Comparative study of tear lipid composition in two human populations with different exposure to particulate matter in La Plata, Argentina

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Abstract
To identify the changes in the lipid profile of the tear film in two human populations exposed to different levels of particulate material, and its relationship with dry eye, by gas chromatography with mass spectrometry (GC-MS) detection. A panel study involving 78 volunteers, who live and work in two locations in Argentina with different pollution levels: urban zone (n = 44) and industrial zone (n = 34). We measured the mean levels of particulate matter (PM) exposure. The tear samples were analyze by gas GC-MS detection and the dry eye was diagnose using Schirmer test, fluorescein breakup time, vital staining with fluorescein and lissamine green, and lid parallel conjunctival folds (LIPCOF). Statistical analysis was performed using Chi-Square, Bartlett’s, Mann-Whitney tests, and Multiple Correspondence Analysis. PM10 level was significantly higher in industrial zone than in urban area (p < 0.05). Subjects exposed to higher levels of PM10 in outdoor air presented more presence of fatty acids (FA) of long chain, a higher proportion of saturated fatty acids (SFA), and lower unsaturated fatty acids (UFA), showing a differentiated profile, which may be associated with a PM level. The incidence of dry eye was greater in the industrial zone (p < 0.001), showing in both populations for this pathology higher FA ω-6 levels, which are responsible for the inflammation process. The lipid profile in populations exposed to higher levels of PM10, like the industrial zone, shows a differentiated profile of FA and more incidence of dry eye with higher FA ω-6 levels, which are responsible for the inflammation process.

Keywords PM · Lipid composition · Tears · Fatty acids · Dry eye · Environmental

Introduction
Air pollution represents an environmental health problem affecting both developed and developing countries all around the world (WHO 2006). Worldwide, there is an increase in the emission of potentially harmful gases and particulates, which not only affect human health but also the environment, as well as the resources needed to achieve sustainable development on the planet.

The tear film is a highly specialized structure, consisting of various secretions arranged specifically, which covers the cornea, the epithelial surface of the bulbar conjunctiva, and the tarsal conjunctiva (Van Haeringen 1981). The tear is comprised of three layers: a lipid or oily layer, an aqueous layer, and a mucin layer (Ohashi et al. 2006; Wolff 1946). However, new studies suggest that mixing between the mucin and aqueous layers occurs, creating a gradient of decreasing mucin concentration into the aqueous layer (Gipson and Argüeso...
Furthermore, the lipid layer can be subdivided into a non-polar, outermost phase and a polar one, adjacent to the mucin-aqueous layer (Korb et al. 1996; Lozato et al. 2001a, b). The primarily responsible for the secretion of this lipid layer is the Meibomian gland and, to a lesser extent, the glands of Zeis and Moll; it is composed of cholesterol esters, lecithin, fatty acids, free cholesterol, and phospholipids (P. A. Lozato et al. 2001a, b; Nicolaides et al. 1981; Tiffany 1979).

The lipid layer is of great importance since it stabilizes the tear film and prevents evaporation by sealing its aqueous layer (Shine We 1998). Additionally, it serves as the frontline protection for the tear film and ocular surface. If the lipid layer is compromised, the rest of the film will experience instability, causing evaporative dry eye (Lam et al. 2011; Walter et al. 2016).

Recent investigations have demonstrated that a compromised tear lipid composition will produce instability, causing evaporative dry eye. Lam et al. (2011) carried out a lipidomic study of meibum from individuals of an Asian ethnicity, which could potentially offer new insights into the higher prevalence of dysfunctional tear syndrome (DTS) observed amongst Asian populations. They observed 27 DTS patients and 10 control subjects, amounting to 256 lipid species from 12 major lipid classes. The findings evinced significantly lower levels of triacylglyceride (TAG) (p < 0.05) in patients under the moderate category of DTS if compared to the mild category; and, notably, a number of (O-acyl)-ω-hydroxy fatty acid (OAHFA) species displayed consistently decreasing levels that correlate with increasing disease severity. In 2014, Lam devoted himself to deepen his studies on the components of the debatable amphiphilic lipid sublayer, which demonstrated the presence of cholesterol sulfate, O-acyl-ω-hydroxy fatty acids, and various sphingolipids and phospholipids in tears. For their part, Walter et al. (2016) conducted a cross-sectional study to explore the relationship between lipid profiles in human tears and dry eye (DE) symptoms and signs. This study also encompasses their connection with ω-3 and ω-6 polyunsaturated fatty acids that modulate inflammatory processes. Results obtained showed that the ratio of ω-3 to ω-6 tear lipids is elevated in DE patients in proportion to the degree of tear film dysfunction and corneal staining. Metabolic deficiency of ω-3 tear film lipids may be a driving force behind chronic ocular surface inflammation in DE.

