Front Matter

Human Exposure to Toxic Metals and Microbiological Pollution via AC-filter Dust from Industrial, Residential and Agricultural Areas: Assessment of Health Risks.

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ABSTRACT

Among others, road traffic, industrial emissions, commercial activities, smoking and cooking are considered as major contributing factors for the increasing levels of pollutants in atmosphere. High levels of potentially toxic metals and microbes in atmosphere, especially in indoor air, may pose serious threat to human health. Therefore, concentration and associated health risks of potentially toxic trace metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) and their risk to human health, and microbial load in indoor air was assessed using air condition (AC) filter dust samples collected from 5 locations representing residential, agricultural and industrial settings of Eastern Province, Kingdom of Saudi Arabia. The levels of trace metals varied considerably among sampling areas, with the highest levels of Cr and Cd recorded in the Industrial-area sites followed by the Agricultural and Urban-Residential sites. The highest levels of Pb and Fe were found in the Agricultural area sites followed by the Industrial and Urban-Residential area sites. The metals in dust sample, especially Cd, Cr and Pb, showed a considerable health risk through dermal pathway. Among the sites, the highest hazard quotient for these metals was found for Al-Qatif-Industrial areas sites and among the metals it was the highest for Cd. The cancer risk from the metals contained in AC filter dust was negligible. Samples collected from Agricultural and Industrial area sites were substantially contaminated with bacteria and fungi, respectively. Bacterial contaminants were mostly Gram Negative, with considerable antibiotic resistance

and haemolytic activity. Thus, indoor air quality as assessed by AC filter dust depicted that a considerable health risk could be posed by the trace heavy metals and microorganisms for a long-term exposure. Furthermore, this study demonstrated that AC filters dust could be a unique and reliable test sample for the assessment of indoor environment.

Key Word: Air-conditioned filter dust, Indoor environment, Heavy metals, Biological contaminants, Risk assessment

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1. Introduction

Depending upon age and work nature, people spend about 70-90 % of their time in indoor environment such as schools, offices, homes and commercial buildings. This is particularly true for dry and hotter regions of the world. Since indoor environment and outdoor environments are in equilibrium, and cannot be isolated from each other, entry of outdoor air having contaminants through doors, windows, exhaust outlets etc. in the buildings may introduce inorganic and organic contaminants to indoor environment. It is reported that movement of human and pets from outside to inside could also be one of the reasons of contaminated soil migration to indoor environment (Hunt et al., 2006). In addition to above mentioned factors, indoor activities such as cooking, heating system, and smoking could also result in the accumulation of contaminants in indoor environment. The contaminants present inside homes or work places become part of indoor dust and thereby becomes a sink of many types of organic and inorganic contaminants and could pose server threat to exposed persons and this is particularly true for workers in commercial buildings and elderly people, children and infants in homes (Hu et al., 2011).

Ingestion of indoor dust could be a major source of human exposure to potentially toxic elements (Layton and Beamer, 2009; Roberts et al., 2009). Additionally, the potentially toxic trace metals in dust may enter human body through direct dermal contact and/or inhalation (Hou et al., 2019). The continued exposure to toxic elements such as cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) through indoor dust even at low concentration could pose serious threats to human health (Ibanez et al. 2010; Praveena et al. 2015; Turner 2011). Indoor environmental conditions (like darkness, humidity) are also conducive for the growth and reproduction of pathogenic and toxin-producing fungi and bacteria. The presence of microorganisms such as bacteria, molds and viruses in indoor air can be major reason for the infections, allergies, and toxicity to the human (Annesi-Maesano, 2013; Khan and Karuppayil, 2012; WHO, 2009).

The presence of inorganic and organic contaminants in dust and their easy transfer to human warrants compositional characterization of indoor dust samples. Monitoring and characterization is critical for assessment and management of the potential risks associated with indoor dust. Indoor air quality can be monitored through collection of dust samples and for this purpose different techniques (such as settled dust and short-term air sampling) have been employed (Haaland and Siegel, 2016; Siddique et al., 2011). The dust settled on the surfaces

such as floor, carpets and/or furniture can be collected either through dusting/wiping or vacuuming off. Unfortunately, this technique has been shown to be ineffective in collection of small sized dust particles. Since size of the dust particles is critical in terms of elemental adsorption on their surfaces, such sampling technique could be misleading especially if trace metals are to be analyzed. This is due to the fact that smaller sized particles, because of their high active surface areas, are more effective in retention of trace metals (Hassan, 2000; Cheng et al., 2018). In short term air sampling, air is drawn through a small diameter filter mounted to pumps having low flow rate of air. Moreover, this method collects air samples for only a short time and therefore does not truly reflect the conditions before and after sampling because literature reveal a large temporal variability in concentrations.

Since indoor air quality composition is related with outdoor activities such as heavy traffic, industrial activities, and to some extent farming practices, it is expected that there would be reasonable compositional differences among dust samples collected from buildings of urban, rural and farmland areas (Mahfouz et al., 2019). In addition, the number of story and height of the building, the frequency and time of windows opening, the number of residents and pets in house and surroundings of buildings (nearness to highways, industrial zones, workshops) could also affect the concentrations of toxic metals in household dust (Ali et al., 2019). Some studies from recent past reveal that dust samples from air-conditioner filters can be used to assess the physical, biological and chemical composition of indoor air quality (Noris et al., 2009, Noris et al., 2011; Haaland and Siegel, 2016; Ali et al., 2018; Ali et al., 2019; Mahfouz et al., 2019). Air conditioning units (windows and split) recirculate the indoor air thus cause mixing and suspension of indoor air and settled dust particles. The dust particles larger than the pore size of commonly used AC filters (mainly coarse fraction of re-suspended particles with size less than 100 μm) get deposited on these filters. These re-suspended dust particles could attach to household items (food, skin, toys and furniture) and thus may enter into human food chain (Huang et al., 2014).

