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# Journal of Environmental Sciences

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***Reprint***

**Volume 48, Number 1 : 1 - 16  
(2019)**





Original Article

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### Article Info

#### Article history:

Received 23/11/2018  
Received in revised form 19/1/2019  
Accepted 30/1/2019

#### Keywords:

Damietta;  
Particulate Matters (PM);  
Aflatoxin;  
Ochratoxin A;  
Outdoor Air;  
Heavy Metals.

### Abstract

Air quality in cities is the result of a complex interaction between natural and anthropogenic environmental conditions. The ambient airborne particulate matter considered as a significant environmental pollutant. The present study aims to focus on isolation and identification of outdoor airborne fungi and heavy metals that were detected in atmospheric particulate matter from different sites in Damietta Governorate. The suspended particulate matter was sampled by using the filtration technique at four locations for three months, April, May and June 2015 in the atmosphere of Damietta Governorate. The average concentration of suspended particulate matter for sampling sites was  $124.653 \pm 34.713 \mu\text{g}/\text{m}^3$ . A total number of  $616 \text{ colony}/\text{m}^3$  was detected in the suspended particulate matter. Eight mold species belonging to three genera were isolated from suspended particulate matter: *Aspergillus*, *Fusarium*, and *Alternaria*. *Aspergillus* was the most frequent and their abundance was greater than the other fungal genera. Within these, *A. flavus* and *A. niger* were the predominant species and comprised 21% of the total fungal count of the isolates. The highest concentrations of aflatoxins (AFs) were 4.1 ng/ml and 2.123 ng/ml, which were produced by *A. parasticus* and *A. flavus* strains, respectively. On the other hand, the isolate of *A. niger* has the ability to produce ochratoxin A (OTA) with mean concentration of 7.775  $\mu\text{g}/\text{ml}$ . The concentrations trend of 6 heavy metals in the suspended particulate matter revealed that  $\text{Fe} > \text{Cu} > \text{Zn} > \text{Mn} > \text{Pb} > \text{Cd}$ .

### Introduction

Air pollution has been one of the major threats to human health and the environment in the past century. Monitoring toxic air pollutants is needed to understand their spatial and temporal distribution and ultimately to minimize their harmful effects (Stafilov *et al.*, 2017). In the past decades, many studies highlighted the role of ambient airborne particulate matter (PM) as an important environmental pollutant for many different cardiopulmonary diseases and lung cancer (Valavanidis *et al.*, 2008). The term 'particulate' refers to all atmospheric substances that are not gases. They can be suspended droplets or solid particles or a mixture of the two aerosols. Individual particles vary in size, geometry, mass, concentration, chemical composition and physical

properties. They may be produced naturally or as a direct or indirect result of human activities (Godish, 1997). Airborne PM is very complex multi-component mixtures originating from both natural and anthropogenic sources. The particulates may include a wide range of chemical species, ranging from metals to organic and inorganic compounds (Yadav and Satsangi, 2013). The natural sources include: suspended terrestrial dust, sea salt spray (mainly at coastal sites) and biomass burning (forest fires), and the major sources of anthropogenic or man-made, particles include transportation, stationary combustion, space heating, biomass burning, and industrial and traffic-related fugitive emissions (street dust) (Stefánsson *et al.*, 2007).

It is hypothesized that the respirable particles (aerodynamic diameter  $< 4 \mu\text{m}$ ) sometimes act as carriers for toxic compounds, such as the mycotoxins. Since these compounds are potentially carcinogenic (Turner *et al.*, 2009) and mutagenic, a better knowledge on exposure to mycotoxins is required due to molds or other causes (Robbins *et al.*, 2000). Airborne

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fungus contaminants are increasingly gaining importance due to the health hazards caused by their spores or microbial metabolites. Molds are fungi that can be found both indoors and outdoors. Molds grow best in warm, damp, and humid conditions, and spread and reproduce by making spores. Mold spores can survive harsh environmental conditions, such as dry conditions, that do not support normal mold growth. Mycotoxins are secondary metabolites produced by molds and microfungi in favorable conditions of pH, humidity, temperature, and substrates, able to cause disease in humans and other animals (Peraica *et al.*, 1999). They can be transported far from their source (Buiarellia *et al.*, 2015). These chemical compounds are lipid-soluble and quickly absorbed by the intestines and skin. Human beings and domestic animals come in contact with them by different routes (diet, dermal contact, respiration); the fungi which produce them occupy different ecological niches, and they span a wide range of critical fungal genera such as (*Aspergillus*, *Penicillium*, *Fusarium*, and other genera) (Hasballah, 2013). Mycotoxins released by molds occurring in damaged buildings in an indoor environment are also hazardous to health. Among these, aflatoxins produced by *A. flavus* are well-known in the world. Other mycotoxins include *citrinin*, *ochratoxin*, *patulin*, *trichothecenes*, and *zearalenone* can also be produced (Rai and Varma, 2010). Fungal spores floating in the open air are exposed to air currents and can travel very long distances (EL-Morsy, 2006). Medrela-Kuder (2003) and Adams *et al.* (2013) found that the numbers and species of airborne fungi vary with the season of the year. The most frequent airborne genera outdoors are *Cladosporium*, *Penicillium*, *Aspergillus*, and *Acremonium* (Gniadek *et al.*, 2005). In addition, Airborne fungi have been found to be associated with specific respiratory illness and allergy, while exposure to fungi and other microbes, their fragments and metabolites may constitute a health risk as increases in asthma attacks and bronchial hyperactivity and other respiratory symptoms such as lung cancer have been correlated to increased microbial and particulate levels in the atmosphere (WHO, 2000).

