

Effect of ambient air PM_{2.5}-bound heavy metals on blood metal(loid)s and children's asthma and allergy pro-inflammatory (IgE, IL-4 and IL-13) biomarkers

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ABSTRACT

Background: We investigated the concentrations of metals in fine particulate matter PM_{2.5} in the outdoor air around the home sites of 123 male children from Ahvaz, average age 7.56, along with their blood samples to measure pro-inflammatory responses (Immunoglobulin E and cytokines: IgE, IL-4 and IL-13).

Methods: We measured 6 metals (As, Cd, Cr, Hg, Ni and Pb) in three Ahvaz's regions including industrial (Padad), vehicle traffic (Golestan) and control (Kianpars).

Results: The higher concentrations of metals in the Padad as the industrial ambient air i.e., arsenic, cadmium, chromium, mercury and nickel coincided with the higher concentrations of those metals in exposed children ($P < 0.05$) versus the controls. Children in Golestan, the high traffic air pollution area had the highest lead concentrations ($p < 0.05$). Also a significant association was shown in Padad between blood arsenic and IgE ($\beta = 26.59$, $P < 0.001$), IL-4 ($\beta = 172.1$, $P < 0.001$) and IL-13 ($\beta = 14.84$, $P < 0.001$), blood chromium and IgE ($\beta = 10.38$, $P < 0.001$), IL-4 ($\beta = 75.27$, $P < 0.001$) and IL-13 ($\beta = 5.27$, $P < 0.001$) and blood mercury and IgE ($\beta = 13.11$, $P < 0.001$), IL-4 ($\beta = 108.09$, $P < 0.001$) and IL-13 ($\beta = 7.96$, $P < 0.001$) and blood lead and IgE ($\beta = 0.92$, $P = 0.025$), IL-4 ($\beta = 7.16$, $P < 0.001$) and IL-13 ($\beta = 0.58$, $P = 0.003$). However, no significant relation was found for Cadmium, Nickel in blood with IgE, IL-4 and IL-13 levels. Moreover, children from industrial areas showed significantly higher concentrations of IgE (mean = 146.44 pg/200land, $P < 0.001$), IL-4 (mean = 548.23 pg/200land, $P < 0.001$) and IL-13 (mean = 52.93 pg/200land, $P < 0.001$) versus Golestan and Kianpars.

Conclusion: Children residing in an industrial area with high concentrations of metals in PM_{2.5} had high metals in blood and high production of IgE, IL-4 and IL-13, reflecting an immune dysregulation and brisk inflammatory responses.

1. Introduction

Air pollution is an important health risk factor all over the world. Results of a global study demonstrated that air pollution was one of the top ten global health risk factors. According to the World Health Organization (WHO), in 2016 about 91% of the entire world's population resided in regions where air pollution was higher than WHO guideline

limits, also annually 4.2 million people die due to ambient air pollution [1]. Epidemiological studies indicated a significant relationship between ambient air particulate matter (PM) and hospitalization, mortality and respiratory diseases [2]. PMs as major pollutants are originated from roadway dust, coal combustion, oil combustion, biomass aerosol and vegetative detritus [3]. 40% of the PM mass in the atmosphere is attributed to fossil fuels [4].

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The ambient air PMs due to their micrometer aerodynamic diameters and prolonged floating time in the atmosphere have high adsorption capacity for scarce and hazardous atmospheric pollutants such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) [5]. Chemically, atmospheric PMs are mixtures of different hazardous, nonhazardous organic and inorganic materials [6]. They can easily go through the respiratory system and deposit in the lung and bronchial tubes. Inhaled metalloids that are bound to fine particulates pass to alveoli or pass through the lung wall into the bloodstream [7]. The result of the 2013 World Environmental Conference in Paris, France, showed that ambient air PMs, especially fine particles (PM_{2.5}) are the main reason for respiratory and cardiovascular system diseases [8]. Several studies indicate that in the industrial cities, metalloids inhalation bound to the PM_{2.5} is increasing [9,10]. Also, an increase in exposure to inhalation of heavy metals had a high significant relationship with lung function decrease in adults [11].

Allergic, atopic and other chronic diseases such as asthma are global illnesses that affect about 150 million children and adults worldwide [12]. Chronic diseases or syndromes are associated with an increase in pro-inflammatory biomarkers. For some reasons, children are more sensitive than adults to the harmful health effects of air pollution [13]. Physiological and biological responses to the chemical in children are differing significantly from adults [14]. Children spend more time in outdoor and have more daily activities; such as soccer and other games. This group is breathing 20 to 50 percent more air and air pollutions than adults [2]. Children don't show similar symptoms even when exposed to higher concentrations of air pollution. It is not known clearly if they ignore or don't feel the symptoms because of being preoccupied with physical activities (Michael T. Kleinman, 2000). Human's immune system is being developed in childhood. It is less poly-functional and produces fewer cytokines in comparison to maturity [15].

Several studies on electronic-waste recycling site in Guiyo, China, indicated a significant association between the increase of blood Pb, Cd, Cr and Mn amounts and cough and wheeze, also blood Pb > 5 µg/dl was significantly associated with asthma [16,17]. A cohort study carried out in New York City from 1998 to 2006 on children of up to 24 Months age, reported an increase in ambient air heavy metals (AAHM) and elemental carbon (EC) which were associated with the increase in the probability of wheezing and coughing respectively. Another study in Mexico City showed the negative impact of ambient air metals in brain injuries of young urbanites [18]. However, there was no significant relation between total atmospheric PM_{2.5} and wheezing or coughing [19]. In a study conducted by [20], an investigation about the effect of heavy metals and PM_{2.5} on Immunoglobulin-E (IgE), eosinophil, and wheezing in children diagnosed with respiratory diseases, showed that raising in the IgE production and lung disease are related to increasing atmospheric PM_{2.5}. IgE is an antibody produced by the immune system, which causes allergic reactions in the nose, lungs, throat, or on the skin. There are some relationships between asthma and IgE that appear to be independent of allergen sensitization [21]. Interleukin- 4(IL-4) and Interleukin-13 (IL-13) are proteins that participate in several immune regulatory and pro-inflammatory activation inductions, such as atopic inflammation and asthma [22]. In addition, IL-4 and IL-13 have some similar properties; both are secreted into plasma by Th2 lymphocytes and mast cells. These cytokines are overproduced and play important roles by inducing IgE synthesis in naive B-lymphocytes in atopic, asthma and allergic reactions [23].

Several studies have investigated the effect of physicochemical characteristics of ambient air PM on inflammation markers in peripheral blood and bronchoalveolar pulmonary of rats [24] and increased pro-inflammatory cytokines IL-6 and IL-8 in lung cells [25].

