



# In vitro cytotoxicity effects of polycyclic aromatic hydrocarbons (PAHs) associated with PM<sub>10</sub> during the Middle Eastern Dust (MED) storms in Ahvaz

Abolfazl Naimabadi<sup>1</sup> · Mohammad Shirmardi<sup>2,3,4</sup> · Gholamreza Goudarzi<sup>5,6</sup> · Ata Ghadiri<sup>7,8</sup> · Vahide Oskoei<sup>9</sup> · Ali Akbar Mohammadi<sup>1</sup> · Gea Oliveri Conti<sup>10</sup> · Margherita Ferrante<sup>10</sup>

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## Abstract

This study aimed to investigate the cytotoxic effects of PAHs extracts by PM<sub>10</sub> from Ahvaz (Iran) during normal and dusty days from December 2012 to June 2015, using the A549 cell line (adenocarcinoma human alveolar basal epithelial cells) and lactase dehydrogenase (LDH) ELISA test. Also, the source of PM<sub>10</sub> will be evaluated using the HYSPLIT Air Q model application. PAHs concentrations associated with PM<sub>10</sub> were evaluated by chromatography-mass spectrometry (GC–MS). Based on the obtained results, PM<sub>10</sub> on both dusty and normal days caused cytotoxicity in the control group cells. According to *t*-test findings, the value of cytotoxicity in normal days was different from dust event days. Similarly, the tests of the normal days illustrated more extreme-level cytotoxicity than dust storm days at the treated dosage. Higher cytotoxicity was observed by increasing the incubation time of cells, which was supported by the ANOVA test. There is a significant relationship between different incubation times based on the ANOVA. Due to their ring structure, the PAHs compounds can exacerbate the toxicity in the samples at longer times.

**Keywords** Middle Eastern Dust (MED) · A549 cell line · Cytotoxicity · PAHs · LDH · HYSPLIT model

## Introduction

Air pollution is rapidly becoming a problem of great concern for the environment and public health across the world; exposure to particulate matter (PM) is considered to be the significant cause of mortality and morbidity, which was

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✉ Ali Akbar Mohammadi  
mohammadi.eng73@gmail.com

<sup>1</sup> Department of Environmental Health Engineering, Neyshabur University of Medical Sciences, Neyshabur, Iran

<sup>2</sup> Social Determinants of Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

<sup>3</sup> Environmental Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

<sup>4</sup> Department of Environmental Health Engineering, School of Public Health, Babol University of Medical Sciences, Babol, Iran

<sup>5</sup> Environmental Technologies Research Center (ETRC), Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>6</sup> Department of Environmental Health Engineering, Health Faculty, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>7</sup> Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>8</sup> Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>9</sup> Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

<sup>10</sup> Environmental and Food Hygiene Laboratory (LIAA) of Department of Medical, Surgical Sciences and Advanced Technologies “G.F. Ingrassia”, University of Catania, Catania, Italy

culpable for the people's death every year in the range of 4.2 to 8.9 million deaths (Kermani et al. 2021; Nabizadeh et al. 2018; Organization 2013). The results of the epidemiological studies show that higher concentrations of airborne PM in the short and long term exposure could lead to detrimental health effects, including a raised chance of lung cancer and leukemia, asthma (Organization 2013), ischaemic heart disease, thrombosis, and strokes (Santo Signorelli et al. 2019). At the same time, there is a dramatic increase in chronic obstructive pulmonary disease (COPD) (Organization 2013), type 2 diabetes mellitus (Bowe et al. 2018), dementia, and thyroid cancer (Ferrante & Conti 2017, Fiore et al. 2019). PM is ranked by its mean aerodynamic diameter, consistently of  $< 10 \mu\text{m}$  (PM<sub>10</sub>),  $< 2.5 \mu\text{m}$  (PM<sub>2.5</sub>), and  $< 0.1 \mu\text{m}$  (PM<sub>0.1</sub>). PM<sub>2.5</sub> and PM<sub>0.1</sub> are attributed to fine and ultrafine PM, respectively, often present at high levels in an urban area. PM at the diameters less than  $10 \mu\text{m}$  are capable of penetrating the respiratory tract; in contrast, respirable particles (Fine PM) can get deep into the lung and potentially to alveoli. While ultrafine PM can cause severe human health outcomes by easily crossing the air-blood barrier and entering into the blood circulation (Santo Signorelli et al. 2019), their harmfulness is also increased by their "Trojan horse" role. In fact, in PM characterizations studies, several authors found that various pollutants coat PM in its surface (Kim et al. 2015; Santo Signorelli et al. 2019). According to the survey, some of the organic matter absorbed on PM is known to be carcinogenic. Also, soluble transition metals such as Cu and Cr, as well as organic compositions such as PAHs that are absorbed in the surface of the particles can lead to the creation of reactive oxygen species (ROS), including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide (O<sub>2</sub><sup>-</sup>), and radical hydroxyl (OH<sup>-</sup>) (Conte et al. 2016; Ferrante et al. 2018).

