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Chemical composition of PM_{10} and its in vitro toxicological impacts on lung cells during the Middle Eastern Dust (MED) storms in Ahvaz, Iran☆

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ABSTRACT

Reports on the effects of PM_{10} from dust storm on lung cells are limited. The main purpose of this study was to investigate the chemical composition and in vitro toxicological impacts of PM_{10} suspensions, its water-soluble fraction, and the solvent-extractable organics extracted from Middle Eastern Dust storms on the human lung epithelial cell (A549). Samples of dust storms and normal days ($PM_{10} < 200\ \mu g\ m^{-3}$) were collected from December 2012 until June 2013 in Ahvaz, the capital of Khuzestan Province in Iran. The chemical composition and cytotoxicity were analyzed by ICP- OES and Lactase Dehydrogenase (LDH) reduction assay, respectively. The results showed that PM_{10} suspensions, their water-soluble fraction and solvent-extractable organics from both dust storm and normal days caused a decrease in the cell viability and an increase in LDH in supernatant in a dose–response manner. Although samples of normal days showed higher cytotoxicity than those of dust storm at the highest treated dosage, T Test showed no significant difference in cytotoxicity between normal days and dust event days ($P_{value} > 0.05$). These results led to the conclusions that dust storm PM_{10} as well as normal day PM_{10} could lead to cytotoxicity, and the organic compounds (PAH_s) and the insoluble particle-core might be the main contributors to cytotoxicity. Our results showed that cytotoxicity and the risk of PM_{10} to human lung may be more severe during dust storm than normal days due to inhalation of a higher mass concentration of airborne particles. Further research on PM dangerous fractions and the most responsible components to make cytotoxicity in exposed cells is recommended.

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1. Introduction

Particulate matter (PM) encompasses an air-suspended mixture of solid and liquid particles that vary in number, shape, size, chemical composition, surface area, solubility and origin

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(Pope and Dockery, 2006). Respirable particulate matters, which are coarse particles, with an aerodynamic diameter equal or less than 10 μm, named as PM_{10}. The coarse fraction (PM_{10}) contains a main component of mineral compounds and certain adsorbed endotoxins. Anthropogenic contributions are greater in urban environments, and particular metals may also be more prevalent in these areas. In an urban setting, approximately 40% of the particle content can be attributed to fossil fuel use (Ghio et al., 2012).

The respiratory system is a barrier and also primarily exposed to airborne particles, which are inhaled and tend to accumulate in the airways. The exposure dose in the tissue depends on the PM atmospheric concentration, the settling rate in the airways, cleaning mechanisms and interaction of particulate matter with lung organ. Many investigations have been conducted to find an association between PM concentration and the increased rate of diseases and deaths in communities (Brook et al., 2010; Pope and Dockery, 2006). Health endpoint for long term exposure to particulate matter was completely different from that for short term exposure, causing lung cancer in the former and bronchitis in the latter (Schwarze et al., 2006). PM originates from anthropogenic and natural sources. The ever changing air quality provides different dimensions of particulate matter with diverse chemical and biological characteristics (Pope and Dockery, 2006). Dust events are defined as natural events with substantial particulate matter (PM) concentrations, usually occurring in arid, semi-arid, or desert areas (Wang et al., 2005, 2006a) and primarily resulting from a low vegetation cover and strong surface winds (Kurosaki and Mikami, 2003). In recent years, the Middle Eastern Dust storm has originated from western neighboring countries causing a drastic increase in serious environmental and socioeconomic problems, affecting both western and central parts of Iran and transporting large amounts of particulates, pollutants, and biological materials for long distances downwind (De Longueville et al., 2010). Some countries like Iraq, Saudi Arabia, and Iran suffer from the MED storms (Brocato et al., 2014; Liao et al., 2011). In Iran an average of 60 dust storm days was recorded in various cities of Khuzestan province resulting in more days-off, flight cancellations, and low visibility (Heidari-Farsani et al., 2014; Soleimani et al., 2013; Zarasvandi et al., 2011).

It should be noted that heavy metals such as lead, chromium, cadmium, as well as cations (such as Fe^{2+}) and anions (such as NO_3^{-2} and SO_4^{-2}), which are transported by particles can cause significant cardiovascular effects and oxidative stress (Alessandria et al., 2014; Ghio et al., 2012; Rezaei et al., 2014). Study on epithelial cells line indicated that PM_{10} extracts had a stronger effect on LDH release and pro inflammatory potential of PM_{10} was remarkable compared with $PM_{2.5}$ (Duvall et al., 2008; Gualtieri et al., 2009; Hetland et al., 2004). Cytotoxicity effect and apoptosis in monocyte cell lines increased due to PM_{10} components (Jomova et al., 2012). Moreover, metals have been proposed to induce ROS formation (Donaldson et al., 2003). Other findings confirm that PM composition plays an important role in particle-induced toxicity. The presence of PM_{10}-induced biological effects at a low polluted site suggests that a reduction of PM_{10} mass does not seem to be sufficient to reduce its toxicity (Schilirò et al., 2015). Accordingly it has been reported in previous studies that A549 cells were particularly peroxide-induced oxidative stress and also resistant to cytotoxicity (Dandrea et al., 2004). Another investigation has also hypothesized that water-soluble or insoluble fractions of PM accompanied with metals can catalyze reactions involving in oxidative stress and it can possibly damage to DNA (Lei et al., 2004). Although physiochemical and biological properties of dust have been well documented (Goudarzi et al., 2013, 2014; Heidari-Farsani et al., 2014; Shahsavani et al., 2012b; Zarasvandi et al., 2011), no studies have been reported to clarify in vitro toxicological impacts of the MED on human pulmonary system. Therefore, the main objective of the present study was to evaluate cytotoxic effects induced by PM_{10} extracts sampled during the MED storms and normal days.