To the best of our knowledge, little or no work has been conducted on the comparative chemical and biological analysis and assessment of associated health risks of indoor air quality using dust of AC filters collected from different land use settings such as rural, urban and farming area. The current study was planned with the objectives: (1) determining levels of potentially toxic trace metals in indoor dust collected from living or work places of residential colonies, industrial and agricultural settings, (2) assessing metal uptake rates for (children and adults) via dermal, inhalation and ingestion of indoor dust, and (3) estimating health risks associated with indoor dust containing trace metals.

2. Materials and Methodology

2.1. Study area and description of sampling sites

For the present study, three sites namely, Al-Qatif, AL-Nabiah and Saihat (small cities) from the Eastern Province of Saudi Arabia were carefully selected to represent residential, industrial and agricultural activities. The table S1 shows the location of sampling sites from where air conditioner filter dust samples were collected. A total of eighteen samples were collected from selected sites reflecting the degree of variations due to commercial/industrial, or agricultural/ rural residential areas activities. Table S1 shows the detail information of AC-dust samples and site description.

2.2. Collection of indoor dust samples using AC filters

To represent maximum loadings of the dust on AC filters, indoor dust sampling was done in dry and humid summer 2018 to obtain representative maximum loadings of the dust on AC filters. For the collection of dust samples from AC filters, the filters were carefully removed from selected AC units. Generally, samples were taken from one AC unit per site with approximately 5–6 grams which is sufficient for analysis. The samples were collected by tapping the filters onto a clean polythene sheet as well as by using a plastic brush to remove the dust. Dust samples from each filter were collected in a sterilized petri dish to avoid contamination of dust microorganisms. The collected samples were stored at -4 °C before digestion and analysis. Table S2 shows the details on each dust sample such as area of the room, time span of filter replacement or cleaning, presence or absence of smoking, and cooking in the building, and the residents were either owners or renter of the building and how many children and elder people living were recorded by interviewing the people living the specific building.

2.3. Analyses of the AC Filter Dust Samples

2.3.1. Determination of potentially toxic metals

A subsample of each dust sample was oven dried at 105 °C for 72 h and around 0.2 g of dried subsample was digested in a 1:3 ratio mixture of HCl and HNO₃ at 150 °C for 2 h (AOAC, 1990). The digests were cooled at room temperature, diluted to 10 mL with double distilled water and filtered through a 0.45-μm filter membrane. The concentration of Cd, Cr, Ni and Pb in the digests were measured by inductively coupled plasma optical emission spectrometer (ICP-OES; Agilent 7700, Agilent Technologies Inc., USA). Standard materials were used between samples to ensure quality of data and the precision of target metals was noted (<2.5 %) by using the standard deviation of repeated readings of standards. Similarly, blanks

(laboratory, filter and reagent) were characterized for concentrations of trace metals to determine metal contamination during analytical procedures.

2.3.2. Health Risk Assessment

Human exposure to potentially toxic metals through indoor dust could be via three pathways, i.e. ingestion, inhalation and dermal absorption. In this part of study, the population was divided into two groups: small children and adults, having 0 to 15 years, and more than 15 years, respectively. Health risk assessment model proposed by United States Environmental Protection Agency (USEPA, 1986; 2005) was used to quantify the average daily dose (ADD) ($\text{mg kg}^{-1} \text{ day}^{-1}$) of the toxic metals contained in AC filter dust employing Eq.1-3.

$$\text{Average daily dose ingestion (ADD}_{\text{ing}}) = \frac{(C \times \text{IngR} \times \text{EF} \times \text{ED})}{\text{BW} \times \text{AT}} \times 10^{-6} \quad (1)$$

$$\text{Average daily inhalation (ADD}_{\text{inh}}) = \frac{(C \times \text{InhR} \times \text{EF} \times \text{ED})}{\text{PEF} \times \text{BW} \times \text{AT}} \quad (2)$$

$$\text{Average daily dose dermal (ADD}_{\text{derm}}) = \frac{(C \times \text{SA} \times \text{AF} \times \text{ABS} \times \text{EF} \times \text{ED})}{\text{BW} \times \text{AT}} \times 10^{-6} \quad (3)$$

Table S3 shows all the factors along with values and symbols applied to calculate the results of the health risk assessment. Where, ADD_{ing} , ADD_{inh} and ADD_{derm} represent average daily dose for ingestion, inhalation and dermal contact, respectively.

To find out the health risks due to potentially toxic metal exposure from indoor dust, the hazards quotients (HQ) for inhalation, ingestion and dermal contact, hazard Index (HI) and cancer risk (CR) from ingestion and inhalation were calculated by Eq. 4 to 6 (Ferreira-Baptista and De Miguel, 2005; Chen et al., 2012).

$$\text{HQ} = \text{ADD}(\text{ingestion, inhalation, dermal})/\text{RfD} \quad (4)$$

$$\text{HI} = \sum \text{HQ}_{\text{ingestion, inhalation, dermal}} \quad (5)$$

$$\text{CR} = \text{ADD}(\text{ingestion, inhalation, dermal}) \times \text{SF} \quad (6)$$

Where RfD is the homologous reference dose, SF is the homologous slope factor and CR is carcinogenic risk. The values of slope factor SF are shown in Table S3. Following classification proposed by the International Agency for Research on Cancer (IARC), toxic metals include Cd and Cr are grouped as Class 1 carcinogenic elements, whereas Pb is classified as Class 2A carcinogenic element. (IARC, 2015). The HQ is used for computing the noncarcinogenic impact of heavy metals in household dust while HI is equal to the sum of the HQs for the three pathways (US EPA, 2009). When $\text{HQ}/\text{HI} > 1$, there is a possibility of non-carcinogenic effects.

