher overall cytotoxicity and genotoxicity than OEM<sub>2.5-0.3</sub> and total PM<sub>2.5-0.3</sub>. Taken together, these results clearly showed that the finest particles are more damaging to BEAS-2B cells than PM<sub>2.5-0.3</sub> because they are richer in organic compounds, thereby inducing more remarkable toxic effects.

**Keywords:** air pollution; Southern Lebanon; fine particles; organic extractable matter; BEAS-2B cells; toxicity

## 1. Introduction

Ranked as the fifth most crucial mortality factor, fine atmospheric particulate matter (PM<sub>2.5</sub>) constitutes one of the main air pollutants that cause millions of deaths every year worldwide [1,2]. According to the World Health Organization (WHO), around 4.2 million people have died from exposure to outdoor air pollution, and those deaths are mainly linked to heart disease, stroke, chronic obstructive pulmonary disease (COPD), lung cancer, and acute respiratory infections in children. Classified in Group 1 in terms of its carcinogenicity to humans by the International Agency of Research on Cancer (IARC) in 2013, PM<sub>2.5</sub> can be formed from precursors by chemical reactions in the atmosphere or emitted directly by natural/anthropogenic sources. It usually consists of a complex and heterogeneous mixture with a direct impact on air quality and human health. PM<sub>2.5</sub> is generally composed of an inorganic (i.e., ions, metals, carbonaceous species), organic (polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOC), polychlorobiphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF)), and biological part (bacteria, fungi) [2–5]. PM<sub>2.5</sub> can induce adverse health effects in humans. It can penetrate deeply into the lung, irritating and corroding the alveolar wall and consequently impairing lung function. PM<sub>2.5</sub> deposits mainly on the bronchial pseudostratified epithelial layer, which provides a chemically, immunologically, and mechanically protective barrier against environmental insults; it can also reach the lower airways. It can be cleared by macrophage-mediated phagocytosis. Nonetheless, the finest particles, especially the ultrafine ones, or their inorganic and organic components, may reach the underlying cells and the blood circulation, therefore exerting their adverse health effects on other organs [6–8]. Until now, limited research has investigated the respective impacts of the different fractions of PM<sub>2.5-0.3</sub> (organic extractable matter fraction (OEM<sub>2.5-0.3</sub>) and non-extractable matter fraction (NEM<sub>2.5-0.3</sub>). Consequently, the comprehension of cellular mechanisms responsible for inducing toxicity associated with exposure to these distinct particle fractions remains limited, especially in the context of BEAS-2B cells.

In vitro toxicology methods have been widely used over the last two decades. They represent a relevant approach for determining and describing the underlying mechanisms involved in the toxicity triggered by air pollution derived from PM<sub>2.5</sub> [4,9–12]. Recent studies, at cellular and molecular scales, have demonstrated that PM<sub>2.5</sub> induces oxidative stress by generating high levels of reactive oxygen species (ROS) and also provokes DNA damage, pro-inflammatory response, cell cycle deregulation, and regulated cell death [4,9,10,13]. The inorganic fraction of PM<sub>2.5</sub> can cause some of these mechanisms, but others have been more related to the organic fraction. It has been determined that the most toxic PM<sub>2.5</sub> components are metals, PAHs, and carbonaceous particles [14]. Several studies evidenced that the co-presence of organic compounds and other inorganic elements may determine the biological effects of PM<sub>2.5</sub> [4,9,15,16]. Badran et al. also supported the view that organic compounds associated with the quasi-ultrafine fraction (PM<sub>0.3</sub>) were the main drivers of fine PM genotoxicity and phase I and II enzymes involved in the metabolic activation of PAHs. This underscores the importance of improving the knowledge of the toxicological impact of ultrafine (PM<sub>0.1</sub>) and quasi-ultrafine (PM<sub>0.3</sub>) particles [4].

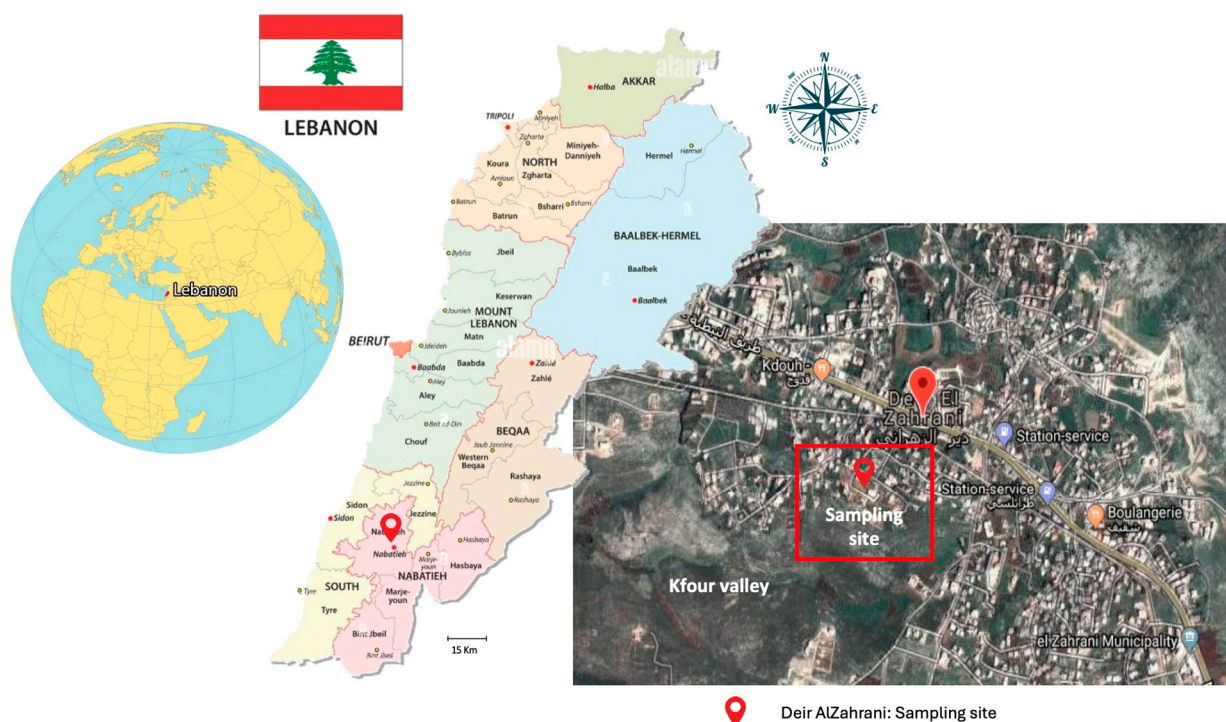
This work aimed to examine the toxicological impact of PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> collected in south Lebanon from the city of Deir AlZahrani on normal human bronchial epithelial cells (BEAS-2B). This city accommodates a diverse array of industries, with certain sectors specializing in aluminum manufacturing and handling, while others are dedicated to the production of real estate materials such as construction supplies and plumbing equipment. Deir AlZahrani is characterized by a significant number of chimneys, indicative of numerous factories within the area capable of emitting particulate matter. This is compounded by emissions from power generators. These factors promote the emission of toxic organic compounds such as PAHs and subsequently increase the environmental risks associated with PM<sub>2.5</sub>. To our knowledge, no toxicological studies have been carried out in southern Lebanon focusing on the sources of PM<sub>2.5</sub> and PM<sub>0.3</sub> and their respective adverse effects on human lung cells. Although the toxicity of atmospheric particles is well known in the

literature, there is a lack of data on the fraction within PM responsible for derived toxic effects. Are fine or ultrafine particles implicated? Is it the entirety of particles or their organic constituents? Therefore, there is an urgent need for new toxicological research to enhance the understanding of the underlying mechanisms of toxicity induced by different chemical fractions of  $PM_{2.5}$  from Southern Lebanon: organic ( $OEM_{2.5-0.3}$  and  $OEM_{0.3}$ ) and inorganic ( $NEM_{2.5-0.3}$ ). The collection site of the present study differs from that of our previous work focusing on Beirut [4]. Indeed, Beirut is characterized by high levels of fine particle pollution, primarily attributable to diesel-powered generators and road traffic. While some studies have been previously conducted in Lebanon, they focused on Beirut and North Lebanon [17–21], and, to the best of our knowledge, this work is the first one focusing on air quality in Southern Lebanon. So, our study represents the first analysis of the air quality in the Deir AlZahrani agglomeration.

## 2. Materials and Methods

### 2.1. Sampling Site

Fine ( $PM_{2.5-0.3}$ ) and quasi-ultrafine ( $PM_{0.3}$ ) particles were collected in Deir AlZahrani ( $33^{\circ}25'14.29''$  N;  $35^{\circ}27'17.9784''$  E), a semi-rural city, located in the south of Lebanon, 75 km from the capital, Beirut (Lebanon). Deir AlZahrani belongs to the district of Nabatiyeh, with 10,000 inhabitants. This area has several industries that fabricate and handle aluminum, while others produce real estate supplies. It also features many fireplaces and diesel-electric generators. The village is crossed by a main highway that links Beirut with Southern Lebanon and is therefore characterized by heavy road traffic (Figure 1). In addition, at 8 km from the sampling site, the Valley of Kfour, known to be the biggest in the region, has several open-cast industrial activities such as tire melting.



**Figure 1.** The location of the sampling site in Southern Lebanon: Deir AlZahrani.

The sampling point was situated on the roof of a 3-story building in Deir AlZahrani, Nabatiyeh, and the PM sampling was performed continuously from the 1st of January to the 3rd of March 2017. It was achieved using a high volume five-stage plus backup cascade impactor (model 235,  $68\text{ m}^3/\text{h}$ , Tisch, Cincinnati, OH, USA), allowing the separation of collected particles according to their equivalent aerodynamic diameter.  $PM_{2.5-0.3}$  was collected on impaction plates (stages 2–5), while  $PM_{0.3}$  was retained on a quartz fiber filter

(Pallflex® Tissuquartz 2500 QAT-UP, Pall, Putnam, NY, USA) placed after the impaction system. The system was changed every 5 days. Plates and backup filters were dried in a laminar flow hood for 48 h. PM<sub>2.5-0.3</sub> were obtained by brushing the plates, and individual PM<sub>2.5-0.3</sub> samples were gathered and carefully homogenized to form an average PM<sub>2.5-0.3</sub> sample. Similarly, average PM<sub>0.3</sub> samples were constituted by producing a proportional mix of pieces of the individual backup filters. Samples were weighted and finally stored at −20 °C. Sampling details have been described elsewhere [4].

## 2.2. Physico-Chemical Analysis of the Collected PM

Numerous analyses were performed to determine the chemical composition of the collected PM. Table A1 summarizes the different types of physico-chemical analysis tests and the samples concerned. Several tests were conducted to determine the inorganic composition of the collected PM, such as X-ray diffraction (XRD), scanning electronic microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) analysis, quantification of major and trace elements using inductively coupled plasma-atomic emission spectroscopy (ICP-AES), and quantification of water-soluble ions using ionic chromatography (IC). Organic compounds such as PAHs, N- and O-PAHs, PCDD, PCDF, and PCB were also quantified, using gas chromatography-mass spectrometry (GC-MS) for PAHs and high-resolution GC-high-resolution MS (HRGC-HRMS) for the other organic compounds. Paraffin, also known as alkanes, was also analyzed using GC-MS. The details related to the methodology are widely explained elsewhere [4].