2. Material and method

2.1. Description the area of study

Ahvaz is one of the metropolitan cities in south-western part of Iran located in an arid area nearby Saudi Arabia, Iraq and Kuwait, the main sources of dust storms in the Middle East. Ahvaz, with coordination of 31° 32N and 48° 68E, an area of 528 km^2 and a population of 1.112 million, is the most polluted city from the viewpoint of PM_{10} annual average (Goudie, 2014). Fig. 1 presents the sampling site, geographical location of Khuzestan province in the Middle East and in relation to the previously mentioned sources of dust events. Khuzestan province is connected to the Persian Gulf and Iraq from south and west, respectively. The sampling station was located at an urban background area in the city.

2.2. PM sampling

A PM_{10} high volume sampler (Tisch Company) equipped with quartz microfiber filters (8 × 10 in, Whatman. USA) was used to collect the samples from December 2012 until June 2013 on both dust event and normal days (Deng et al., 2007; Geng et al., 2006). The sampler was placed on the roof top of the Health School at the height of 10 m above the ground level, and there were no obstacles to minimize the potential effects of natural and anthropogenic features on the air flow as well as particle concentrations. The sampler was operated with a flow rate of 1.3–1.7 m^3/min (and finally the average flow rate was calculated) for 24 h (Meng and Zhang, 2007). The exact flow rate was calculated daily and corrected for variations in atmospheric pressure and actual differential pressure across the quartz filter. The filters were pre- and post-conditioned in a dry and dark place for 48 h and weighed with an analytical balance (±10 μg) before and after sampling to calculate the PM mass trapped on the filter (Shahsavani et al., 2012a).

2.3. Dust storm classification and the origin of dust

Dust event days were defined based on visibility, wind speed and PM_{10} concentration following the criteria defined by Hoffmann et al. (2008) and shown in Table 1. We defined criteria to distinct between normal and dusty days based on hourly average concentration of PM_{10}. Exceeding from 200 μg/m^3 was considered as dusty days and lower than this value could be supposed as normal days. The concentration of PM_{10} in Ahvaz is too high because of existing industries and high frequency of occurrence of dust storms. National Oceanic and Atmospheric Administration (NOAA) conducted a research on transport, dispersion and transformation of each dust storm occurrence throughout the world. Their efforts offered a unique tool entitled “Hybrid Single-particle Lagrangian Integrated Trajectory (HYSPLIT)”model (Draxler and Rolph, 2006). Backward trajectory of HYSPLIT was used to realize the approximate origin of dust storm in the present study.

2.4. Particles extraction

After gravimetric analysis, each chosen filter loading samples was divided into four parts. To prepare total particle (complete sample) suspensions, one-fourth of the exposed quartz micro-fiber