Polycyclic aromatic hydrocarbons (PAHs) are typical hydrocarbons present in automotive and industrial fuel and their emissions, including PM<sub>10</sub> and PM<sub>2.5</sub>. These compounds are vital due to their high persistence in marine environments and their capability of bioaccumulation (Conte et al. 2016; Dehghani et al. 2022; Ferrante et al. 2018) and toxicity in aquatic and terrestrial organisms (Conti et al. 2012; Copat et al. 2012). The PAHs are the groups of primary and stable compounds that have two or more benzene rings bonded in the linear arrangements and cluster and angular ones. For their high toxicity, 16 PAHs have been included in the category of primary pollutants by USEPA (US Environmental Protection Agency) (Ali et al. 2016; Chirino et al. 2015; Farmer et al. 2003; Lıbalova et al. 2014). Some evidence reported mutagenic and carcinogenic effects of some PAHs, including Benzo[a]Pyrene (Hassanvand et al. 2015; Soltani et al. 2015).

The main target organs for PAHs include the nervous system, liver, kidneys, skin, lungs, and mucous membranes,

airways, eyes, and male fertility (Farmer et al. 2003). The PAHs can reach all lipid-containing tissues. These compounds have a high tendency to be stored in the kidney and liver, but small amounts of them are also stored in the spleen and adrenal gland. With different types and severity, the PAHs possess short-term and long-term impacts on health. PAHs induce cellular oxidative stress (OxS). OxS is an imbalance between reactive species and antioxidant defenses. The production of these free radical species leads to cardiovascular diseases and oxidative stress effects in the lung system and increases oxidative DNA damage (Conte et al. 2016; Ferrante et al. 2018).

Currently, increasing dust storm events due to their continued atmospheric persistence and carrying a high level of PM<sub>10</sub> in a large area can cause the exert adverse impacts on atmospheric pollution and human well-being (Khaniabadi et al. 2017; Zarei et al. 2019). Dust storms in Middle Eastern countries like Iran have a higher concentration of PM, so their diverse effects on inhabitants are considered to be a common phenomenon every year (Atafar et al. 2019).

There has been no study on the potential genotoxicity of PAHs in the Ahavz air on human lung epithelial cells (A549). Hence, it is necessary for us to study it. Therefore, this study aimed to investigate the cytotoxic effects of PAHs extracts by PM<sub>10</sub> from Ahvaz (Iran) during normal and dusty days using the A549 cell line (adenocarcinoma human alveolar basal epithelial cells) and LDH ELISA test. Also, the source of PM<sub>10</sub> will be evaluated using the HYSPLIT Air Q model application.