Similarly, the CR is used to estimate an individual's exposure to carcinogenic hazards during a lifetime. Carcinogenic risk is negligible when CR value is $< 1 \times 10^{-6}$, acceptable or tolerable when it ranges from 1×10^{-6} to 1×10^{-4} and $CR > 1 \times 10^{-4}$ means that 1 in 10,000 people may develop cancer from lifetime exposure to carcinogenic hazards. Keeping in view the behavior, sensitivity and physiological differences, risk assessment attributes were calculated for adults and children independently.

2.3.3. Isolation and Characterization of Bacteria

For comparative analysis of microbial contaminants in AC filter dust, samples were collected from three different habitats in Al-Qatif city namely; Al-Qatif-Agriculture, Residential and Industrial areas. For bacterial isolation, purification and bacterial counts as well as strains preservation, nutrient agar medium was prepared using composition (g L⁻¹) lab-lemco powder 1.0, yeast extract 2.0, peptone 5.0, sodium chloride 5.0 and agar 15.0. A known weight of each dust sample was suspended in sterile distilled water, which was then diluted and sub-cultured on nutrient agar medium. All petri dishes were incubated at 37 °C for 24 - 48 h. Separate colonies were selected, purified and kept in a nutrient agar and stored in refrigerator.

Purified bacterial strains were tested for antibiotic sensitivity using Diagnostic Sensitivity Test Agar (ThermoFisher Scientific, USA) which was prepared according to manufacturer's protocol. Four antibiotics (ampicillin (10 µg), kanamycin (30 µg), doxycycline (30 µg) and neomycin (30 µg) were used in this study. All antibiotics were purchased from BD BBL™, USA. At the end of incubation period of 18 h at 37 °C, size of colony inhibition zone was determined. The zone width was measured and compared against reference standards for each bacterium and antibiotic, and then bacterial strains were categorized into highly, moderately and low sensitive with respect to each antibiotic (Sarker et al., 2014).

Morphological characterization and identification of purified isolated bacterial strains were conducted through Gram Staining Test. This stain is used to differentiate bacterial species into two large groups namely gram-negative and gram-positive. In first step, crystal violet was applied to heat the fixed smear of intended bacterial culture for 1 minute and fixed by iodine for 1 minute also, to form a complex. Subsequently, the smear was subjected to rapid decolourization by ethanol for 5 seconds. Thereafter, Safranin was added for 1 minute. After each step, culture was washed with water gently to remove excess. Finally, the bacterial smear was left to dry at room temperature and examined under oil immersion. For toxicity assessment, hemolytic activity of contaminant's bacteria was isolated from air conditioning dust, and were tested by cultivation on Blood agar medium (Bioworld, USA).

2.3.4. Isolation and Characterization of Fungi

For isolation and purification of fungi from AC filter dust of the three different areas in Al-Qatif city, Sabouraud dextrose agar (SDA) medium was used. The SDA medium had the following composition (g L⁻¹): mycological peptone 10.0, glucose 40.0 and agar 15.0. The medium was prepared according to the manufacture's protocol. A known weight of each dust sample was suspended in sterile distilled water, further diluted and sub-cultured on SDA medium. All the petri dishes cultured with the dust samples were incubated at 20-25 °C for 5 to 7 days. Separate colonies were selected, further purified and kept in SDA medium contained in petri dishes and stored in refrigerator for further analysis. The fungal isolates were examined by the use of wet mount technique. A known mass of fungal growth was placed on clean slide containing droplets of water, subsequently covered and examined under microscope.

2.3.5. Data analyses

Statistical analyses were performed using Microsoft Office Excel (Office 2016) or Minitab 17 (Minitab Inc., State College, PA, USA) with significance correlation set at *p value* ≤ 0.05.

3. Results and Discussion

3.2. Concentration of Heavy Metals

The concentration of potentially toxic metals (Cd, Cr, Cu, Fe, Mn, Pb and Zn) in AC filter dust is presented in Table 1. Cadmium concentration in the AC filter dust samples varied from 0.12 to 510 mg kg⁻¹. Among the sampling sites, the highest mean Cd concentration (129.7 mg kg⁻¹) was recorded in AC filter dust samples collected from Al-Qatif-Industrial area, whereas the lowest Cd concentration (4.50 mg kg⁻¹) was recorded in dust samples of AC-filters collected from Al-Nabiah area. The areas on the basis of average Cd concentration in dust of AC filters followed the decreasing order of: Al-Qatif-Industrial > Al-Qatif-Residential > Al-Qatif-Agriculture > Saihat > Al-Nabiah. The higher levels of Cd in air-conditioner filter dust of Al-Qatif-Industrial regions may be explained by the industrial activities like preparation of paints and Cd batteries in the area. However, higher levels of Cd in Al-Qatif-Agriculture site could be attributed to the use of Cd containing pesticides and non-cleaning of AC filters for long periods of time. Moreover, the survey of the households depicted that ACs operating in Al-Qatif-Agriculture area had not been cleaned since long time as shown in Table S2. High levels of Cd in the Al-Qatif-residential may be ascribed to nearby workshops specialized for plating and painting of alloys and metal using Cd containing pigments. Some of the Cd concentration values were found consistent with the study reported for Riyadh city of Saudi Arabia (Al-Rajhi et al., 1996), but mean value reported by these authors is much lower than the mean values found at all the sites in our study. Lower value as reported in these studies may be due to lower

industrial activities 20 years ago as compared to present day situation. Moreover, we found that presence of Cd in AC filter dust had not correlation with the presence of any other metal in the filter dust (Table 1).