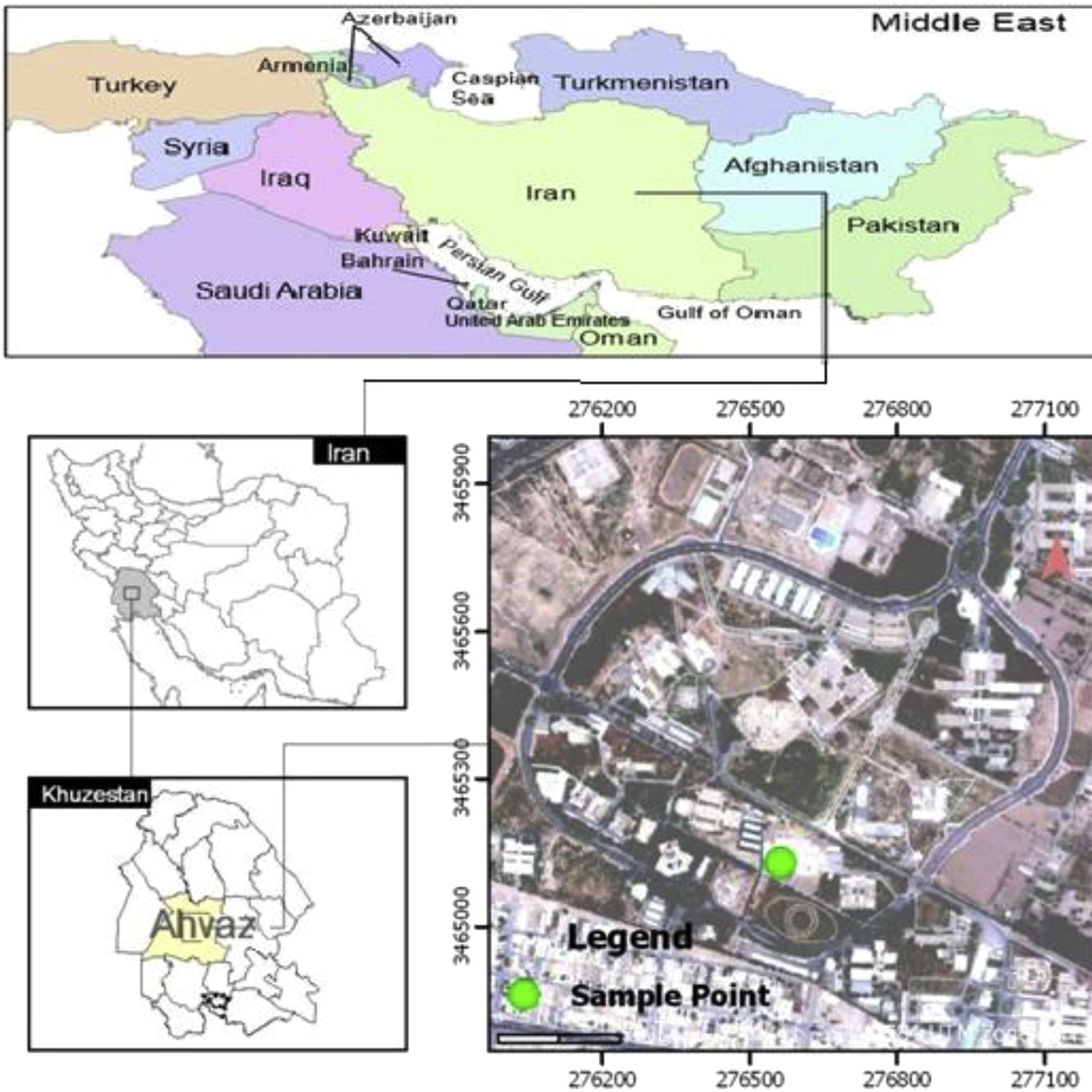

Fig. 1. Location of the study area and sampling station.

Table 1
Dust storm classification (Hoffmann et al., 2008).

Category	Visibility (m)	Wind speed (m s^{-1})	Hourly averaged PM_{10} (μg m^{-3})
Dusty air	haze	–	50–200
Light dust storm (DS1)	<2000	–	200–500
Dust storm (DS2)	<1000	>17	500–2000
Strong dust storm (DS3)	<200	>20	2000–5000
Serious strong DS (DS4)	<50	>25	>5000

filters was cut into pieces, suspended in Milli-Q water, and sonicated for 0.5 h followed by 10 min of vortex flow. The suspension solutions were divided into two parts one part of which was transferred into a new pre weighed beaker and frozenly dried in vacuum. The weights of the particles were obtained and the average harvesting ratio was calculated. The particles were suspended in RPMI1640 without fetal bovine serum (FBS) at concentrations of 5000 μg/mL, and stored at −20 °C. Before using, the suspensions were surged for 10 min. To isolate water-soluble fraction of the PM_{10} samples, another part of the suspension was transferred and filtrated with 0.45 μm Whatman filter paper. The rest fraction (insoluble components) was abandoned. After freeze drying over the solutions containing water-soluble fraction, the concentrated components were weighed, diluted with RPMI1640 without FBS and stored at −20 °C. The solution was prepared with 5000 μg/mL concentrations (Meng and Zhang, 2007; Schilirò et al., 2015).

2.5. Chemical analysis of water-soluble ions

The second part of the filter was used for determination of the ionic species, including four anions (F^-, Cl^-, NO_3^-, and SO_4^{2-}) and five cations (Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}). Water soluble inorganic ions were determined by extracting samples in 25 mL of ultra-pure water (Millipore Milli Q water system) for 20 min in an ultrasonic bath (Bandelin, Germany). After being shaken for 10 min, the extract was then filtered (0.45 μm PTFE filters, Alltech, USA) to remove insoluble fractions and then the ionic components were determined by using the Waters ion chromatography (Model: Alliance 2695, USA) with a 432 Conductivity Detector that was operating at a flow rate of 1 mL/min. Cationic and anionic solvents used were nitric acid 3.2 mM and sodium hydrogen carbonate 1.5 mM combined with sodium carbonate 1.6 mM, respectively. For each analysis, 20 μL of the anionic sample and 20 μL of the cationic sample were injected into the ion chromatography. It is noteworthy that the recovery rate for each ion was in the range of 70–110%. The relative standard deviation of each ion was less than 3% for the reproducibility of the tests. In order to determine the background concentrations of each element, blank samples (i.e., unexposed control filters) were chemically analyzed routinely. Then, the real concentration of each species was calculated by subtracting the blank values from the results of the chemical analysis conducted on the exposed filters (Shahsavani et al., 2012b; Shen et al., 2009).