Up to now, no study has been done about the effect of the ambient air PM_{2.5} bound metals on children's pro-inflammatory cytokines IL-4 and IL-13 and IgE. This work was conducted for the first time in Ahvaz, Iran as a highly industrialized city to fulfill this gap. this study was aimed to investigate: (1) determination of heavy metal levels in PM_{2.5} in different

areas in Ahvaz city as the capital of Khuzestan province; (2) determination of atmospheric heavy metals in industrial, vehicle traffic and reference areas and its relation to schoolchildren blood heavy metals; (3) investigation the effects of heavy metals in the ambient air and children blood on the synthesis of IL-4 and IL-13 and IgE.

2. Methodology

2.1. Description of the study area

Ahvaz city is located in the south-west of Iran and has a tropical climate with a limited downfall period. Characteristics of the region are high humidity and temperature during the year, with strong surface winds. Ahvaz has a surface area of about 220 km² and a total population of about 1.3 million [26] with an about density of 4815.73 people per square kilometer [27]. Because of the existence of many industrial factories, oil extraction and refineries, petrochemical industries and power plants, the region is industrially polluted. In 2013, WHO named Ahvaz as the most air-polluted city in the world [28]. Fig. 1 illustrates the prevalence of wind direction and wind speed during this study. Wind direction and speed data were obtained from Iran Metrological Organization. The prevailing wind direction is from northwest to southeast. The humidity, temperature and pressure were evaluated by a digital device (Humidity/Barometer/Temperature PHB-318) during this study. Air humidity during summer was within 42 to 100% and in the winter was 32 to 75% and air temperature during summer and winter were 46 °C/31 °C and 22 °C/12 °C respectively.

2.2. PM Sampling site description

PM_{2.5} samples were collected from three locations in Ahvaz: 1) Health center No. 18 in Padad region (industrial area) (31°17'32.49"N, 48°42'18.01"E) which is located in the east of Ahvaz and adjacent to steel, carbon black, pipeline manufacturing industries and Karun industrial area. 2) Department of Environmental Protection of Khuzestan in Golestan region(vehicle traffic area) (31°18'30.80"N, 48°38'37.00"E) located in southwest of Ahvaz which industries including pipe-rolling, steel and plant, pipeline manufacturing are located in the southern part of the site. 3) Rajaei dormitory located in Kianpars region (reference area) (31°20'36.45"N, 48°41'5.00"E). Fig. 1 shows the location of sampling sites and wind rose plots during the study demonstrated in (Fig. 2).

2.2.1. PM Sampling

The high volume (HIVOL) samplers (Tisch- USA) coupled with glass fiber filters (grade G653 and media 8 by 10 inch or 203 × 254 mm) for PM and heavy metals sampling [29] were installed 8-15 m above the ground to minimize the effects of natural features, such as trees, buildings and other barriers on sampling. The average flow of the pumps was 1.3 m³/min (50 ft³/min) and the total pump working time per sampling was 24 h. The sampling method and HIVOL calibration and control were done according to the United States Environmental Protection Agency Method IO-2.1 ([5]; [30]). A total of 72 PM_{2.5} 24 h samples (24 for each site) were collected in the cold season from November to January 2018 (12 samples at each site) and warm season from May to August 2019 (12 samples at each site). Taken samples were stored at -20 °C until digestion. The filters were placed for 48 h before and after sampling in a desiccator and weighted [31].

2.3. Study participants description

A total of 123 children aged 6 to 9 years old were sampled twice, once in each cold and warm season. All of the participants based on income level belonged to the middle-class family and within a 500-meter radius of PM_{2.5} sampling sites. Students were selected from schools (49 male students from Padad, 49 male students from Golestan

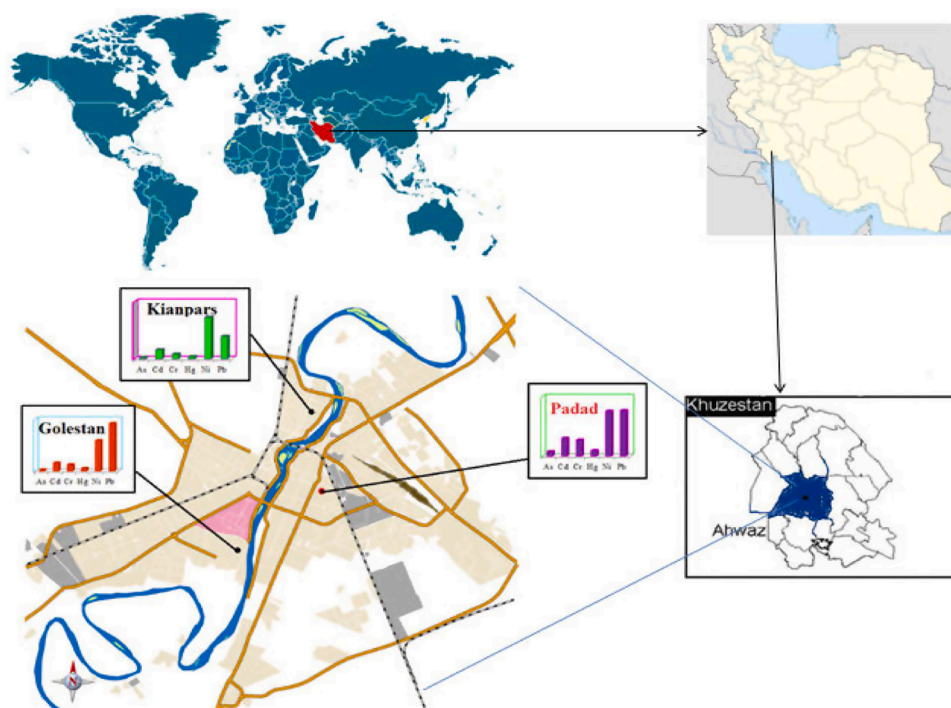


Fig. 1. Map of location study (ambient air and blood sampling) area.

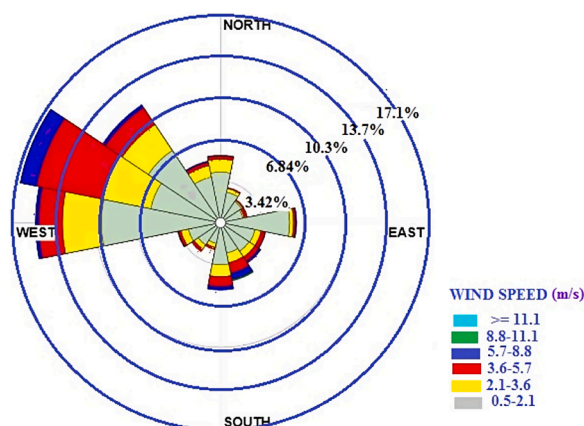


Fig. 2. Wind rose plots during the study.

and 25 male students from Kianpars). The most important inclusion criteria for children to be included in the study were: 1) No history of allergies, respiratory and other chronic illnesses; 2) No colds during one month to sampling; 3) No history of respiratory and other chronic illnesses in parents; 4) Lack of parental smoking; 5) A history of five years of living in the chosen sampling area; 6) School and home located inside the sampling region; 6) belonging to a same socioeconomic group and have a similar dietary intake

Participants were joined into a WhatsApp group to receive dietary information, also height and weight for each participant were measured to calculate body mass index (BMI, in kg/m^2) [32].