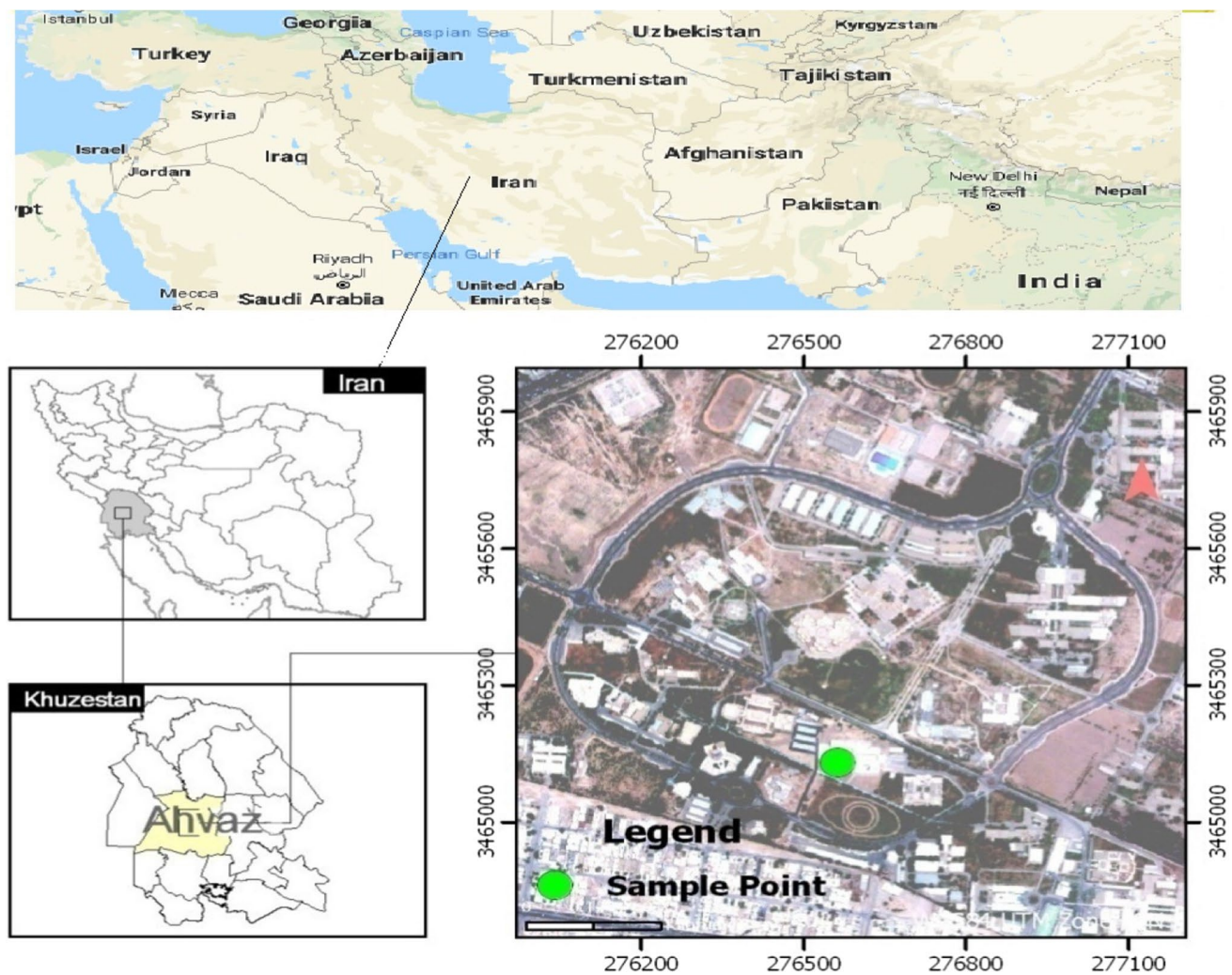
## Materials and methods

### Sampling location

Ahvaz is the capital of Khuzestan Province, Iran (Fig. 1). It is a famous industrial city located in arid southwestern areas of Iran (31° 32'N and 48° 68'E). Large industrials such as South Oil wealthy Zones Company, official and industrial facilities, and National Iranian Drilling Company have made Ahvaz a large industrial center of Iran. The city possesses a surface area of about 220 km<sup>2</sup> that has a population of about 1.3 million people (Farsani et al. 2018; Karimi et al. 2019; Marzouni et al. 2017; Shahsavani et al. 2012). This region is distinguished by little plant life, surface winds with reliable power, and higher temperatures and humidity. This city has been facing severe Middle Eastern Dust storms during the last decade.

### PM sampling

Urban background concentrations of PM<sub>10</sub> over both dust events and normal days are sampled at the height of 10 m on



**Fig. 1** Study area Location and the measurement station

the higher altitudes of Health School buildings. The high-volume air sampler (Tisch Company) was placed at a position far from any point sources of pollution and significant obstacles to reducing the effect of any potential contributor. Samplings were carried out using quartz fiber filters ( $\varnothing$  90 mm) and a pump with a 1.7 m<sup>3</sup>/min airflow rate within a 24-h period from December 2012 to June 2014. During this period, 30 samples were obtained (15 samples for both situations, normal and dusty days). The procedure used to prepare each filter before and after sampling has been described previously (Naimabadi et al. 2016; Zou et al. 2017).

### Dust storm categories and source identification

Dust storms days were classified according to the principles provided by Hoffma, into five categories. “Dusty air” with hazy visibility and average PM<sub>10</sub> per hour ranging from 50 to 200  $\mu\text{g m}^{-3}$  was the first category. In the class of “Serious

strong D.S. (DS<sub>4</sub>),” visibility and average PM<sub>10</sub> concentration had the highest severity with less than 50 m and more than 5000  $\mu\text{g m}^{-3}$  while “Strong dust storm (DS<sub>3</sub>)” was in the next level with less than 200 m and 2000–5000  $\mu\text{g m}^{-3}$ , respectively. The third category of data, which was “Dust storm (DS<sub>2</sub>),” had visibility less than 1000 m and PM<sub>10</sub> concentration ranging from 500 to 2000. Furthermore, for the wind speed category, “Serious strong D.S. (DS<sub>4</sub>)” was the first while “Dust storm (DS<sub>2</sub>)” was the last: the former with more than 25 m s<sup>-1</sup> and the latter with more than 17 m s<sup>-1</sup>. The field of view, wind speed, and particle concentration are essential parameters. In addition to field observations, the level of PM<sub>10</sub> particles was used as a critical parameter to divide the sampling days into normal days and dusty days. A PM<sub>10</sub> in higher concentrations of 200  $\mu\text{g m}^{-3}$  was acknowledged as a dust event day, and the level below this limit was considered as normal days (Asl et al. 2018; Khaniabadi et al. 2017).

