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ARTICLE

Effects of particulate matter under behavioral, hematological and biochemical parameters in Wistar rats

Samanta Cristina-Siebel-de-Moraes[1], Victória Branca Moron[1], Aline Belem-Machado[1], Paula Schmitt[1], Daniela Montanari-Migliavacca-Osorio[2] & Daiane Bolzan-Berlese[1]

1 Department of Biochemistry, Post-Graduation Program in Environmental Quality, Feevale University, Brazil.

2 Department of Chemistry, Post-Graduation Program in Environmental Quality, Feevale University, Brazil.

Correspondence
A. Belem-Machado
E-mail: linebmachado@hotmail.com
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Resumen

Efectos del material particulado en los parámetros de comportamiento, hematológicos y bioquímicos en las ratas Wistar

El Material Particulado (MP) puede alterar los procesos cognitivos, el comportamiento depresivo y hematológico en el modelo animal. El objetivo fue evaluar los efectos causados por MP en los parámetros conductuales, hematológicos y bioquímicos en un modelo animal. Se observaron alteraciones en la actividad locomotora de las ratas, donde hubo una disminución significativa en la locomoción del Grupo 3 (MP_{10}) en comparación con los Grupo 1 (Control) y Grupo 2 ($MP_{2,5}$). Con respecto al comportamiento de ansiedad, el Grupo 3 pasó significativamente más tiempo en brazos abiertos en comparación con el control y $MP_{2,5}$. No se observaron cambios hematológicos y bioquímicos. En este estudio, se concluye que, la exposición a la MP puede causar daño neurológico y, en consecuencia, afectar a otros sistemas.

Palabras clave: Hematología; Contaminación del aire; Pruebas de comportamiento; Bioquímica.

Abstract

Particulate matter (PM) can alter the cognitive processes, the depressive behavior, and the hematological profile in animals. This study aimed to evaluate the effects on behavioral, hematological, and biochemical parameters caused by PM in an animal model. A significant decrease in the locomotion activity of Group 3 (PM_{10}) in relation to Group 1 (Control) and Group 2 ($PM_{2.5}$) was observed. Regarding the anxiety behavior, Group 3 remained significantly the most part of the time in the open arms when compared to the control and $PM_{2.5}$ groups. No hematological or biochemical alterations were observed among groups. In this study, we concluded that the exposure to particulate matter can cause neurological damages, and consequently, affect other systems.

Key words: Hematology; Atmospheric pollution; Behavioral tests; Biochemistry

Introduction

Particulate matter (PM) is the most noticeable pollutant in the atmosphere, being referred to as a consolidation of atmospheric particles. Aerosols, in solid or liquid form, are particles suspended in the air, found in urbanized centers, industrial poles, and highways (Seinfeld & Pandis 2006, Nicodemos *et al.* 2009, Chithra & Nagendra 2013, Manahan 2013).

Particulate matter is classified according to its size and shape in total suspended particles (TSP), smoke, and inhalable particulates (IP). They are divided into coarse inhalable particles (PM_{10}), with aerodynamic diameter between 2.5 µm and 10 µm, and fine inhalable particles ($PM_{2.5}$), with aerodynamic diameter up to 2.5 µm. The IP acts as catalysts because they increase the speed of the chemical reactions, transforming the primary pollutants into secondary pollutants, which is harmful (Mello *et al.* 2010, Guerra & Miranda 2011).

The smaller particles (2.5 µm) are derived from the combustion of mobile sources, such as vehicles, and stationary, or fixed sources, such as industries. Carbon, lead, vanadium, bromine, sulfur, and nitrogen oxides are the main pollutants of this fine particle fraction. Carbon monoxide (CO), carbon dioxide (CO_2), and water are the result of biomass burning, which at the final stage releases products from the incomplete combustion, such as organic particles, also present in the PM (Donaldson *et al.* 2001).

The differential of PM is characterized by the transport of gases aspirated on the surface that migrate to the distant points of the respiratory tract, responsible for the gas exchange in the lungs. When the particles are settled in the upper respiratory tract, the body's defense system is activated and tries to remove it through mechanisms such as sneezing, caused by macroparticles that do not pass beyond the nasal orifice due to their size. As for the particles that reach the lower respiratory system, the defense mechanism is through coughing (Braga *et al.* 2001).

The effects of the exposure to particulate matter also affect the cardiac system, manifesting through alterations such as a change in heart rate, myocardial ischemia, ineffective blood coagulation, increased oxidative stress, and inflammatory induction (Fernandes *et al.* 2010). Demonstrating that the inflammatory process after the exposure to atmospheric pollution is a potent mechanism associated with metabolic alterations.