2.4. Blood sampling and preparation procedure

On an appointed day, participants were taken the blood samples. Firstly, written informed consent was obtained from the parents. 7 ml of venous blood obtained from every participant by two skillful nurses using vacutainer needles. Previous studies have shown these needles do

not add Cr, Cd, Mn, Ni and Pb to the blood [33]. Each blood sample was divided into two parts (3 and 4 ml), isolated and thereafter transferred into lead-free tubes. Blood sample tubes with a volume of 3 ml were centrifuged at room temperature for 10 min at 3000 RPM [34], aliquoted (200 μL serum supernatant was taken and discharged into microtube), and stored at -80°C until the IgE, IL-4 and IL-13 analyses. Blood sample tubes with a volume of 4 ml were stored at -25°C until the analysis of the heavy metals [35,36].

2.5. $\text{PM}_{2.5}$ -bound metals analysis

To determine the heavy metals in ambient air $\text{PM}_{2.5}$, the wet digestion method was applied. In the first step, one quartile of the fiberglass filters was cut into fragments by a Teflon scissor and put into the Teflon (PTFE) tubes. Then a mixture of 6 mL HNO_3 (65%), 2 mL HClO_4 (70%) and 1 mL HF (48%) was added into the Teflon digestion vessel, the vessel lid was closed tightly. After completing the digestion process, the mixture was placed for 4 h at 170°C . At the end of the due time and after cooling of the vessels, the lid of Teflon vessels was opened and the solution was dried on a hot plate at 95 to 100°C for 30 min to 1 h. Following that, a mixture of concentrated nitric acid and Milli- Q water 18.2 ($\text{M}\Omega\cdot\text{cm}$) was added to the dried solution (the ratio 1:9) and the obtained solution was shaken by a shaker (Ika Ks 130 basic) for 15 min. At last, the digested solution was filtered through a Whatman Grade 42 filter into a 50 mL Falcon Conical Tubes and brought to a volume of 25 mL by distilled water (Milli- Q). The tubes were kept at 4°C until samples were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) [37,38]. Blank solutions were prepared at the same time.

2.6. Extraction of heavy metals from whole blood samples

4 mL of the whole blood sample was taken into a digestion Pyrex flask. Then 20 mL of distilled concentrated HNO_3 (72%) was added to this sample and the content was heated for 30 min at 80°C on a hot plate. After cooling, 6 mL of HClO_4 (70%) was added into the matrix and heated again at 250°C until white fume was evolved. At last, the

vapor became clear and the solution was almost colorless. The solution was cooled and brought to 10 ml by adding de-ionized water (specific resistance $\geq 18.2 \text{ M}\Omega\cdot\text{cm}$). Some blank samples were prepared to increase Accuracy Criteria (AC) and Precision. Samples were kept at 4°C until analyzing by ICP-OES [39,40].

2.7. Heavy metals analysis

The digested blood and PM samples were injected into the ICP-OES (Spectro Arcos) (capture in the 130-770 nm wavelength range, RF generator: 1400 W, Plasma Gas Flow Rate: 14.5 l/min, Pump Rate: 30 RPM), instrument to identify 6 elements of Arsenic (As), Cadmium (Cd), Chrome (Cr), Mercury (Hg), Nickel (Ni) and Lead (Pb). Limit of detections (LOD) and limit of quantitations (LOQ) were calculated based on the Center for Disease Control and prevention (CDC) guideline [41]. LOD, LOQ, blank samples (BS), the deionized water (DW) and element detection wave length (WL) specification for all elements are indicated in (Table 1).

2.8. Cytokines (IL-4, IL-13) and Immunoglobulin E (IgE) measurements

IgE, IL-4 and IL-13 factors were measured by enzyme-linked immunosorbent assay (ELISA). To defrost serum samples those were taken from the freezer (-80°C) and kept at room temperature for 30 min. For uniformity of cell supernatants, microtubes were shaken several times. Preparation of IgE, IL-4 and IL-13 were fulfilled using eBioscience sandwich ELISA Kits - Germany (product codebase Kit: 15501647; IgE: 15521757; IL-4: 15511777; IL-13) according to the manufacturer's protocol. Lastly, an ELISA Rader (BioTek; USA; ELx808) was used for sample determination at 450 nm as Measurement and 620 nm as Reference wavelength [42].

2.9. Statistical analysis

The Excel and SPSS v.21 and STATA-15 software were applied for data analysis. Firstly, the mean and standard deviation were calculated for all data. Shapiro Wilks test, Kruskal-Wallis (nonparametric test) and spearman correlation were used to evaluate the normality of the data and significance among sampling areas and to determine the relationship of heavy metals in $\text{PM}_{2.5}$ with children blood. Mixed Model analyses by controlling effects of BMI, season and region were used to study the relation between blood heavy metals and three pro-inflammatory biomarkers (IgE, IL-4 and IL-13) in children.

3. Results

3.1. Ambient air $\text{PM}_{2.5}$ concentration

The box plot for ambient air $\text{PM}_{2.5}$ mass for three studied sites has been shown in Table 2. A total of 72 PM 24 h samples (24 for each site) were collected for measurement of the concentration of $\text{PM}_{2.5}$ and heavy metals. The $\text{PM}_{2.5}$ mean concentrations were obtained $68.56 \mu\text{g}/\text{m}^3$ in

Table 1
The LOD, LOQ, DW and BS of ICP-OES for heavy metals analysis.

Element	LOD ¹ (ng mL ⁻¹)	LOQ ² (ng mL ⁻¹)	DW ³ (ng mL ⁻¹)	BS ⁴ (ng mL ⁻¹)	WL(nm)	Recovery (%)
As	0.358	1.074	<0.358	<0.358	189.042	97-110
Cd	0.49	1.47	<0.49	1.4	228.802	71-87
Cr	0.96	2.88	<0.96	3.78	267.716	96-120
Hg	0.351	1.053	<0.351	<0.351	253.652	88-99
Ni	0.286	0.858	0.356	2.93	231.604	88-98
Pb	2.2	6.6	<2.2	4.3	283.305	84-101

¹LOD: limit of detection; ²LOQ: limit of quantitation; ³DW: deionized water; ⁴BS: blank sample; WL: wave length

Table 2

$\text{PM}_{2.5}$ bound heavy metal, blood heavy metal levels and the asthma biomarkers of children from Padad, Golestan and Kianpars.