## 2.3. Sample Preparation for Toxicological Studies

In order to study the implications of each PM fraction in the induction of toxic effects, the toxicity of the whole PM<sub>2.5-0.3</sub>, its OEM<sub>2.5-0.3</sub>, and NEM<sub>2.5-0.3</sub> were evaluated and compared. In addition, evaluation and comparison of the toxicity of the OEM<sub>2.5-0.3</sub> and OEM<sub>0.3</sub> were performed. Regarding the preparation of the samples, 200 mg of native PM<sub>2.5-0.3</sub> and their equivalent of this mass in PM<sub>0.3</sub> filters were placed in glass centrifuge tubes containing 10 mL of dichloromethane (DCM) and subjected to sonication (10 min, 20 °C). After extraction, the mixture was centrifuged at 1000 g, 20 °C for 10 min, allowing the separation of the extracted organic part from the PM or filters freed from this fraction. The supernatant was then recovered, and the extraction was repeated 3 times successively with fresh DCM to ensure the recovery of all the extractable organic compounds. For each sample, the solutions resulting from successive extractions of PM by DCM were combined, and the DCM was evaporated under a nitrogen stream and replaced with dimethyl sulfoxide (DMSO), which is compatible with toxicity studies.

At the end of the extraction, OEM<sub>2.5-0.3</sub> (supernatants) were obtained from PM<sub>2.5-0.3</sub> in addition to NEM<sub>2.5-0.3</sub> (kept in the centrifugation tube). From the PM<sub>0.3</sub> collected on filters, only OEM<sub>0.3</sub> was prepared. Concentrations of the final OEM<sub>2.5-0.3</sub> and OEM<sub>0.3</sub> solution were 98.1 and 96.4 mg PM-equivalent/mL (PM-eq/mL), respectively. For more details, please see [4].

## 2.4. In Vitro Toxicity of PM<sub>2.5</sub>

### 2.4.1. Cell Culture and Exposure

Normal human bronchial epithelial BEAS-2B cells, and an Ad12-SV40 hybrid virus-transformed human bronchial epithelial cell line (ATCC® CRL-9609™) were cultured as published elsewhere [22]. Just before reaching the confluence (<80%), BEAS-2B cells were exposed to the different fractions of PM: native PM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub>, and OEM<sub>0.3</sub>, prepared in LHC-9 culture medium, at concentrations ranging from 3 to 96 µg PM/cm<sup>2</sup> or µg PM-eq/cm<sup>2</sup>. Negative controls were represented by BEAS-2B incubated either with LHC-9 culture medium when exposed to PM and NEM or with dimethylsulfoxide (DMSO) at 0.5% (v/v) when exposed to OEM. In addition, benzo[a]pyrene (BaP) (25 mM), 1-nitropyrene (1-NPyr) (25 mM), and 9 fluorenone (9-FluO) (25 mM) were used as positive controls of PAHs, O-, and N-PAHs effects, respectively.

#### 2.4.2. Global Cytotoxicity Evaluation: Intracellular Adenosine Triphosphate (ATP) and Extracellular Lactate Dehydrogenase (LDH) Assays

Quantification of ATP, indicating the presence of metabolically active cells, was performed using the CellTiter-Glo<sup>®</sup> Luminescent Cell Viability Assay purchased from Promega. In addition, the global cytotoxic effects were evaluated through the quantification of LDH, which was carried out using the Cytotoxicity Detection Kit (LDH), purchased from Sigma-Aldrich (St. Louis, MO, USA). For these tests, BEAS-2B cells were seeded on a 96-well plate and exposed to PM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub>, and OEM<sub>0.3</sub> at different concentrations (3, 6, 12, 24, 48, and 96 µg PM/cm<sup>2</sup> or µg PM-eq/cm<sup>2</sup>) for 24 and 72 h. At the end of exposure, intracellular ATP and extracellular LDH were quantified according to the supplier's instructions.

#### 2.4.3. Metabolic Activation of PAHs: Evaluation of the Aryl Hydrocarbon Receptor (AHR) Cell Signaling Pathway

The gene expression of enzymes implicated in the metabolic activation of PAHs was evaluated using quantitative real-time PCR (TaqMan<sup>™</sup>). BEAS-2B cells were exposed for 6 and 24 h to the different fractions of PM at 3 and 12 µg/cm<sup>2</sup> or µg PM-eq/cm<sup>2</sup>. At the end of exposure, total RNA was isolated using the miRNeasy Mini Kit (Qiagen, Courtaboeuf, France). After the reverse transcription of 1 µg of total RNA in single-stranded cDNA using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher scientific, Illkirch-Graffenstaden, France), gene expression relative quantitation was carried out using Taqman<sup>™</sup> gene expression assays: *18S ribosomal RNA (RNA18S)* (Housekeeping gene, Hs9999990), *AHR (Hs00169233)*, *aryl-hydrocarbon receptor repressor (AhRR)* (Hs01005075\_m1), *aryl-hydrocarbon receptor nuclear translocator (ARNT)* (Hs01121918\_m1), *cytochrome P450A1 (CYP1A1)* (Hs01054797\_g1), *cytochrome P450B1 (CYP1B1)* (Hs00164383\_m1), *epoxyde hydrolase 1 (EPHX-1)* (Hs01116806\_m1), and *glutathione S-transferase alpha 4 (GSTA4)* (Hs01119249\_m1). The StepOnePlus<sup>™</sup> Real-Time PCR System and the Expression Suite Software v2.3 (ThermoFisher scientific) were used (for more details see [9]).

#### 2.4.4. PM-Induced Genotoxicity

The capacity of PM and its different fractions to induce genotoxicity was evaluated through the Milliplex Map Kit-7-plex DNA damage/Genotoxicity based on the Luminex xMAP technology (Merck-Millipore, Saint-Quentin-en-Yvelines, France). The Milliplex Kit is used to detect changes in phosphorylated checkpoint kinase 1 (CHK-1) (Ser 345), phosphorylated checkpoint kinase 2 (CHK-2) (Thr68), phosphorylated H2A histone family member X (H2AX) (Ser 139), and phosphorylated protein 53 (pP53) (Ser 15), as well as total protein levels of ataxia telangiectasia and Rad3-related protein (ATR), mouse double minute 2 homolog (MDM2), and protein 21 (P21) in cell lysates, according to the manufacturer's recommendations.

#### 2.4.5. Inflammation: TNF-α and IL-6 Secretion

Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) secretion were evaluated and quantified in the cell-free culture supernatants of BEAS-2B cells using MILLIPLEX<sup>®</sup> MAP Human Cytokine/Chemokine Magnetic Bead Panel-Immunology Multiplex Assay (Merck-Millipore, Saint-Quentin-en-Yvelines, France). This assay used lipopolysaccharides (LPS) as a positive control of cytokine secretion.

### 2.5. Statistical Analysis

Results were normalized to control and presented as mean values and standard deviations. For each incubation time, results from BEAS-2B cells exposed to PM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub>, and OEM<sub>2.5-0.3</sub> were compared to those obtained in non-exposed cells using the Mann-Whitney U test (SPSS software, v26). Significant differences were reported with *p* values < 0.05.

### 3. Results and Discussion

#### 3.1. PM<sub>2.5</sub> Atmospheric Concentration

The site's average daily PM<sub>2.5</sub> atmospheric concentration was 26 µg/m<sup>3</sup>. This concentration exceeds the new daily guideline value recommended by the WHO in 2021, which is 5 µg/m<sup>3</sup>. The concentration of PM<sub>2.5</sub> collected in the same period in the southern suburb of Beirut was much higher (54 µg/m<sup>3</sup>). This can be explained by the more intense road traffic at this site, the number of inhabitants, which is higher in Beirut, and the number of private diesel generators used to resolve the electricity crisis. In Lebanon, the annual average of PM<sub>2.5</sub> often exceeds the WHO guidelines [23]. A significant relationship between short-term variations in ambient concentrations of PM<sub>2.5</sub> and emergency hospital admissions in Beirut has been demonstrated, and the exposure of the Lebanese population to fine particles for up to 48 h can repress DNA repair mechanisms [24,25]. It was shown that Lebanese rural/suburban zones are more polluted than urban ones due to the usage of more traditional heating equipment and chemical products in agriculture, such as pesticides and fertilizers [26].

#### 3.2. The Chemical Analysis of the Collected PM

##### 3.2.1. Elemental, Water-Soluble Ions, and Total Carbon

Table 1 represents the average concentrations of major and trace elements, water-soluble ions, and total carbon in fine (PM<sub>2.5-0.3</sub>) and quasi-ultrafine particles (PM<sub>0.3</sub>). Results showed that Ca and NO<sub>3</sub><sup>−</sup> were the most abundant elements in both PM<sub>2.5-0.3</sub> (24.46% and 22.74%) and PM<sub>0.3</sub> (20.40% and 19.32%), respectively. In addition, elements and ions such as Al, SO<sub>4</sub><sup>2−</sup>, Fe, NH<sub>4</sub><sup>+</sup>, Mg, Cl<sup>−</sup>, K, and Na were predominant in both PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub>, with different percentages. Scanning electronic microscopy (SEM) analysis also showed the presence of silica (Si), which seems to be predominant in PM<sub>2.5-0.3</sub> particles (Figure A1). Other elements and ions such as Ti, Cu, P, Mn, Sr, Ba, Pb, Zn, Cr, Sn, Mo, NO<sub>3</sub><sup>−</sup>, SO<sub>4</sub><sup>2−</sup>, NH<sub>4</sub><sup>+</sup>, and Cl<sup>−</sup> were also detected in both PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub>, with a percentage ranging from 0.1% to 1%. Sn (0.01%), Co (0.005%), As (0.005%), and Cd (0.002%) were only detected in the quasi-ultrafine fraction (PM<sub>0.3</sub>). The predominance of elements such as Ca, Al, Fe, Mg, and Ti can be related to the resuspension of soil dust [27]. From XRD analysis, Ca appears in the form of calcite and gypsum. The presence of Ca in high quantities could also be linked to the soil type in Lebanon, which is calcareous. XRD analysis also showed the presence of kaolinite, one of the characteristic minerals of the Lebanese soil type [4,28] (Figure A2). It can explain the presence of Al in PM in addition to the possible influence of Al factories close to the sampling site. On the other hand, elements influenced by vehicular emissions, such as Ba, Ni, Cu, and Zn, were observed. The presence of these elements is linked to the road traffic and probably to the existence of a principal highway in Deir AlZahrani, connecting the capital to all the cities of the South. Particularly, Cu is related to brake emissions, and Zn is linked to tire emissions [29] and the use of lubricating oil [30]. Otherwise, two major ions, NO<sub>3</sub><sup>−</sup> and SO<sub>4</sub><sup>2−</sup>, were detected in both types of PM. These ions could originate from the gas-to-particle conversion of NO<sub>x</sub> and SO<sub>2</sub> precursor gases and long-range transport [4,31].

**Table 1.** Elements, water-soluble ions, and total carbon concentrations in PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> collected at Deir AlZahrani site.

