Airborne pollen and spores' deposition in alveolar tissues as a tool in drowning forensic diagnosis.

Abstract

We report the results of a histological study of lung samples where an unusual quantity of airborne pollen and fungal spores were found in drowned rats. Pollen and spores were found in lungs of drowned rats but not in the post-mortem submerged ones. Another control group consisting of rats that underwent 60 minutes exposure to a highly pollen loaded atmosphere also recorded negative for the presence of pollen or fungal spores. Pollen types coincided with plants growing at the surrounding gardens flowering during the days of the experiment, performed during spring, that were detected by the aerobiological trap located at the city. The pollen observed at the lower airways' tissues were Chenopodiaceae, *Cupressus*, Ericaceae, *Jasminum*, *Olea europaea*, *Plantago*, *Pinus*, Poaceae, *Quercus* and Urticaceae. Regarding fungal spores *Alternaria, Aspergillus, Cladosporium cladosporoides, Cladosporium herbarum, Leptosphaeria, Polythrincium* and *Phitomyces*.

Pollen and spores' penetration into deeper regions of the respiratory tract is an unusual phenomenon not happening in regular breathing conditions. Our results revealed that these particles appeared in a significant number in lung samples of drowned animals probably pushed down from upper airways by the force of water inhalation during drowning. Their presence into alveolar spaces offer a useful forensic evidence in doubtful drowning autopsies, favored by the characteristic of the sporopollenin (pollen wall) and chitin (fungal spore wall) resistance. Moreover, the presence of these particles in alveoli areas of drowned bodies can help forensics to obtain information about pre-mortem dates and places.

Keywords: drowning; pollen; spores; lower airways

1 Introduction

Forensic botany is defined as the study of plants and plant tissues in relation to criminal and civil investigation. The first known legal case in which botanical information was admitted as key evidence for the resolution of a crime was the resolution of the Morrow-Lindbergh kidnapping and murder case in 1935, which was solved thanks to the analysis of wood from a ladder (Graham, 1997). Within the forensic botany, the forensic palynology plays an important role, being the most applied area in the science of applying modern and fossil pollen and spores (palynomorphs) analyses to help solve legal problems either civil or criminal (Bryant 2014; Bock and Norris 2016). Pollen analysis has been used as a validated tool in forensics since the second half of the XX century (Bryant et al. 1990; Wiltshire and Black 2006). Pollen and spores can be obtained from an extremely wide range of surfaces, including bodies, but also paleopalynological and even aerobiological data can help to clarify legal issues. Pollen and spores provide clues as to the source of the items and the characteristics of the environments. The use of the pollen and spores' data analysis in forensics lies in the combined study of biological particles abundance, dispersal mechanisms, resistance to mechanical and chemical destruction, microscopic size and morphology (Mildenhall et al. 2006). Also, the relation of aerobiological data with palynomorphs found at different investigated scenes, objects, or even corpses offer the possibility of establishing the season and place of death in case of murders, or the presence of persons in a suspected offence scene (Erdtman 1969; Horrocks et al. 1999). The first well-documented case of the use of palynology to solve a crime occurred in Austria in 1959. Thanks to pollen samples from living and extinct plant species found in the mud adhered to the shoes of a murder suspect, the suspect was framed and the exact place where he buried his victim was located on the banks of the Danube River (Erdtman 1969). Pollen recovered from dirt, clothing, hair, rope, baskets, and materials used as packing can reveal geographical origin or can link an individual or item with the scene of a crime. Likewise, the palynomorphs found in illegal drugs, can link those drugs with their source area and can show which shipments of drugs originated from the same, or from different, source areas (Dominguez-Vilches 1994). Different studies indicate that pollen and spores we inhale from the air are mostly retained in the upper airways and rarely pass into the lower airways, bronchi and bronchioles; more specifically only particles of 0.5 to 5 μm are believed to reach the alveoli (Wilson et al. 1973; Michel et al. 1977; Novey and Wilson 1977; Driessen and Quanjer 1991; Lei and Grammer 2019). Few researchers have noticed presence of pollen in distal parts of the lung, including alveoli, in very particular conditions. Michel et al. (1977) detected 1.1% of radioactivity in the

pulmonary zone of 10 subjects after inhalation of tree pollen particles labelled with ^{131}I, as well as an average of 3.3 pollen grains per g of lung tissue in fragments of right lung of 5 subjects who underwent a pneumonectomy for cancer. Moreover, Steerenberg et al. (1999) localized pollen grains in the alveoli although they were forced to enter by using both intranasal and intratracheal artificial administration.