Owing to rapid urbanization and the increase in vehicles and industries, the concentrations of heavy metals that are purely anthropogenic, such as Cd and Zn, are increasing in ambient air. On the other hand, the strict implementation of unleaded gasoline usage has reduced the concentration of Pb remarkably (Suvarapu and Ok Baek, 2017). Heavy metals have toxic effects when they are involved in biochemical reactions in living organisms. Typical responses include growth inhibition, suppression of oxygen consumption and impairment of reproduction and tissue repair (Air Pollution Information System, 2016). Pb and Cd have attracted considerable interest because of their

persistent, bioaccumulative and toxic natures. In urban areas, road traffic is recognized as an essential source of both particles and certain metals. Road traffic involves numerous potential sources of metals, e.g., combustion products from fuel and oil, wear products from tires, brake linings, bearings, coach and road construction materials, and resuspension of soil and road dust (Sternbeck *et al.*, 2002). Heavy metals in the atmosphere can accumulate in various plants and animals and enter humans through the food chain. Most studies reported the major sources of the particulate matter and heavy metals in the atmosphere to be industrial emissions, vehicular emissions and secondary aerosols (Suvarapu and Ok Baek, 2017).

Air pollution in Damietta Governorate, which is categorized as urban site, comes from a great variety of light industries; such as shoes, dairy, textile, sweets industries, and in addition to the different activities of workshops; furniture and painting workshops, as well as from fuel combustion in motor vehicles (Al-Asmar, 2006). In addition to oil and gas exploration, refining and tourism. The area also benefits from a growing fishing industry, which obtains most of its catch from the Mediterranean Sea. Also, sewage and solid wastes, which flow from cities, villages and tourist resorts which either have partial or non-existent drainage treatment plants, So Damietta branch receive nutrients and high organic loads (Hasballah, 2013). Since there is a lack of knowledge on the pathogenic fungi in outdoor air of Damietta Governorate, so the isolation and identification of outdoor airborne fungi and heavy metals in an atmospheric PM from different sites of Damietta Governorate is the aim of this study.

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## Methodology

### Background of Study Area

Damietta Governorate lies between latitude 31.26 N and longitude 31.48 E and locates in the north of the Delta near the seashore at the end of the River Nile. It lies 15 km from the Nile estuary, and the River Nile "Damietta sector" separates it into two parts surrounded by The Mediterranean Sea to the north, Al-Manzala Lake to the east, and the Delta farms and plains to the south and the west. Damietta Governorate consists of four administrative districts. These are Damietta City, Faraskour, Kafr-Saad, and El-Zarka. It is boarded on all sides by Dakahleya Governorate except for its northern border, which faces the Mediterranean Sea (Damietta Governorate, the administration of the public relations, 2015).

### Sampling Strategy

In the present study, particulate matters were collected at four sites in Damietta Governorate for three months (April-May-June 2015) using the filtration method. The air sampler was placed on a platform at the height of  $6 \pm 2$  m above ground level, rooftop (ambient air). Average sampling time was about 24-h periods at least once a week/site. Samples were collected from selected sites using Global Positioning System (GPS), as follow: El-Zarka (Site 1), Kafr El-Battekh (Site 2), New Damietta City (Site 3) and Damietta City (Site 4). The samples locations are characterized by their strategic positions with various traffic density and commercial activities. Description of the selected sites is summarized in **Map (1)**. SPM sampler was employed using cellulose membrane fiber filters (Whatman: pore size:  $0.45 \mu\text{m}$ ; diameter: 47 mm) to collect air samples. The stacked filter unit constituted a part of the sampling set-up, also consisting of a vacuum pump and a flow meter, calibrated to draw 1.5 L/min. The volume of air sampled was calculated from the total sampling time multiplied by the mean flow rate (average of pre and post-sampling rates) (**Harrison and Perry, 1986**).

### Determination of Suspended Particulate Matter (SPM) Concentrations

The concentration of SPM matter was calculated in microgram per cubic meter ( $\mu\text{g}/\text{m}^3$ ) (**Katz, 1986**), by using the following relation:

$$\text{SPM Conc. } (\mu\text{g}/\text{m}^3) = \frac{W(\mu\text{g}) \times 1000}{R(\text{L}/\text{min}) \times T(\text{min})}$$

Where: W = the weight of collected SPM ( $\mu\text{g}$ ),

R = the mean flow rate (l/min),

T = the sampling time (min).

### Isolation and Identification of Fungi

Fungal species that isolated from SPM were collected on filter papers according to **Harrigan and Margaret (1966)**. Weighted filter papers of SPM were mixed separately with sterilized distilled water to a final volume of 10 ml. One ml of the diluted sample was spread on to Czapek's agar or potato dextrose agar (PDA) media and incubated at 28-30°C for 3-5 days. Subculturing was repeated successively until pure fungi were obtained and were identified according to their cultural morphology and spores description (**Raper et al., 1968** and **Domsch et al., 1980 a and b**). The fungal culture was grown on (PDA) slants at 28 °C for about seven days or until good sporulation was observed. Spores were harvested by adding 10 ml of a sterilized

aqueous solution of Tween 80 (0.05% v/v) to cultures (**Ramakrishna et al., 1996**). Spore suspensions were then centrifuged at 20,000 rcf for 5 min and the supernatants discarded. The spore concentrations were adjusted to yield a final count of 105 spores/ml, and the ensuing preparations were used as spore inocula.

### Growth Media

The culture media used in this study was yeast extract sucrose (YES) to produce aflatoxins. The yeast extract sucrose culture was carried out according to the method of (**Munimazi and Bullerman, 1998**) at following (2% yeast extract and 15% sucrose/liter distilled water) were poured into 500 Erlenmeyer flask, and autoclaved at 121°C for 15 min.