To identify the source of dust, the National Oceanic and Atmospheric Administration of the United States carried out various surveys on the movement, dispersion, and change of the dust storm across the world, resulting in an Air Qmodel called Hybrid Single-particle Lagrangian Integrated Trajectory or *HYSPLIT* model.

This model is a dual distribution and distribution model for calculating the trajectory of dust motion, dispersion, and simulation using PUFF and particle approaches. This model calculates the path and concentration of pollutants using the minimum meteorological parameters. The computational model is an integration from the perspectives of Eulerian and Lagrangian models. The backward trajectory model at different altitudes can determine the source of dust entering each area according to the testing period. In this inquiry, one of the sampling periods was evaluated by the *HYSPLIT* model, and the results of this model show the source of dust by using meteorological knowledge. As illustrated in Fig. 2, the source of dust in this period is the internal Iraqi territory and the evaporating sediments of the Tigris and Euphrates rivers (Xiao et al. 2015).

### Particles extraction and PAHs analysis

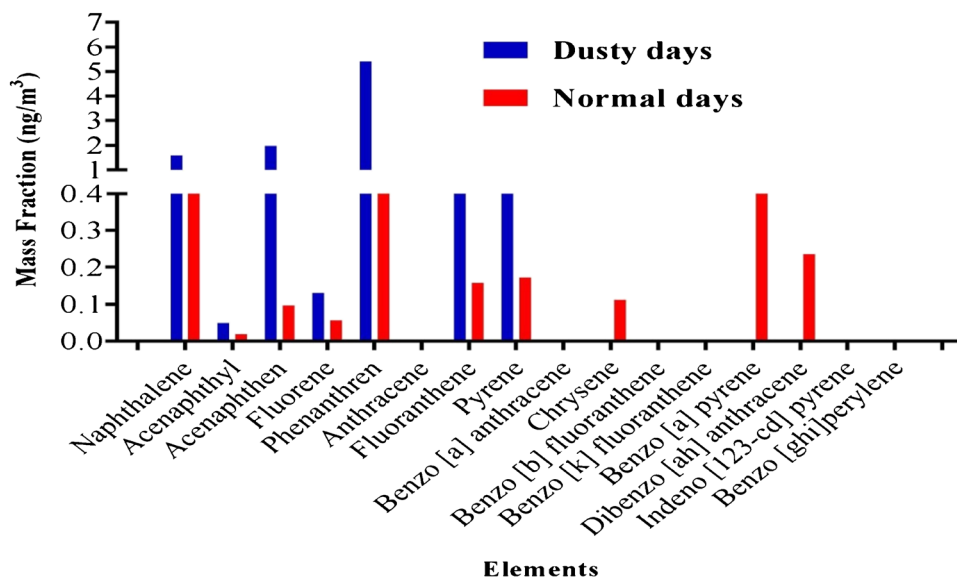
For PAHs extraction, we adopted two separate steps; firstly, we cut one-eighth of the quartz filter into the small pieces; finally, we mixed it in an Erlenmeyer flask containing 10 mL of dichloromethane and acetone (1:3) HPLC grade. Twenty minutes after extraction time to ultrasonic waves, we used a shaker for about an hour to stir the resulting mixture and then passed it through the 0.45  $\mu\text{m}$  syringe filter. Then it was dried under nitrogen gas flow, and 2 mL of this solution covered by aluminum foil was carried to the laboratory, and the measurement of 16 lists of PAHs (naphthalene, Acenaphthyl,

Acenaphthen, Fluorene, Phenanthren, Anthracene, Fluoranthene, Pyrene, Benzo [a] anthracene, Chrysene, Benzo [b] fluoranthene, Benzo [k] fluoranthene, Benzo [a] pyrene, Dibenzo [ah] anthracene, Indeno [123-cd] pyrene, Benzo [ghi]perylene) was carried out using a GC-MS (Agilent, USA) instrument. The stationary phase was the Capillary, HP-5MS (30 m  $\times$  0.25 mm i.d.). The inlet temperature was 250  $^{\circ}\text{C}$ , and Helium as the carrier gas controlled at the stable velocity (1 mL/min) (Meng & Zhang 2007, Schilirò et al. 2015). Every needed chemical was obtained from Sigma, St. Louis, MO, USA. The attained organic solvent-extractable suspensions were rotary dried to measure the organic compound's impacts on the cells and then mixed with 1 mL of dimethyl sulfoxide (DMSO). DMSO is used as a solvent for organic matter in many research works so that it may have some toxicity, but because this organic substance was used as a negative control in the LDH assay kit, this effect was eliminated, and the created cytotoxicity was only related to PAH compounds. Finally, we need to store the resulting solution in the laboratory freezer at the degree of  $-70^{\circ}\text{C}$  till the test time (Alessandria et al. 2014, Meng & Zhang 2007, Schilirò et al. 2015).