The open field test is the most used test to analyze the animal's behavior to a certain chemical, which evaluates the locomotor and rearing of the animal. These are also the most sensitive responses to prolonged or repeated exposures (Rojas-Carvajal *et al.* 2018). The elevated plus maze test is another behavior test and is widely used to evaluate the anxiety behavior in rats. The test is based on the number of entries in the closed (protection site) and open (unprotection site) arms of the apparatus, in which it is assumed that the animal approaches or avoids conflict, respectively (Carobrez & Bertoglio 2005).

Therefore, the present study aimed to evaluate the effects caused by the coarse and fine particulate matter on behavioral, hematological, and biochemical parameters in male Wistar rats.

Materials and methods

Particulate matter

The particulate matter used in this research was collected in 2017, from the metropolitan area of Porto Alegre, in the municipalities of São Leopoldo and Canoas/RS – Brazil. Sample collections occurred once a month at each collection point for 6 months. The precise geographical coordinates are expressed in table 1, with a justification of the locations, the points are shown in the map of figure 1.

Sampling points	Municipality	Geographical coordinates	Justification
Point 1	São Leopoldo	-29° 46'19,01" -51° 9' 7,17"	Urban area. Estimated population at 225.5 thousand inhabitants and demographic density of 2083,8 inhabitants per km^2. Point located approximately 200 m from BR 116.
Point 2	Canoas	-29° 55'19,79" -51° 10' 43,05"	Urban area. Estimated population at 338.5 thousand inhabitants and demographic density of 2470,1 inhabitants per km^2. Point located approximately 60 m from BR 116.

Tabla 1. Localización y descripción de los puntos de muestreo de MP.

Table 1. Localization and description of the sampling points of PM.