### Preparation of Fungi for Production of Aflatoxins

Two hundred mL of yeast extract sucrose was transferred into 250 Erlenmeyer flasks and autoclaved at 121°C for 15 min. The yeast extract sucrose medium was inoculated with fungal strain and incubated at 28°C for 14 days.

### Extraction of Aflatoxins from Liquid Media

Aflatoxins were extracted according to the method described by **El-Banna et al. (1987)**. Extraction was carried out using 20 ml of chloroform (twice with 10 ml media), and homogenization for 3 min in a separation funnel. The chloroform phase was filtered through filter paper Whatman No. 3 and concentrated to dryness under a nitrogen stream.

### Determination of Aflatoxins by HPLC

100  $\mu\text{l}$  of trifluoroacetic acid was added to the dry residue of each sample and standard and vortexed for 30 sec. Kept for 15 min at 40°C in a water bath, then evaporated to dryness under nitrogen. 900  $\mu\text{l}$  water/acetonitrile mixture (9/1, v/v); was added to each vial to dissolve residue and vortexed and used for HPLC analysis. The HPLC system consisted of Waters Binary Pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Waters 2475 Multi-Wavelength Fluorescence Detector, and a data workstation with software Breeze. A phenomenon C18 (250 x 4.6 mm i.d.), 5 m from Waters Corporation (USA). The mobile phase consists of acetonitrile/water/methanol (1:6:3, by volume). The separation was performed at an ambient temperature at a flow rate of 1.0 ml/min. The injected volume was 20  $\mu\text{l}$  for either the standard solutions or the sample extracts. The fluorescence detector was operated

at an excitation wavelength of 365 nm and an emission wavelength of 440 nm. AFs concentration in samples was determined from the standard curve, using peak area for quantity estimation (AOAC, 2000).

### Determination of (OTA) by HPLC

Ten milliliters of culture medium was filtered through a 0.2µm syringe filter and extracted with 20 ml of chloroform. The chloroform phase was filtered with sodium sulfate anhydrous and concentrated to dryness under a nitrogen stream. The precipitate was dissolved in 1 ml water: acetonitrile (3: 1 v/v) and mixed well by vortex for 30 s. The mixture was used for HPLC analysis. An isocratic system with acetonitrile: water: acetic acid (55: 43: 2) was used. The separation was performed at an ambient temperature at a flow rate of 1.0 ml/ min. The injection volume was 20µL for both standard solutions and sample extracts. The fluorescence detector was operated at a wavelength of 335 nm for excitation and 465 nm for emission. OTA concentrations in samples were determined from the standard curve using peak area for quantification (AOAC, 2000).

### Determination of Metallic Constituents

Six elements were measured to study their levels and sources. The measured heavy metals were: Pb, Mn, Fe, Zn, Cd, and Cu. Particulate samples were collected on filters, and acid digested (using a mixture of acids, HNO<sub>3</sub>-HCl, 1:2 respectively), to oxidize the organic matrix and to dissolve the metals present in the sample. Flame atomic absorption spectrophotometry subsequently makes the analysis (A.A.S). Perkin-Elmer double beam 2380 atomic absorption spectrometer was used with adapted Perkin-Elmer hollow-cathode lamps and conventional 10-cm slot burner head for an air-acetylene flame (Lodge and Editor, 1998).

### Assessment of Metals Contamination

The levels of contamination of the heavy metals in the SPM were assessed by determining the contamination factors (Cf) of each metal according to the method developed by Håkanson as reported by Yekeen and Onifade (2012).

$$Cf = C_m / C_{ref} \text{ -----1}$$

Where C<sub>m</sub> is the measured concentration of the heavy metal in the SPM and C<sub>ref</sub> is the reference value of heavy metal used. Furthermore, each area was evaluated for the extent of metal pollution by employing the method based on the Pollution Load Index (PLI) developed by Thomilson *et al.* (1980), as follows:

$$PLI = (Cf_1 \times Cf_2 \times Cf_3 \times Cf_4 \dots \times Cf_n)^{1/n} \text{ ----- 2}$$

Where n is the number of metals studied, and Cf is the contamination factor calculated for each metal as described in equation 1. The PLI provides simple but comparative means for assessing a site quality (Thomilson *et al.*, 1980). The categories of pollution level indices developed for the description of SPM contamination include PLI < 1 represents perfection, PLI = 1 represents baseline level of pollution and PLI > 1 denotes deterioration of site quality (Thomilson *et al.*, 1980).

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## Results

### 1. Evaluation of PM Concentration

The average concentration of SPM for sampling sites in Damietta Governorate atmosphere was 124.653±34.713 µg/m<sup>3</sup> (Table (1)). This concentration is about 0.524 times lower than the maximum allowable level that given by the Egyptian Environmental Law No. 4, 1994 (230 µg/m<sup>3</sup> 24 h). Furthermore, most of the recorded daily levels of SPM highly exceeded the NAAQS of 150 µg/m<sup>3</sup> set as a maximum concentration over 24 h. Table (2) represents that the mean levels of SPM at investigated sites were 164.352, 114.352, 82.562, and 137.346 µg/m<sup>3</sup> at sites 1, 2, 3 and 4, respectively.

### 2. Fungal Species

The results in Table (3) indicated that SPM contaminated with eight species belonging to three genera with total fungal count 616 colony/m<sup>3</sup>. *Aspergillus*, *Fusarium*, and *Alternaria* were the most prevalent fungi in all examined samples. Where *A. flavus* and *A. niger* constituted 21% of the total fungal count of the isolates, followed by *Aspergillus parasiticus* (16%). *A. ochraceus*, *Fusarium equiseti* and *Alternaria alternata* (11%) for each. While *A. fumigatus* and *A. terreus* represented the lowest occurrence of 5%.