### Cell line

A549 cells are adenocarcinomic human alveolar basal epithelial cells in nature; these cells are squamous and have the responsibility of distributing substances, including electrolytes and water, throughout the pulmonary alveoli. They grow in the in vitro environment as a stick and are positioned in the body as a layer. They are creatine positive cells and are evident with the immune peroxidase staining. Furthermore, these cells can synthesize lecithin and contain high levels of unsaturated fatty acids. In this analysis, the A549 cells

**Fig. 2** The concentration of PAHs associated with PM10 samples in Ahvaz



were first obtained from the Cell Bank of the Pasteur Institute of Iran. The cells were then cultured in an RPMI 1640 medium containing 10% FBS, 1% penicillin/streptomycin, and 2 mM of L-glutamine in a humid atmosphere containing 95% air, 5% CO<sub>2</sub> at 37 °C. In the next step, we utilized a 96-well plate to incubate for 24 h to stick the cells to the bottom of the dish. After 24 h, the cells were observed under the microscope with higher power more than the human eye. When the cells were found to be suitable for testing, the Supernatant would be discarded (Alessandria et al. 2014; Goudarzi et al. 2018; Schilirò et al. 2015).

### Treatment of the A549 cells with solvent-extractable organic

The first solution was first thawed and brought to room temperature and then thoroughly mixed by ultrasound to treat the cell; PM<sub>10</sub> extract samples were diluted at a ratio of 1:10. After preparing the cells in the previously described 96-well plate, 100 µl of fresh culture medium and 100 µl of the sample after passing through a 25-µm filter were added to each well aseptically. The incubation periods were 12, 24, and 48 h. Finally, the LDH test was performed. It must be emphasized that the separate 96-well dish was considered for every incubation period. The tests were carried out in triplicates to increase the accuracy of the work (Meng & Zhang 2007, MohseniBandpi et al. 2017).

### Lactate Dehydrogenase enzyme (LDH) assay

LDH assay was utilized to measure the effect of particle extracts on A549 cells. The amount of LDH release can be used as a marker of cytotoxicity and cell viability. The LDH assay is a kind of colorimetric method to the reduction and breakdown of the yellow crystal of tetrazolium by using Succinate dehydrogenase (SDH) and the production of insoluble blue crystals by utilizing the LDH Cytotoxicity Detection Kit (Alessandria et al. 2014; Corsini et al. 2013) of Takara Bio (Takara, Japan). Briefly, after the cell exposure with PM<sub>10</sub> samples extracts, we incubated treated cells at times of 12, 24, and 48 h. The Supernatant of each well was extracted by centrifugation at the speed of 250 rpm for 10 min (sigma 3–161) (benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene (B[a] P), dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene). Then, 100 µl of Supernatant and C solution at the amount of 100 µl were mixed and then incubated for 30 min by keeping at room temperature. Completely, the optical density (OD) of the samples was read by an ELISA reader at a wavelength of 490 nm, and the cytotoxicity percentage was estimated according to Eq. 1:

$$\text{Cytotoxicity}(\%) = \frac{\text{exp.value} - \text{lowcontrol}}{\text{highcontrol} - \text{lowcontrol}} \times 100 \quad (1)$$

In this equation, we have three controls as follows:

- Positive control: Rather than particle suspension, the Cells were treated with Triton X-100, illustrating the highest amount of LDH released from the cell (A549 cells + 100 µl of culture medium + 100 µl of Triton X-100);
- Negative control: Untreated cells (A549 cells + 200 µl of culture medium), and
- Background control: 200 µl of cell-free culture medium (Alessandria et al. 2014; Hoffmann et al. 2008; Schilirò et al. 2010).

### Statistical analysis

The average and standard deviation of the measured values were estimated. Independent *t*-test and ANOVA were used to compare groups ( $p < 0.05$ ). All the statistical analyses were performed using the SPSS 17 software.