Chromium concentration in the AC filter dust samples varied from 0.74 to 236 mg kg⁻¹. The highest mean concentration of Cr (76.2 mg kg⁻¹) in AC filter dust was found in samples collected from Al-Qatif-Industrial region, whereas the lowest Cr (10.7 mg kg⁻¹) was found in dust samples collected from Al-Nabiah area (Table 1). The areas on the basis of average Cr concentration in AC filter dust followed the decreasing order of: Al-Qatif-Industrial > Al-Qatif-Agriculture > Al-Qatif-Residential > Saihat > Al-Nabiah. As expected, Al-Qatif-Industrial area showed Cr levels higher than the permissible limit and therefore warrants regular monitoring. The higher levels of Cr in Al-Qatif-Industrial area could be attributed to use of Cr in industries as a catalyst, in plating to prevent corrosion of metal and in paints and pigments manufacturing industries. The concentration of Cr in AC filter dust had significant positive correlation with the concentration of Fe in the filter dust (Table 1) indicating their contribution from same sources.

Copper concentration in the AC filter dust samples varied from 7.8 to 224 mg kg⁻¹. The highest mean concentration of Cu (147 mg kg⁻¹) was found in dust samples collected from AC filters of Al-Qatif- Agricultural area, whereas the lowest Cd (48.2 mg kg⁻¹) was found in AC filter dust samples collected from Saihat area. Cu concentration in AC filter dust follow the decreasing order of: Al-Qatif-Agricultural > Al-Qatif-Industrial > Al-Qatif-Residential > Al-Nabiah > Saihat. The higher levels of Cu in AC dust samples collected from Al-Qatif-Agricultural area could be attributed to the common use of Cu compounds in agriculture to treat plant diseases, like mildew, or for water treatment (Xiong et al., 2010). Moreover, Cu is also used in the manufacturing of wire, sheet metal, and other metal products in industrial areas. The concentration of Cu in AC filter dust was positively correlated with the concertation Zn in the dust (Table 1).

Results show that the AC filter dust collected from Saihat-Residential area contained the lowest mean concentration of Fe (796 mg kg⁻¹), that could be due to the reason that this area is far away from industry and agriculture zones. On the other hand, the higher concentration of Fe in AC filter dust samples collected from Al-Qatif Residential (6667 mg kg⁻¹) and Al-Nabiah (4212 mg kg⁻¹) areas may be explained by the presence of scrap plants for collected waste Fe products and a workshop for wilding and dying in these areas, respectively. Moreover, in Al-Qatif-Residential region, use of Fe containing fertilizers and pesticides may have contributed

to Fe load of dust. Iron concentration in the AC filter dust had positively correlation with Cr, Cu, Mn and Zn concentration in the dust (Table 1).

With a mean value of 55 mg kg⁻¹, Pb concentration varied among the studied areas between 38.1-93.2 mg kg⁻¹. Pb include paint, lead glazes of pottery, smelters and use of Pbarsenate as an insecticide (Mathee et al., 2006). The highest concentration of Pb in Al-Qatif-Agriculture area may be attributed to the use lead arsenate pesticides with irrigation water and via foliar sprays (Holmgren et al., 1993). It can be inferred that the Pb in soil is generally much higher in agriculture area and can pose a significant health threat if not properly addressed. This situation calls urgent attention to monitoring where potentially toxic metals shows upward trend especially for Pb and Cr.

The mean concentration of Mn in AC filter dust was recorded 97 mg kg⁻¹, with minimum and maximum mean concentration values of 57.2 mg kg⁻¹ recorded for Al-Nabiah and 197 mg kg⁻¹ for Al-Qatif-Agricultural, respectively. The most common use of Mn in fertilizers and fungicides for agricultural purposes is a probable reason of its higher concentration in agriculture area (Ferraz et al., 1988). The concentration of Mn in Al-Qatif-Industrial area was also appreciable, probably due to the reason that it is used in manufacturing of dry cell batteries and steel preparation (Emara et al., 1971).

The concentration of Zn in AC filter dust collected from Al-Qatif-Agricultural area was more than double of the highest value recorded for other areas. This may be explained by the extensive use of Zn as fertilizer and Zn containing pesticides at agriculture farms. Second higher concentration of Zn was found at Al-Nabiah, and it could be attributed to the presence of scrap plant in this area. In industrial areas, the galvanizing steel, automobile and rubber industry increase the percentage of zinc in industrial aerosols.