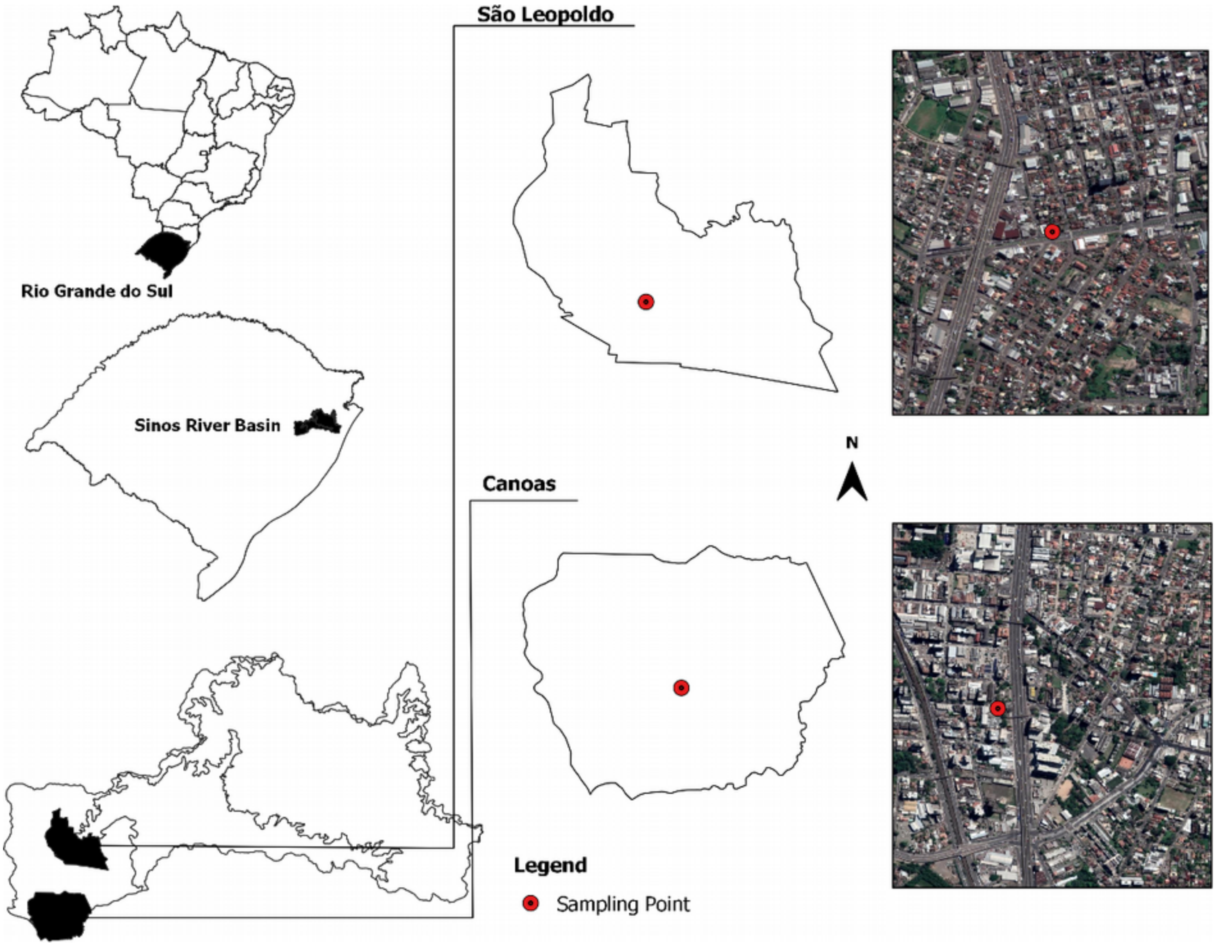

Figura 1. Mapa de los puntos de muestreo en São Leopoldo y Canoas.
Figure 1. Map with the sampling points in São Leopoldo and Canoas.

The PM samples were collected monthly, by a period of 24 hours, from January to July 2017 (Summer until Winter), according to the Protocol for Collection of Atmospheric Particulate Matter from the National Institute for Space Research (INPE 2012). The atmospheric particles, fine and coarse, were collected utilizing a fine and coarse matter sampler Stacked Filter Unit (SFU) (Maenhaut *et al.* 1993, Hopke *et al.* 1997) in which the quartz or borosilicate filters were placed in series, allowing the separation of particles into two bands of size (particles with a diameter between 10 µm and 2.5 µm and particles smaller than 2.5 µm).

Quantification of metal elements in $PM_{2.5-10}$ and $PM_{2.5}$

The metals (Al, Cd, Pb, Cu, Cr, Fe, Mn, and Ni) were quantified by graphite furnace atomic absorption spectrometry (ZEEnit 600, Analytik Jena AG) and Zn was determined by flame atomic absorption spectrometry (SpectrAA 110, Varian) in the solubilized fractions of $PM_{2.5-10}$ and $PM_{2.5}$.

Animal model

For the animal model protocol, adapted from the protocol of Unsal *et al.* (2018) and Binoki (2010), thirty male Wistar rats were used (± 250 grams) from Feevale University Vivarium. The animals were exposed to a 12 hours light/dark cycle in a climatized and humidified room (22 ± 1 °C and 50 ± 10% RH). The animals received water ad libitum and a reference industrialized food for rats (Nuvilab CR1-Nuvital®). The rats were randomly divided into 3 groups composed of 10 rats each. Control Group: animals submitted to the instillation of 50 µl of distilled water in each nostril. Group PM_{10}: animals submitted to the instillation of 50 µl of an aqueous suspension of PM_{10} in each nostril. Group $PM_{2.5}$: animals submitted to the instillation of 50 µl of an aqueous suspension of $PM_{2.5}$ in each nostril. The experiment was performed during 8 consecutive weeks of nasal instillations of distilled water, or PM_{10}, or $PM_{2.5}$. During each week three interventions were promoted on alternate days. A calibrated micropipette was used for the nasal instillations. Thus, the animals were submitted to behavioral, hematological, and biochemical analyses (Binoki 2010).

The animal model protocol was approved by the Ethics Committee for Animal Research of Feevale University under the number 01.14.027.

Behavioral parameter analysis

Determination of locomotor parameters: the open field test (OFT) consists of placing the animals in the left posterior quadrant of the arena of the open field, size 50x50x39 cm (length/height/depth), with high walls, white plywood, and a frontal glass wall. The field floor is divided into 12 equal quadrants by black lines. The animal is placed in the right corner, then the test occurs for 5 minutes. The number of times the animal crosses the black lines (crossings=locomotor response) and the number of times the rat rises on its rear paws (rearings=exploratory response) were registered as long as the free exploration of the environment was allowed. The fecal bolus corresponds to the number of animal feces for 5 minutes. After the test with each animal, the box was sanitized with 30% alcohol to prevent that the urine and feces smell interfere in the test. An observer, blind to the experimental condition of the experimental animal, recorded the behavioral data using the protocol described above (Nahas 1999).