### 3. Detection of (AFs) in SPM

The ability of isolated fungi to produce AFs in yeast extract sucrose (YES) media was examined. The results in Table (4) indicated that an isolate of *A. flavus* isolated from SPM collected from the site (1) can produce AFs with concentrations of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub> with values of 1.33, 0.268, 0.347 and 0.178 ng/ml, respectively. While *A. flavus* isolated from SPM collected from the site (4) produced AFB<sub>1</sub> and AFB<sub>2</sub> with values of 0.146 and 0.112 ng/ml, respectively. Whereas the other strain of *Aspergillus fumigatus* isolated from SPM collected from the site (4)

was found to produce AFs with values of 0.153, 0.278 and 0.357 ng/ml for AFB<sub>1</sub>, AFB<sub>2</sub>, and AFG<sub>2</sub>, respectively. On the other hand, the strain of *A. parasiticus* isolated from SPM collected from the site (3) was found to produce AFB<sub>2</sub> and AFG<sub>2</sub> with concentrations of 1.85 and 2.26 ng/ml, respectively. Other strain of *A. niger* isolated from SPM collected from site (3) and site (4) was found to produce OTA with concentrations of 5.73 and 9.82 ng/ml, respectively. The isolates of *Aspergillus terreus*, *Fusarium equiseti* and *Alternaria alternata* isolated from SPM did not produce any types of aflatoxins. Many known toxigenic *Fusarium* and *Aspergillus* spp. were found to be nontoxic in this paper, which could be as a result of mutation as the repeated process of subculturing may lead to loss of mycotoxins producing ability in the organism. That could also be to the strains of these toxigenic fungi do not have the genetic capacity to elaborate toxins (Makun *et al.*, 2010).

#### 4. Evaluation of Heavy Metals

Table (6) shows that the average lead (Pb) concentration was  $1.62 \pm 1.96 \mu\text{g}/\text{m}^3$ . This result slightly exceeded the Egyptian limit concentration of Pb at  $0.5 \mu\text{g}/\text{m}^3$  (EEAA, 1995). The average manganese (Mn) concentration was  $1.64 \pm 1.15 \mu\text{g}/\text{m}^3$ . This result exceeded WHO guideline value for manganese  $0.15 \mu\text{g}/\text{m}^3$  (Fernández Espinosa *et al.*, 2002). It was noticed that the mean Mn concentrations for sampling sites were 2.36, 0.02, 1.61 and  $2.55 \mu\text{g}/\text{m}^3$  at sites 1, 2, 3 and 4, respectively. The average iron (Fe) concentration was  $18.27 \pm 9.89 \mu\text{g}/\text{m}^3$ . This result exceeded UNEP's permissible limit of  $0.005 \mu\text{g}/\text{m}^3$  (NESREA, 2009). The mean levels of Fe for sampling sites were 30.25, 29.61, 10.83 and  $15.38 \mu\text{g}/\text{m}^3$  at sites 1, 2, 3 and 4, respectively. The average Zinc (Zn) concentration was  $4.99 \pm 3.18 \mu\text{g}/\text{m}^3$ .

The average cadmium (Cd) concentration was  $0.25 \pm 0.15 \mu\text{g}/\text{m}^3$ . This result exceeded WHO guideline value for cadmium  $0.005 \mu\text{g}/\text{m}^3$  (Fernández Espinosa *et al.*, 2002). It was noticed that the mean Cd concentrations were 0.36, 0.39, 0.10 and  $0.14 \mu\text{g}/\text{m}^3$  at sites 1, 2, 3 and 4, respectively. The average Copper (Cu) concentration was  $11.49 \pm 7.09 \mu\text{g}/\text{m}^3$ . This result exceeded The Occupational Safety and Health Administration (OSHA) for copper in the air in workplaces ( $0.001 \mu\text{g}/\text{m}^3$ ) (ATSDR, 2004). In Damietta Governorate, the trend of the concentrations of these heavy metals in the SPM revealed that  $\text{Fe} > \text{Cu} > \text{Zn} > \text{Mn} > \text{Pb} > \text{Cd}$ .

#### 5. Contamination Factors of Heavy Metals

The contamination factors of the heavy metals in Damietta Governorate showed very high environmental contamination by Cd followed by Cu and then Mn (Fig.

2). The CF reflects the metal enrichment in the SPM. According to Yekeen and Onifade (2012), classification of the contamination factor (Cf) of heavy metals was classified into four groups indicated that the  $\text{Cf} < 1$  refers to low contamination;  $1 \leq \text{Cf} < 3$  means moderate contamination;  $3 \leq \text{Cf} \leq 6$  indicates considerable contamination and  $\text{Cf} > 6$  shows very high contamination, respectively. The Cf in the study sites where metal concentration was high. Consequently, it can be concluded that, in Damietta Governorate, a Cf for Cd, Cu, and Mn represent very high contamination, and a Cf for Zn, Fe, and Pb represent moderate contaminations.

#### 6. Pollution Load Indices of Heavy Metals

The result of the pollution load indices of the heavy metals in SPM of Damietta Governorate shows that there is potential deterioration of the site quality (Fig. 3). According to the model of Thomilson *et al.* (1980), a Pollution Load Index (PLI)  $> 1$  denotes deterioration of site quality. Consequently, it can be concluded that a Pollution Load Index (PLI) of Damietta Governorate denotes deterioration of site quality. The concentrations of the heavy metals recorded in this study exceeded the ambient air permissible limits. The similar trend of the contamination of SPM observed by Thomilson *et al.* (1980), and they suggested that this is maybe because several natural and anthropogenic sources introduced a varying degree of trace metals into the atmosphere which may be transported over a long distance within an area.