3.3. Health Risk Assessment

The values of HQing were well below 1.0 (Table 2), indicating that non-carcinogen hazard of potential toxic metals from dust ingestion was low. The values of HQing for all the metals were much higher for children than adults. For children, maximum values of HQing, for Cr and Cd were recorded in AL-Qatif Industrial area, for Mn, Pb and Zn in Al-Qatif Agricultural area and for Cu in Al-Nabih area. Irrespective of area and age group, HQing for Cr was the highest among the metals, followed by Pb and then Cd. Similar to our finding, among the various toxic metals studied, Ali et al. (2017), Shi et al. (2008) and Wei et al. (2015) reported the highest value of HQing from road dust for Cr in different regions of China. Moreover, Ali et al. (2017) reported that HQing values of Cr was higher for road dust collected from industrial area as compared to residential or agricultural areas. On the other hand, likewise results obtained in

this study, HQing of Pb, Zn and Cu were reported to be higher for urban areas indicating that these metals can have severe adverse health effects in populated areas. The values of HQinh were much lower than HQing for all the metals (Table 2), indicating a negligible hazard of the metals through inhalation pathway. For the obtained results, Cd and Mn had higher HQinh values than the other metals. The values of HQderm, except for Mn, were very high, reaching to 200 for Cd in adults (Table 2). Except for Zn, for all the metals values of HQderm for children 16-17 times higher than for adults in all areas. For Zn, HQderm for children was 8 to 18.5 times higher than for kids. The higher values of HQderm for children are because of their playing habits whereby they ingest dust through directly or through hand licking or from toys (Mohmand et al., 2015). In our study, with respect of HQderm, the metal followed the ranking of: $Cd > Cr > Pb > Cu > Zn > Mn$. The values of HQinh for Cd were well below 1.0, in agricultural, residential and industrial areas of Al-Qatif region for adults, for Cd and Cr in all regions for both adults and children, and in Pb for agricultural and industrial areas of AL-Qatif region. We found that among the three exposure pathways, the highest risk was associated with dermal pathway, with ingestion and inhalation ranked to be second and third in risk, respectively. Similarly, Liu et al. (2012) and Mohmand et al. (2015) reported that potential non-carcinogenic health risk through inhalation of road dust was negligible compared to other exposure routes. Accordingly, some other researchers reported HQinh to be 10 times lower than others exposure routes (Li et al., 2013; Zheng et al., 2010; Wang et al., 2016). However, in contrast to our results, Ali et al. (2017) and Shi et al. (2008) reported the highest risk from ingestion pathway instead of dermal.

Table 3 presents the Hazard index (HI) or non-carcinogenic risk of the potentially toxic heavy metals in AC filter dust. The values of HI, total non-carcinogenic risk through all the three pathways, followed the same trend as that of HQderm (Table 2). Among the metals, the highest values of HI were recorded for Cd, followed by Cr and then Pb, while lowest values were recorded for Zn. Among areas, the highest values depended upon the metal, for Cd and Cr, the highest value was recorded in Al-Qatif-Industrial area. The values of HI for Cd were well above 1.0, in agricultural, residential and industrial areas of Al-Qatif region for adults, for Cd and Cr in all regions for both adults and children, and for Pb in agricultural and industrial areas of AL-Qatif region. The values of HI for Cd, Cr and Pb were above for either all or some of the studied areas, in contrast to other studies reporting HI values < 1 from road dust (Wei et al., 2015), are quite alarming and represent significant non-carcinogenic hazard of metals, especially through dermal contact.

The cancer risk (CR) values from ingestion of Pb in all the areas were negligible (less than 1×10^{-6}) for both adults and children except for Saihat-residential area in adult (0.14845) (Table 4). Higher level of CR value in adult at Saihat-Residential is associated with exposure of Pb due to their occupations such as welding, battery manufacturing, printing and stained glass. In addition, the major sources of Pb to the human population are inhalation of air-born Pb from vehicle emission and resettlement of coarse particle pollutants (Baird, 2000). Exposure to Pb can cause development of congenital malformations and affect the nervous system, which leads to impairment in the newborn's motor and cognitive abilities (Garza et al., 2006; Bellinger, 2005). The high CR value of Pb for ingestion at Saihat-Residential area warrant close attention. For others area, results of the present study are in accordance with early findings for various regions of the world whereby CR values for As, Co, Cr, and Ni in road dust were reported to be lower than the safe level of 1×10^{-6} to 1×10^{-4} (Table 4) (Liu et al., 2014; Hu et al., 2011; Keshavarzi et al., 2015; Liu et al., 2012; Alamdar et al., 2016).

As expected, overall results depict that health risk for children from trace metals from indoor dust exposure were higher compared to adults and dermal contact seems to be the major pathway of exposure to trace metals from indoor dust followed by inhalation and ingestion. However, trace metals have tendency to bio-accumulate upon exposure to an extended period and could pose severe health impacts to human especially children. This is particularly true in the case of closed environment installed with cooling units that circulate the suspended dust particles, hence results of the present investigation, the health risks associated with indoor dust could not be ignored.

3.4. Biological characterization of air-conditioners filters dust samples

2.5.1. Population Density of Bacteria

The highest bacterial population density was recorded in dust samples collected from Al-Qatif-Agricultural area (4.5×10^5 cfu 100 mg⁻¹), followed by Al-Qatif-Residential (4.5×10^4 cfu 100 mg⁻¹) and –Industrial areas (88×10^3 cfu 100 mg⁻¹), respectively. The higher bacterial population density in AC dust filter of Agricultural-Residential may be attributed to the higher moisture content and possible existence of organic dust particles than the other areas (Lee et al., 2006). The presence of microbial contaminants in dust samples from air conditioners are in agreement with earlier research reporting bacterial population density in the range of 120 to 2300 cfu m⁻³ (Stryjakowska-Sekulska et al., 2007). They revealed that number of microorganisms continuously changes due to change in environmental conditions and other parameters related to microbial growth.