Determination of anxiety parameters: the elevated plus maze test (EPM) is formed by 4 opposed and elevated arms. Two arms have walls, being called closed arms (40x40x10 cm). The other two arms do not have walls, being called open arms (40x10 cm, with acrylic side edges of 1 cm height). The maze was placed 50 cm from the floor. The use of maze is based on the natural aversion of the rats to open fields. The number of entries and time spent on the open arms are used as indicators regarding the anxiety, the more intense the exploration of the open arms, the smaller the anxiety. The rodents remained in the EMP for 5 minutes, and the sessions were registered concomitantly. When the experiment started, the rats were always placed in the center of the maze with its head facing one of the open arms. After, the time spent and the total numbers of entries on the open and closed arms were analyzed. The entry was registered when the animal placed its four paws inside one of the arms. An observer, blind to the experimental condition of the experimental animal, recorded the behavioral data using the protocol described above (Hogg 1996).

Biochemical and hematological parameters analysis

After the behavioral evaluation, the animals were anesthetized and sacrificed by guillotine for the blood sample collections. The biochemical parameters were glycemia, total cholesterol, HDL, and triglycerides, and the hematological parameters were erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, leukocytes, platelets, segmented, monocytes, and lymphocytes.

Statistical analysis

For the data analysis, descriptive statistics were initially used, followed by non-parametric tests due to the non-normality of the data (KOLMOGOROV-SMIRNOV test). For the comparison of data between two properties, the ANOVA test was performed followed by the Tukey test, in which $p<0.05$ was considered significantly different. Data were processed in the *Statistical Package for the Social Sciences Software* (SPSS), version 24.0.

Results and Discussion

Particulate Matter

The fine and coarse particulate matter were collected from January to July 2017, totalizing 24 samples, 12 for the fine PM, and 12 for the coarse PM. The collections were performed on non-rainy days.

The figure 2 presents the concentrations of $PM_{2,5-10}$ and $PM_{2,5}$ detected at São Leopoldo and Canoas (Figs. 2a and 2b) sampling points, as well as the indications of international air quality standard (United States Environmental Protection Agency [US EPA] and European Commission [EC]) for $PM_{2.5}$ (24 hours). The results demonstrated that $PM_{2.5-10}$ exceeded the quality standards in all samples, except for July 2017. However, $PM_{2.5}$ only exceed the quality standards in January and February 2017 at São Leopoldo (Fig. 2a). Similar behavior was observed for the $PM_{2.5-10}$ from Canoas, which did not exceed the quality standards only in March and July 2017. And the $PM_{2.5}$ was exceeded only in April 2017.