Species (mg/g)	PM <sub>2.5-0.3</sub>	(%)	PM <sub>0.3</sub>	(%)
Ca	87	24	85	20
NO <sub>3</sub> <sup>−</sup>	81	23	80	19
Al	43	12	26	6
SO <sub>4</sub> <sup>2−</sup>	35	10	16	4
Fe	22	6	13	3
NH <sub>4</sub> <sup>+</sup>	19	5	44	10
Mg	16	4	25	6

Table 1. Cont.

Species (mg/g)	PM <sub>2.5-0.3</sub>	(%)	PM <sub>0.3</sub>	(%)
Cl <sup>-</sup>	9	2	15	4
K	10	3	15	4
Na	8	2	57	14
ThTi	2	0.7	1	0.2
Cu	2	0.6	2	0.5
P	0.9	0.2	2	0.5
Mn	0.4	0.1	0.2	0.05
Sr	0.3	0.08	0.2	0.05
Ba	0.3	0.08	0.2	0.05
Pb	0.2	0.06	1	0.2
Zn	0.2	0.06	1	0.2
Cr	0.1	0.03	0.08	0.02
V	0.1	0.03	0.4	0.1
Sn	<DL *	-	0.04	0.01
Ni	0.1	0.03	0.2	0.05
Mo	0.1	0.03	1	0.2
Co	<DL *	-	0.02	0.005
As	<DL *	-	0.02	0.005
Cd	<DL *	-	0.01	0.002
Total carbon	18	5	31	7

\* DL: detection limit.

### 3.2.2. Organic Composition

The 16 priority PAHs listed by the United States Environmental Protection Agency (US EPA) were detected in both PM<sub>2.5-0.3</sub> and (mostly) PM<sub>0.3</sub> (Table 2). Indeed, the content of PAHs in PM<sub>0.3</sub> was about 28 times higher compared to that in PM<sub>2.5-0.3</sub> (1007.3 µg/g of PAHs in PM<sub>0.3</sub> versus 35.8 µg/g of PAHs in PM<sub>2.5-0.3</sub>). This difference is the result of anthropogenic activities and typical combustion processes that mainly emit dUFP [4]. In addition, the most abundant PAHs were BbF, InPyr, BghiP, Chry, BaP, and BbK. These PAHs emissions are generally linked to combustion sources, including gasoline motor vehicles, biomass burning, and industrial processes [32]. To better investigate the emission sources of these PAHs, diagnostic ratios were calculated based on already published studies [33,34]. The Fla/(Fla + Pyr) ratio was lower than 0.5 for both PM samples, showing the predominance of diesel emissions (<0.5) [33,34]. In addition, the Fla/Pyr ratio was greater than 0.6, which suggests that the emissions could be related to road traffic (Table A2). Moreover, to evaluate the carcinogenic risk of environmental exposure to these PAHs, concentrations were converted to a BaP-toxic equivalent factor (BaP-TEQ), using factors published elsewhere [35,36]. The potential carcinogenicity of these PM<sub>2.5</sub> (PM<sub>2.5-0.3</sub> + PM<sub>0.3</sub>) was equal to 3 ng/m<sup>3</sup> BaP-TEQ, higher than the BaP WHO guideline (0.12 ng/m<sup>3</sup>; cancer risk 1/100,000). The highest BaP-TEQ values were for dibenz[a,h]anthracene: 1.6 ng/m<sup>3</sup> BaP-TEQ, Benzo[a]Pyrene: 0.6 ng/m<sup>3</sup> BaP-TEQ, Benzo[b]fluorethene: 0.3 ng/m<sup>3</sup> BaP-TEQ, and Indeno [1,2,3-c,d]Pyrene: 0.25 ng/m<sup>3</sup> BaP-TEQ. Although this value exceeded the BaP-TEQ values set by the WHO, these differences showed and validated the influence of the presence of important industrial activities and traffic in Deir AlZahrani. An even higher value was previously detected for particles sampled in the same period but on another site in Lebanon (in the southern suburb of Beirut; 8 ng/m<sup>3</sup> BaP-TEQ) [4]. These differences show and validate the influence of the presence of more dynamic industrial activities and busier traffic in the suburbs of Beirut.



**Table 2.** Concentrations of PAHs, N-PAHs, and O-PAHs (in  $\mu\text{g/g}$ ) detected in  $\text{PM}_{2.5-0.3}$  and  $\text{PM}_{0.3}$  collected at Deir AlZahrani.

	Abbreviation	$\text{PM}_{2.5-0.3}$	$\text{PM}_{0.3}$
PAHs ( $\mu\text{g/g}$ )			
Naphthalene	Nap	0.1	1
Acenaphthylene	Acy	0.06	1
Acenaphthene	Ace	0.01	0.3
Fluorene	Flu	0.07	0.6
Phenanthrene	Phe	2	11
Anthracene	Ant	0.05	1
Fluoranthene	Fla	3	28
Pyrene	Pyr	2	27
Benz[a]anthracene	BaA	2	50
Chrysene	Chry	4	63
Benzo[b]fluoranthene	BbF	9	268
Benzo[k]fluoranthene	BbK	2	51
Benzo[a]pyrene	BaP	2	54
Indeno[1,2,3-c,d]pyrene	InPyr	4	226
Dibenz[a,h]anthracene	DahA	0.4	29
Benzo[g,h,i]perylene	BghiP	5	196
$\Sigma$ PAHs		35.8	1007.3
N-PAHs ( $\mu\text{g/g}$ )			
2-nitrofluorene	2-NFlu	0.4	0.9
6-nitrochrysene	6-NChry	0.3	1
7-nitrobenz(a)anthracene	7-NBaA	<DL *	3
3-nitrofluoranthene	3-NFluor	<DL *	2
1-nitropyrene	1-NPyr	0.4	1
1,3-dinitronaphthalene	1.3-DNNAp	<DL *	<LD *
5-nitroacenaphthene	5-NAce	0.1	0.7
9-nitroanthracene	9-NAnt	0.1	3
3-nitrophenanthrene	3-NPhe	<DL *	1
2-nitroanthracene	2-NAnt	<DL *	1
$\Sigma$ N-PAHs		1.3	13
O-PAHs ( $\mu\text{g/g}$ )			
9-fluorenone	9-FluO	0.7	5
9,10-anthraquinone	9,10-AntQ	3	11
Benzo(a)fluorenone	BaFluO	0.7	5
7H-benz(de)anthracen-7-one	1,9-BAntO	1	33
Benzo(a)anthracen-7,12-dione	7,12-BaAQ	<LD *	18
1,8-naphtalic anhydride		4	63
1-naphtaldehyde		<LD *	<LD *
Phenanthrene-9-carboxyaldehyde		<LD *	<LD *
$\Sigma$ O-PAHs		9.2	136

\* DL: detection limit.

O- and N-PAHs: 5 O-PAHs and 5 N-PAHs were detected in  $\text{PM}_{2.5-0.3}$ , and 6 O-PAHs/9 N-PAHs in  $\text{PM}_{0.3}$  (Table 2). The total concentrations of N- and O-PAHs were higher in  $\text{PM}_{0.3}$  vs.  $\text{PM}_{2.5-0.3}$  (O-PAHs: 136  $\mu\text{g/g}$  vs. 9.2  $\mu\text{g/g}$ , respectively; N-PAHs: 13  $\mu\text{g/g}$  vs. 1.3  $\mu\text{g/g}$ , respectively). Among the detected O-PAHs, the most abundant in  $\text{PM}_{2.5-0.3}$  were 1,8-naphtalic anhydride (3.7  $\mu\text{g/g}$ ; 40% of the total O-PAHs content); followed by 9,10-anthraquinone (2.9  $\mu\text{g/g}$ ; 32%) and 7H-benz(de)anthracen-7-one (1.1  $\mu\text{g/g}$ ; 12%). However, in  $\text{PM}_{0.3}$ , the most abundant O-PAHs was 1,8-naphtalic anhydride (63  $\mu\text{g/g}$ ; 46%); followed by 7H-benz(de)anthracen-7-one (33.5  $\mu\text{g/g}$ ; 25%); benz(a)anthracen-7,12-dione (18.3  $\mu\text{g/g}$ ; 13%), which was not detected in the case of  $\text{PM}_{2.5-0.3}$ ; and 9,10-anthraquinone (11  $\mu\text{g/g}$ ; 8%). Concerning the N-PAHs, the major component in  $\text{PM}_{2.5-0.3}$  was 1-nitropyrene

(0.4 µg/g; 34%), followed by 2-nitrofluorene (0.3 µg/g; 29%) and 6-nitrochrysene (0.3 µg/g; 23%). However, in PM<sub>0.3</sub>, the most abundant N-PAHs was 7-nitrobenz(a)anthracene, which is not detected in PM<sub>2.5-0.3</sub> (2.6 µg/g; 20%); followed by 9-nitroanthracene (2.5 µg/g; 19%); 3-nitrofluoranthene (1.8 µg/g; 14%), also not detected in the PM<sub>2.5-0.3</sub>; 2-nitroanthracene (1.3 µg/g; 10%); and 6-nitrochrysene (1 µg/g; 8%). It should be noted that 9-nitroanthracene, 1-nitropyrene, 2-nitrofluoranthene, and 3-nitrofluoranthene were shown to be 25% as potent as BaP [37].

Compared to PAHs, O- and N-PAHs have a higher molecular weight, lower vapor pressure, and a higher tendency to be adsorbed onto particulate matter [38,39]. Moreover, these components can be emitted directly into the atmosphere. Also, they can be formed as secondary pollutants via heterogeneous or homogeneous photo-oxidation reaction of PAHs with atmospheric oxidants, photolysis, and thermal transformation [40–42]. The emission sources of each O- and N-PAHs are poorly identified and documented in the bibliography. Their determination is challenging because of the coexistence of their primary and/or secondary sources. However, some studies have tried to associate compounds with emission sources, such as the case of 1,8-Naphthalic anhydride, which was reported as a major O-PAHs emitted by residential wood combustion [43–45]. Unlike anhydrous 1,8-naphthalic emitted directly from a combustion source, the formation of quinones, including 9,10-Anthraquinone, has been demonstrated in certain studies as products of the oxidation of PAHs by photolysis or reactions with O<sub>3</sub>, NO<sub>3</sub>, and OH [39,46–48]. Among N-PAHs, 1-NP is formed and emitted directly into the atmosphere during fossil fuel combustion at high temperatures and has been proposed as a chemical marker for diesel exhaust particles [49]. IARC also categorized it in Group 2 A (probably carcinogenic to humans). Concerning 9-NAnt, it has been reported to be emitted from direct sources, predominantly diesel engines. It can also be formed in the atmosphere because of reactions with OH radicals followed by heterogeneous nitration reactions with anthracene during the day and reactions with NO<sub>3</sub> at night [50].