Parameter	regions			P- Value
	Padad	Golestan	Kianpars	
Heavy metals in $\text{PM}_{2.5}$	N = 24	N = 24	N = 24	
$\text{PM}_{2.5}$ ($\mu\text{g}/\text{m}^3$)	68.56 $\pm$ 11.02	67.05 $\pm$ 12.26	55.30 $\pm$ 14.74	<0.001
As (ng/ m^3)	99.03 $\pm$ 37.17	40.36 $\pm$ 9.3	17.15 $\pm$ 10.61	<0.001
Cd (ng/ m^3)	5.01 $\pm$ 0.84	4.80 $\pm$ 0.94	2.38 $\pm$ 0.32	<0.001
Cr (ng/ m^3)	15.76 $\pm$ 1.32	14.83 $\pm$ 1.32	11.99 $\pm$ 0.61	<0.001
Hg (ng/ m^3)	4.25 $\pm$ 0.95	3.31 $\pm$ 0.28	2.24 $\pm$ 0.2	<0.001
Ni (ng/ m^3)	12.30 $\pm$ 1.22	8.76 $\pm$ 0.93	6.12 $\pm$ 1.03	<0.001
Pb (ng/ m^3)	105.34 $\pm$ 28.3	143.26 $\pm$ 29.45	86.34 $\pm$ 16.34	<0.001
Blood heavy metals	N = 49	N = 49	N = 25	
BAAs (ng/mL)	18.7 $\pm$ 14.9	7.6 $\pm$ 0.78	6 $\pm$ 5.2	0.003
BAAs > 12 ng/mL n, %	27 (55)	8 (16.3)	2 (8)	
BCd ($\mu\text{g}/\text{dL}$)	5.53 $\pm$ 2.47	3.15 $\pm$ 1.63	2.14 $\pm$ 1.11	<0.001
BCd > 5 $\mu\text{g}/\text{dL}$ n, %	31 (63.2)	9 (18.3)	1 (4)	0.001
BCr ($\mu\text{g}/\text{dL}$)	4.89 $\pm$ 2.99	2.42 $\pm$ 1.54	1.18 $\pm$ 0.69	<0.001
BCr > 3 $\mu\text{g}/\text{L}$ n, %	37 (75.5)	17 (34.6)	0 (0)	0.033
BHg (ng/mL)	16 $\pm$ 14	8.2 $\pm$ 7.3	5.3 $\pm$ 6.1	<0.001
BHg > 10 ng/mL n, %	34 (69.3)	18 (36.7)	9 (36)	0.07
BNi ($\mu\text{g}/\text{dL}$)	9.48 $\pm$ 6.12	6.77 $\pm$ 3.51	5.61 $\pm$ 3.12	0.002
BPb ($\mu\text{g}/\text{dL}$)	11.71 $\pm$ 9.13	12.7 $\pm$ 8.51	5.72 $\pm$ 3.29	<0.087
BPb > 5 $\mu\text{g}/\text{dL}$ n, %	40 (81.6)	40 (81.6)	9 (36)	1.000
blood cytokines and Immunoglobulin E	N = 49	N = 49	N = 25	
IgE (pg/200 μL)	146.34 $\pm$ 123.77	90.89 $\pm$ 96.74	56.48 $\pm$ 56.19	<0.001
IL-4 (pg/200 μL)	548.23 $\pm$ 468.93	229.5 $\pm$ 280.34	156.73 $\pm$ 193.87	<0.001
IL-13 (pg/200 μL)	52.93 $\pm$ 45.86	26.72 $\pm$ 32.05	15.69 $\pm$ 16.42	<0.001

Padad, $67.05 \mu\text{g}/\text{m}^3$ in Golestan and $55.30 \mu\text{g}/\text{m}^3$ in Kianpars areas. The results of this study demonstrated that $\text{PM}_{2.5}$ concentrations were exceeded the WHO air quality guideline levels.

3.2. Ambient air heavy metals levels

Ambient air heavy metals concentrations during the investigated seasons have been shown in Table 2, the mean concentrations of As, Cd, Cr, Hg and Ni in the industrial region (Padad) were higher than two other sites. This indicated emissions from the nearby industrial region had a large role in the emission of heavy metals. However, the Pb mean concentration in the vehicle traffic area (Golestan) was higher than two other sites. At the reference site (Kianpars) the heavy metals and $\text{PM}_{2.5}$ concentrations were lower than two other sites.

3.3. Heavy metals concentration in children venous blood

Table 2 shows the average concentration levels of blood arsenic (BAAs), blood chromium (BCr), blood cadmium (BCd), blood mercury (BHg), blood nickel (BNi) and blood lead (BPb) in children. The BAAs, BCd, BCr, BHg and BNi of children blood samples from Padad region were higher than those from Golestan and Kianpars regions, and, the BPb was higher in Golestan. The BAAs, BCr and BHg of children from Padad ($P < 0.001$) were higher than those of two other regions, however, there were no significant differences in BCd, BNi and BPb ($P > 0.05$) among

the regions. Abnormal BAs concentrations (>12 ng/mL) were observed in 55, 16.3 and 8% of children From Padad, Golestan and Kianpars respectively. Furthermore, 69.3; 36.7 and 36 % children had (BHg > 10 ng/mL), and 75.5; 34.6 and 0 % had (BCr > 3 μ g/L) in Padad, Golestan and Kianpars regions respectively ($P < 0.05$). The threshold values of BPb > 5 μ g/dL, BCD > 5 μ g/L and Bni > 10 ng/mL indicated no significant differences among regions' results ($p > 0.05$).

3.4. Pro-inflammatory biomarkers concentration in children

Pro-inflammatory biomarkers were measured in order to determine the allergic and inflammatory status in children. Table 2 has indicated the average concentration of IgE, IL-4 and IL-13 pro-inflammatory biomarkers in children blood serum of the ages (6 to 9 olds) in different sampling areas. The results showed IgE ($P < 0.001$), IL-4 ($P < 0.001$) and IL-13 ($P < 0.001$) of Padad children were higher than those from Golestan and Kianpars regions. Fig. 3 shows the relationship of IgE with IL-4 and IL-13 in three sampling regions. The results indicated that by

increasing the over-synthesis of pro-inflammatory factors, IgE relationship with IL-4 and IL-13 has increased.

3.5. Association between heavy metal levels in ambient air and children blood

Table 3 indicates the association between ambient air and blood heavy metals. The results of Spearman correlation showed a significant relationship between AAs-BAs, ACD-BCd, ACr-BCr, AHg-BHg, ANi-BNi and APb-BPb ($P < 0.001$). Stronger correlations were evident between ACD-BCd, ACr-BCr and AAs-BAs respectively.