2.5.2. Morphological characterization of bacteria by Gram's stain

Morphological characterization of bacterial contaminants isolated from air-conditioner dust collected from different location was carried out by Gram's staining of pure colonies. In the dust samples collected from all the locations, gram positive bacteria were abundant than gram negative bacteria, and mainly belonging to the genera *Bacillus* and *Staphylococcus* (Table 5). From Al-Qatif-Industrial area, only gram-positive bacteria were isolated. There was great variation in number of gram-positive bacteria in Al-Qatif-Residential and Al-Qatif-Agricultural areas, being 75% and 25% abundant, respectively. Interestingly, Borrego et al. (2010) reported that gram-positive bacteria were more abundant in air than gram negative bacteria. Also, gram-positive cocci and bacilli and gram-negative bacteria were isolated from air conditioning units (Ross et al., 2004). Moreover, existence of bacterial contaminants in parts of air conditioners especially air filters has been recorded by many scientists (Khalfallah et al., 2016; Ross et al., 2004).

2.5.3. Antibiotic sensitivity test

In order to test the possible health risk that could be encountered from dust containing the bacterial contaminants, antibiotic sensitivity test was carried out. In this test 4 different antibiotic were used. Most of the bacteria isolated from the three areas, especially those collected from Al-Qatif-Residential, were resistant to ampicillin (average size of inhibition zone: 10.8 mm) (Table 6). On the other hand, most of the bacteria, especially those collected from Al-Qatif-Agricultural area, showed sensitivity to doxycycline and kanamycin (average size of inhibition zone: 25.2 mm and 26.9 mm, respectively) (Sarker et al., 2014). Results collectively indicated that most of the bacterial contaminants are most sensitive to doxycycline, intermediate sensitive to kanamycin and least sensitive to neomycin that disrupt protein synthesis in bacteria. Contrarily, most of bacterial contaminants showed clear resistance to the antibiotic ampicillin that has an effect on cell wall synthesis. Similar antibiotic profiling results was recorded by Gibbs et al. (2006). In the current study, results of antibiotic resistance collectively indicated the potential health risks of air conditioning dust bacterial contaminants to human (Safatov et al., 2008).

2.5.4. Hemolysis activity test

In this experiment the possible toxicity and health risk of bacterial contaminants isolated from air conditioners dust, was determined based upon hemolytic activity. Results indicated that 67% of bacteria isolated from Al-Qatif-Agricultural area showed potential beta-hemolytic activity while rest 33% were alpha-hemolytic (Table 7). Half of the bacteria collected from Al-Qatif-Residential area were non-hemolytic, 33% alpha-hemolytic and 17% beta-hemolytic.

About 67% of bacteria collected from Al-Qatif-Industrial area were non-hemolytic while rest were beta-hemolytic. The bacteria collected from industrial area showed potential beta-hemolytic activity due to augmented selective pressure caused by increased toxic chemical levels on many microbes. This means, only highly resistance micro-flora with versatile biochemical activities and antibiotic resistance mechanisms can grow. The presence of both alpha- and beta-hemolytic bacteria in agriculture area could be due to the irregular and delayed cleaning of the AC filters. The delay in filter cleaning could have health hazardous effect to human, thus needs regular inspection and cleaning. Finally, bacterial contaminants from residential zone showed more variation in hemolytic activities, and recorded the lowest percent of isolates with beta-hemolytic activity. Generally, pathogenicity of many bacteria is due to a vast number of virulence factors among of which the hemolytic toxins e.g. hemolysins that produced during hemolytic activity and poses high threat to human health (Chopra et al., 2000; Janda and Abbott, 2010).

2.5.5. Population density and community structure of Fungi

On the other hand, the highest fungal population density was recorded in dust samples from Al-Qatif-Industrial area (4×10^4 cfu 100 mg⁻¹), followed by Al-Qatif-Agricultural area (953 cfu 100 mg⁻¹) and the lowest from Al-Qatif-Residential (76 cfu 100 mg⁻¹). Interestingly, the dust samples from the same area were found to contain the highest heavy metal contents which reflect the higher possible resistance of the isolated fungi to the heavy metals' toxicity. The common air contaminant fungi *Stachybotrys chartarum* and *Cladosporium* sp recorded in air dust from farm area (Table 5). In Al-Qatif-Industrial area the normal fungal contaminants belong to *Penicillium* sp and *Aspergillus* sp were recorded. Interestingly, yeast was only recorded in Al-Qatif-Residential area. Results indicated that the most common indoor fungi contaminants are recorded in this study as previously recorded by Aydogdu et al. (2005) and Nevalainen and Morawaska (2009) in indoor air. Nevertheless, most of isolated fungal candidates has been recorded as potent pathogens and can induce diverse effects on human health such as allergic asthma, rhinitis, hypersensitivity pneumonitis and carcinogenic effects due to aflatoxins (Annesi-Maesano, 2013; Khan and Karuppayil, 2012; WHO, 2009).

Conclusion

It may be concluded that indoor air of sites located in industrial areas were more loaded with trace metals including Cd and Cr as compared to agricultural residential area sites which contained high Cu, Pb and Zn levels. The highest non-carcinogenic risk was through dermal pathway, the highest for Cr and Cd than other metals, and higher in children than adults. The

carcinogenic risk was negligible for all the metals and in all the areas based on all exposure pathways. Moreover, AC dust was substantially loaded with bacterial and fungal communities, a significant fraction of the former was found to contain antibiotic resistance and hemolytic activity. The study proved that indoor assessment of indoor air quality and health risk assessment from trace heavy metals and microorganism associated with dust particles using AC filter dust is a reliable method. Nevertheless, it is worth mentioning that the current study comprises of relatively a small number of samples collected from one region during a particular season hence, more studies are recommended to explore more aspects such as more trace metals in both indoor and outdoor environments.

Back matter

References

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Table 1. Concentrations (mg kg⁻¹) and pearson correlation among different heavy metals found in the dust of AC filters collected from urban and peri-urban areas of Eastern Saudi Arabia.