PCB, PCDD, and PCDF: All these chemical compounds were also analyzed and quantified in both PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub>. The results showed that PM<sub>2.5-0.3</sub> was more concentrated in PCDD and PCB than PM<sub>0.3</sub>. However, PM<sub>0.3</sub> was more concentrated in PCDD and PCB indicators than PM<sub>2.5-0.3</sub> (Table 3). Concentrations of PCDD, PCDF, and PCB were also quantified on two other sites in Lebanon (Zouk Mikael and Fiaa) by [51]. The total concentrations of these compounds in the whole PM<sub>2.5</sub> were much higher in Deir AlZahrani compared to the two other sites (PCDD: 399.43 fg/m<sup>3</sup> vs. 81.1 ± 38.7 and 52.0 ± 21.7 fg/m<sup>3</sup>; PCDF: 333.33 fg/m<sup>3</sup> vs. 82.5 ± 35.1 and 71.8 ± 36.7 fg/m<sup>3</sup>; PCB: 7043.95 fg/m<sup>3</sup> vs. 454.8 ± 202 and 586.3 ± 128 fg/m<sup>3</sup>, respectively). These compounds are generally produced from anthropogenic activities such as forest fires or domestic and hospital waste incineration [52]. In this study, the presence of PCDD, PCDF, and PCB could be attributed to the uncontrolled domestic waste incineration in the Kfour valley, which is relatively close to the sampling site.

Paraffins (n-alkanes): Paraffins, also known as n-alkanes, were quantified in PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub>, and the results are presented in Figure A3. Concentrations of paraffins were about 5 times higher in PM<sub>0.3</sub> (906 µg/g; 14 ng/m<sup>3</sup>) than in PM<sub>2.5-0.3</sub> (181 µg/g; 2.7 ng/m<sup>3</sup>). This is also true for other organic compounds, such as PAHs and their derivatives.

The carbon preference index (CPI), defined as the concentration ratio of odd to even carbon number n-alkanes and the carbon maximum number (C<sub>max</sub>), has been widely used in the literature to evaluate biogenic and anthropogenic contributions to organic aerosol [4,53–55]. C<sub>max</sub> for PM<sub>2.5-0.3</sub> was C<sub>34</sub> and C<sub>36</sub> for PM<sub>0.3</sub>, indicating that the n-alkanes primarily originated from terrestrial plants, especially epicuticular waxes. Values of overall and high CPI were equal to 1.18 and 1.51 for PM<sub>2.5-0.3</sub>, respectively, and to 1.61 and 1.29 for PM<sub>0.3</sub>, respectively. These data suggested that the n-alkanes detected in the two samples originated from both natural and anthropogenic sources (1.5 < high CPI < 3 and overall CPI > 1).

**Table 3.** Concentrations of PCDD, PCDF, and PCB (ng/g and fg/m<sup>3</sup> of PM) detected in PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> collected at Deir AlZahrani.

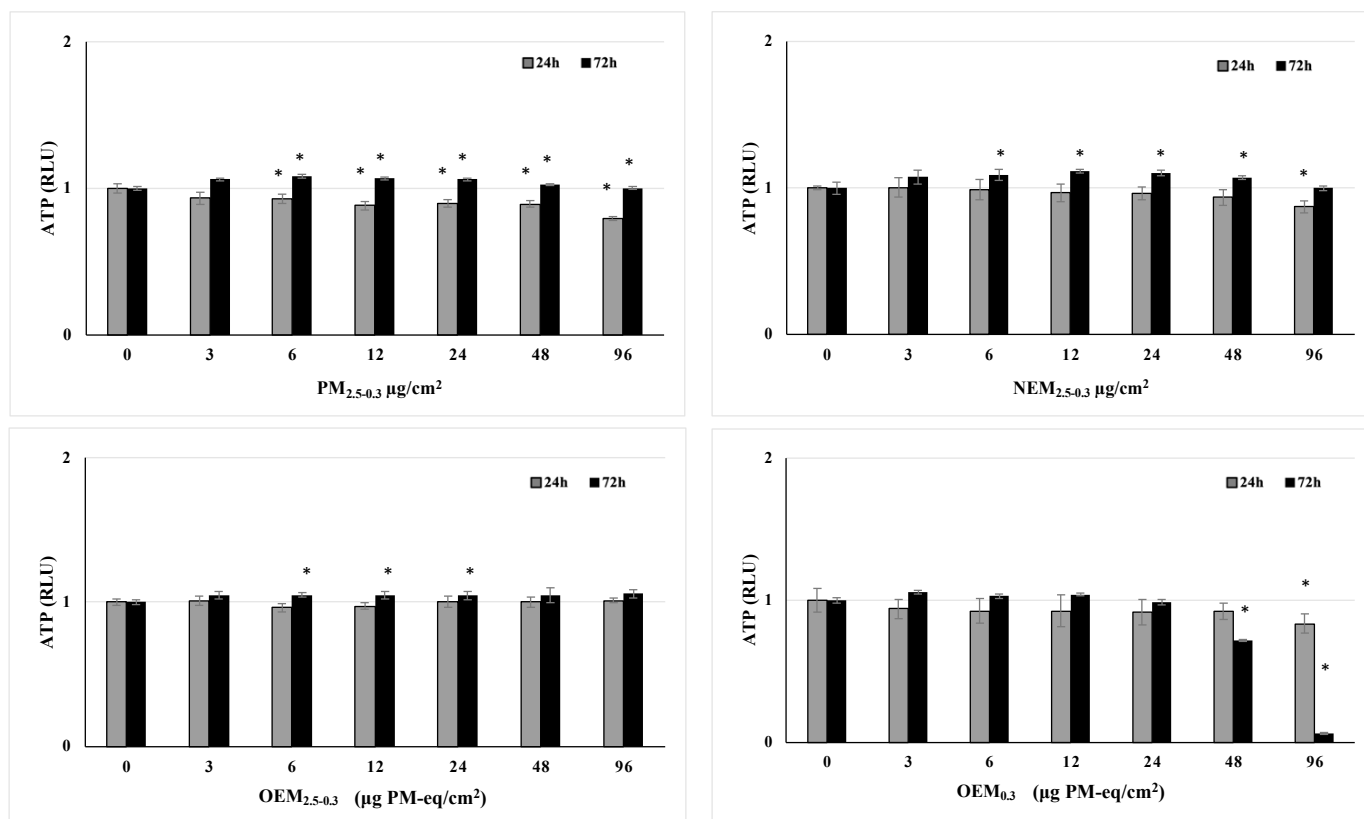
	PM <sub>2.5-0.3</sub>	PM <sub>0.3</sub>
PCDD	ng/g	ng/g
2,3,7,8 TCDD	0.02	0.04
1,2,3,7,8 PeCDD	0.07	0.2
1,2,3,4,7,8 HCDD	0.09	0.2
1,2,3,6,7,8 HCDD	0.3	0.4
1,2,3,7,8,9 HCDD	0.2	0.3
1,2,3,4,6,7,8,9 HpCDD	3	3
OCDD	17	9
ΣPCDD	20	13
PCDF		
2,3,8,7 TCDF	0.3	0.4
1,2,3,7,8 PeCDF	0.2	0.6
2,3,4,7,8 PeCDF	0.4	1
1,2,3,4,7,8 HCDF	0.5	1
1,2,3,6,7,8 HCDF	0.4	1
2,3,4,6,7,8 HCDF	0.5	2
1,2,3,7,8,9 HCDF	0.1	0.4
1,2,3,4,6,7,8 HpCDF	2	5
1,2,3,4,7,8,9 HpCDF	0.3	0.7
OCDF	5	5
ΣPCDF	9	17
PCB		
PCB 81	0.08	0.2
PCB77	2	2
PCB 123	0.7	0.6
PCB 118	19	17
PCB 114	0.8	0.2
PCB 105	10	7
PCB 126	0.09	0.2
PCB 167	2	1
PCB 156	4	4
PCB 157	0.8	0.3
PCB 169	0.09	0.3
PCB 189	1	1
ΣPCB	40	33
<i>PCB indicators</i>		
PCB 28	28	90
PCB52	44	74
PCB101	26	43
PCB138	45	34
PCB153	54	39
PCB180	42	28
ΣPCB indicators	239	308

### 3.3. Evaluation of PM<sub>2.5</sub> Toxicity:

#### 3.3.1. ATP Production and LDH Activity

In this study, cell viability was evaluated through the quantification of intracellular ATP produced by mitochondria, which are special double-membraned intracellular compartments, popularly known as the ‘powerhouses of the cell’ because they provide ATP used to fuel cellular biochemical reactions [56]. Alterations in cellular metabolism are linked to impaired mitochondrial functions such as a decrease in ATP production and an increase in reaction oxygen species production [57]. In this study, BEAS-2B cells were exposed to the different prepared suspensions (PM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub>, and OEM<sub>0.3</sub>) at different

concentrations (3, 6, 12, 24, 48, and 96  $\mu\text{g}/\text{cm}^2$  or  $\mu\text{g PM-eq}/\text{cm}^2$ ) for 24 and 72 h (Figure 2). A significant decrease in ATP production was observed following BEAS-2B exposure to 6, 12, 24, 48, and 96  $\mu\text{g}/\text{cm}^2$  of  $\text{PM}_{2.5-0.3}$  for 24 and 72 h.