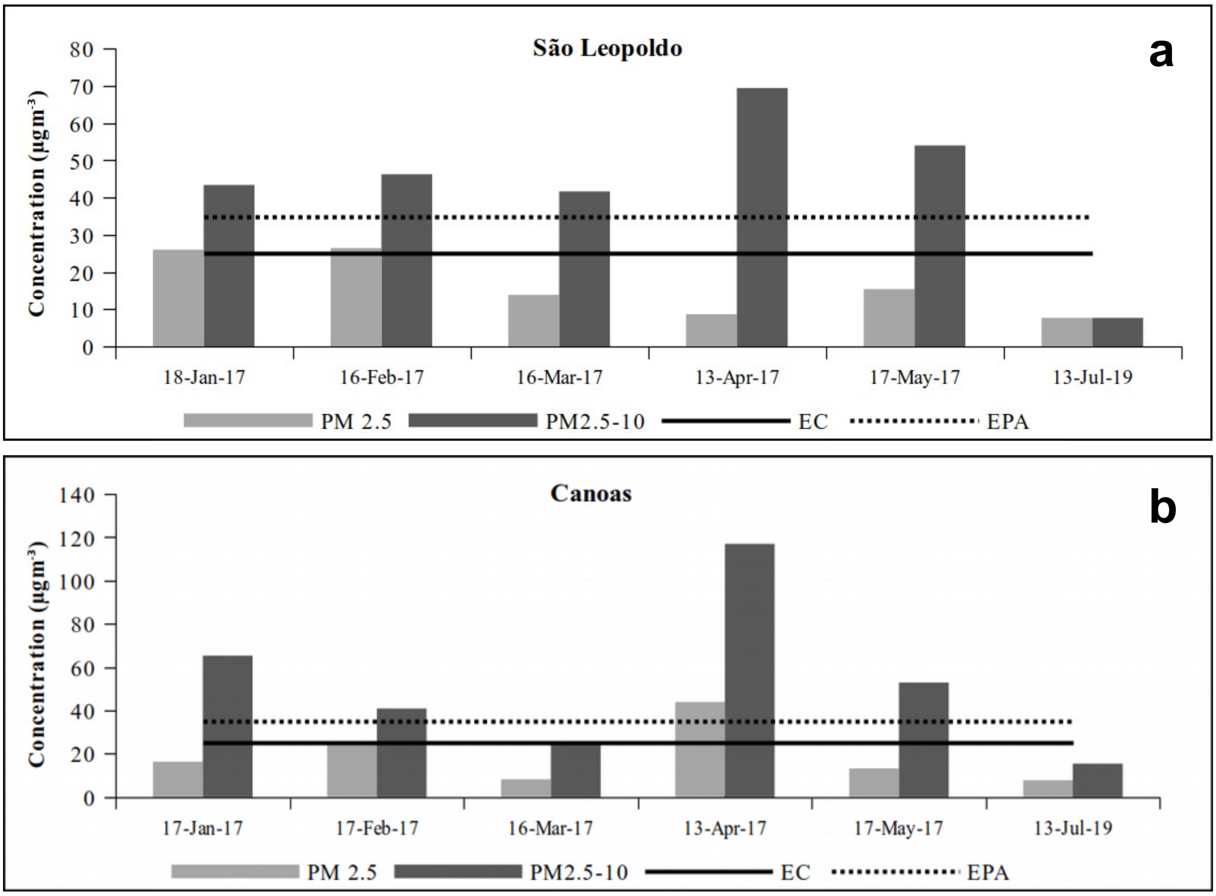

Figura 2. Concentraciones de $MP_{2.5\text{-}10}$ y $MP_{2.5}$ en los puntos de muestreo. **a:** São Leopoldo; **b:** Canoas. EC: Comisión Europea; EPA: Agencia de Protección Ambiental.

Figure 2. $PM_{2.5\text{-}10}$ and $PM_{2.5}$ concentrations in the sampling points. **a:** São Leopoldo; **b:** Canoas. EC: European Commission; EPA: Environmental Protection Agency.

The mean concentrations and standard deviation (SD) of $PM_{2.5\text{-}10}$ and $PM_{2.5}$ at São Leopoldo sampling point were 43.76 (SD=20.37) and 16.37 (SD=8.20) μgm^{-3}, respectively, and at Canoas sampling point were 52.67 (SD=36.37) and 18.97 (SD=13.66) μgm^{-3}, respectively.

Metal elements in $PM_{2.5\text{-}10}$ and $PM_{2.5}$

Table 2 presents the mean concentrations of metal elements found in $PM_{2.5}$ and $PM_{2.5\text{-}10}$. The elements Al and Fe presented the highest concentrations among the metal elements analyzed for the study period. The elements Cd, Ni, and Cr presented below the limits of quantification of the analysis method.

The metallic elements Al, Fe, and Mn are constituents of Earth's crust and occur naturally in the PM through the resuspension of the soil (Alleman *et al.* 2010, Loyola *et al.* 2012, Alves *et al.* 2015), although anthropogenic activities, such as metal smelting and the construction, can contribute to the increase of the levels of these elements in the atmosphere (Hieu & Lee 2010, Lim *et al.*, 2010). Pb is considered toxic and usually related to the fine fraction of PM (Espinosa *et al.* 2001). Xu *et al.* (2012) related vehicular emissions as one of the main sources of Pb in the environment, even after the prohibition of the use of compounds of this element as an additive in gasoline. Migliavacca *et al.* (2012) identified that the occurrence of Pb in the metropolitan region of Porto Alegre, Southern Brazil, is associated with a combination of two anthropogenic sources: siderurgy/smelting activity and vehicular emissions. Zn is used as zinc oxide (ZnO) by the rubber industry in the tire vulcanization process, therefore, the presence of Zn in areas influenced by vehicular traffic can be associated with tire wear (Adachi & Tainosho 2004, Thorpe & Harrison 2008, Alleman *et al.* 2010, Loyola *et al.* 2012). The Cu can be emitted through burning and improper disposal of solid