3.6. Association between blood heavy metal levels and pro-inflammatory biomarkers

The adjusted regression mixed model results showed a relation of blood As, Cd, Cr, Hg, Ni and Pb with IgE, IL-4 and IL-13 levels. Table 4 indicates a significant relationship between IgE and blood As ($P <$

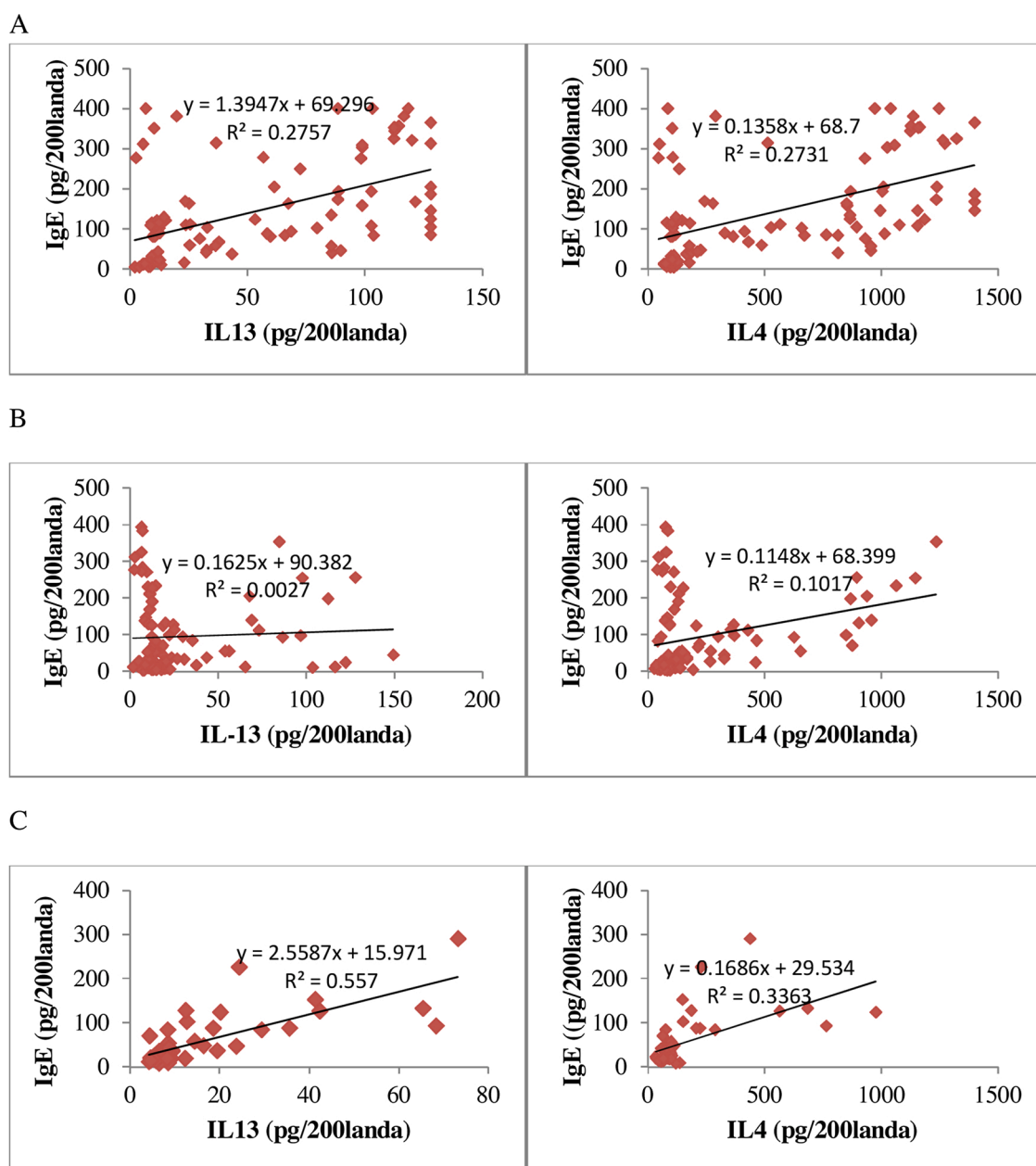


Fig. 3. The relation of IgE with IL-4 and IL-13 in three regions (A) Padad (B) Golestan (C) Kianpars.

Table 3

Spearman correlation coefficients among heavy metals in the atmosphere and children's blood.

	BAs	BCd	BCr	BHg	BNI	BPb	AAs	ACd	ACr	AHg	ANi	APb
BAs	1											
BCd	0.59	1										
BCr	0.75	0.61	1									
BHg	0.65	0.42	0.66	1								
BNI	0.28	0.27	0.34	0.36	1							
BPb	0.37	0.24	0.4	0.27	0.1	1						
AAs	0.48	0.58	0.59	0.41	0.32	0.17	1					
ACd	0.47	0.57	0.59	0.43	0.29	0.29	0.9	1				
ACr	0.39	0.50	0.52	0.37	0.28	0.32	0.77	0.89	1			
AHg	0.37	0.49	0.51	0.32	0.29	0.25	0.87	0.85	0.85	1		
ANi	0.42	0.53	0.58	0.39	0.28	0.34	0.82	0.96	0.96	0.83	1	
APb	-0.09	-0.05	-0.01	-0.01	-0.03	0.27	-0.14	0.17	0.21	0.29	0.27	1

BAs: blood arsenic; BCd: blood cadmium; BCr: blood chromium; BHg: blood mercury; BNI: blood nickel; BPb: blood lead.

AAs: atmospheric arsenic; ACd: atmospheric cadmium; ACr: atmospheric chromium; AHg: atmospheric mercury; ANi: atmospheric nickel; APb: atmospheric lead.

Table 4

Adjusted regression mix model analysis for the relation between blood heavy metals with IgE, IL-4 and IL-13.