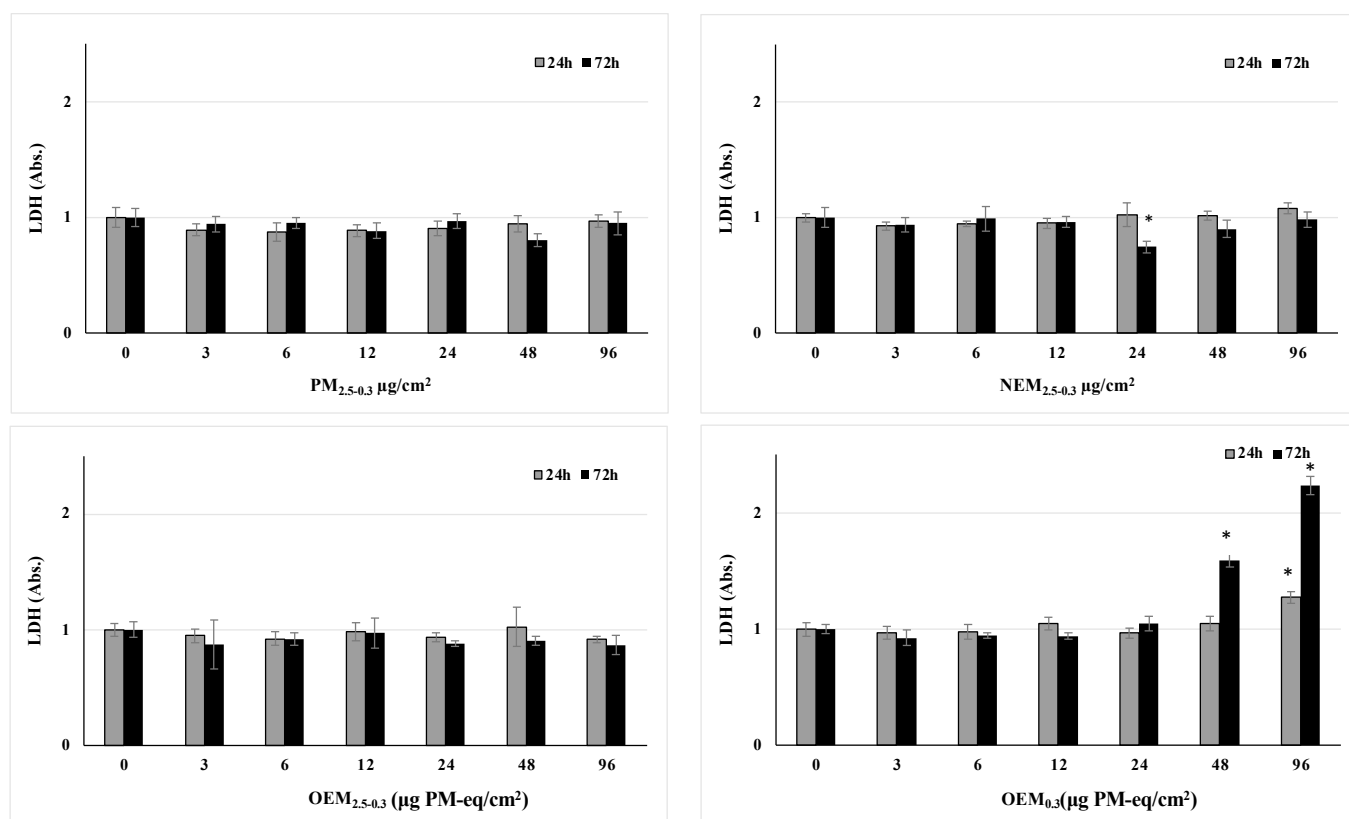


**Figure 2.** Adenosine triphosphate (ATP) concentrations in BEAS-2B cells, normalized to control. Cells were exposed for 24 and 72 h to increasing concentrations (3, 6, 12, 24, 48, and 96  $\mu\text{g PM-eq}/\text{cm}^2$ ) of  $\text{PM}_{2.5-0.3}$  (**upper left**), organic extractable matter from  $\text{PM}_{2.5-0.3}$  ( $\text{OEM}_{2.5-0.3}$ ) (**lower left**), non-extractable matter fraction from  $\text{PM}_{2.5-0.3}$  ( $\text{NEM}_{2.5-0.3}$ ) (**upper right**), and organic extractable matter from the quasi-ultrafine fraction ( $\text{OEM}_{0.3}$ ) (**lower right**). The results are described by their means and standard deviations (16 replicates for non-exposed cells and 8 replicates for exposed cells (Mann–Whitney U-test vs. controls (non-exposed cells); \* =  $p < 0.05$ ).

These data showed that  $\text{PM}_{2.5-0.3}$  affected cellular metabolism, resulting in a decrease in the cellular ATP concentration at short times (24 h), and this perturbation was more pronounced when exposed to the highest concentration of  $\text{PM}_{2.5-0.3}$  (96  $\mu\text{g}/\text{cm}^2$ ). However, after 72 h of exposure, BEAS-2B cells were trying to compensate for the decrease in ATP and to eliminate  $\text{PM}_{2.5-0.3}$ , which can explain the increase in cellular ATP. In addition, exposure of BEAS-2B to the  $\text{NEM}_{2.5-0.3}$  and  $\text{OEM}_{2.5-0.3}$  fractions in the same conditions showed different cellular responses compared to the whole  $\text{PM}_{2.5-0.3}$ . Concerning the exposure to the  $\text{NEM}_{2.5-0.3}$  fraction, a significant increase of the cellular ATP was observed following 72 h of exposure to 6, 12, 24, and 48  $\mu\text{g PM-eq}/\text{cm}^2$ , in addition to a significant decrease following 24 h of exposure to the highest concentration (96  $\mu\text{g PM-eq}/\text{cm}^2$ ). Regarding  $\text{OEM}_{2.5-0.3}$ , only a significant increase was observed following the exposure to 6, 12, 24, and 48  $\mu\text{g PM-eq}/\text{cm}^2$  for 72 h. Taken together, these results showed different cellular responses when exposed to the whole PM vs. its fractions ( $\text{NEM}_{2.5-0.3}$  and  $\text{OEM}_{2.5-0.3}$ ). In our conditions, alteration in cellular metabolism was mostly observed in the case of the native  $\text{PM}_{2.5-0.3}$ , and the effect was shown to be dose-dependent in the case of  $\text{PM}_{2.5-0.3}$  (for 24 and 72 h) and  $\text{NEM}_{2.5-0.3}$  (for 72 h). Regarding  $\text{OEM}_{0.3}$ , a decrease in cellular ATP was observed following 24 h of exposure to 96 Eq.  $\text{PM}/\text{cm}^2$  and 72 h to 48 and 96 Eq.  $\text{PM}/\text{cm}^2$ .

These results showed a time and concentration-dependent effect of OEM<sub>0.3</sub>. In addition, the effect of OEM<sub>0.3</sub> was more pronounced than that of OEM<sub>2.5-0.3</sub>. [4] demonstrated similar findings following the exposure of BEAS-2B cells to OEM<sub>2.5-0.3</sub> and OEM<sub>0.3</sub> collected from Beirut. This difference could be related to the organic composition of these two fractions, which are more concentrated in OEM<sub>0.3</sub> (cf. 3.2.2. organic composition).

Global cytotoxicity was also established by studying LDH activities in cell-free culture supernatants (Figure 3). The data show that the exposure of cells to different pollutants induces a decrease in the activity of LDH after exposure to OEM<sub>0.3</sub>, 24 and 72 h to 96 µg PM/cm<sup>2</sup> or µg PM-eq/cm<sup>2</sup> and only 72 h to 48 Eq. PM/cm<sup>2</sup> showed slight and transitory cytotoxicity after their exposure to the highest concentrations of OEM<sub>0.3</sub>. Like ATP activities, the effect of OEM<sub>0.3</sub> was more pronounced than that of OEM<sub>2.5-0.3</sub>, related to the presence of more organic compounds and explained by chemical organic fraction characterization.



**Figure 3.** Lactate dehydrogenase (LDH) concentrations in supernatants of BEAS-2B cells, normalized to control. Cells were exposed for 24 and 72 h to increasing concentrations (3, 6, 12, 24, 48, and 96 µg/cm<sup>2</sup> or µg PM-eq/cm<sup>2</sup>) of PM<sub>2.5-0.3</sub> (**upper left**), organic extractable matter from PM<sub>2.5-0.3</sub> (OEM<sub>2.5-0.3</sub>) (**lower left**), non-extractable matter fraction from PM<sub>2.5-0.3</sub> (NEM<sub>2.5-0.3</sub>) (**upper right**), and organic extractable matter from the quasi-ultrafine fraction (OEM<sub>0.3</sub>) (**lower right**). The results are described by their means and their standard deviations (8 replicates for non-exposed cells and 8 replicates for exposed cells (Mann–Whitney U-test vs. controls (non-exposed cells); \* =  $p < 0.05$ ).

### 3.3.2. Metabolic Activation of PAHs

PAHs represent toxic compounds, and their toxicity occurs after their metabolic activation. Depending on their nature, metabolites obtained after this activation will interact with cellular macromolecules and induce dysfunction in the body. Briefly, once in the cell, the PAHs binds to its cytoplasmic receptor, the AhR, inducing its activation and migration into the nucleus, where it will bind with another nuclear protein called ARNT (AhR nuclear translocator). Thus, the new complex is formed, is bonded to specific regions of DNA called “xenobiotic response elements” (ERX) and induces the expression of specific

target genes involved in the PAHs metabolism process (e.g., *CYP1A1*, *CYP1B1*, *EPHX1*, and *GSTA4*).

In this study, we evaluated the expression of the above-mentioned genes in addition to *AHRR*, which functions as a negative feedback modulator by repressing AhR-dependent gene expression following BEAS-2B exposure to C1 = 3 and C2 = 12  $\mu\text{g}/\text{cm}^2$  or  $\mu\text{g eqPM}/\text{cm}^2$  for 6 and 24 h. The results are represented in Table 4. Exposure of BEAS-2B to the prepared fractions showed different responses concerning the gene expression of *AHR*, *ARNT*, *AHRR*, *CYP1A1*, *CYP1B1*, *EPHX1*, and *GSTA-4*. First, after 6 h of exposure to the whole  $\text{PM}_{2.5-0.3}$ , significant increases in the gene expression of *AHRR* and *CYP1B1* were observed for the highest concentration (C2 = 12  $\mu\text{g}/\text{cm}^2$ ). Then, after 24 h of exposure, a significant increase in the gene expression of *CYP1A1* was observed when exposed to C1. Moreover, all the genes studied showed a significant increase in gene expression when exposed to a C2 concentration for 24 h. These results showed that BEAS-2B cells represented metabolically competent cells, since they were able to phagocytose the  $\text{PM}_{2.5-0.3}$  and induce the metabolic activation of organic compounds, as supported by other authors [4,9,58,59]. However, the cellular response to the  $\text{NEM}_{2.5-0.3}$  and  $\text{OEM}_{2.5-0.3}$  fractions was entirely different from that of  $\text{PM}_{2.5-0.3}$ . No significant increase in the expression of any of the studied genes was observed following exposure to these two fractions. This is quite normal in the case of  $\text{NEM}_{2.5-0.3}$ , which must not contain any organic compounds following DCM extraction. Nevertheless, the absence of induction of the metabolic activation pathway by  $\text{OEM}_{2.5-0.3}$ , which normally contained all the organic compounds extractable with DMSO, could be somewhat disturbing. However, it can be assumed that this absence could be due to the difference between the bioavailability of the organic compounds in the organic extract versus that in the total particles. Here, two hypotheses can be drawn. The first one is based on earlier metabolic activation of PAHs adsorbed on the PM, as the particles could represent a vector facilitating the entry of PAHs into the cells. The second one is based on the earlier bioactivation of PAHs in the organic extract, where they were free in the medium and could also penetrate easily and quickly, unlike when the PAHs were adsorbed on the particles and also required more time to be detached from the core particles to enter the biotransformation cycle [4,60,61].

Concerning the effect of the  $\text{OEM}_{0.3}$ , the results showed a significant increase in *AHRR* and *CYP1B1* gene expression after 6 h of exposure to C2, of *CYP1A1* after 24 h of exposure to C1, and of *ARNT*, *CYP1A1*, *CYP1B1*, and *GSTA4* after 24 h of exposure to C2. The results therefore showed a difference in the cellular response to the two organic extracts  $\text{OEM}_{0.3}$  and  $\text{OEM}_{2.5-0.3}$ . This could be explained by an earlier or prolonged metabolic activation of  $\text{OEM}_{0.3}$  vs.  $\text{OEM}_{2.5-0.3}$ , since it contained many more organic compounds (28-fold increases of PAHs in  $\text{PM}_{0.3}$  vs.  $\text{PM}_{2.5-0.3}$ ) [4].

Taken together, these results showed the capacity of organic compounds to induce the AHR metabolic activation pathway in BEAS-2B cells. This induction was only shown by fractions containing organic compounds (whole  $\text{PM}_{2.5-0.3}$  and OEM). Knowing that the concentration of organic compounds is the same in  $\text{PM}_{2.5-0.3}$  and OEM, it was important to consider that the induction of this pathway by each fraction is linked to the bioavailability of the organic compounds in each fraction rather than their concentrations. [4] demonstrated similar findings following exposure of BEAS-2B cells to  $\text{PM}_{2.5-0.3}$  and  $\text{OEM}_{0.3}$ , with more pronounced results observed for particles from Beirut compared to those from Deir AlZahrani. This variance aligns with findings regarding the chemical composition of  $\text{PM}_{2.5-0.3}$  and  $\text{PM}_{0.3}$  collected in Beirut by [4]. Particles collected in Beirut exhibited a higher concentration of toxic organic compounds compared to those sampled in Deir AlZahrani [4].