However, the fact that particles of similar size such as diatoms, under conditions of strong water inhalation, such as drowning conditions, reach these lower areas, made us consider the possibility that water inhaled in drowning could push pollen grains and spores retained in the upper parts (nostrils, mouth, nasopharynx, pharynx, glottis, epiglottis) to the terminal lower airways. This hypothesis was reinforced by the presence of pollen grains in some cases involving drowned persons (Martínez et al., 2015) and in patients who underwent bronchoalveolar lavage (Dankaart et al. 1991).

The present research analyses for the first time the usefulness of pollen and fungal spores for drowning diagnosis, a cause of death highly discussed in legal medicine due to the absence of pathognomonic findings allowing a reliable diagnosis of drowning indicative of drowning in comparison with other causes of death (Farrugia and Ludes 2011; Piette and De Letter 2006; Van Hoyweghen et al. 2015). Other bioindicator used as a complementary test for this purpose in forensics, is the presence of diatoms algae in tissue analysis of different organs, such as lungs, muscle and even bones of corpses, due to their silica cell wall (Badu et al. 2015). Similar to diatoms, pollen and spores can remain with the same morphology after years, and even resist to physical and chemical treatments. The cell wall composition of these resistance cells, and specially the presence of sporopollenin in pollen grains, a highly stable biopolymer, allows this material to be recovered from different locations and to provide useful information to palynologists in different areas as palaeontology, archaeology, botany, and melissopalynology (Halbritter et al. 2018). Moreover, although the main objective of the work is to show how pollen and spores can help to drowning diagnosis, another interesting point analyzed is how the pollen and spores types found into lungs can give us information about the period and location of bodies just before the death. Our study aimed to establish evidence of pollen and spores present in lower airways in association with drowning by analysing drowned and non-drowned rats' lung tissues to investigate the effects of water on inhaled pollen and spores and their possible use as a drowning diagnosis sign in forensics.

2 Material and methods

During the period April 25th -May 8th 2017, a total of 27 rats were divided into 3 groups of 9 animals for the experimental procedure. Group 1 was subdivided in three subgroups of 3 animals that were immersed for drowning in 3 different water samples. Group 2 animals were sub-grouped and sacrificed before immersion (sham drowned) in the respective different water samples. Finally, a control group (Group 3) of the remaining 9 rats were sacrificed, but not immersed, after 60 minutes of exposure to a highly pollen loaded atmosphere artificially recreated in a box full of three different pollen types. Lung samples were harvested from all the animals after the euthanasia and histologically analysed.

2.1 Water samples

Water samples were obtained from three sites of South Spain between April 12th –27th: the Mediterranean Sea at San Luis de Sabinillas (Manilva, Malaga), Coordinates W5°13'40.91" N36°22'4.12"; Lake Navallana (Alcolea, Cordoba), W4°40′23″ N37°55′56″, and Guadalquivir River in Cordoba city, W4°46'46" N37°52'44. Samples were obtained with plastic bottles from the seashore and close to the banks of the lake and river respectively. The sampling was done at a depth of about 50 cm below the surface but careful not to scrape the bottom.

2.2 Animals

27 adult Wistar rats of average weight of 453.9±52.6 grams were randomly assigned to one of 3 groups, each consisting of 9 animals. First group were rats drowned in river water (n= 3), in lake water (n= 3) and in sea water (n= 3); second group were rats sham drowned -submerged after death- in river water (n= 3), in lake water (n= 3) and in sea water; and a third group of 9 rats not drowned

but sacrificed after undergoing a 60 minutes period of breathing in a highly pollen loaded atmosphere. High concentrations of three pollen types, *Cupressus*, *Pinus* and *Olea,* were loaded into a 1 m^3 volume box for this control group. These rats remained for 60 minutes in the cardboard box breathing high pollen concentrations that were sampled with a volumetric Hirst type portable air trap placed into the box. At the same time another portable Hirst sampler monitored the indoor natural atmosphere of the laboratory, while the city atmosphere was monitored at the same time with another aerobiological Hirst trap located outdoor at the University Campus.

All the rats were maintained under the same laboratory conditions of the animal facility of the School of Medicine and Nursing (University of Cordoba), under the supervision of an animal technician prior to the experiment.