2.6. Heavy metal analysis

The third part of the exposed quartz micro-fiber filter, was cut and put in a Teflon container; then a mixture of nitric acid, hydrochloric acid, and hydrofluoric acid was added to it, and the filter was digested in a hot oven at 170 °C for 4 h. After that time elapsed, we opened the cap of the Teflon container on a heater at 95–100 °C to evaporate all the remaining acids inside. After cooling, in the next stage, the concentrated nitric acid and distilled water (ratio of 1–9 V %) were added and shaken well for 15 min. The obtained solution was filtered through a Whatman-42 filter paper. The resultant solution was then diluted to 25 mL with distilled water and stored in a clean, sterile, and plastic bottle at 4 °C until further analyses. The digested samples were analyzed for target metals by inductively coupled plasma optical emission spectroscopy a SPECTRO ICP-OES model Spectra arcos (Germany). Detection limits for analyzed metals are: As (0.179 $\mu g kg^{-1}$), Cd (0.049 $\mu g\ kg^{-1}$), Cr (0.096 $mg\ kg^{-1}$), Cu (0.306 $\mu g kg^{-1}$), Ni (0.28 $\mu g kg^{-1}$), Pb (2 $\mu g kg^{-1}$), V (0. 5 $\mu g kg^{-1}$), Zn (0.3 $\mu g kg^{-1}$), Fe (0.16 $\mu g kg^{-1}$) and Al (0.17 $\mu g kg^{-1}$). Calibration curves were obtained using 6 points with certified standards (High Purity Standards). Blanks were analyzed by completion of the full analytical procedure without samples. After each analytical run, the calibration curve was displayed on the screen, and a visual check was made for linearity and replication. The chemicals were analytical grade and were purchased from Merck, Germany (Heidari-Farsani et al., 2014; López et al., 2005; Schilirò et al., 2015).

2.7. Cell culture

The human lung epithelial cell line, A549, was obtained from Pasteur Institute at National Cell Bank of Iran (NCBI). These cells were considered as a model for human epithelial lung cells. The cells were grown as monolayer adhering to flask and were maintained and treated in RPMI-1640 supplemented with 10% (v/v) FBS, 2 mM glutamine and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 cell culture (Schilirò et al., 2015).

2.7.1. Lactate Dehydrogenase enzyme (LDH) assay

To evaluate PM_{10} cytotoxicity, LDH activity was determined in cell-free supernatants using a commercially available kit (Takara Bio Inc., Japan) modified for adherent cells. Briefly, A549 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and exposed to PM_{10} extracts containing 250,125 and 62.5 μg/mL particle concentrations that always induced a significant effect in previous studies (Alessandria et al., 2014; Corsini et al., 2013; Schilirò et al., 2010). The cells were treated for three times (12-24-48 h). Then, LDH activities were measured in the supernatants. The percentage of cytotoxicity was reported by using the following equation:

$$Cytotoxicty(\%) = \frac{\text{exp.value} - \text{low control}}{\text{high control} - \text{low control}} \times 100$$

where experimental value is LDH activity released from treated normal cells in samples Non-exposed cells and Triton X-100 (2%, v/v) exposed cells were considered as negative (low) and positive control (high), respectively. Low control is LDH activity released from untreated normal cells and high control is the maximum LDH activity induced by addition Triton X-100.

2.8. Statistical analysis

The statistical analysis was performed using the SPSS Package (version 17.0) for Windows. The mean values were compared using the Independent student T Test, and the one way ANOVA, and Duncan test was used to assess the relationships between variables. The mean difference and correlation were considered significant at ($P_{value} < 0.05$).

3. Results and discussion

3.1. PM_{10} concentrations, its classification and trajectories

A total of 15 PM_{10} filters for normal day and 15 PM_{10} filters during the occurrence of MED storms in Ahvaz were analyzed. PM_{10} mean mass concentration was 483 $\mu g/m^3$ during the entire period of the study. The highest value for dust days was observed in spring with 4443 $\mu g/m^3$ concentration which was classified as DS3 in Table 1, whereas the lowest was 54 $\mu g/m^3$ in winter during a normal day. Moreover, significant differences ($P_{value} < 0.05$) were observed in case of PM_{10} concentrations between normal and dust days. PM_{10} concentrations of dust days for the two studied seasons are presented in Table 2. The results showed that mean concentrations of PM_{10} during dusty days in the winter and spring were 510 and 457 $\mu g/m^3$, respectively. According to Table 2, mean concentration of PM_{10} on dusty days during the spring was 9 times higher than the 24-h mean concentrations 50 $\mu g/m^3$ (WHO) and the corresponding value for dusty days in the winter was about 10.2 times higher than the WHO value. Therefore, most of the MED storms occurring during study period were classified in DS2 and DS3. It should be noted that the occurrence of serious strong dust storms (DS4) in Ahvaz were also recorded in previous works (Derakhshandeh et al., 2014; Shahsavani et al., 2012a). These high PM_{10} concentrations are attributed to the large deserts located at

Table 2
Summary statistics of PM_{10} concentrations (μg/m^3) in the ambient air of Ahvaz during two seasons.