**Table 4.** Gene expression of *AHR*, *AHRR*, *ARNT*, *CYP1A1*, *CYP1B1*, *EPHX*, and *GSTA4* in BEAS-2B cells exposed for 6 and 24 h to different treatment: PM<sub>2.5-0.3</sub>, organic extract matter (OEM<sub>2.5-0.3</sub>), non-extractable matter fraction (NEM<sub>2.5-0.3</sub>), and organic extractable matter from the quasi-ultrafine fraction (OEM<sub>0.3</sub>) at C1 = 3 and C2 = 12 µg/cm<sup>2</sup> or µg eqPM/cm<sup>2</sup>. These values are depicted as mean values and standard deviations of 3 replicates for controls and 3 replicates for exposed cells (Mann–Whitney U-test vs. controls, \*: *p* < 0.05).

		Ahr		AhRR		ARNT		CYP1A1		CYP1B1		EPHX1		GSTA4	
		6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h
(-) control		1.00 ±0.02	1.00 ±0.02	1.00 ±0.08	1.00 ±0.04	1.00 ±0.02	1.00 ±0.06	1.00 ±0.07	1.00 ±0.03	1.00 ±0.04	1.00 ±0.13	1.00 ±0.12	1.00 ±0.02	1.00 ±0.10	1.00 ±0.14
PM <sub>2.5-0.3</sub>	C1	0.87 ±0.07	1.74 ±0.34	1.6 ±0.33	1.11 ±0.33	0.93 ±0.09	1.37 ±0.29	1.00 ±0.13	3.70 * ±0.94	1.59 ±0.06	2.04 * ±0.36	0.54 ±0.07	1.45 ±0.57	0.68 ±0.11	1.49 ±0.57
	C2	1.45± 0.26	2.69 * ±0.61	2.48 * ±0.64	2.18 * ±0.64	0.87 ±0.04	2.69 * ±0.70	1.57 ±0.14	3.45 * ±0.68	2.35 * ±0.52	4.96 * ±1.35	0.86 ±0.08	3.10 * ±0.51	0.89 ±0.04	0.63 * ±0.14
NEM <sub>2.5-0.3</sub>	C1	0.93 ±0.18	0.96 ±0.03	1.37 ±0.37	1.16 ±0.37	0.93 ±0.09	1.06 ±0.60	1.07 ±0.2	1.22 ±0.24	1.13 ±0.28	1.66 ±0.58	0.90 ±0.25	1.33 ±0.54	1.11 ±0.33	1.30 ±0.34
	C2	0.87 ±0.10	1.57 ±0.55	0.90 ±0.23	1.15 ±0.23	0.90 ±0.05	1.55 ±0.39	0.83 ±0.09	1.87 ±0.34	1.11 ±0.28	1.64 ±0.51	0.84 ±0.01	1.35 ±0.32	0.82 ±0.09	1.22 ±0.22
OEM <sub>2.5-0.3</sub>	C1	0.65 ±0.12	0.94 ±0.45	0.91 ±0.04	0.97 ±0.04	0.88 ±0.06	1.28 ±0.40	1.02 ±0.18	1.00 ±0.12	0.75 ±0.06	1.48 ±0.13	0.78 ±0.02	1.06 ±0.30	0.88 ±0.01	0.93 ±0.14
	C2	0.61 ±0.13	1.22 ±0.59	1.05 ±0.35	1.19 ±0.35	0.99 ±0.03	1.40 ±0.57	0.90 ±0.02	1.63 ±0.36	1.35 ±0.48	1.88 ±0.87	0.95 ±0.26	1.80 ±0.25	0.95 ±0.01	1.20 ±0.59
OEM <sub>0.3</sub>	C1	0.80 ±0.10	1.33 ±0.23	1.26 ±0.62	1.27 ±0.62	0.87 ±0.11	1.38 ±0.23	1.26 ±0.36	0.93 ±0.03	2.84 * ±0.32	0.84 ±0.12	1.33 ±0.38	1.18 ±0.20	0.83 ±0.09	1.53 ±0.28
	C2	1.46 ±0.51	1.70 ±0.33	2.11 * ±0.37	1.70 ±0.37	1.18 ±0.33	0.65 ±0.03	2.11 * ±0.85	1.21 ±0.54	3.91 * ±0.42	3.40 * ±0.98	2.81 * ±0.81	1.81 ±0.57	1.50 ±0.28	2.21 * ±0.80
BaP	25 µM	0.9 ± 0.13	1.50 *±0.1	0.94 ±0.2	2.50 * ±0.93	1.05 ±0.2	1.63 * ±0.10	1.03 ±0.2	7.70 * ±1.64	0.69 ±0.26	6.02 * ±1.57	0.82 ±0.06	1.70 * ±0.50	0.81 ±0.2	1.46 ±0.26
1.50 *±0.1	25 µM	4.18 * ±0.4	2.48 * ±0.72	3.15 * ±1.05	1.64 ±0.11	2.36 * ±0.54	1.34 ±0.60	2.28 * ±0.35	1.86 * ±0.46	2.55 * ±0.67	1.67 * ±0.17	2.44 * ±0.45	1.75 ±0.26	3.04 * ±1	1.29 ±0.2
9-FluO	25 µM	277.7 * ±64	152.1 * ±25	61.3 * ±12.9	30.2 * ±5.23	134.8 * ±39	117.0 * ±27	37.3 * ±3.71	18.1 * ±3.14	46.4 * ±16.1	17.1 * ±3.16	114.5 * ±19	58.1 * ±9.65	202.7 * ±34	94.9 * ±18

Background color, Significant expression (\*: *p* < 0.05).

### 3.3.3. Genotoxicity Induced by Atmospheric PM

PM<sub>2.5</sub> is classified as carcinogenic to humans [62]. Since its composition is extremely complex, it was challenging to identify which fraction from the fine particles could be mainly responsible for the genotoxic effects. In this context, we have studied protein expression of several biomarkers involved in the genotoxicity pathway, such as phosphorylated p-CHK1, p-CHK2, p-H2AX, and p-P53, and total proteins, ATR, MDM2, and P21 following the exposure of BEAS-2B to two concentrations C1 = 3 and C2 = 12 µg/cm<sup>2</sup> for 24 and 72 h (Table 5). The results showed that exposure to the entire PM<sub>2.5-0.3</sub> induced only a significant decrease in phosphorylated H2AX in BEAS-2B exposed for 24 h to C1 and C2. In addition, BEAS-2B exposed to the NEM<sub>2.5-0.3</sub> fraction also reduced its ATR expression when exposed to C1 for 72 h and in phosphorylated H2AX when exposed for 72 h to C2. Conversely, no effect was observed when BEAS-2B cells were exposed to OEM<sub>2.5-0.3</sub>. However, a significant protein expression increase was observed only when the cells were exposed to OEM<sub>0.3</sub>. Results showed a significant increase in ATR and CHK1 by BEAS-2B exposed to C2 for 72h; of P21, P53, and MDM2 by BEAS-2B exposed to C2 for 24 and 72h; and for H2AX by BEAS-2B exposed to C2 for 72 h. These results showed that the extractable organic fraction of PM<sub>0.3</sub>, the most concentrated in organic compounds, was the only one capable of inducing the expression of proteins related to cell cycle arrest and DNA damage under the studied conditions. On the other hand, the decrease in these biomarkers in the case of exposure to PM<sub>2.5-0.3</sub> and its two fractions could be attributed to a greater repair activity of the cells. The same results concerning the decrease in protein expression have been recently supported by other authors [4,9,59,63]. Keeping DNA stable in eukaryotic cells involves a network of processes such as copying DNA, solving DNA issues, and managing cell cycles. The ATM and ATR kinases were key in overseeing this network when DNA was damaged. Both in vivo and in vitro investigations indicated that ATM and ATR exhibited distinct

DNA damage-specific responses. ATM predominantly responded to double-stranded DNA breaks (DSBs), while ATR’s activation encompassed a wider range of DNA damage, including DSBs and various lesions that disrupt DNA replication. These results showed a significant increase in ATR expression after exposure to OEM<sub>0.3</sub>, and subsequently CHK1, P21, P53, MDM2, and H2AX expression, involved in cell cycle arrest, apoptosis, and DNA damage repair [4,64,65]. [4] showed also that the quasi-ultrafine particles were the most genotoxic due to the predominance of organic compounds such as PAHs in a fraction of this size [4].

**Table 5.** Genotoxicity: protein expression of ataxia telangiectasia and Rad3-related protein (ATR), phosphorylated checkpoint kinase 1 (p-CHK-1), phosphorylated checkpoint kinase 2 (p-CHK-2), phosphorylated H2A histone family member X (p-H2AX), total proteins of mouse double minute 2 homolog (MDM2), total protein 21 (P21), and phosphorylated protein 53 (p-P53), in BEAS-2B cells exposed for 6 and 24 h to different treatment: PM<sub>2.5-0.3</sub>, organic extractable matter (OEM<sub>2.5-0.3</sub>), non-extractable matter fraction (NEM<sub>2.5-0.3</sub>), and organic extractable matter from the quasi-ultrafine fraction (OEM<sub>0.3</sub>) at C1 = 3 and C2 = 12 µg/cm<sup>2</sup> or µg eqPM/cm<sup>2</sup> and positive controls (BaP, 1-NPyr and 9-FluO). These values are depicted as mean values and standard deviations of 3 replicates for controls and 3 for exposed cells (Mann–Whitney U test vs. controls, \*: *p* < 0.05).