Model		IL-4				IL-13				IgE			
		coefficient	95%CI		P value	coefficient	95%CI		P value	coefficient	95%CI		P value
BPb		7.16	3.31	11.01	<0.001	0.58	0.19	0.96	0.003	0.92	0.11	1.73	0.025
Region (3 = base)	1	235.11	86.08	348.15	0.002	24.76	8.33	41.2	0.003	69.79	20.07	119.51	0.006
	2	-8.2	-152.52	136.13	0.911	4.52	-11.36	20.41	0.577	24.01	-23.87	71.89	0.326
Season*		58.96	42.17	93.74	0.001	4.66	1.26	8.06	0.007	12.71	5.90	19.52	<0.001
BMI		104.29	63.92	144.63	<0.001	8.25	3.81	12.70	0.000	13.37	-0.05	26.78	0.051
BAs		172.1	142.1	202.1	<0.001	14.84	11.69	17.99	<0.001	26.59	19.82	33.35	<0.001
Region (3 = base)	1	100.8	-8.83	210.41	0.072	13.01	-0.273	26.3	0.055	48.25	2.29	94.21	0.040
	2	25.32	-76.24	126.88	0.625	7.17	-5.24	19.59	0.258	27.98	-15.67	71.65	0.209
Season*		17.72	-17.8	53.25	0.328	0.972	-2.32	4.26	0.563	5.65	-0.41	11.71	0.068
BMI		67.04	37.3	96.8	<0.001	5.00	1.40	8.59	0.006	7.32	-5.07	19.72	0.247
BCd		10.04	-4.88	24.95	0.187	1.41	-0.08	2.91	0.064	-2.3	-5.34	0.75	0.139
Region (3 = base)	1	232.37	7.41	393.32	0.005	22.57	5.56	39.59	0.009	81.20	29.62	132.78	0.002
	2	28.42	-119.46	176.3	0.706	6.90	-8.80	22.61	0.389	32.25	-16.39	80.90	0.194
Season*		86.01	54.01	118.01	<0.001	6.86	3.70	10.02	<0.001	16.18	10.14	22.23	<0.001
BMI		114.99	73.68	156.29	<0.001	9.07	4.68	13.46	<0.001	15.12	1.51	28.75	0.030
BCr		75.27	60.75	89.79	<0.001	5.22	3.66	6.79	<0.001	10.38	7.14	13.61	<0.001
Region (3 = base)	1	29.07	-94.34	152.48	0.64	10.81	-4.37	25.99	0.163	41.07	-6.32	88.46	0.089
	2	-43.31	-154.49	67.86	0.44	2.63	16.51	-11.25	0.711	18.73	-25.82	63.29	0.410
Season*		12.25	-22.17	46.67	0.485	1.74	-1.72	5.21	0.325	6.02	-0.51	12.56	0.071
BMI		76.43	44.62	108.24	<0.001	6.48	2.52	10.43	0.001	9.44	-3.12	22.01	0.141
BHg		108.09	80.08	136.1	<0.001	7.96	5.06	10.86	<0.001	13.11	7.25	18.96	<0.001
Region (3 = base)	1	159.07	30.1	288.05	0.016	19.37	4.47	34.27	0.011	60.80	12.24	109.36	0.014
	2	8.76	-112.4	129.9	0.887	6.11	-7.94	20.17	0.394	26.41	-19.88	72.71	0.263
Season*		40.05	5.45	74.63	0.023	3.47	0.027	6.92	0.048	10.62	4.2	17.05	0.001
BMI		107.21	73.21	141.2	<0.001	8.57	5.06	10.86	<0.001	13.81	0.82	26.79	0.037
BNI		5.44	-1.31	12.21	0.114	0.07	-0.61	0.76	0.828	-0.14	-1.57	1.3	0.852
Region (3 = base)	1	242.44	86.36	398.53	0.002	26.89	10.06	43.72	0.002	74.24	23.65	124.82	0.004
	2	31.49	-116.03	179.01	0.676	8.19	-7.74	24.14	0.313	30.16	-18.06	78.38	0.220
Season*		83.35	51.31	115.38	<0.001	6.82	3.65	9.98	<0.001	16.25	10.07	22.44	<0.001
BMI		117.67	76.31	159.02	<0.001	9.24	4.77	13.71	<0.001	14.85	1.37	28.32	0.031

* Season has been classified into two layers, in the mix model we have used the ratio of cold season to warm season.

0.001), Cr ($P < 0.001$), Hg ($P < 0.001$), and Pb ($P < 0.001$), IL-4 and blood As ($P < 0.001$), Cr ($P < 0.001$), Hg ($P < 0.001$), and Pb ($P = 0.003$), and between the IL-13 and blood As ($P < 0.001$), Cr ($P < 0.001$), Hg ($P < 0.001$), and Pb ($P = 0.025$). The model didn't show any significant relationship between IgE, IL-4 and IL-13 with BCd and BNI. In this model, region, BMI and season were used as the control group to study the impact of each blood heavy metal on pro-inflammatory biomarkers.

4. Discussion

Present study was conducted for the first time on evaluation and association among ambient air heavy metals, blood heavy metals and (IgE, IL-4 and IL-13) responses as allergic and pro-inflammatory biomarkers in children in Ahvaz, as a highly industrialized city in Iran. As the results of Table 2 show achieved daily and annually concentration of

PM_{2.5} in each site exceeded the WHO daily (50 µg/m³) and annually (10 µg/m³) standard reference values [5]. PM_{2.5} exposure levels from Padad region were higher than those from Golestan and Kianpars regions. High concentrations of PM_{2.5} in Ahvaz are related to vehicular traffics, vicinity to many industrial factories and Proximity to local and external dust sources [43]. In 2012 high-level concentrations of PM have been reported in Ahvaz [26], which shows that Iran should develop a strategic plan to improve air quality in Ahvaz.

We found that the As, Cd, Cr, Ni and Hg levels in ambient air and children blood from Padad with significant difference ($p < 0.001$) were higher than those of Golestan and Kianpars areas. These might be due to a large number of industries' (steel, carbon black, pipe production, oil refinery, reactor production, etc.) proximity to this region.

Pastuzka reported that the As, Cd, Cr, Hg, Ni and Pb concentrations were higher in industrial sites [44]. Results of a study in Singapore showed that metallurgical industries, diesel vehicles and industrial

sources with fossil fuel combustion emit Cd, Cr, Co and Ni into the atmosphere [45]. In a study, road dust sample analyses in Ahvaz city showed that the heavy metal ranges across different sites were from 4.35–3577.06 mg kg⁻¹ for Pb, 20–4580 µg kg⁻¹ for Hg, 32.40–1220.40 mg kg⁻¹ for Ni, 4.40–36.10 mg kg⁻¹ for Co, 22.40–312.40 mg kg⁻¹ for Cr, < 0.01–721.29 mg kg⁻¹ for Cu, 1.80–51.60 mg kg⁻¹ for As, 0.15–78 mg kg⁻¹ for Cd. The results of this study have shown that in industrial road dust of Ahvaz, the concentration of heavy metals were higher than those levels of the reference area [43]. In addition, the average levels of Hg, Cr, V, Sb, Co, Mn, Ni, Zn, Pb, Cu and Mo were higher than world soil values [46]. However, this work showed the ambient air and BPb concentrations in Golestan were higher ($p < 0.001$) than Padad. It could be due to high traffic jam of vehicles in Golestan. A study has shown that Pb is emitted from gasoline and diesel fuels [47].