## Results and discussions

### PM<sub>10</sub> concentrations, its classification, and trajectories

The levels of PM<sub>10</sub> particles at various climatic conditions are shown in Table 1; it is clear that PM<sub>10</sub> particles on dusty days have a higher concentration than normal days. The mean PM<sub>10</sub> concentration was 1,421 µg m<sup>-3</sup> on dusty days, and the highest value reported on these days was 3649 µg m<sup>-3</sup> (Table 1) and, in all cases is exceeded the WHO limit (50 µg m<sup>-3</sup>). Also, more dust storms that occurred during the study period can be categorized as DS<sub>2</sub> and DS<sub>3</sub>. By and large, some severe storms have been reported in previous studies in the city of Ahvaz. The immense deserts located south of the town are responsible and the main source of the particles and dust storms in the area. Based on the vertical velocity method utilizing backward trajectory modeling, the aerosols produced in Ahvaz originate from areas such as Iraq, Saudi Arabia, and Kuwait (Zarasvandi et al. 2011). Other places were acknowledged

**Table 1** The statistical review of the PM<sub>10</sub> value (µg/m<sup>3</sup>) in the ambient air, Ahvaz city

	Average	Min	Max	SD (+)
Normal days	95.5	47	144	68.58
Dusty days	1421	342	3649	1235

in previous studies, including North Africa, especially the Sahara Desert. Besides, the study area is surrounded by many marshlands. In recent decades, the proportion of desert areas are dramatically boosted as the result of low precipitation in the Middle East, improper management of the existing water resource, and climatic changes which increased the drying process of these marshlands; therefore, the dried bottoms of these marshlands are considered to be as the momentous sources of dust storms (Naimabadi et al. 2018; Neisi et al. 2018).

### The concentrations of PAH compounds on dusty and normal days

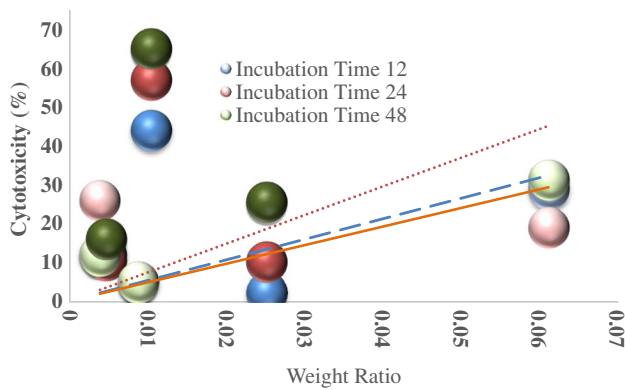
The mean concentration of PAHs in  $PM_{10}$  particles on dusty and normal days is shown in Fig. 2. The compounds of phenanthrene and acenaphthene had the highest concentrations (about 5.41 and 1.98  $ng\ m^{-3}$  in  $PM_{10}$  particles, respectively) on the dusty days. The total level of high-molecular-weight PAHs ( $\Sigma HMWPAHs$ ) in the  $PM_{10}$  particles was 9.38  $ng\ m^{-3}$  on the dusty days, while the corresponding value for the normal days was 5.98  $ng\ m^{-3}$ . The total level of low molecular weight PAHs in the  $PM_{10}$  particles for dusty and normal days was 1.77  $ng\ m^{-3}$  and 1.55  $ng\ m^{-3}$ , respectively. The concentration of PAHs during the normal days was further than that of dusty days, and this difference was significantly based on the independent *t*-test ( $P = 0.000$ ). In the category of “Not human carcinogens  $\Sigma PAH_s$ ” and “Human carcinogens  $\Sigma PAH_s$ ” dusty days had the most number of 7.4  $ng\ m^{-3}$  and 1.04  $ng\ m^{-3}$ , respectively, while normal days were in the next levels with 6.5  $ng\ m^{-3}$  and 0  $ng\ m^{-3}$ . Halek et al. (2006) carried out a study in Tehran and found a concentration of 4.17  $ng\ m^{-3}$  for  $PM_{7.2-10}$  particles, 5.6  $ng\ m^{-3}$  for  $PM_{3-7.2}$  particles, 11.95  $ng\ m^{-3}$  for  $PM_{1.5-3}$  particles, 9.18  $ng\ m^{-3}$  for  $PM_{0.95-1.5}$  particles, 2.82  $ng\ m^{-3}$  for  $PM_{0.49-0.95}$  particles, and 47.91  $ng\ m^{-3}$  for  $PM < 0.49\ \mu m$  particles. In this inquiry, 87.6% of PAHs had a lower molecular weight (128.18 to 178.28), and 12.4% of PAHs had a higher molecular weight (202.66 to 276.34). Bi et al (2003) conducted a study in Guangzhou city, China, in 2001, and reported that the mean concentration of the total PAHs measured was 134.4–298.5  $ng\ m^{-3}$ , which had the highest levels in April. The amount of PAHs measured at the height of 25 m was about 30–50% less than its value on a ground surface (Bi et al. 2003). Dallarosa et al. (2005) conducted a study in the city of Alger, Brazil, to identify and quantify the principal sources of PAHs. Three diverse stations were selected to collect  $PM_{10}$  samples from November 2001 to November 2002. Next, they measured the concentration of 16 fundamental components of PAHs. The mean concentration of PAHs was 0.4–2.3  $ng\ m^{-3}$ . Based on the obtained results, it is obvious to consider motor vehicles, burning hospital waste, industries, and burning coal at power