		ATR		CHK1		CHK2		P21		P53		MDM2		H2AX	
		24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
(-) CONTROL		1.00 ±0.07	1.00 ±0.14	1.00 ±0.08	1.00 ±0.15	1.00 ±0.09	1.00 ±0.12	1.00 ±0.10	1.00 ±0.24	1.00 ±0.17	1.00 ±0.20	1.00 ±0.11	1.00 ±0.12	1.00 ±0.12	1.00 ±0.13
PM <sub>2.5-0.3</sub>	C1	0.92 ±0.06	1.19 ±0.04	0.87 ±0.39	1.05 ±0.06	0.87 ±0.03	1.11 ±0.02	0.69 ±0.001	1.08 ±0.04	0.89 ±0.06	0.96 ±0.09	0.96 ±0.88	1.18 ±0.20	0.68 * ±0.12	0.81 ±0.03
	C2	0.94 ±0.14	1.02 ±0.03	0.86 ±0.13	1.15 ±0.37	0.85 ±0.14	1.18 ±0.4	0.50 ±0.09	1.27 ±0.65	1.08 ±0.13	1.06 ±0.35	1.04 ±0.16	1.35 ±0.37	0.63 * ±0.14	1.10 ±0.48
NEM <sub>2.5-0.3</sub>	C1	1.22 ±0.2	0.76 * ±0.05	1.13 ±0.26	1.03 ±0.11	1.22 ±0.27	1.06 ±0.08	0.87 ±0.18	1.09 ±0.33	1.35 ±0.3	0.58 * ±0.11	1.17 ±0.26	0.90 ±0.10	1.11 ±0.23	0.47 * ±0.45
	C2	1.03 ±0.16	0.81 ±0.16	0.96 ±0.26	1.08 ±0.21	0.99 ±0.27	1.05 ±0.21	0.56 ±0.16	0.70 ±0.20	1.27 ±0.43	0.46 ±0.26 *	1.08 ±0.22	0.99 ±0.24	0.99 ±0.22	0.46 * ±0.12
OEM <sub>2.5-0.3</sub>	C1	1.08 ±0.21	1.01 ±0.20	1.12 ±0.34	1.41 ±0.32	1.16 ±0.37	1.52 ±0.35	1.09 ±0.2	0.88 ±0.20	1.38 ±0.63	1.15 ±0.40	1.13 ±0.30	1.11 ±0.21	0.92 ±0.24	0.82 ±0.38
	C2	1.09 ±0.15	0.87 ±0.12	1.17 ±0.18	1.06 ±0.10	0.98 ±0.11	1.03 ±0.08	1.31 ±0.12	0.61 * ±0.04	1.34 ±0.19	1.09 ±0.09	0.83 ±0.72	0.90 ±0.18	1.00 ±0.09	0.49 ±0.05
OEM <sub>0.3</sub>	C1	0.89 ±0.08	1.09 ±0.19	0.86 ±0.14	1.35 ±0.18	0.92 ±0.10	1.38 ±0.23	1.00 ±0.12	1.08 ±0.10	1.10 ±0.08	1.02 ±0.17	0.94 ±0.11	0.96 ±0.11	0.86 ±0.16	0.49 ±0.14
	C2	0.99 ±0.07	3.41 * ±0.60	1.15 ±0.14	0.78 * ±0.09	1.20 ±0.12	0.65 ±0.03	2.45 * ±0.26	20.21 * ±4.5	1.78 * ±0.39	9.10 * ±3.7	1.26 * ±0.09	3.45 * ±1.22	0.75 ±0.10	1.03 ±0.12 *
BAP	25 µM	1 ±0.224	1.1 ±0.15	1.23 ±0.2	1.56 * ±0.16	1.34 * ±0.19	1.65 * ±0.17	1.42 * ±0.08	0.94 ±0.38	1.24 ±0.20	1.03 ±0.11	0.88 ±0.15	1.40 ±0.20	4.29 * ±0.47	3.51 * ±0.56
1-Npyr	25 µM	1.06 ±0.01	0.93 ±0.33	1.16 ±0.08	1.24 ±0.21	3.63 * ±0.29	1.31 ±0.27	1.19 ±0.24	0.65 ±0.15	1.15 ±0.03	0.80 ±0.193	0.95 ±0.07	0.88 ±0.26	1.71 * ±0.09	1.42 * ±0.17
9-FluO	25 µM	1 ±0.209	0.88 ±0.14	1.03 ±0.21	1.37 ±0.32	1.04 ±0.22	1.46 ±0.37	0.83 ±0.27	0.46 ±0.11	1.06 ±0.25	0.79 ±0.15	1.17 ±0.26	0.94 ±0.25	1.12 ±0.29	1.51 ±0.63

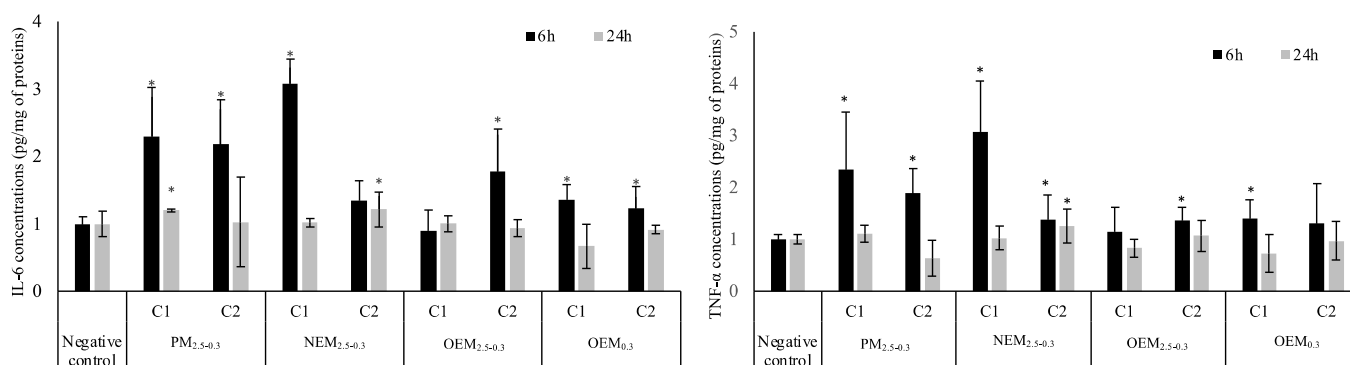
Background color, Significant expression (\*: *p* < 0.05).

### 3.3.4. Inflammation: TNF-α and IL-6 Secretion

The inhalation and deposition of PM<sub>2.5</sub> in the lungs trigger oxidative stress and inflammatory responses that provokes a wide range of cytokine secretion [66–68]. As PM represents a mixture of organic and inorganic components, an evaluation of the capacity of each fraction (PM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub>, and OEM<sub>0.3</sub>) to stimulate the production of inflammatory cytokines was carried out. BEAS-2B cells were exposed to two concentrations C1 = 3 µg/cm<sup>2</sup> or µg eqPM/cm<sup>2</sup> and C2 = 12 µg/cm<sup>2</sup> or µg eqPM/cm<sup>2</sup> of each suspension, for 6 and 24 h (Figure 4). Results showed the capacity of all fractions to stimulate the production of two cytokines, TNF-α and IL-6, at 6 h rather than at 24 h. The organic extracts (OEM) containing only organic compounds, as well as the NEM containing the inorganic fraction, both provoke the production of IL-6 and TNF-α by BEAS-2B. Numerous experimental studies conducted both in vitro and in vivo have highlighted the detrimental



pulmonary health impacts caused by PM<sub>2.5</sub> derived from air pollution. These effects predominantly arose from PM<sub>2.5</sub> capacity to instigate essential mechanisms of toxicity, notably oxidative stress-induced inflammation in the airways [4,10,69,70]. Exposure of BEAS-2B, NHBE, and particularly COPD-DHBE to PM<sub>2.5</sub> showed a cytosolic ROS overproduction, inducing oxidative damage and activating oxygen-sensitive NRF2 and NF-κB signaling pathways [69]. Accordingly, exposure to a mixture of PAHs and diesel exhaust particles induced an increase in gene and protein expressions of IL-6 and TNF-α [68,71,72]. In addition, a previous study demonstrated that exposure to certain metals (Ag, Al, Ba, K, Li, Ni, Sn, and V) was significantly associated with increased inflammatory markers such as IL-6 as well as oxidative stress markers [73]. Taken together, the already published studies together with this one show that all the components of PM can participate in the inflammatory process [66,74,75].



**Figure 4.** Inflammation response through IL-6 and TNF-α quantification in BEAS-2B cells exposed for 6 and 24 h to different treatment: PM<sub>2.5-0.3</sub>, organic extractable matter (OEM<sub>2.5-0.3</sub>), non-extractable matter fraction (NEM<sub>2.5-0.3</sub>), and organic extractable matter from the quasi-ultrafine fraction (OEM<sub>0.3</sub>) at C1 = 3 and C2 = 12 μg/cm<sup>2</sup> or μg eqPM/cm<sup>2</sup>. The results are described by their means and standard deviations (8 replicates for controls and 8 replicates for exposed cells (Mann–Whitney U-test vs. controls (non-exposed cells); \* = *p* < 0.05). Variations were normalized and compared to the negative control. \*: *p* < 0.05 (Mann–Whitney test).

#### 4. Conclusions

The main objectives of this study were to compare the physico-chemical properties and toxicity of fine and quasi-ultrafine particles and to target the fraction within PM responsible for the known toxic effects.

To achieve these objectives, PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> were sampled in Southern Lebanon's Deir AlZahrani, and an extraction of the different parts of PM<sub>2.5-0.3</sub> was carried out while obtaining native PM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub>, and NEM<sub>2.5-0.3</sub>. The chemical analysis of both PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> showed the presence of elements related to the resuspension of soil dust (Ca, Al, Fe, Mg, and Ti) in addition to other elements, related to traffic emissions (Zn and Cu). In addition, the concentration of organic compounds such as PAHs, O-, and N-PAHs was higher in PM<sub>0.3</sub> than PM<sub>2.5-0.3</sub>, showing the influence of anthropogenic activities and combustion on the emission of ultrafine particles.

Toxicological studies showed that the induction of the metabolic activation of PAHs was delayed by PM<sub>2.5-0.3</sub> rather than OEM<sub>2.5-0.3</sub>. This delay is probably related to the bioavailability of organic compounds. Furthermore, only OEM<sub>0.3</sub> was able to induce an increase in the protein expression of ATR, P21, P53, MDM2, and H2AX, all implicated in the genotoxic pathway. This could be related to the organic composition of PM<sub>0.3</sub>, which is more concentrated in PAHs and derivatives, compared to PM<sub>2.5-0.3</sub>.

These results indicate that UFPs seem to be of great concern to human health because they are concentrated in organic compounds and much more toxic than fine particles. These PM<sub>2.5-0.3</sub> and their toxicity must be taken with caution into consideration when setting emission limits for concentrations of particles.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available as it is a part of a thesis study of a student.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

1-NPyr	1-nitropyrene
9-FluO	9-Fluorenone
Ace	Acenaphthalene
Acy	Acenaphthylene
Ant	Anthracene
ATP	Adenosine triphosphate
BaA	Benz[a]anthracene
BaP	Benzo[a]pyrene
BbF	Benzo[b]fluoranthene
BghiP	Benzo[g,h,i]perylene
BkF	Benzo[k]fluoranthene
Chr	Chrysene
DahA	Dibenz[a,h]anthracene
Fla	Fluoranthene
Flu	Fluorene
InPy	Indeno[1,2,3-c,d]pyrene
LDH	Lactate dehydrogenase
N-PAHs	nitroated-PAHs
Nap	Naphthalene
OEM	organic extractable matter
NEM	non-extractable matter
O-PAHs	oxygenated-PAHs
PAHs	polycyclic aromatic hydrocarbons

Phe Phenanthrene  
 PM<sub>2.5-0.3</sub> particles with an equivalent aerodynamic diameter between 0.3 and 2.5 mm  
 PM<sub>0.3</sub> particles with an equivalent aerodynamic diameter below 0.3 mm (quasi-ultrafine particles)  
 Pyr Pyrene  
 ROS reactive oxygen species;