Table 3 shows a significant relationship between the blood heavy metals and ambient air heavy metals. The emitted heavy metals can float in the atmosphere or settle on the soil surface and enter children's bodies through respiration and direct contact. Children's outdoor play patterns in Ahvaz city indicated that children spend most of their after-school hours in outdoor playing different hobbies [48]. Therefore, children are highly susceptible to inhalation and contact with these contaminants. Table 4 shows that the impact of heavy metals on the increase of pro-inflammatory biomarkers in the Padad (industrial) area is greater than Golestan (vehicle traffic) and Kianpars (reference) areas, which can be due to the high concentration of hazardous and heavy metal pollutants in Padad region. Cao et al. have shown that blood Cr, Mn, Pb, As and Sb levels in children who live in the neighborhood of a coking plant were very high, with the non-carcinogenic risks of 3 to 10 times higher than the admissible levels (1.0×10^{-6}) [35]. Other studies on e-waste areas in China reported that due to the open burning mismanagement of electronic waste sites, a significant correlation between heavy metal levels in PM_{2.5} and children's blood was shown [17,34]. In addition, as shown in Table 2 the serum IgE, IL-4 and IL-13 levels of children from padad region were higher than two other studied areas which this is due to increased levels of BAs, BCB, BCr, BHg, BNi. Several studies have shown that the IL-6, IL-8 and IgE levels of the human population living in industrial areas were high [25,42]. Table 4 showed a significant relation of BAs, BCr, BHg and BPb with IgE, IL-4 and IL-13 levels, but no significant relationship was observed between IgE, IL-4 and IL-13 and BCd and BNi.

The ambient air As average levels from Padad, Golestan and Kianpars regions were 99.3, 40.36 and 17.15 (ng/m³) respectively. The WHO guideline estimate of arsenic lifetime risk is 1.5×10^{-3} for 1 µg/m³ in ambient air [49]. We found the lifetime risk associated with atmospheric arsenic in Padad, Golestan and Kianpars were 1.5×10^{-4} , 6.1×10^{-5} and 2.6×10^{-5} respectively. MAYO clinic laboratories have reported that the reference value of blood arsenic concentration in all ages is 0–12 ng/mL and blood arsenic level upper than 12 ng/mL indicates significant exposure [50]. Current study showed 55.1%, 39.2% and 8% of the Padad, Golestan and Kianpars children had BAs > 12 ng/mL, and the average concentration of arsenic in the blood of children was 18.73, 7.61 and 6.2 ng/mL respectively. It showed that Arsenic pollutant in Padad is a very serious problem. We found when BAs > 25 ng/mL the IgE, IL-4 and IL-13 levels increased severely.

In this work, chromium had a significant relation with synthesis of IgE, IL-4 and IL-13 in children. Due to the results of mean BCr from studied areas in Table 2, nearly 74% of the children from Padad compared to 35% of Golestan and 6% of Kianpars children had BCr > 3 µg/L (the normal range of blood chromium is 1–3 ng/mL) [17]. Our results indicated that in children living in high levels of ambient air chromium, the synthesis of IgE, IL-4 and IL-13 were increased. To date, no study has investigated the effect of chromium on serum IgE, IL-4 and IL-13 in the human, however, few researches have shown the effect of chromium on levels of serum IL-2 and IL-6 in pigs [51]. An in vitro experimental research showed that chromium could increase the level of IL-4 and IFN-γ [52]. In addition, Richard Hayse reported a significant association between chromium concentrations in ambient air and lung

cancer in industrial workers [53]. In this work a significant association was observed between BCr and IgE, IL-4 and IL-13, as when the BCr > 3 ng/dl the IgE, IL-4 and IL-13 were synthesized extraordinarily.

In the present study the average concentration of BHg in Padad children was > 16 ng/mL, however for Golestan and Kianpars this number was < 9 and < 6 ng/mL respectively. In addition, about 71% of the Padad Children had BHg > 10 ng/mL. MAYO clinic laboratories have reported that the normal level of BHg for everyone is < 10 ng/mL. A significant association was observed in our study between children BHg concentration with IgE, IL-4, and IL-13. Moreover, the average concentration of ambient air Hg was < 4.5 ng/m³ which is less than the WHO regional office for Europe air quality guidelines of mercury in outdoor (0.005–0.01 µg/m³) [49]. Nuttall reported that high doses of mercury cause acute symptoms and repeated exposures to low doses can produce chronic symptoms over time [54]. Wo et al. found that increasing the concentration of blood HgCl₂ induces and enhances the IL-4 gene's activities in the rat mast cell line RBL-2H3 [55]. Due to the high concentration of BHg in padad children, it seems necessary to continue monitoring the mercury sources and exposure paths.

Table 2 showed that lead concentration in the children's blood and ambient air were higher in Golestan as a vehicle traffic region, also Table 3 indicated a significant relationship between ambient air lead and children's blood lead. This result might be due to burning low quality vehicle fuel in Ahvaz. Pb is the only heavy metal registered in Iran's national atmosphere quality standards with an annual average of 0.5 µg/m³. In the 1990s due to local regulations the use of leaded gasoline reduced in the developed countries and in 2002, the consumption of leaded gasoline in Iran was prohibited [5]. However, Our study showed the Pb concentration in roadway and vehicle traffic areas was higher than other areas. Several studies have indicated that resuspension of soil previously polluted with leaded fuel was the main source of Pb enrichment in many urban areas [47,56]. The ambient air Pb concentration in this study was < 0.5 µg/m³, although nearly 43% of the children in our study had BPb > 5 µg/dl [17]. In our study, a significant relationship was observed between BPb and IL-4 ($P > 0.001$), IL-13 ($P > 0.003$) and IgE ($P > 0.025$).

In this work, the attributed risk of nickel and cadmium in the ambient air according to the EPA guideline [49] was 2.16×10^{-5} and 9×10^{-6} respectively. Besides, about 43% and 100% of the participants had BCd > 5 µg/L (the toxicity threshold level for blood Cadmium) and BCd > 0.2 µg/L (the lowest toxicity threshold for blood cadmium) respectively [57]. In our results no significant relationship was observed between BNi and BCd and IL-4, IL-13 and IgE.

The levels of PM_{2.5} bound As, Cd, Cr, Hg, Ni and Pb from the industrial area in the present study were higher than the several Asian and European industrial cities [29,37]. Therefore, highly industrial cities such as Ahvaz, needs strict and controlling local policies.

Our study showed living in the industrial area was associated with increased concentrations of BAs and BCr, and BHg, and accelerated synthesis of pro-inflammatory biomarkers (IL-4, IL-13 and IgE). According to the linear regression analysis, children with BAs > 12 ng/mL, BCr > 3 µg/L, BHg > 10 ng/mL and BPb > 5 µg/dl were more susceptible to high levels of IgE, IL-4 and IL-13. Nevertheless, in children with high levels of BNi and BCd, the increased concentrations of IgE, IL-4 and IL-13 weren't observed.