	Metals	Canoas				São Leopoldo			
		Mean	Median	Minimum	Maximum	Mean	Median	Minimum	Maximum
$PM_{2.5}$	Al	320.97	321.49	132.90	508.00	5736.52	829.15	9.32	21278.46
	Pb	16.72	14.78	1.53	35.80	32.90	23.53	1.44	83.09
	Cu	14.06	13.44	3.35	26.03	18.01	16.70	4.18	34.44
	Fe	304.72	8.13	1.25	1201.38	495.79	286.52	71.79	1338.32
	Mn	9.79	2.79	0.06	33.54	8.41	4.66	0.27	24.06
	Zn	23.98	16.47	4.95	58.05	52.00	64.28	5.79	73.65
$PM_{2.5\text{-}10}$	Al	873.26	502.75	322.28	2165.27	854.48	296.43	181.59	2643.48
	Pb	2.88	1.53	1.44	7.01	4.78	2.87	1.30	12.06
	Cu	39.14	33.54	18.32	71.16	23.26	15.67	1.05	60.64
	Fe	363.12	77.95	1.25	1295.34	35.99	36.49	1.25	69.75
	Mn	7.41	3.45	0.27	22.48	7.18	7.70	0.27	13.06
	Zn	24.13	21.88	5.79	46.99	68.82	78.95	0.81	116.55

Tabla 2. Estadística descriptiva de $MP_{2.5}$ Y $MP_{2.5\text{-}10}$ y concentraciones de elementos metálicos (ng m^{-3}) determinadas en las muestras recolectadas en Canoas y São Leopoldo. n=12.

Table 2. Descriptive statistics of $PM_{2.5}$ and $PM_{2.5\text{-}10}$ and metallic element concentrations (ng m^{-3}) determined in the samples collected at Canoas and São Leopoldo. n=12.

	Crossing	Rearing	Fecal Bolus
Group 1 (Control)	58.20 ± 12.22	18.60 ± 6.75	3.80 ± 2.20
Group 2 ($PM_{2.5}$)	52.10 ± 9.51	18.40 ± 6.24	2.90 ± 2.18
Group 3 (PM_{10})	39.60 ± 10.58*	13.00 ± 8.52	2.40 ± 1.50

Tabla 3. Resultados de campo abierto. Los datos se expresan como media ± desviación estándar. n = 10 animales por grupo. * $p < 0.05$ ANOVA seguido de la prueba de Tukey. *Diferencia significativa ($p<0{,}05$) del grupo 3 *versus* grupo 1 y grupo 2, para actividad locomotora.

Table 3. Open Field Results. Data are expressed as mean ± standard deviation. n=10 animals per group. *$p < 0.05$ ANOVA followed by Tukey test. *Group 3 differs from group 1 and group 2 for locomotor activity.

waste, and by burning fossil fuel (Kabata-Pendias & Mukherjee 2007).

Open Field

After 8 weeks of nasal instillation, the animals were submitted to the behavioral tests. Table 3 presents the results obtained from the open field test, which evaluated the locomotor activity (crossing), exploratory (rearing), and fecal bolus.

According to table 3, a significant difference between the group that received PM_{10} in relation to the Control Group and Group $PM_{2.5}$ can be observed. The data demonstrated that Group PM_{10} had a smaller locomotor activity in relation to the Control and $PM_{2.5}$ groups. There was no significant difference regarding the exploratory activity (rearing) and fecal bolus.

The coarse fraction of the particulate matter is the fraction that is retained on the upper airways, which can be eliminated through physiological mechanisms such as a sneeze or nasal cleaning. When the particles of this particulate matter are not totally eliminated or removed, the risk of exposure increases, and consequently the damages increase as well. The particles of the particulate matter can have some different paths when they remain in the organism, where they can escape from the reticuloendothelial system (Wang *et al.* 2017), and be deposited in the lungs and be phagocyted by alveolar macrophages, which can trigger an inflammatory response, including the release of cytokines (Luo *et al.* 2017). Some particles can transit through the epithelial barrier and reach the blood circulation or reach the Central Nervous System (CNS) (Wang *et al.* 2017).

Studies performed by Costa *et al.* (2017) in adult mice demonstrated that the exposure to particulate matter, even in low amounts, can cause damages to the CNS, such as neurotoxicity and neuroinflammation, even though it has not been elucidated how these mechanisms occur (Block & Calderón-Guarcidueñas 2009). These damages on the CNS can lead to cognitive, neuroinflammatory, and neurodegenerative alterations.

Our experiment corroborates to the study abovementioned, considering that the decrease in the locomotor activity may have been influenced by the contact with the particulate matter, affecting mainly the region of the brain responsible for walking or locomotion.