**Appendix A**

**Table A1.** A summary of the different physico-chemical analyses.

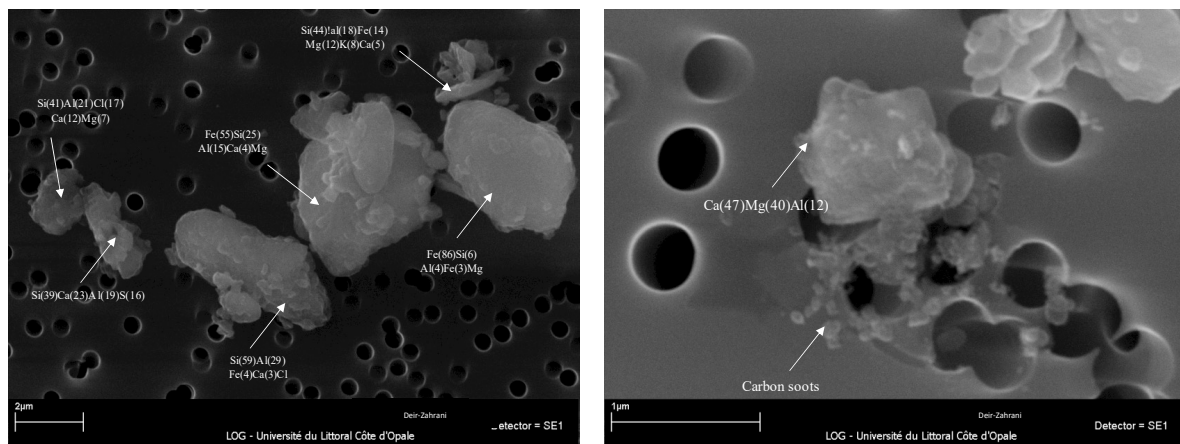
Physico-Chemical Analysis	Techniques	Analyzed Samples		
		PM <sub>2.5-0.3</sub>	PM <sub>0.3</sub>	
PM morphology and individual particle composition	Scanning electron microscopy coupled with energy-dispersive X-ray (SEM-EDX)	✓	-	
Cristalline phases	X-ray diffraction	✓	-	
Elements and water-soluble ions	Inductively coupled plasma atomic emission spectroscopy and ionic chromatography	✓	✓	
Organic compounds	PAHs	Gas chromatography–mass spectrometry (GC–MS)	✓	✓
	N- and O-PAHs	High-resolution gas chromatography and high-resolution mass spectrometry	✓	✓
	PCDD, PCDF, and PCB	High-resolution gas chromatography and high-resolution mass spectrometry	✓	✓
	n-alkanes	Gas chromatography–mass spectrometry (GC–MS)	✓	✓

✓: Applied to.

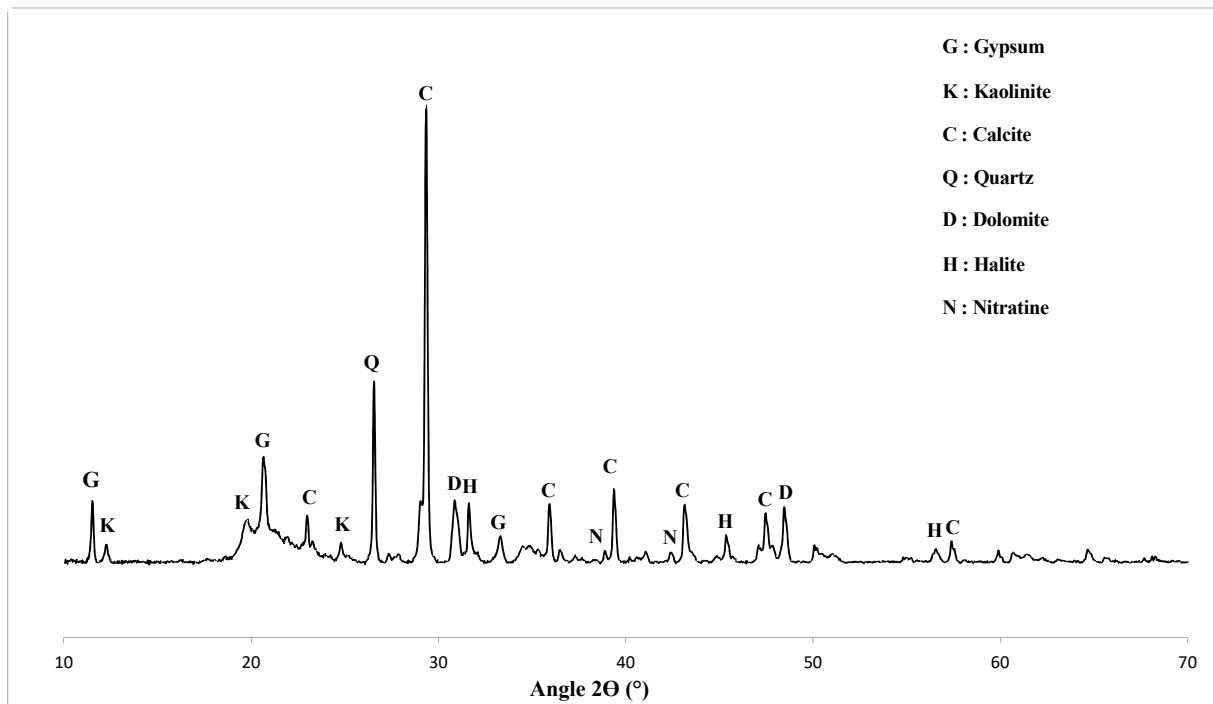
**Table A2.** PAHs diagnostic ratios calculated for PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> collected at Deir Al Zahrani and compared to the bibliography to determine their possible emission sources.

PAHs Diagnosis Ratio	PM <sub>2.5-0.3</sub>	PM <sub>0.3</sub>	Characteristic Ratio Values and Sources		
InPy/(InPy+B(g,h,i)Pe) <sup>(a)</sup>	0.59	0.50	0.18: Cars	0.62: Wood burning	0.35–0.70: Diesel burning
Fla/(Fla+Pyr) <sup>(a)</sup>	0.47	0.45	<0.5: Gasoline	>0.5: Diesel	
B[a]P/(B[a]P+Chry) <sup>(b)</sup>	0.29	0.38	>0.35: Vehicular emission	0.2–0.35: Coal combustion	
B[b]F/B[k]F <sup>(a)</sup>	4.01	2.89	>0.5: Diesel		
B[a]P/B[g,h,i]Pe <sup>(a)</sup>	0.53	0.47	0.5–0.6: Traffic emission	>1.25: Brown coal or lignite	
Anth/(Anth+Phe) <sup>(b)</sup>	0.09	0.30	>0.1: Pyrogenic	<0.1: Petrogenic	
InPy/B(g,h,i)Pe <sup>(a)</sup>	1.42	1.00	<0.4: Gasoline	~1: Diesel	
Fla/(Fla+Pyr) <sup>(b)</sup>	0.48	0.46	<0.4: Petrogenic	0.4–0.5: Fossil fuel combustion	>0.5: Grass wood coal combustion
Fla/Pyr <sup>(b)</sup>	0.94	0.84	<0.6: Non-traffic emission	>0.6: Traffic emission	
CPAHs/TPAHs <sup>(a)</sup>	0.92	0.95	≈1 (Combustion)		

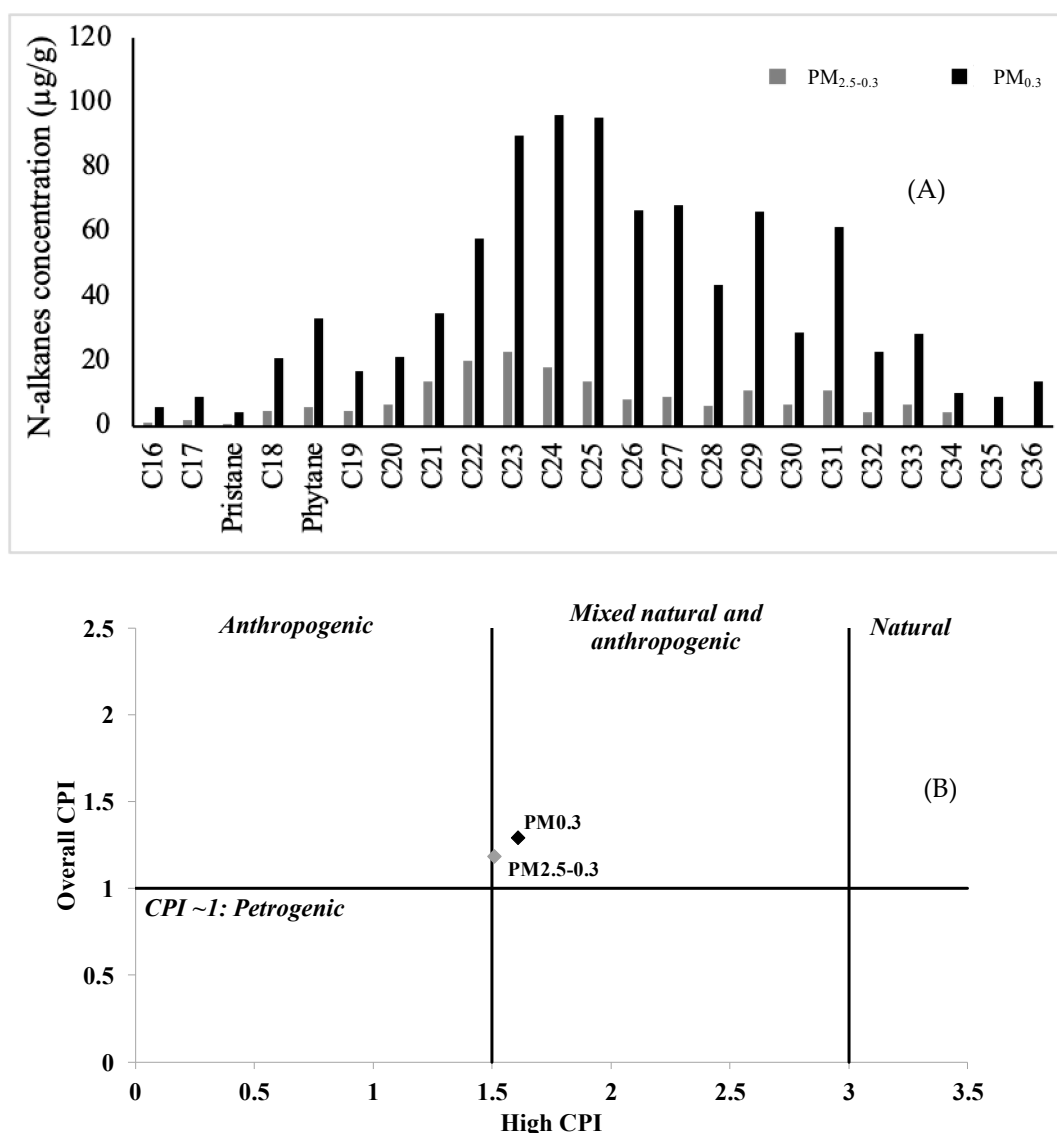
(a): [33]; (b): [34].



**Figure A1.** Particles (PM<sub>2.5-0.3</sub>) observed by scanning electron microscopy coupled with energy-dispersive X-ray (**left**) and individual particle composition (**right**) (mass % into brackets). Si: silicium. Al: aluminum. Fe: iron. S: sulfur. Ca: calcium. Mg: magnesium. K: potassium. Cl: chlorine.



**Figure A2.** X-ray diffraction patterns of PM<sub>2.5</sub> collected at Deir AlZahrani.



**Figure A3.** N-alkane concentrations ( $\mu\text{g/g}$ ) determined in  $\text{PM}_{2.5-0.3}$  and in  $\text{PM}_{0.3}$  (A), and identification of n-alkane sources using overall CPI and high CPI in  $\text{PM}_{2.5-0.3}$  and  $\text{PM}_{0.3}$  (B).

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