#### 7. Statistical Analysis

The correlation matrix for various metals and SPM is shown in Table (7). High correlation coefficients indicate the existence of common emission patterns and source types, and in that case, the source which is effective over the three locations is vehicular traffic. SPM was correlated significantly with Fe and Cd ( $r > 0.5$ ). Fe and Cd had the highest correlations ( $r = 0.656$  and  $0.640$ ), respectively with SPM. Pb has a functional association with Fe and Cu ( $r = 0.583$  and  $0.606$ ), respectively. This is due to that the major sources at these locations are motor vehicles, while Pb has a low association with Mn ( $r = 0.361$ ), this can be attributed to the fact that Mn in the atmosphere of these sites was mainly emitted from mechanical workshops for repairing cars, while Pb source is motor-vehicles distributed in these areas. Moreover, Pb has a low association with Zn and Cd ( $r = 0.085$  and  $0.486$ ), respectively. Manganese has a functional association with Cu ( $r = 0.522$ ). The result indicates similar sources of origin for most of the elements. On the other hand,

Mn has a negative association with Fe, Zn, and Cd, reflecting a different emission source. Iron was highly correlated with Cd and Zn ( $r = 0.991$  and  $0.855$ ), respectively, this indicates similar sources of origin for most of the elements. On the other hand, Fe has a negative association with Cu. Zinc was highly correlated with Cd ( $r = 0.898$ ), this can be attributed to the fact that Zn and Cd in the atmosphere of these sites

were mainly emitted from motor vehicles. On the other hand, Zn has a negative association with Cu. Furthermore, Cd has a negative association with Cu, and that in agreement with **Huang et al. (2009)** who stated that the strong positive correlations among elements indicate that the characteristics and origins of emission for these elements might be similar.

**Table (1):** Concentrations of SPM Detected at Damietta Governorate Compared with Air Quality Standard for 24 h ( $\mu\text{g}/\text{m}^3$ ).

Location	Concentration ( $\mu\text{g}/\text{m}^3$ )	Reference
<b>Egypt: Damietta Governorate</b>	124.653	<b>The Present Work</b>
<i>WHO Air Quality guideline (WHO).</i>	120	<b>WHO, (1997).</b>
<i>Air Quality Standard (U.S.A).</i>	260	<b>Dara, (1993)</b>
<i>Egypt</i>	230	<b>EEAA, (1995)</b>
<i>Poland</i>	200	<b>Shakour, (1982)</b>
<i>California</i>	50	<b>Shakour, (1982)</b>
<i>South Africa</i>	100	<b>Farmer, (1997)</b>
<i>USSR</i>	150	<b>Shakour, (1982)</b>

**Table (2):** Mean Concentrations $\pm$ S.D. of SPM ( $\mu\text{g}/\text{m}^3$ ) for 24 hr., at the Investigated Sites at Damietta Governorate, Egypt from April-June 2015.

Site Season	1	2	3	4	Mean	Max.	Min.	S. D.
<b>April 2015</b>	146.991	120.833	47.454	164.352	119.908	164.352	47.454	51.508
<b>May 2015</b>	138.889	62.500	130.787	121.528	113.426	138.889	62.500	34.684
<b>June 2015</b>	207.176	159.722	69.445	126.157	140.625	207.176	69.445	57.936
<b>Mean</b>	164.352	114.352	82.562	137.346	124.653	164.352	82.562	34.713
<b>Max</b>	207.176	159.722	130.787	164.352				
<b>Min.</b>	138.889	62.500	47.454	121.528				
<b>S. D.</b>	37.307	48.934	43.187	23.502				

**Table (3):** Isolated Fungal Species and Percentage of Occurrence in SPM.

Fungal Sp.	Number of Occurrence	Frequency Ratio (%)
<i>Aspergillus flavus</i>	4	21
<i>Aspergillus fumigatus</i>	1	5
<i>Aspergillus niger</i>	4	21
<i>Aspergillus ochraceus</i>	2	11
<i>Aspergillus terreus</i>	1	5
<i>Aspergillus parasiticus</i>	3	16
<i>Fusarium equiseti</i>	2	11
<i>Alternaria alternate</i>	2	11
<b>Total fungal count (colony/m<sup>3</sup>)</b>	616	



**Table (4):** AFs Production by Some Toxicogenic Fungi Isolated from SPM.

Fungi Producing Toxins	Site	Concentration of AFs (ng/ml)				
		AFB <sub>1</sub>	AFB <sub>2</sub>	AFG <sub>1</sub>	AFG <sub>2</sub>	Total AFs
<i>Aspergillus flavus</i>	1	1.33	0.268	0.347	0.178	<b>2.123</b>
<i>Aspergillus flavus</i>	4	0.146	0.112	ND	ND	<b>0.258</b>
<i>Aspergillus fumigatus</i>	4	0.153	0.278	ND	0.357	<b>0.788</b>
<i>Aspergillus terreus</i>	3	ND	ND	ND	ND	ND
<i>Aspergillus parasticus</i>	3	ND	<b>1.85</b>	ND	<b>2.26</b>	<b>4.11</b>
<i>Fusarium equiseti</i>	1-4	ND	ND	ND	ND	ND
<i>Alternaria alternata</i>	1-2	ND	ND	ND	ND	ND

ND = Not Detected

**Table (5):** OTA production by some toxicogenic fungi isolated from SPM.

Fungi Producing Toxins	Site	Concentrations of OTA (µg/ml)
<i>Aspergillus niger</i>	3	5.73
<i>Aspergillus niger</i>	4	9.82

**Table (6):** Mean Concentrations±S.D. of Heavy Metals (µg/m<sup>3</sup>) for 24 hr., at the Investigated Sites at Damietta Governorate.