Elevated Plus Maze

According to the data shown in table 4, regarding the anxiety behavior evaluated in the elevated plus maze test, a significant difference was observed in relation to the time spent in the open and closed

	No. E in open	T in open	No. E in closed	T in closed	Rearing
Group 1 (CTL)	2.5 ± 1.0	84.8 ± 58	2.5 ± 0.7	218.6 ± 53	5.2 ± 3.0
Group 2 ($PM_{2.5}$)	2.7 ± 1.3	116.5 ± 36.5	3.0 ± 1.7	155.2 ± 52.5	8.0 ± 4.5
Group 3 (PM_{10})	3.5 ± 1.50	143.6 ± 40.8 *	3.5 ± 1.6	151.8 ± 39.4	7.9 ± 4.7

*Group 3 differs from Group 1 only in T in open ($p<0.05$, ANOVA followed by Tukey test)

Tabla 4. Resultados del laberinto elevado. No. E in open: número de entradas en los brazos abiertos; T in closed: Tiempo pasado en los brazos abiertos; No. E in closed: número de entradas en los brazos cerrados. T in closed: tiempo pasado en los brazos cerrados. Los datos se expresan como media ± desviación estándar; tiempo (T) en segundos. n=10 animales por grupo.

Table 4. Elevated Plus Maze Results. No. E in open: number of entries in the open arms; T in open: Time spent in the open arms. No. E in closed: number of entries in the closed arms. T in closed: time spent in the closed arms. Data are expressed as mean ± standard deviation. Time (T) is expressed as seconds. n= 10 animals per group.

arms. In the other parameters, no statistical differences were noted. Control Group stayed more time in the closed arms and less time in the open arms when compared to Group PM_{10}. These data demonstrate that the animals that received nasal instillation of PM_{10} appeared to be less anxious in relation to the control and $PM_{2.5}$ groups. However, this characteristic can be associated with the decrease in the locomotor activity, as shown in table 3.

The modulation of anxiety, pain, and emotion is related to the periaqueductal gray matter (PGM), which is a brain area located in the mesencephalon of mammals (Keay & Bandler 2004). Regarding its function, studies in vivo were performed and demonstrated that the PGM is related to anxiety, besides maintaining the cardiovascular and respiratory system in homeostasis; because it is not a structure that works homogeneously, it is divided into different subregions capable of controlling the markers abovementioned (Depaulis & Bandler 1991, Carrive 1993, Behbehani 1995, Keay & Bandler 2004, Benarroch 2012, Carrive & Morgan 2012, Linnman *et al.* 2012, Holstege 2014).

The results of this experiment differ from other studies performed by the authors abovementioned, which demonstrate that the rats, when exposed to the elevated plus maze test, presented a high level of anxiety, remaining more time in the closed arms. The exposure to particulate matter causes respiratory damages, and possibly the decrease of the anxiety is associated with the fact that the rodents decreased their locomotor activity, spending more time in the open arms. This decrease in the locomotor activity may be related to neuroinflammation and neurodegeneration of the CNS, associated with the periaqueductal gray matter, in which one of its functions is to control the respiration in mammals, resulted in the uncontrolled respiratory function, caused by the instillation of the particulate matter. Thus, to remain in the open arms seems to be associated with a congested airway, in which the rats attempt to obtain a better respiratory pattern.

Alterations in the respiratory pattern are in agreement with studies performed by Mazzoli-Rocha *et al.* (2014), which demonstrated that intranasal instillations of particulate matter, when repeated, cause an alveolar collapse and pulmonary bronchoconstriction, making breathing difficult corroborating with studies conducted by Dubowsky *et al.* (2006), which affirms that the pulmonary inflammation is linked to air pollution, causing an imbalance in the autonomous system, activating the sympathetic nervous system, and modifying the respiratory pattern.

Hematological Parameters

In this study, no significant alterations were observed regarding the hematological and biochemical parameters (Table 5), although there was evidence that the particles from the particulate matter reached the cardiovascular system through inflammatory mediators produced by the lungs. These mediators can reach the bloodstream, causing hematological and biochemical alterations (Brito 2014). However, these alterations were not evidenced, possibly due to the instillation time, and/or protocol, or even in relation to the animal model used. Similarly, another study with nasal instillation of $PM_{2.5}$ in rats also did not have alterations in the red blood cells count (Yi *et al.* 2017).