Seasons		Average	Min	Max	SD
Winter	Normal	117	54	195	38
	Dust	510	204	2862	664
Spring	Normal	129	58	195	40
	Dust	457	204	4443	586

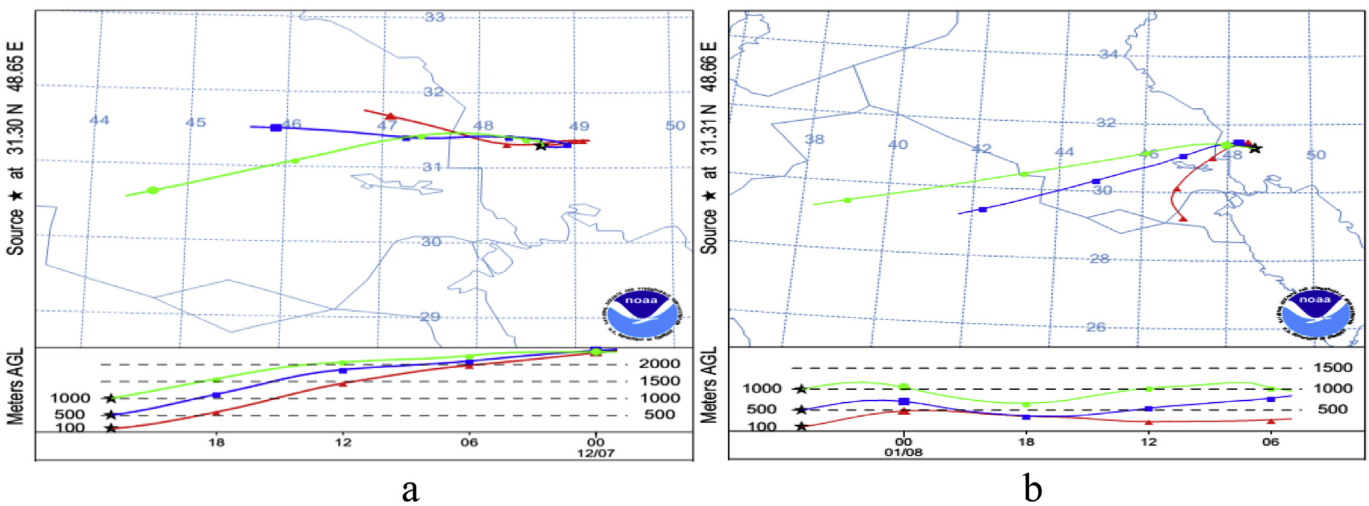

Fig. 2. Backward trajectories of occurred MED storms during the study to realize dust origins using HYSPLIT model on December 1, 2012 (a) and January 8, 2013 (b).

the west of the city, which are known as the major sources of dust storms in this region. According to vertical velocity method using backward trajectory of HYSPLIT, the aerosol generated over Ahvaz originated from Iraq (Fig. 2a), Saudi Arabia and Kuwait (Fig. 2b). Other sources such as North Africa particularly the Sahara Desert were also mentioned in previous investigations (Soleimani et al., 2015). Previous studies revealed that the occurrence of the MED over Ahvaz and the increasing rate of particulate matter in the ambient air were due to the lack of precipitation in the Middle East region, improper management of water bodies, adjacency to an arid area, (Sahara Desert and Iraq) and climate change (Heidari-Farsani et al., 2014; Shahsavani et al., 2012b; Soleimani et al., 2015).

3.2. Chemical composition of PM_{10} samples

3.2.1. Inorganic ions

Ionic components of PM_{10} in normal and dust days are shown in Table 3. According to the results, only 9.7% of the total mass of $PM_{10}$was related to ionic components, indicating that the ionic components were more concentrated in the coarse fraction of the MED particles. This is similar to the results of other studies, in which the ionic contribution to PM_{10} mass were reported to be in the range of 5–15% during dust storm days (Heidari-Farsani et al., 2014; Shahsavani et al., 2012b; Zarasvandi et al., 2011). Fig. 3a–b illustrates the relative contribution of ionic components to the total mass of PM_{10} during dust storm and normal days. As shown in Fig. 3a, ionic components in PM_{10} during dust storm days were in the following order of $SO_4^{2-} > Cl^- > NO_3^- > Ca^{2+} > Na^+ > Mg^{2+} > K^+$; while the ionic components in PM_{10} in normal days were $SO_4^{2-} > NO_3^- > Na^+ > Ca^{2+} > Cl^- > K^+ > Mg^{2+}$ (Fig. 3b). It should be noted that SO_4^{2-}, Cl^-, NO_3^-, and Ca^{2+} were the most frequent ionic components of PM_{10} during the MED storms. Components including Ca^{2+}, Mg^{2+}, and Na^+ are believed to be crustal ions (Shahsavani et al., 2012b; Wang et al., 2006b) Considerably high concentrations of SO_4^{2-} in PM_{10} could be associated with proximity of the sampling location to oil extraction and refinery plants, which are frequently found in this area. High Concentrations of NO_3^- may originate from the combustion of fossil fuel by motor vehicles. In addition, High Cl^- concentrations could originate from sea salt particles, since the study area is close to marine environment such as the Persian Gulf, the Karun River and many other dams and rivers. Na^+ ion can also originate from sea salt particles. However, the mass concentrations of NH_4^+ was not measured in this study, but based on the previous works, its concentrations were closely related to those of NO_3^-, implying that NH_4^+most probably originated from the neutralization between acidic components and NH_3 (Shahsavani et al., 2012b; Wang et al., 2006b). Zarasvandi et al. (2011) also observed that the most important oxide compositions of airborne particles were SiO_2, Al_2O_3, Fe_2O_3, CaO and MgO.

Table 3
Summary statistics of the ionic components in PM_{10} concentration (μg/m^3) during the period of study.