In the present study, children from Padad had higher IgE, IL-4 and IL-13 compared to volunteers from the two other areas, which may point out that Padad children from were more at risk of allergic, atopic, asthmatic and other chronic diseases. Several studies have shown an association between pro-inflammatory biomarkers (IgE, IL-4 and IL-13) and allergic, atopic dermatitis, skin fibrosis and asthmatic diseases [58–61]. Fig. 3 indicated the relation of IgE with IL-4 and IL-13 in sampling areas. According to the figure the concentration of IgE, IL-4 and IL-13 in the reference region was lower. In this region, because of low levels of blood metal(loid)s, the rate of pro-inflammatory biomarker synthesis in plasma is lower and the IgE relationship with IL-4 and IL-13

is more meaningful. In Golestan area, the pro-inflammatory levels in children were increased, and due to abnormal synthesis of these factors the IgE relationship with IL-4 and IL-13 decreased. In Padad region, the IgE relationship with IL-4 and IL-13 has increased significantly again. This phenomenon might be due to the sharp increase in the synthesis of pro-inflammatory biomarkers, in which the precision of this factor should be surveyed. Several studies demonstrated that living in industrial areas poses harmful long-term effects on the health of the present and future generations [62,63]. In addition, several studies showed the role of IgE, IL-4 and IL-13 secretion on allergic patients, childhood asthma and other chronic diseases [22,64].

It is worth mentioning that other confounder factors such as anionic and cationic ions, polychlorinated biphenyl (PCBs), polycyclic aromatic hydrocarbon (PAHs) and other elements can impact on lung function and respiratory symptoms through epigenetic mechanisms. Several studies have reported that PAHs exposure was found in industrial region [47,65], also some studies found that there was a correlation between heavy metals and PAHs in industrial zones [66,67] and blood of children [17]. Furthermore, numerous studies have shown a significant association between PAHs and allergic, lung function and chronic disease [67, 68].

5. Conclusions

This was the first study that simultaneously surveyed the effect of ambient air PM_{2.5}-bound metals on blood metal(loid)s, and children pro-inflammatory lung biomarkers (IL-4, IL-13 and IgE) in industrial, vehicle traffic and reference areas. Our findings suggested that Ar, Cd, Cr, Hg, Ni levels in PM_{2.5} and blood were higher in the industrial exposed area compared with the vehicle traffic and reference areas and there was a significant relationship between Ar, Cr, Hg and BPb level and IgE, IL-4 and IL-13. Our results indicate the children residing at industrial areas are at very high risk of the allergic, asthmatic, atopic diseases, and early death.

CRediT authorship contribution statement

Amir Zahedi: Conceptualization, Methodology, Investigation, Resources, Writing - Original Draft. **Mohammad Sadegh Hassanvand:** Conceptualization, Methodology, Investigation, Writing - Original Draft. **Nematollah Jaafarzadeh:** Conceptualization, Methodology, Resources, Investigation, Supervision, acquisition, Project administration. **Ata Ghadiri:** Methodology, Investigation, Resources, Review & Editing. **Mansour Shamsipour:** Validation, Formal analysis. **Mohamad Ghasemi Dehcheshmeh:** Methodology, Resources

Declaration of Competing Interest

The authors declare they have no conflict of interest in preparation of the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jtemb.2021.126826>.

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A) Calculation for PM2.5

PMs Concentration was obtained by following equation

$$PM\left(\mu\frac{g}{m^3}\right)=\frac{(W_f - W_i) * 10^6}{V}$$

Where, W_f and W_i are the final & initial weight of the filters. V shows the volume of air sampled $V = (\text{elapsed time} * 60 * \text{flow rate})$, and 10^6 is a factor to convert grams to micrograms(1).

B) Calculation for ambient air metal(loid)s

To determine the heavy metals in ambient air PM_{2.5}, the wet digestion method was applied and samples were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Results were obtained in part per billion (ppb) and converted to (ng/m³) per liter using the following equation.

Ambient air PM_{2.5}-bound heavy metals concentration was obtained by following equation:

$$PM\left(n\frac{g}{m^3}\right)=\frac{C}{V}$$

Where, C is the ambient air PM_{2.5}-bound heavy metals concentration (ppb) these concentrations are obtained by the ICP-OES. V shows the volume of air sampled $V = (\text{elapsed time} * 60 * \text{flow rate})$.

Particles enter the atmosphere from various natural sources (salts from the oceans and seas, volcanoes, meadows, deserts, etc.), fossil fuels, industries, vehicles, etc. According to the emission source, Particulate materials in the atmosphere are characterized by a range of morphological, chemical, physical and thermodynamic properties(2) and there can be various toxic materials including : metal(loid)s(3), PAHs, NPAHs, OPAHs(4), microplastics (MP)(5) PCBs ..., and non-toxics including : pollens(6), cationic, anionic and nonionic(7) and thousands of compounds in the particle structure. The selected elements in this study are classified in different risk groups according to EPA Integrated Risk Information System (IRIS)

classification(8). And in very low concentrations (ng/m³), they can pose carcinogenic and pathogenic risks to humans. Numerous studies have shown that they are bound to atmospheric particles at low concentrations. But based on the degree of toxicity in low concentrations, they also cause health problems. Numerous studies show that heavy metals concentrations (ng/m³) in the structure of PM_{2.5} (µg/m³) (9-11). Metal(loid)s in the present study are in the category of hazardous minor elements and in very low concentrations (ng/m³) pose health risk to humans.

C) Blood metal(loid)s:

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4 mL of the whole blood sample was taken into a digestion Pyrex flask and at last the brought to 10 ml by adding deionized water (specific resistance ≥ 18.2 MΩ.cm) was prepared. Samples were analyzing by ICP-OES.

Blood metal(loid)s concentration was obtained by following relation:

Concentrations of chemicals in liquids are typically measured in units of the mass of chemical (milligrams, mg or micrograms, ug) per volume of water (liter, ml). for liquid or water 1 ppm= approximately 1 mg/L (also written as mg/l) of contaminant in water, and 1 ppb =1 ug/L (also written as ug/l). A measurement of 1 mg/L is the same as 1 ppm or 1,000 ppb, which is equal to 1,000 ug/L (1,000 ng/ml). Numerous studies show that the concentration of heavy metals in the blood is expressed in ng/mL and µg/dL (9, 12).

D) Blood Immunoglobulin E (IgE) and cytokines (IL-4 and IL-13):

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IgE, IL-4 and IL-13 were obtained by an ELISA Rader (BioTek; USA; ELx808) at 450 nm as Measurement and 620 nm as Reference wavelength.

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