Biochemical Parameters

According to the biochemical data expressed in table 6, no significant differences were observed

	Group 1 (CTL)	Group 2 ($PM_{2.5}$)	Group 3 (PM_{10})
Erythrocytes (mm³)	$8.3x10^6$ ± 268214.2	$8.1x10^6$ ± 599837.0	$8.2x10^6$ ± 353712.0
Hemoglobin (g%)	14.30 ± 0.4784	14.28 ± 0.4158	14.12 ± 0.5922
Hematocrit (%)	44.66 ± 1.5255	45.54 ± 1.6372	43.77 ± 1.7683
MCV (U³)	53.89 ± 0.4864	53.24 ± 0.4624	53.49 ± 0.5152
MCH (pg)	17.25 ± 0.2014	17.22 ± 0.2300	17.21 ± 0.2514
MCHC (%)	32.02 ± 0.1874	32.26 ± 0.2271	32.21 ± 0.2558
Leukocytes (mm³)	70400 ± 24631.50	63900 ± 16244.31	64500 ± 31099.30
Platelets (mm³)	$8.9x10^5$ ± 68555.65	$9.2x10^5$ ± 51633.75	$9.1x10^5$ ± 177835.87
Segmented (mm³)	15.4 ± 7.19	14.8 ± 4.46	16.4 ± 7.12
Monocytes (mm³)	4.4 ± 6.31	6.5 ± 4.76	3.7 ± 5.49
Lymphocytes (mm³)	80.1 ± 5.48	76.7 ± 9.45	77.9 ± 5.89

Tabla 5. Parámetros hematológicos. No hubo diferencias significativas (p>0.05, ANOVA). MCV: Volumen corpuscular medio; MCH: Hemoglobina corpuscular media; MCHC: Concentración de hemoglobina corpuscular media. Los datos se expresan como media ± desviación estándar; n = 10 animales por grupo.

Table 5. Hematological Parameters Results. There were no significant differences (p>0.05, ANOVA). MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration. Data are expressed as mean ± standard deviation; n=10 animals per group.

	Group 1 (CTL)	Group 2 ($PM_{2.5}$)	Group 3 (PM_{10})
Glucose (mg/dL)	237.6 ± 44.36	224.8 ± 33.27	268.3 ± 59.79
Total Cholesterol (mg/dL)	62.6 ± 5.35	65.3 ± 5.39	64.1 ± 5.23
HDL (mg/dL)	23.7 ± 2.58	23.2 ± 1.75	25.7 ± 1.88
Triglycerides (mg/dL)	108.4 ± 38.93	87.6 ± 25.10	91.3 ± 23.74

Tabla 6. Resultado de parámetros bioquímicos. No hubo diferencias significativas (p> 0.05, ANOVA). Los datos se expresan como media ± desviación estándar; n = 10 animales por grupo.

Table 6. Biochemical parameters results. There were no significant differences (p>0.05, ANOVA). Data are expressed as mean ± standard deviation. n=10 animals per group.

in the analyses of the biochemical parameters beween the groups, which agree with another study performed by Yan *et al.* (2014). These results suggest that the negative effects caused by the particulate matter are from neurological order and not systemic.

Therefore, new studies should be performed to comprehend the behavioral and biochemical mechanisms and their association with air pollution.

Conclusions

Particulate matter is one of the most harmful pollutants that are present in our atmosphere, composed of coarse and fine particles, which cause neurological diseases and pulmonary reactions. These particles are launched daily in the open air and carried by air currents, then these fractions can be inhaled and deposited in the lower and upper respiratory system.

The coarse fraction, characterized by PM_{10}, is retained in the upper airways when inhaled and the body is responsible for eliminating it through sneeze and nasal cleaning. The fine fraction, which is classified as $PM_{2.5}$, has high toxicity, capable of depositing in the lower respiratory system, in the alveolus region which are small structures that perform the gas exchange.

These alterations were perceived throughout this study, during the evaluation of locomotion and anxiety, which demonstrated that the rats were less anxious, because they remained most of the time in the open arms. This permanence in the open arms probably occurred because the animals presented respiratory difficulties, which lead to a decrease in the locomotion and, not necessarily due to an absence of anxiety. Regarding the hematological and biochemical parameters, no alterations were observed in the animals exposed to $PM_{2.5}$ and PM_{10}. From these results, we can conclude that the particulate matter when was inhaled by the rats caused some alterations in their behavior, however, no blood alterations were observed, which can be related to the time of exposure. Therefore, the behavioral alterations were associated with air pollution, nevertheless, more studies involving particulate matter are necessary to comprehend the risks that these pollutants can cause in health.

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