Species	Ionic composition of PM_{10} in dusty days				Ionic composition of PM_{10} in normal days			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Mass	708	849	300	4443	168	69	54	298
Na^+	9.78	3.48	7	14.4	8.24	1.35	7.29	9.2
Ca^{2+}	9.86	1.57	8.25	12	5.2	0.49	4.86	5.55
K^+	0.82	0.20	0.57	1.01	1.07	0.7	0.57	1.56
Mg^{2+}	0.75	0.43	0.4	1.38	0.23	0.06	0.19	0.27
SO_4^{2-}	19.24	12.4	11	37.2	9.99	3.07	7.8	12.2
NO_3^-	11.3	12.4	4.35	29.8	9.54	4.91	6.07	13
Cl^-	16.85	17.58	4.17	41.7	3.38	1.35	2.43	4.34

3.2.2. Elements

The concentrations of metals and metalloids during dust and normal days are shown in Table 4. The average concentration of metals and metalloids such as Al, Fe, Zn, Pb, Cr, Cu, V, As, Ni and Cd during dust event days were 4.1, 12.3, 2.1, 2.06, 8.8, 1.7, 12, 1.5, 5.5

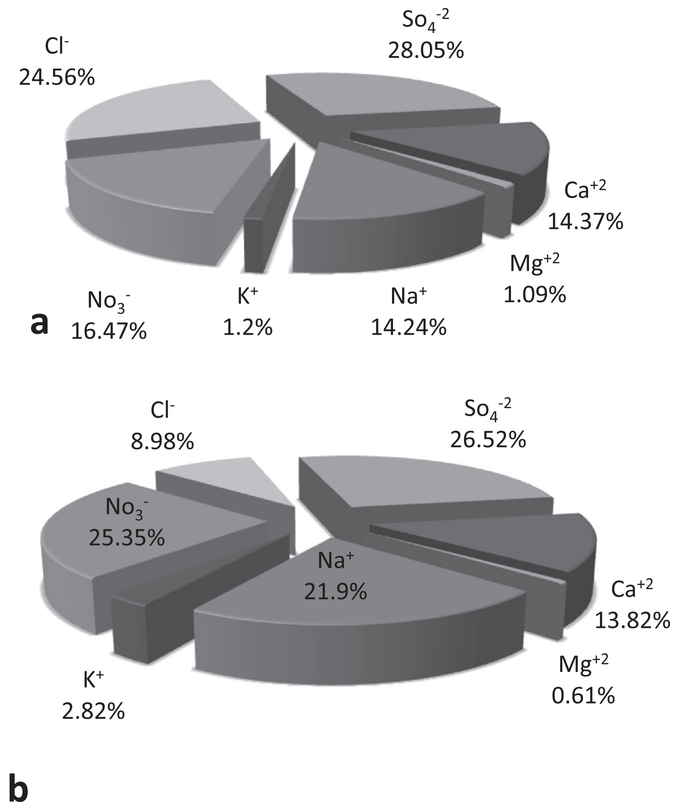

Fig. 3. Relative contributions of the ionic components to the total mass of PM_{10} during dust storm days (DSDs) and normal days (NDs): ions percentage in PM_{10} during DSDs (**a**); and ions percentage in PM_{10} during NDs (**b**).

and 1.04 times higher than their concentration in normal days. Heidari-Farsani et al. (2014) have reported that the mean concentration of Zn, Pb, and Cd during dusty days were 1.43, 1.3, and 2.7 times higher than normal ones, respectively. In another research, Shahsavani et al. (2012b) reported that the concentration of heavy metal during the Middle Eastern Dust storms were 1.5–3 times higher than the normal ones. Our findings are in good agreement with the previous studies. This could be possibly due to the high mass of PM_{10} released into the air during this condition (Najafi et al., 2014; Prabhakar et al., 2014). In the case of dust storm conditions, the order of concentration of metals and metalloids was as follows: Al > Zn > Fe > Cu > V > Ni > Cr > Pb > Cd > As. The average concentration of metals and metalloids during the occurrence of the Middle East dust (MED) storms was higher than the standard values proposed by the World Health Organization. The weight ratio was defined and used to evaluate the weight of each metal ($\mu g/m^3$) based on the average PM_{10} concentration ($\mu g/m^3$) during normal and dusty air (Metal/$PM_{10,}$ dimensionless) (Fig. 4).

Fig. 4 shows that the weight ratio of Al, Zn, Pb, Cu, As and Cd in normal days was higher than that in dusty days. This may be due to existence of anthropogenic sources such as combustion of fossil fuel and high activity of industries amid the city (Prabhakar et al., 2014; Zarasvandi et al., 2011). The air stability conditions and regular temperature inversions provided air accumulation over the city by limiting the dilutions and dispersions (Heidari-Farsani et al., 2014; Kurosaki and Mikami, 2003; Sorooshian et al., 2012) This parameter (weight ratio) for the elements including Fe, Cr, V, and Ni in dusty days was higher than that in normal days indicating Cr, V and Ni are typically associated with fossil fuel combustion and oil industrial practices (Nriagu, 1989; Zarasvandi et al., 2011). Since Ahvaz is located near the Middle East countries such as Iraq, Kuwait, and Saudi Arabia, which are rich of oil fields and oil refineries, the dust storms originating from the desert of these countries may be the reason for the increased ratio of these