2.3 Drowning procedures and euthanasia of experimental animals

Experimental procedures were performed during the period April 25th -May 8th of 2017 for the first and second group, and on the 1st June for the third group. The drowning process was conducted in batches according to the allocated groups. Each rat from the first group was caged and completely submerged in plastic container filled with water for a total period of 60 minutes. No anaesthetic was used as the purpose of this experiment is to simulate actual drowning conditions.

Animals belonging to the second group were anaesthetized by injection of Ketamine 75mg/kg and killed by elongation of the cervical spine before the post-mortem immersion for 60 minutes. Rats belonging to the third group were caged in a cardboard box let them breathe in a highly pollen loaded atmosphere for 60 minutes before being anaesthetized by injection of Ketamine 75mg/kg and sacrificed by elongation of the cervical spine. For each rat, 3 lung samples were removed for histological analysis.

2.4 Samples procedures

After necropsy, lungs' samples for histological examinations were removed under a laminar flow hood to avoid external particle contamination. Then the obtained samples were immediately fixed by immersion in 10% buffered formaldehyde before being included in a paraffin wax block. Different sets of surgical instruments for the dissection of external and internal organs for each rat were used to prevent cross-contamination among animal tissues. Indeed, all glassware used for the experiment were thoroughly washed with detergent and rinsed several times with deionised water. Subsequently, 3 histological samples per paraffin block were cut with microtome into sections with a surface of around 100 mm2 and a thickness of 6μm. Each section was put over a slide and afterwards, during the staining process, they pass through at least 15 washings dyes and different alcohol dilutions (Suvarna et al., 2019). All those repeated washings would eliminate any risk of potential pollen and spores' contamination, so no particle except for those included into the animal tissues could appear on the samples. Slides were stained in Haematoxilin and Eosin (H-E). Sections were examined under bright-field illumination on a Nikon Eclipse 1000 light microscope coupled with a high-definition camera at magnifications of x100 and x400.

2.5 Aerobiogical sampling

An outdoor aerobiological study of the pollen and spores' content of the air of the city of Córdoba was performed during all the sacrifice periods by using a Hirst spore trap (Hirst 1952) placed at the Rabanales University Campus of the University of Córdoba. Moreover, an indoor pollen/spores sampling was conducted inside the laboratory of the animal facility where the Group 3 of rats were sacrificed. In this experiment, two Hirst type portable air collectors, Lanzoni® Manufacturer were used (Levetin 2004). One of them was placed into a box (1m x1m x1m) where high quantities of pollen grains from 3 different plant types (*Cupressus, Pinus* and *Olea*) were introduced in order to sample the pollen-rich air that rats breathed before their sacrifice. Pollen was continuously been stirred due the rats moving around the box. The other one was placed out of the box in the opposite end of the laboratory in order to sample the natural indoor biological content. Both portable samplers were placed at the same height of 120 cm over the floor. Box and indoor aerobiological sampling were performed at the same time for 60 minutes, 9:00- 10:00h of June 1st.

Data about the pollen/spores sampling outside the box were compared with the aerobiological data of that date, June 1st, obtained by the outdoor Hirst sampler located at the Rabanales Campus. Data from indoor and outdoor samplers were analyzed according to the protocol proposed by the Spanish Aerobiology Network (REA) (Galán et al. 2007) and the minimum European requirements for aerobiological analysis (Galán et al. 2014). Biological particles were analysed under a Nikon Eclipse 1000 light microscope at x400 magnifications.

In order to obtain comparable data from indoor and outdoor samplers, apart from selecting data from the same time periods, correction factors were applied to pollen and spore raw data to calculate biological particles concentrations/m^3 recorded by all the traps during the sampling time (9:00-10:00h) This factor considers the suction volume of air sampled (10 liters / minute), sampling duration (60 minutes) and microscope characteristics (Galán et al. 2014).

Pollen and spores detected at the Cordoba city atmosphere, during the sacrifice of all rats' groups (G1, G2 and G3) were compared with those found into rats' lower airways. Moreover, in the case of G3 these data were also compared with aerobiological data obtained from the laboratory.

3 Results

Tissues from lower airways of both lungs of all the animals were analysed through the histological study of the alveolar tissue. A variety of pollen and fungal spores were found in lungs of the first group of drowned rats, but not in lung samples from the other two groups of not drowned rats. It was also remarkable the fact that most of palynomorphs were found into alveolar spaces. The average size of a rat one is around 200 microns, whereas the average size of palynomorphs rounds about 20-40 microns.