Area (n*)	Cd	Cr	Cu	Fe	Mn	Pb	Zn
Al-Nabiah (4)	4.6 ± 3.0	10.7 ± 3.6	52.3 ± 19.3	1674 ± 630	57.2 ± 13.0	38.1 ± 21.5	344 ± 185
Al-Qatif-Agricultural (3)	29.7 ± 13.4	39.8 ± 13.1	147 ± 28.0	6667 ± 2590	197 ± 73.4	93.2 ± 30.8	962 ± 330
Al-Qatif-Industrial (4)	130 ± 127	76.2 ± 53.3	105 ± 25.9	4212 ± 980	106 ± 12.8	64.2 ± 13.7	380 ± 29.1
Al-Qatif-Residential (3)	71.0 ± 55.3	15.6 ± 12.5	107 ± 56.3	3109 ± 1440	81.7 ± 39.8	42.2 ± 18.2	275 ± 130
Saihat-Residential (4)	8.7 ± 4.5	11.4 ± 4.3	48.2 ± 19.6	796 ± 380	66.3 ± 25.2	42.6 ± 10.4	160 ± 55.9
Mean	49	31	88	3114	97	55	403
Median	3.17	18.3	87.8	2278	91.3	51.6	296
Min	0.12	0.74	7.8	4.50	4.17	1.67	26.5
Max	510	236	224	11200	334	137	1410
Pearson correlation							
Heavy Metal	Cd	Cr	Pb	Cu	Fe	Mn	
Cr	-0.059						
Pb	-0.127	0.348					
Cu	-0.053	-0.052	0.023				
Fe	0.007	0.542*	0.792*	0.163			
Mn	0.024	0.258	0.837*	0.159	0.858*		
Zn	-0.002	0.218	0.641*	0.487*	0.743*	0.714*	

*Number of samples

Value indicated with asterisk represent significant correlation (*p value* ≤ 0.05)

Table 2. Hazard quotient of ingestion (HQing), inhalation (HQinh) and dermal (HQderm) pathways for the potentially toxic heavy metals in the dust of AC filters collected from urban and peri-urban areas of Eastern Saudi Arabia.

Age Group	Area	Cd	Cr	Cu	Mn	Pb	Zn
HQing							
Adult	Al-Nabiah	0.001	0.008	0.016	0.005	0.025	0.003
	Al-Qatif-Agricultural	0.005	0.023	0.006	0.014	0.047	0.006
	Al-Qatif-Industrial	0.030	0.059	0.006	0.010	0.043	0.003
	Al-Qatif-Residential	0.012	0.009	0.005	0.005	0.021	0.002
	Saihat-Residential	0.002	0.009	0.003	0.006	0.028	0.001
Children	Al-Nabiah	0.005	0.039	0.077	0.026	0.119	0.012
	Al-Qatif-Agricultural	0.024	0.109	0.030	0.067	0.219	0.026
	Al-Qatif-Industrial	0.142	0.278	0.029	0.048	0.201	0.014
	Al-Qatif-Residential	0.058	0.042	0.022	0.028	0.099	0.007
	Saihat-Residential	0.009	0.041	0.013	0.030	0.133	0.006
HQinh							
Adult	Al-Nabiah	0.0001	1.23E-06	2.43E-06	0.0004	3.76E-07	3.96E-06
	Al-Qatif-Agricultural	0.0008	3.44E-06	9.52E-07	0.0010	6.9E-07	8.31E-06
	Al-Qatif-Industrial	0.0045	8.77E-06	9.02E-07	0.0007	6.34E-07	4.37E-06
	Al-Qatif-Residential	0.0018	1.34E-06	6.96E-07	0.0004	3.12E-07	2.37E-06
	Saihat-Residential	0.0003	1.31E-06	4.16E-07	0.0005	4.20E-07	1.84E-06
Children	Al-Nabiah	0.0001	1.09E-06	2.16E-06	0.0004	3.33E-07	3.52E-06
	Al-Qatif-Agricultural	0.0007	3.05E-06	8.44E-07	0.0009	6.12E-07	7.37E-06
	Al-Qatif-Industrial	0.0040	7.77E-06	8E-07	0.0006	5.62E-07	3.88E-06
	Al-Qatif-Residential	0.0016	1.19E-06	6.17E-07	0.0004	2.77E-07	2.1E-06
	Saihat-Residential	0.0003	1.16E-06	3.69E-07	0.0004	3.73E-07	1.63E-06
HQderm							
Adult	Al-Nabiah	0.427	0.067	0.022	1.16E-05	0.041	0.001
	Al-Qatif-Agricultural	2.085	0.187	0.009	3.01E-05	0.075	0.002
	Al-Qatif-Industrial	12.15	0.476	0.008	2.15E-05	0.069	0.001
	Al-Qatif-Residential	4.989	0.073	0.006	1.25E-05	0.034	0.001
	Saihat-Residential	0.810	0.071	0.004	1.35E-05	0.046	0.001
Children	Al-Nabiah	6.998	1.091	0.360	1.91E-04	0.668	0.018
	Al-Qatif-Agricultural	34.15	3.056	0.141	4.92E-04	1.226	0.037
	Al-Qatif-Industrial	199.0	7.790	0.134	3.53E-04	1.126	0.019
	Al-Qatif-Residential	81.70	1.194	0.103	2.04E-04	0.555	0.010
	Saihat-Residential	13.27	1.163	0.061	2.21E-04	0.747	0.008

Table 3. Hazard index (HI) of the potentially toxic heavy metals in the dust of AC filters collected from urban and peri-urban areas of Eastern Saudi Arabia.