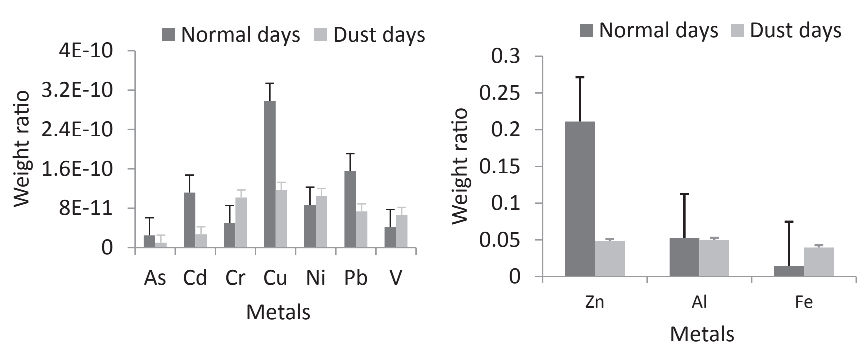

Fig. 4. Comparison of weight ratio for metals in two weather conditions.

Table 4
Summary statistics of metal contents in PM_{10} during the study period.

	Metals contents of PM_{10} in dust event days (ng/m^3)				Metals contents of PM_{10} in normal days (ng/m^3)			
Species	Mean	SD	Min	Max	Mean	SD	Min	Max
Al	35137.76	20751.96	4769.79	50917.43	8463.54	5462.89	4600.69	12326.40
Fe	28387.17	26348.50	9434.70	62293.50	2309.10	1743.20	1076.39	3541.66
Zn	34202.83	44192.53	9475.96	100347	16276	1534.50	15190.97	17361.10
Pb	52.06	61.31	0.74	139.90	25.17	6.14	20.83	29.51
Cr	72.62	81.36	12.35	192.66	8.24	9.20	1.73	14.75
Cu	83.81	40.74	37.50	125	48.18	10.43	40.79	55.55
Cd	19.50	15.49	8.68	42.43	18.66	7.98	13.02	24.30
As	6.67	12.06	0.59	24.77	4.25	5.03	0.69	7.81
V	82.96	93.32	27.08	222.47	6.27	0.30	6.51	6.94
Ni	74.34	52.77	28.42	135.59	0.99	1.16	0.17	1.82

elements (Shahsavani et al., 2012a). In addition, Ahvaz itself is one of the major industrial centers of Iran and many oil fields and oil refineries are located around this city. Owing to the high density of steel smelter factories and smelting operations in the region, it is hypothesized that the concentration of metals commonly associated with these activities (Fe, Cu, Zn, Al and Pb) increases simultaneous with fine soil (Sorooshian et al., 2012). Prabhakar et al. (2014) reported that airborne particles originating from smelting activities are enriched with high levels of metals and metalloids that can settle and contaminate the local top soil. Particles on soil dust particles that are subsequently transported via wind or of windblown particles originating from contaminated soil. Tabatabaei et al. (2015) showed the source of the deposited particles in the occurrence period of dust in Bushehr, a harbor in the south of Iran, indicating that dust originated from Syria and Iraq, and entered Iran after-ward. The mineral characteristics of dusts and other aerosols from the Middle East were also studied by Engelbrecht et al. (2009) and their results showed that geological dust, smoke from pits burning and heavy metal condensates (possibly from metals smelting and battery manufacturing facilities) were the main air pollutants. High concentrations of trace metals in Baghdad, Balad, and Taji in Iraq were associated with normal days. Enrichment factors (EF) for V and Sr elements estimated to be less than ten (EF $<$ 10), implying natural sources or crustal origin, whereas Na, Ni, Co, Ba and Cr, with EF $>$ 10, resulted from anthropogenic origin (Zarasvandi et al., 2011).

3.3. Lactate Dehydrogenase activity

An increase in cytoplasmic extracellular Lactate Dehydrogenase enzyme (LDH) activity reflects an increase in the amount of membrane-damage in cells (Brook et al., 2010). The release of the LDH enzyme into the culture supernatant was aimed to measure the cytotoxicity of the PM_{10} extracts. Extracts from no load filters (blank) had no significant effect on LDH release. The damaging effects were induced by PM_{10} suspensions, their water-soluble fractions and solvent-extractable organics from normal days. LDH release attributed to PM_{10} in normal days was slightly higher than that induced by dust storm PM_{10} in Ahvaz. Three times incubation of A549 cells with PM_{10} extracts at concentrations of 250, 125, 62 μg/mL and solvent-extractable organics during normal and dust event days are illustrated in Fig. 5a–c. As shown in this Fig, at the highest concentration (250 μg/mL), the percentage of toxicity in samples of normal days incubated for 12 h, was slightly higher than that of dust event days (31 v 30%). This may be partly explained by organic substances bound on the particle surface, such as PAHs, nitro-polycyclic aromatic compounds as well as metals (Jin et al., 2004; Müller et al., 2006; Schilirò et al., 2015). At the same concentration, no difference was observed in the percentage of toxicity for incubation time of 24 h between normal and dust event days (46 v 46%), but with increasing the incubation time to 48 h the percentage of toxicity was the highest in normal days (48 v 42%). Although lactate Dehydrogenase enzyme activity or cytotoxicity effect at 250 μg/mL during normal days was higher than that in dust event days, no statistically significant differences for both circumstances were observed in extracellular LDH activities after cell exposure at 250, 125, 62 and organic solvent (Fig 5). Student T Test showed that cytotoxicity did not differ by considering all concentrations for both normal and dusty days ($P_{value}>0.05$). Nevertheless, Duncan test suggested a significant difference between 250 μg/mL and other concentrations (125, 62 and organic extracts) in extracellular LDH activities after cell exposure ($P_{value}<0.05$) for normal days. The PM_{10} extracts induced 1.7%–48% as the percentage release of LDH in exposed cells in comparison with control cells. No statistically significant difference was observed in incubation time (12, 24 and 48 h) in the LDH release induced by the PM_{10} extracts for both weather conditions ($P_{value}>0.05$). The LDH increased in A549 due to either the organic or aqueous extract and did not correlate with the PM_{10} concentration or with metals and ions parameters. Both types of PM_{10} extracts have a significant impact on cells, and it is likely that water-soluble components could cross the membranes and enforce rapid inflammatory responses; organic