Site Element	1	2	3	4	Mean	Max.	Min.	S. D.
<b>Pb</b>	0.12	1.67	4.37	0.3	1.62	4.37	0.12	1.96
<b>Mn</b>	2.36	0.02	1.61	2.55	1.64	2.55	0.02	1.15
<b>Fe</b>	30.25	29.61	10.83	15.38	18.27	30.25	10.83	9.89
<b>Zn</b>	7.25	7.89	3.72	1.09	4.99	7.89	1.09	3.18
<b>Cd</b>	0.36	0.39	0.10	0.14	0.25	0.39	0.10	0.15
<b>Cu</b>	10.64	3.16	20.47	11.68	11.49	20.47	3.16	7.09

**Table (7):** Correlation Coefficients between Heavy Metals and SPM over Damietta Governorate.

	SPM	Pb	Mn	Fe	Zn	Cd	Cu
<b>SPM</b>	--	-0.758	-0.281	<b>0.656</b>	0.269	<b>0.640</b>	<b>-0.940</b>
<b>Pb</b>		--	0.361	<b>0.583</b>	0.085	0.486	<b>0.606</b>
<b>Mn</b>			--	-0.354	-0.600	-0.474	<b>0.522</b>
<b>Fe</b>				--	<b>0.855</b>	<b>0.991</b>	<b>-0.817</b>
<b>Zn</b>					--	<b>0.898</b>	<b>-0.565</b>
<b>Cd</b>						--	<b>-0.831</b>
<b>Cu</b>							--

The values in bold indicate a significant correlation between those two parameters ( $\alpha=0.5$ ).



Map (1) : Location Map of the Study Area (Damietta Governorate) Showing the Sites of Investigations.

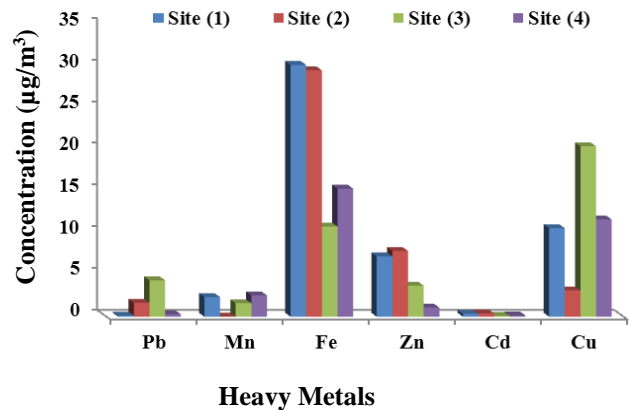


Figure (1): Mean Concentrations of Heavy Metals over the Sites under the Investigation at Damietta Governorate.

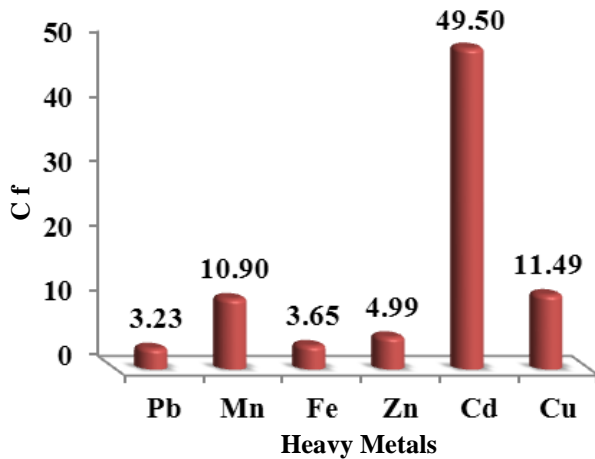


Figure (2): Contamination Factors of the Heavy Metals.

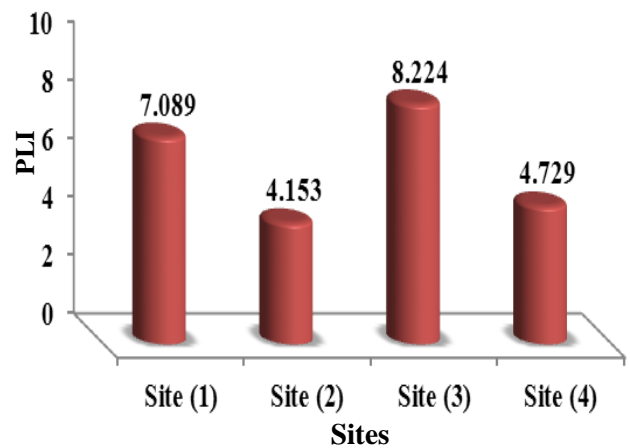


Figure (3): Pollution Load Index of the Heavy Metals.

## Discussion

### 1. Evaluation of PM Concentration

The maximum mean concentration of SPM (Site 1) that may be attributed to that this site is characterized by commercial activities, crop residue burning activities and high density of motor vehicles. Site (1) is located at the southern part of Damietta Governorate; it is chiefly affected also by northern winds loaded with natural dust and sea spray beside the high concentration of particulate matter emitted from traffic, especially diesel emission. This is in agreement with **Yatkin and Bayram (2008)** who found that urban traffic contributes more than 70% for particulate matter as well as heavy metals in ambient air of Izmir, Turkey. Also, **Singh et al. (2017)** observed a significant increase in the levels of SPM at an agricultural site in Patiala during April-May and October-November as compared to other sampling months due to crop residue burning activities in these months. The obtained results of SPM were above or

nearly similar with **Basahi et al. (2017)** who found that the extreme values of TSP occurred on days when dust storms passed through the western coast of Saudi Arabia.