Age Group	Area	Cd	Cr	Cu	Mn	Pb	Zn
Adult	Al-Nabiah	0.428	0.075	0.038	0.006	0.066	0.004
	Al-Qatif-Agricultural	2.09	0.210	0.015	0.015	0.121	0.008
	Al-Qatif-Industrial	12.2	0.535	0.014	0.011	0.112	0.004
	Al-Qatif-Residential	5.00	0.082	0.011	0.006	0.055	0.002
	Saihat-Residential	0.813	0.079	0.006	0.007	0.074	0.002
Children	Al-Nabiah	7.00	1.13	0.437	0.027	0.787	0.030
	Al-Qatif-Agricultural	34.2	3.16	0.171	0.069	1.44	0.063
	Al-Qatif-Industrial	199	8.07	0.162	0.049	1.33	0.033
	Al-Qatif-Residential	81.8	1.24	0.125	0.028	0.654	0.018
	Saihat-Residential	13.3	1.20	0.075	0.031	0.881	0.014

Table 4. Cancer risk from ingestion of Cd, Cr and Pb contained in the dust of AC filters collected from urban and peri-urban areas of Eastern Saudi Arabia.

Metal	Age group	Al-Nabiah	Al-Qatif-Agricultural	Al-Qatif-Industrial	Al-Qatif-Residential	Saihat-Residential
Pb	Adult	5.52E-10	1.01E-09	9.32E-10	4.59E-10	0.14845
	Children	4.90E-10	8.99E-10	8.26E-10	4.07E-10	5.48E-10
Cd	Adult	9.92E-09	4.84E-08	2.82E-07	1.16E-07	1.88E-08
	Children	8.8E-09	4.29E-08	2.5E-07	1.03E-07	1.67E-08
Cr	Adult	1.55E-07	4.33E-07	1.1E-06	1.69E-07	1.65E-07
	Children	1.37E-07	3.84E-07	9.79E-07	1.5E-07	1.46E-07

Table 5. Characteristics of the bacterial candidates (gram staining) and fungi isolated from the dust of AC filters collected from urban and peri-urban areas of Eastern Saudi Arabia.

	Area	Isolate code	Stain	Comment
Bacteria	Al-Qatif-Agricultural (NA1)	1/NA 1	G. negative	single rods, producing mucus or exopolymer
		NA 1/2	G. positive	long rods in chain
		3/NA 1	G. negative	single short rods
	Al-Qatif-Residential (NA2)	1/NA 2	G. positive	<i>Bacillus</i> , thick and long rods in chain
		2/NA 2	G. positive	long rods in chain
		3/NA 2	G. positive	<i>Bacillus</i> sp & long rods
		4/NA 2	G. negative	thick and short rods
		5/NA 2	G. negative	short rods
		6/NA 2	G. positive	rods in chain with endospores
	Al-Qatif-Industrial (NA3)	1/NA 3	G. positive	<i>Staphylococcus</i> sp
		2/NA 3	G. positive	<i>Staphylococcus</i> sp
		3/NA 3	G. positive	short rods and endospore forming
	Area	Isolate code	Comment	
Fungi	Al-Qatif-gricultural NA1)	1/SD 1	<i>Stachybotrys chartarum</i> , black molds and <i>Cladosporium</i>	
	Al-Qatif-Residential (NA2)	1/SD 2	Yeast and candida	
	Al-Qatif-Industrial (NA3)	SD 3/1	Green molds, <i>Cladosporium</i> sp, <i>Penicillium</i> sp and <i>Aspergillus</i> sp	
		2/SD 3	Black <i>Aspergillus</i> sp	
		3/SD 3	Green molds	

Table 6. Effect of different antibiotics on bacterial candidates isolated from dust of AC filters collected from urban and peri-urban areas of Eastern Saudi Arabia.

Area	Isolate code	Size of inhibition zone (mm)			
		Neomycin	Kanamycin	Doxycycline	Ampicillin
Al-Qatif-Agricultural (NA1)	1/NA 1	19.5	26.3	35.3	17.6
	NA ½	18	23.3	23.1	18.3
	3/NA 1	23.3	26	22.3	22.0
Al-Qatif-Residential (NA2)	1/NA 2	18.3	13.6	13.6	16.3
	2/NA 2	18.3	27.6	18.3	14.0
	3/NA 2	16.6	No effect	15.3	18.3
	4/NA 2	19.5	9.6	12.3	16.3
	5/NA 2	21.6	27.3	16.0	No effect
	6/NA 2	15.3	17.5	22.0	No effect
Al-Qatif-Industrial (NA3)	1/NA 3	18.3	24.3	23.3	25.3
	2/NA 3	13.0	8.0	16.3	No effect
	3/NA 3	18.6	23.6	25.16	16.3

Table 7. Hemolytic activity of some bacterial candidates isolated from the dust of AC filters collected from urban and peri-urban areas of Eastern Saudi Arabia.

	Area	Isolate code	Hemolytic activity
Bacteria	Al-Qatif-Agricultural (NA1)	1/NA 1	α -hemolytic
		NA 1/2	β -hemolytic
		3/NA 1	β -hemolytic
	Al-Qatif-Residential (NA2)	1/NA 2	Non- hemolytic
		2/NA 2	α -hemolytic
		3/NA 2	Non- hemolytic
		4/NA 2	Non- hemolytic
		5/NA 2	α -hemolytic
		6/NA 2	β -hemolytic
	Al-Qatif-Industrial (NA3)	1/NA 3	Non- hemolytic
		2/NA 3	β -hemolytic
		3/NA 3	Non- hemolytic

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