Types and quantity of pollen and fungal spores observed at the lower airways' tissues of rats belonging to drowned rats are indicated at Table 1. The figures per rat contain the summatory of the all the analysed samples per rat. The origin of submersion water is indicated for each sample. In this table it is possible to observe the unusual quantities of airborne pollen and fungal spores that were found into drowned rats' lungs.

		Drowned in River Water			**Drowned in Lake Water**			**Drowned in Sea Water**		
		DRW1	**DRW2**	**DRW3**	**DLW1**	**DLW2**	**DLW3**	**DSW1**	**DSW2**	**DSW3**
Pollen	*Ericaceae*	2								
	Olea		4		1					
	Pinus	2				1				
	Plantago	3	2							
	Poaceae			1						
	Urticaceae			1						
	Quercus			1		1	1		1	
Spores	*Alternaria*	1								
	Aspergillus	3	4	1			1			
	C. cladosporioides	1			1		1	15	1	
	C. herbarum	2	2		2					
	Leptosphaeria								1	
	Polythrincium	1					1			
Total		15	12	4	4	2	4	15	3	0

Table 1 Types and quantity of pollen and fungal spores observed at the lower airways' tissues of the different subgroups (1,2 and 3) of Drowned (D) rats in different waters: River Water (RW), Lake Water (LW) and Sea Water (SW).

Regarding pollen grains found at the lower airway's tissues of drowned rats, it was possible to observe different pollen types: Chenopodiaceae, *Cupressus*, Ericaceae, *Jasminum, fruticans, Olea europaea*, *Plantago*, *Pinus,* Poaceae, *Quercus* and Urticaceae. Pictures of some of them located into lung tissues are showed at Figure 1.

Pollen types found in the alveolar tissues of drowned rats coincided with those found at the Cordoba atmosphere during the study period (April 25th -May 8th) detected by the Hirst trap located at the city (Figure 2). Also, it was possible to observe some pollen types coming from the ornamental vegetation growing at the School of Medicine and Nursing gardens, as *Jasminum fruticans* which grows in the perimetral hedge of the faculty or *Cupressus*, largely planted at the school gardens.

Regarding fungal spores in drowned rats' tissues, it was possible to observe different types as *Alternaria, Aspergillus, Cladosporium cladosporoides, Cladosporium herbarum*, *Leptosphaeria*, *Polythrincium* and *Phitomyces*. Pictures of some of them are showed at Figure 3.

Fungal spore types found in the alveolar tissues of drowned rats coincide with those found at the Córdoba atmosphere during the study period (Figure 4).

Lung tissues of the rats belonging to the second group (submerged after sacrificed) and third group (breathing so high pollen concentrations before sacrificed), were also histologically analysed. None of the samples showed any palynomorph. Pictures from control tissues' samples are showed in Figure 5.

Of the 9 drowned rats we observed the appearance of pollen and spores in the bronchio-alveolar tissues in 8 of them (88.9%), whereas in the 100% of the control rats, neither pollen nor fungal spores was found. We discovered at least one pollen grain in the pulmonary tissues of most of the drowned rats, with the highest recorded number of pollen in a single tissue at seven. A similar result was observed for spores. Incidentally, fragments of pollen and spores were seen.

Comparing the number of pollen grains found in the drowned rats' lungs, to the relative amounts of pollen grains in the Córdoba air during the same period, it may be remarkable that no pollen of *Urtica membranaceae* were found in the histological samples, despite its small size. Regarding fungal spores, it is noticeable that none of the most abundant spores detected in the Córdoba city atmosphere such as *Coprinus*, *Epicoccum* and *Stemphylium*., was observed in the lung tissues of the control rats.

Table 2 shows the obtained results during the aerobiological sampling performed at the same time on June 1st outdoor, indoor and into a fulfilled pollen box where the Group 3 rats (control group) were placed for 60 minutes just before being sacrificed. Pollen grains introduced in the box belonged to different size pollen types in order to observe possible entrance difference. *Olea europaea* pollen grains vary in size from 20-25 µm; *Cupressus* from 25-30 µm; and *Pinus* from 40-50 µm.

Table 2 Biological particles concentrations (pollen/spores/m3) during the simultaneous aerobiological samplings performed outdoor, indoor and into the fulfilled pollen box, on June 1st during the third control group experiment and sacrifice.