plants as the primary sources of PAHs. In Dellarosa et al.’s study, indeno [1, 2, 3-cd] pyrene and benzo [g, h, i] perylene had the highest concentration among PAHs compounds, and their primary source was motor vehicles in urban areas with higher rates of traffic congestion. Kong et al. considered the concentration of PAHs in  $PM_{2.5}$  and  $PM_{2.5-10}$  particles in five cities of Liaoning province, China, from 2004 to 2005. The concentration of total PAHs was 75.32–190.89  $ng\ m^{-3}$  in the  $PM_{2.5}$  particles and was 16.74–303.24  $ng\ m^{-3}$  in the  $PM_{2.5-10}$  particles. Moreover, 90% of the total PAHs in the  $PM_{2.5}$  particles had 3–5 rings. The ratio of the amount of PAHs in the  $PM_{2.5-10}$  particles in the winter toward summer was 1.7–37.6. In the concentration of the total PAHs between residential areas in comparison with commercial spaces, the obtained results illustrated the higher proportion for residential areas, with the higher value of  $PM_{2.5}$  and  $PM_{2.5-10}$  particles for industrial areas, and in urban areas, the concentration of PAHs was more than presumed. The PCA analysis revealed that the increased concentrations of PAHs were attributed to vehicles and urban traffic and the usage of residential heating appliances. In Kong et al.’s study, after quantifying the PAHs in different cities, the risk of carcinogenesis of these compounds was evaluated, and the risk of BaP was higher than the other compounds (Kong et al. 2010). The results of investigating the hydrocarbons associated with PM are different in various parts of the world, but, as highlighted in the research mentioned above, traffic congestion and industrial areas have maximum concentrations of these compounds, and their concentration increases predominantly in winter, probably due to the temperature inversion. The purpose of the presented study is the measurement of the PAHs concentration in a particulate phase, and the study of the cytotoxic effects of PAHs contained by  $PM_{10}$  in each sample. The results demonstrated the higher proportions of these compounds in the dusty days than on the normal days, which can be justified due to the high volume of particles on the dusty days.

The concentration of PAHs at the particulate phase of every test was split by the number of particulates in the same test. The obtained proportion was utilized as the scale of measurement to determine the intensity of the effect of the mentioned compounds on cells (Ali et al. 2016; Goudarzi et al. 2017, 2018; Liu et al. 2016; Naimabadi et al. 2016).

### PAHs cytotoxic effects assessment on A549 cell line

According to Naimabadi research, Cytotoxicity of PAHs adsorbed to  $PM_{10}$  was analyzed by an indicator estimated by weight ratio, which was calculated by summing the mean levels of whole PAH compounds for a testing day to particles levels on the same day (Naimabadi et al. 2016). The calculated weight ratios of the PAHs are shown in Fig. 3. The percentage of cytotoxicity increased by boosting the proportions of the

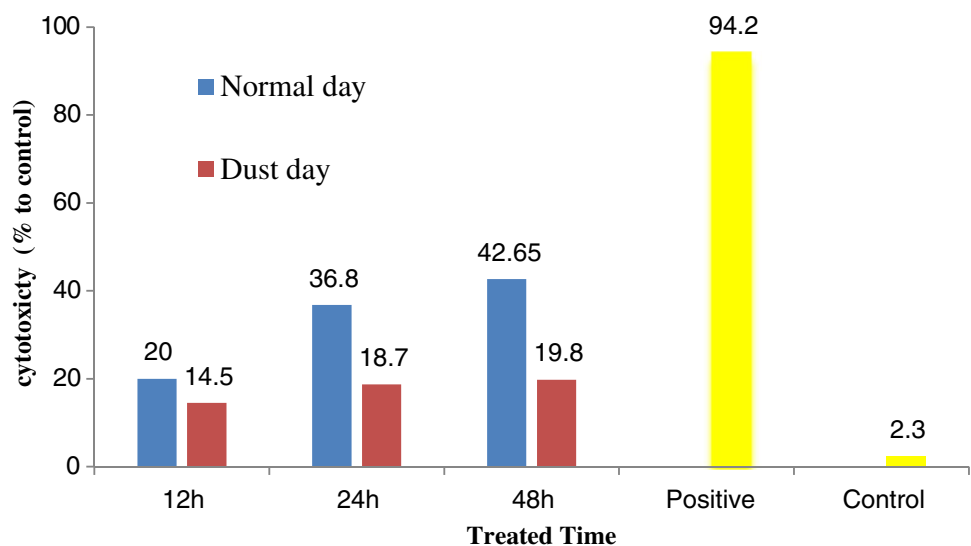


**Fig. 3** The Correlation of the particulates’ weight ratio and cytotoxicity intensity of extracts of PM10 at different incubation times