### 2. Fungal species and (AFs) in SPM

Fungi are common in indoor and outdoor environment. Nearly 10% of people worldwide have fungal allergy. Numerous studies have shown that exposure to fungi may be associated with acute toxic effects, allergies, and asthma (**Pavan and Manjunath, 2014**). Our results of fungal species were in agreement with **Pei-Chih et al. (2000)**; **Gniadek et al. (2005)** and **EL-Morsy, (2006)** who detected that the most frequent airborne genera outdoors are *Cladosporium*, *Penicillium*, *Aspergillus*, and *Acremonium*. The presence of these species in air may be attributed to weather condition such as warm and moist condition as spores of certain species may survive for long periods, while others may

decline very rapidly (Flannigan and Miller, 1994 and Adams *et al.*, 2013). Fungal spores floating in the open air are exposed to air currents and can travel very long distances. The concentration of airborne fungal spores has been related to wind, humidity, temperature, rainfall, altitude, vegetation, and some specific reservoirs of contamination. Also, fungal propagative units may be dispersed in the air by insects (Asan *et al.*, 2003). In addition, (Rosas *et al.*, 1992) demonstrated that *Aspergillus* and *Penicillium* spores are the most widespread aeroallergens in the world. According to Asan *et al.* (2003), many molds, especially *Cladosporium* and *Aspergillus* species can occur naturally in the exterior environment and enter the indoor environment as spores or active fungi attached to dust particles. *Alternaria*, *Penicillium*, *Aspergillus*, and *Fusarium* were found to be the dominant types in some studies such as (Savino and Caretta, 1992; Mentese *et al.*, 2009; Hanson *et al.*, 2016 and Rostami *et al.*, 2017). Also the results in agreement with Pavan and Manjunath (2014) they isolated 12 genera from the outdoor environment of the cowshed and they found that the dominant fungal species were *Cladosporium* sp., *Aspergillus* sp., and *Alternaria alternata* and seasonal occurrence of fungal spores in both indoor and outdoor of the cowshed revealed that maximum spores were recorded in summer season. The results in Table (4) were in agreement with Mahmoud *et al.* (2016) they found that *A. flavus* had the highest ability to produce toxin in comparison to other *Aspergillus* species. Moreover, they added that because of this high potential for toxin production such as AFB<sub>1</sub> and AFB<sub>2</sub>, this species is one of the most important fungal species related to human health and animals. Other strain of *A. niger* isolated from SPM was found to produce OTA this result is in agreement with Abdel Hameed *et al.* (2012) who stated that *Fusarium*, *Aspergillus* and *Penicillium* are the main mycotoxin producers, *Penicillium verrucosum* and *A. ochraceous* produce OTA which is often higher in grains. Teren *et al.* (1996) mentioned that *Aspergillus carbonarius* has been identified as a third major source of OTA, together with a low percentage of isolates of the closely related species of *A. niger* (Table 5). The isolates of *Aspergillus terreus*, *Fusarium equiseti* and *Alternaria alternata* isolated from SPM did not produce any types of aflatoxins. The results are in agreement with Pei *et al.* (2009) who stated that AFs are a group of toxic chemical compounds produced by the species of *A. flavus*, *A. parasiticus* and rarely by *A. nomius*. AFB is primarily produced by *Aspergillus flavus*, while the other two species produce both B and G aflatoxins. Furthermore, Mphande *et al.* (2004) investigated 32 isolates of *A. flavus* for their ability for mycotoxins production. They found that 11 isolates of them did not produce detectable AFs, eight isolates produced only AFB<sub>1</sub> and AFB<sub>2</sub>, and 13 produced all four aflatoxins

(AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub>) in varying amounts. Razzaghi-Abyaneh *et al.* (2006) isolated *A. flavus* strains from soil and observed that 27% of the strains were AFs producers. Furthermore, these results are in accordance with El-Samawaty *et al.* (2012) who demonstrated that *Aspergillus* were the most predominant myco-pollutants of outdoor air in Riyadh city, also found that *A. flavus* was the highest producer of B<sub>1</sub> (6.6 ppb), G<sub>1</sub> (3.82 ppb) and G<sub>2</sub> (2.82 ppb).

### 3. Evaluation of Heavy Metals

The results of Pb in our study were lower than concentrations of lead in the city atmosphere, 6.81 to 4.15 µg/m<sup>3</sup> recorded by El-Henawy (2005). Cho *et al.* (2011) concluded that the decline in the concentrations of Pb in ambient air was due to the ban on tetraethyl lead in gasoline. This is in agreement with Busheina *et al.* (2017) who reported that about 90% of lead in the atmosphere comes from the exhaust gases of motor vehicles, due to the application of lead in fuels. Moreover, spring months characterized by the passage of "Khamasin" dust-laden winds, which is a local hot, dry, and dust-laden winds and are mainly southerly and easterly winds, occur frequently during the period of February through June (El-Zeiny, 2010). Jaradat and Momani (1999) documented that the major sources of atmospheric manganese could be suspension of road dust by vehicles, wind erosion and suspension of soil. Traffic emissions, suspension of road dust by vehicles, wind erosion and suspension of soil seem to be the primary source of Mn pollution. Sekhavatjou *et al.* (2010) indicated that the traffic of vehicle highly influences the concentration level of Fe. Moreover, Islam *et al.* (2015) documented that Fe exhibited relatively higher values because Fe may originate from soil dust, poorly managed transport, and building construction, among others, the major sources of Fe are both anthropogenic and crustal in origin, including iron and steel manufacturing units and the weathering of exposed Fe in urban areas. The results of Zn exceeded the averaged concentration of element Zn (1.060 µg/m<sup>3</sup>) obtained in traffic junction from Taichung, Taiwan by Fang *et al.* (2005). The mean levels of Zn for sampling sites were 7.25, 7.89, 3.72 and 1.09 µg/m<sup>3</sup> at sites 1, 2, 3 and 4, respectively. That in agreement with El-Henawy (2005) detected that the maximum annual mean concentration of zinc in Damietta City was 1.99 µg/m<sup>3</sup>. Furthermore, Islam *et al.* (2015) documented that zinc is an element commonly found in the Earth's crust. It is released to the environment from both natural and anthropogenic sources; however, releases from anthropogenic sources are greater than those from natural sources. Fernández-Espinosa and Ternero-Rodríguez (2004) and da Silva *et al.* (2008) documented that elements such as Cd are traffic related