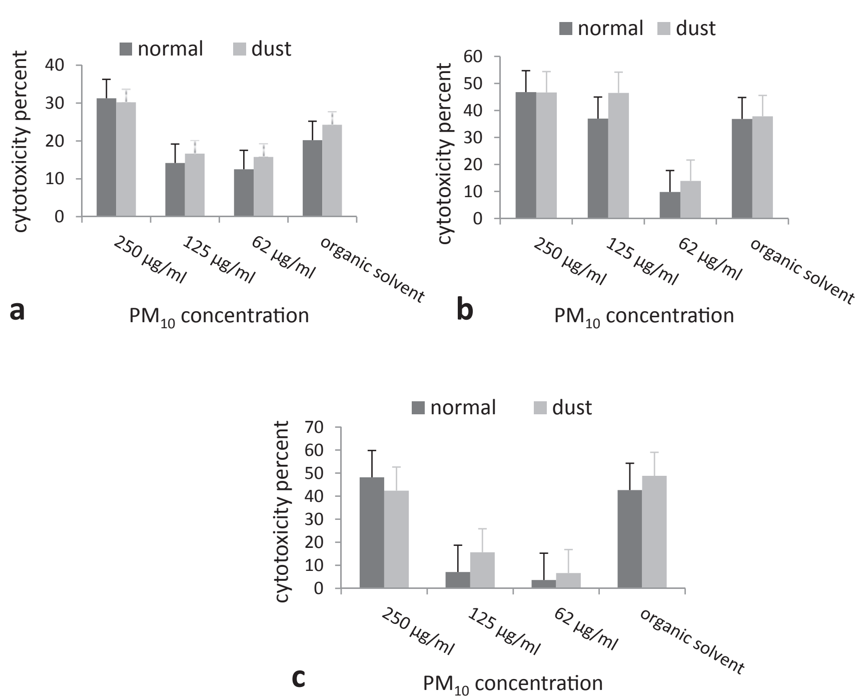

Fig. 5. Cytotoxicity effects of PM_{10} suspensions, their water-soluble fraction at concentrations of 250,125, 62 μg/ml and solvent-extractable organics for both circumstances in different treated time. A: 12 h incubation; B: 24 h incubation and C: 48 h incubation.

compounds still cross the membranes but have a more injurious impact on the cells (Alessandria et al., 2014). Many other studies have investigated the cytotoxicity of PM_{10} in lung epithelial cells line and different PM induced biologic effects considering the sampling site (Pérez et al., 2007), fraction size (Alfaro-Moreno et al., 2002; Hetland et al., 2004; Osornio-Vargas et al., 2003), sampling season (Frampton et al., 1999) and content of contaminants adsorbed on the particulate matter (Baulig et al., 2003; Billet et al., 2007; Calcabrini et al., 2004; Müller et al., 2006).

4. Conclusions

Dust storms have been brought about due to improper management in water bodies, droughts and climate change in the Middle East region, worsening life in west of Iran particularly in Ahvaz during last two decades. The current study focused on the chemical characterization and the cytotoxic effects of particulate matter on A549 cell line in two weather conditions of Ahvaz city. The results of this study showed that the heavy metals concentrations in the dust event days were higher than those in the normal days, indicating more complications associated with metals and also water soluble fraction on lung cells during a MED event. Cytotoxicity effect of PM_{10} may be due to PAHs and oxidizing substances of water-soluble fraction as well as the insoluble PM_{10} particle-core. In addition, the water-soluble fraction with a concentration of 250 μg/mL during both normal days and MED occurrence showed higher cytotoxicity than the solvent extractable organics. Physiologically, soluble components had a remarkable impact on the cell line to increase the LDH enzyme activity. Moreover, the lack of correlations in some cases indicates that specific chemical parameters should be detected in upcoming studies. Although dust storm PM_{10} showed a decreased cytotoxicity in A549 cell line than normal days PM_{10} at the highest treatments, the risk of health threats may be greater during dust storm events in real life because of increasing the airborne PM_{10} mass concentration at the time of dust occurrence.

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