Pollen	**Outdoor**	**Indoor**	**Box**
Apiaceae	8	3	0
Cupressus	0	17	19364
Olea europaea	16	13	386
Pinus	3	7	476
Rosaceae	0	3	0
Urtica	2	3	0

Spores			
Alternaria	16	3	283
Aspergillus	28	37	0

In this experiment, third group of control rats were not drowned but only sacrificed by elongation of the cervical spine after anesthesia. The objective was to analyze if this period breathing in an overloaded pollen atmosphere could increase the likelihood of pollen intake into lower the airways even without drowning. Results indicated that, despite the very high concentrations of pollen, hundredfold greater than the outdoor and indoor atmosphere, no pollen was found at the lungs of this group of rats, even the smaller size pollens. Histological samples were completely clean of pollen neither spores as it can be observed in Figure 5.

4 Discussion

A recent work proving the effectiveness of diatoms in drowning forensic diagnosis was performed at the Legal Medicine Laboratory of the University of Córdoba, by comparing tissue analyses of true drowned rats, with the results of previously sacrificed and post-mortem submerged rats' tissues. After acid digestion, drowned rats' tissues, and especially alveolar surrounding ones, showed a significant higher concentration of diatoms in comparison with the control post-mortem submerged rat group (Badu et al. 2015). Similar scenario is observed in the case of pollen and spores. The performance of experimental procedures during spring allowed that rats breathed higher pollen concentrations and determined that animals that died under drowning conditions presented pollen and spores into their lower airways, but not those that died before being submerged. Moreover, a control experiment was made in an artificially overloaded pollen atmosphere in order to assure this fact. Nevertheless, no pollen neither spores were found in control animals, despite of the high pollen concentrations that they could inhaled just before death. The quantities of pollen and spores found in drowned rats lungs indicate that, the inhaled water during the drowning process forced palynomorphs usually filtered at the upper airways to enter into lower airways. Although the number of palynomorphs found into lungs was not so high, it was so outstanding in comparison with the null quantity expected into animal lungs (Driessen and Quanjer 1991; Lei and Grammer 2019). Observing histological samples, although external contamination can never be completely excluded, it is highly unlikely due to the sterile conditions during histological procedures and the high number of dyes, alcohol and water washings suffered by tissue samples. Analysing lung samples' slides it was noticed as pollen and spores mostly appear into alveolar spaces of respiratory bronchi but not embedded in samples' tissues, although it occurs in some cases (i.e. Figure 3 a) and d)), probably due to the dragging effect of the microtome steel blade on those tissues where there is no stroma to facilitate the O_2/CO_2 gaseous exchange in lungs. This dragging could have affected affect pollen and spore position into histological samples. This possibility is perfectly possible from the point of view of alveolar spaces' size. Considering that the average alveolar spaces' size rounds the 200 microns is perfectly possible to contain pollen, fungal spores, or diatoms with an average size of around 20-40 microns. The possibility that pollen grains and spores were into water samples would be also excluded because of fresh pollens from wind pollinated plants usually float in water but not appear into water streams, lakes or into the sea. It is due to their physical characteristics, usually spherical, small and with a cytoplasm full of air vesicles, designed to be dispersed floating in the air (Agashe and Caulton 2019; Ackerman 2000). In any case, water samples were taken from a depth of 50cm below the surface in order to avoid any potential suspended biological particles on surface water. Furthermore, we did not find pollen grains or fungal spores of plants growing in foreign places (outside Córdoba city and surroundings).

Diagnosis of drowning is still a challenge in forensic medicine due to the absence of autopsy findings which are highly indicative of this cause of death (Farrugia and Ludes 2011; Piette and De Letter 2006). Although not always are present in presumptive drowning cases, diatoms are one of the proposed bioindicators of drowning because they are frequently found after autopsy in tissue analysis of different organs of drowned corpses (Martínez et al., 2015). The tissues where they usually appear are lungs, muscle and

even bones. The ability of penetration of these algae is quite high due to their reduced size and their silica cell wall persistence (Thakar and Singh 2010).