elements, which can be found in the atmosphere of the cities. ATSDR (2012) observed that the major sources of Cd in ambient air are dust particles, volcanic eruptions, forest fires and industrial activities involving Cd. The major sources for human exposure through inhalation are cigarette smoke and occupational impacts. Pujol *et al.* (2016) indicated that the main source of Cu was road traffic, but a significant contribution was the result of industrial activity. Copper enters air, mainly through release during the combustion of fossil fuels and can be released into the environment by both natural sources such as, wind-blown dust, decaying vegetation, and sea spray, by precipitation and particles into the atmosphere; they settle out on soil and water surfaces, other sources of the atmosphere are Cu-containing fungicides, metalworking factories and electroplating materials (Baptista *et al.*, 2008). The results of contamination factors of heavy metals indicated that Damietta Governorate is very high contaminated concerning Cd. Chen *et al.* (2015) revealed that the enrichment factor values for Zn and Cd were found to be higher than 10. Most of the studies (Basha *et al.*, 2010; Tan *et al.*, 2014; Zhang *et al.*, 2014) reported that the enrichment factor for Cd was always highest, which indicates the major sources for Cd into the atmosphere were anthropogenic. In contrast, the major sources of Fe and Zn were mostly natural because their enrichment factor values were lower than 1.

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## Conclusion

The results indicated that the ambient air contaminated by eight mold species belonging to three genera, *Aspergillus*, *Fusarium* and *Alternaria*. *A. parasticus* and *A. flavus* strains could produce aflatoxins (AFs) in addition to *A. niger* has the ability to produce (OTA). Many known toxigenic *Aspergillus*, *Fusarium* and *Alternaria spp.* were found to be nontoxic in this study. The results revealed that the major sources of heavy metals in ambient air of Damietta Governorate are as follows: soil and suspended dust for Fe and Mn; vehicular emissions for Pb, Mn, Cu, Zn, Cd and Fe and brake wear for Cu, Fe, Zn, and Mn. The trend of the concentrations of these heavy metals in the SPM revealed that Fe > Cu > Zn > Mn > Pb > Cd.

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**Acknowledgments:** The authors express their deep thanks to the staff members and colleagues of the Environmental Sciences Department, Faculty of Science, Damietta University, for their continuous cooperation and encouragement.

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الملخص العربي

الكشف عن التلوث بالفطريات الممرضة والمعادن الثقيلة في الهواء الخارجي لمحافظة دمياط ، مصر.

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يعدُّ تلوث الهواء المحيط الذي يتكون من تركيزات مرتفعة من الجسيمات الصغيرة والدقيقة، أكثر المخاطر البيئية إضراراً بالصحة. وتعتبر الجسيمات العالقة أخطر الجسيمات الملوثة للهواء حيث من الممكن ان تصل للرئتين وتستقر هناك. وتتراكم هذه المواد العالقة في الهواء في الجهاز التنفسي وينجم عنها تأثيرات صحية متعددة. فعند التعرض للمواد العالقة الدقيقة ينجم عنها عدة مشكلات أهمها زيادة الحالات الإسعافية، والتنويم بالمستشفيات المتعلقة بأمراض القلب والرئتين، وتدني في كفاءة عمل الرئتين، وأحياناً الموت المبكر. ويتعدى تأثير هذه المواد العالقة المشكلات الصحية ليشمل تدني الرؤية، وما تسببه من مشكلات، وتدمير للألوان والدهانات ومواد المباني.

وتهدف الدراسة الحالية إلى التركيز على عزل وتحديد الفطريات المحمولة في الهواء والمعادن الثقيلة التي اكتشفت في الجسيمات العالقة من مواقع مختلفة في محافظة دمياط. وقد تم أخذ عينات الجسيمات العالقة باستخدام تقنية الترشيح في أربعة مواقع لمدة ثلاثة أشهر ، أبريل ومايو ويونيو 2015 في أجواء محافظة دمياط . وبلغ متوسط تركيز الجسيمات العالقة في مواقع أخذ العينات  $124.653 \pm 34.713$  ميكروجرام/م<sup>3</sup>. وتم عزل عدد إجمالي 616 مستعمرة / م<sup>3</sup> من الجسيمات العالقة . وقد تم عزل ثمانية أنواع من الفطريات التي تنتمي إلى ثلاثة أجناس من الجسيمات العالقة *Aspergillus* ، *Fusarium* ، و *Alternaria*. كان *Aspergillus* الأكثر تكرارا ووفرة أكثر من الأجناس الأخرى من الفطريات . وضمن هذه الفصائل ، كان الفطر *A. flavus* و *A. niger* هما النوعان السائدان وكانا يشكلان 21٪ من إجمالي عدد الفطريات للعزلات . وكان أعلى التركيزات من الأفلاتوكسين (AFs) كانت 4.1 نانو غرام/مل و 2.123 نانو غرام/مل ، والتي أنتجت من سلالات *A. parasticus* و *A. flavus* ، على التوالي . من ناحية أخرى ، فإن لعزلة *A. niger* القدرة على إنتاج *ochratoxin A (OTA)* بمتوسط تركيز 7.775 ميكروجرام/مل . كما أظهر اتجاه تركيزات ست معادن ثقيلة في الجسيمات العالقة أن ترتيبهم في محافظة دمياط كالتالي  $Fe > Cu > Zn > Mn > Pb > Cd$ .