weight and decreasing on the dusty days compared to the normal days. The findings of the one-way ANOVA demonstrated an essential contrasting between the different incubation times and the percentage of cytotoxicity, and this difference was more significant between the incubation times of 12 and 48 h ( $P$  value  $< 0.05$ ). Yet, there is no meaningful discrepancy between the incubation times of 24 and 48 h as well as between times of 12 and 24 h. Figure 4 shows these changes. The increase in incubation time and the longer contact time with these compounds are essential factors in causing damage to the cells. Goudarzi et al. (2019) evaluated organic and chemical properties of the  $PM_{2.5}$  particle, and then in vitro toxic impacts on lung cells during the Middle Eastern Dust storms that occurred in Ahvaz, Iran, during winter and spring 2015. Goudarzi’s study results showed that the amount of PAHs increases with increasing particle concentration. The main point is that the species with more toxic effects (such as Benzo [a] pyrene) were observed in samples of normal days. The percentage of cytotoxicity of particles on these days also

shows higher values. The one-way ANOVA results revealed a substantial divergence among the incubation time and percentage of cytotoxicity, so that the percentage of cytotoxicity increases with increasing the incubation time (Goudarzi et al. 2019). Longhin et al. (2013) showed the seasonal response for fine and quasi-ultrafine particles in Milan, which was cultured in human cells (THP-1 and A549 cell lines). The chemical composition of  $PM_{10}$  and  $PM_{0.4}$  particles was evaluated, and the results showed that toxic effects are mainly due to the existence of the organic compound (Longhin et al. 2013). Dergham et al. in 2015, evaluated seasonal variations of the physicochemical properties of airborne particulate matter ( $PM_{10-0.3}$ ) and their toxicological effects on human bronchial epithelial cells (BEAS-2B). The study was conducted in different seasons and three areas, including rural, urban, and industrial in Dunkerque, France (Dergham et al. 2015). The results showed that physicochemical characteristics are approximately referred to the season and sample sites, and the concentration of particles ( $PM_{10-0.3}$ ) is significantly associated with places with traffic congestion caused by heavy vehicles. Likewise, the result revealed that the concentration of particles alone could not justify the cytotoxic effects, and the source of particles and season possess a considerable function in creating undesirable effects. In the existing inquiry, the organic compounds carry more impact on the cytotoxicity of particles than the mineral compounds. The higher percentage of cytotoxicity can be because of the presence of organic compounds, which are the leading reason for DNA damage, on normal days compared to dusty days (Goudarzi et al. 2017, Meng & Zhang 2007, Naimabadi et al. 2018). Gualtieri in 2010 investigated the cytotoxicity differences in the inflammatory potential of  $PM_{10}$  particles on the A549 human lung epithelial cells in Rome, Italy, to examine the effect of variations in chemical compositions on the cytotoxic effects (Gualtieri et al. 2010). Samples were gathered from in the two seasons of winter and summer. Some

**Fig. 4** Cytotoxicity effects of PM10 extracts of normal and dusty days at different incubation times



samples had a higher PAH concentration, and some had more metal content. The findings illustrated that tests with the highest amount of PAHs compared to metal elements had a high potential for toxicity. In general, the biological effect is associated with the production of intracellular ROS, which results from PM suspensions. They can have destructive effects on proteins, providing structure and supporting cell membranes related to lipids and cellular components. Besides, they indicated that the expression of cytokines from human alveolar macrophages, obtained from incubation of insoluble PM<sub>10</sub>, was relatively 50 times more than the water-insoluble fraction. Some studies attribute the toxic effect on organic compounds relative to the aqueous compounds (Schilirò et al. 2015).

## Conclusion

The occurrence of dust storms in western parts of Iran during recent years is mainly due to recent droughts and improper water resource management, affecting the lives of individuals in the area and reducing the number of healthy days throughout the year. The present study focused on the chemical properties of particulate matter carried by these dust storms and their toxic effects on the A549 cell line in vitro exposure. The findings demonstrated that the amount of PAHs increases with increasing particle size. However, the main point is that the species with more toxic effects (Benzo [a] pyrene) were observed in samples on normal days. The percentage of cytotoxicity of PM<sub>10</sub> on these days also shows higher values. The one-way ANOVA results revealed a meaningful divergence between incubation times and the percentage of cytotoxicity, so that the percentage of cytotoxicity increases with prolonging the incubation time. Although the cytotoxicity of the particles for normal days was higher on the A549 cell line, due to the reasons stated above, the hazards of dust storms on the environment and health are inevitable because people receive a higher particle concentration while breathing air during dusty days.

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## Declarations

**Conflicts of interest** The authors declare no competing interests.

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