It is probable that this penetration is not an event which happens very frequently, and certainly not massively as it has been observed in some drowning cases in humans (Martínez et al., 2015). So, as it is supposed that inhalation into deeper regions of the respiratory organ is not a common or normal phenomenon, the occurrence of diatoms in lung samples can be taken as a forensic evidence of drowning. Following the same reasoning, the finding of other palynomorphs, such as pollen and spores, in alveolar spaces could also be taken as a forensic evidence of drowning. This idea is reinforced because pollen and spores do not easily enter into lower airways, except for special circumstances. It has been proved that all particles with an aerodynamic diameter in excess of 10 μm are trapped in the upper airways (Bates et al. 1966; Novey and Wilson 1977). Anemophilous pollen grains present a diameter of 10-50 μm, mostly between 20-30 μm (Charpin et al. 1962; Stanley and Linskens 1985). Our results, supported by the experiment performed with the control third group, reinforced this idea. Even when rats were forced to breath so high pollen concentrations of different size pollen grains into a closed box, histological analyses of their lungs did not show any pollen evidence.

The fact that particles of similar size such as diatoms, under conditions of strong water inhalation, such as drowning conditions, reach these lower areas, made us consider the possibility that inhaled water in drowning could push down pollen grains and spores retained in the upper airways (nostrils, mouth, nasopharynx, pharynx, glottis, epiglottis) to the terminal lower airways.

Their specific morphology allows identification to an individual parent plant taxon that can be related to a specific ecological habitat or a specific scene. Although identification and interpretation of pollen is a specialist job, sub-sampling and preparing pollen samples for analysis may be carried out by non-specialists (Mildenhall et al. 2006). Pollen and spore assemblages characterise different environments and scenes, nevertheless they can be easily be picked up and transported away from scenes of interest without providing any visual clue to a suspect as to what has occurred (Halbritter et al. 2018).

In our study, data from airborne pollen monitoring have demonstrated to be useful to obtain inferences about different environmental issues of the death circumstances. All the pollen grains and spores found in animal carcasses during the experiment, coincide with those found in the Córdoba city atmosphere or with species growing at the School gardens. The comparison of pollen and spores recovered from corpses with aerobiological data, could help to confirm the suspicion about place of death, and even the dates. In conclusion, the analysis of pollen and spores in lower airways of bodies will be so useful to the forensic report providing associative evidence, assisting to prove or disprove causes of death or even links between people and places, dates or other people.

5 Conclusions

Pollen and spores' penetration into deeper regions of the respiratory tract is not a common phenomenon. Our results revealed that these particles appeared in a significant number in lung samples of drowned animals probably pushed down from upper airways by the force of water inhalation during drowning. The observed rats' histological samples in the present study would indicate that inhaled water during drowning can force pollen and spores filtered at the upper airways to penetrate into alveolar spaces. The presence of these biological particles into alveolar spaces offer a useful forensic finding in doubtful drowning autopsies, favoured by the characteristic of the sporopollenin (pollen wall) and chitin (fungal spore wall) resistance. Finally, the comparison of these data with aerobiological ones can help forensics to obtain information about pre-mortem places and dates.

Compliance with Ethical Standards:

Funding

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

The experiment was performed according to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and was ethically approved by the University of Córdoba bioethics committee before commencement.

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Table captions

Table 1 Types and quantity of pollen and fungal spores observed at the lower airways' tissues of the different subgroups (1,2 and 3) of Drowned (D) rats in different waters: River Water (RW), Lake Water (LW) and Sea Water (SW).

Table 2 Biological particles concentrations (pollen/spores/m3) during the simultaneous aerobiological samplings performed outdoor, indoor and into the fulfilled pollen box, on June 1st during the control group experiment and sacrifice

Figure captions

Fig. 1 Pollen grains found in the alveolar tissue of first group of drowned rats (arrows). *Jasminum fruticans* (a), *Olea europaea* (b), *Pinus* (c), *Plantago* (d), *Poaceae* (e) and *Quercus* (f). Stain: H&E. Magnification: x400

Fig. 2 Pollen grains in Cordoba city atmosphere during the period April 25th-May 8th

Fig. 3 Fungal spores embedded in the tissues of the lower airways of first group of drowned rats (arrows) *Aspergillus* (a), *Cladosporium cladosporoides* (b); *Cladosporium herbarum* (c); *Phytomyces* (d). Stain: H&E. Magnification: x400

Fig. 4 Fungal spores in Córdoba city atmosphere during the period April 25th-May 8th

Fig. 5 Rats of the third group were maintained in a pollen-rich atmosphere for an hour before being sacrificed (a). Normal alveolar structure (b-c). Microscopic analysis of collected air sample reveal high concentrations of pollen (d). Stain: H&E. (b-c); Unstained (d) Magnification: (b-d) x400