The particulate matter (PM) is a pollutant in the atmosphere that can come into direct contact with our eyes, hence represents a risk factor. Thus, the effects of atmospheric pollution at the eye level have been studied with different ocular diseases such as dry eye (Alves et al. 2014), conjunctivitis (Fu et al. 2017b), and meibomian glands dysfunction (MGD), as well as other pathologies such as blepharitis (Malerbi et al. 2012) and pollution-induced alterations in the external segment (Fu et al. 2017a).

In our region, you will find La Plata city, the capital of the province of Buenos Aires, which has a high vehicle-to-person ratio. Fourteen kilometers from this location is the municipality of Ensenada, a city with increased air pollution levels that are mainly the product of industrial activity, principally related to the petrochemical sector.

Since, there are no studies that relate the levels of PM with the lipid profile of the tear film, as a trigger for the destabilization of the tear and promoter of a dry eye. That is why the research objective is to identify changes in the lipid profile of the tear film in two human populations exposed to different levels of particulate matter, and determine its relation to dry eye, utilizing gas chromatography with mass spectrometry (GC-MS) detection for this purpose.

### Patients and methods

#### Study population

The study involved 78 volunteers (n), whose ages range between 18 and 62 years old, who live and work in two locations with different pollution levels: La Plata (n = 44) and Ensenada (n = 34). La Plata is considered as an urban area (U), while Ensenada as an industrial zone (I) (Fig. 1). The mean ± SD age of the 34 volunteers in Ensenada was 34 ± 13, while the one from the 44 volunteers in the urban center of La Plata was 29 ± 6. The sex of the study subjects corresponding to both areas was as follows: U (26F:18M) and I (14F:20M). More information about the study subjects is published in previous work (Gutierrez et al. 2016). The research protocol was approved by the Comité Consultivo Central de Bioética (Central Advisory Committee on Bioethics) of the Universidad Nacional de La Plata (National University of La Plata), and all subjects gave their informed consent before registering for the study. Exclusion criteria included retinal detachment or recent eye surgery, the use of contact lens, pregnancy or breastfeeding (in female volunteers), participation in any other clinical study as a volunteer in the last 30 days, intake of medicine during the week previous to the study, drug addictions, and alcohol intake within 48 hours before the test. At the time of the study, none of the participants was under medical treatment involving drugs of any kind. The study was conducted during February and October 2015. The monitoring was conducted throughout the study period. With regard to patient expiation, we worked with patients who live and work in the study areas to ensure the degree of chronic exposure.
PM in study area

In our study area, available PM monitoring data concerning the last few years (Colman Lerner 2013; Orte et al. 2015; Wichmann et al. 2009) is presented in Table 1.

PM$_{10}$ level was significantly higher in area I than in area U ($p < 0.05$), confirming the difference between the areas of study. Furthermore, a slight increase in PM$_{2.5}$ level was observed, but it was not statistically significant.

Removal of tear samples

Removal of the tear samples was performed in both eyes of the patient as described in the Schirmer I test (Gil del Río 1981; Lam et al. 2014). The strips containing the tear samples were then placed in Eppendorf tubes. One hundred microliters of sterile saline was added to each tube in order to extract the sample embedded in the strip and centrifuged at 10,000 rpm for 5 min (Legend Micro 17/17b centrifuge, Thermo scientific). The extracted samples were placed in clean Eppendorf tubes and stored at $-20^\circ$C until analysis.

Analysis of tear film lipid composition

The fatty acids present in the tear fluid were determined via gas chromatography coupled with a mass spectrometer (CGMS Clarus SQ 8S), prior to their derivatization to methyl esters (FAMEs).

The derivatization was performed following the protocol described by Ichihara and Fukubayashi (2010), according to which 100 μL of the tear samples is first extracted with 130 μL of toluene, 200 μL of HCl/Methanol 8% P/V, and 1.0 mL of methanol in a glass tube and then heated for 1 hour at 100 °C. After cooling down to room temperature, 1 mL of distilled water and 1 mL of hexane were added to extract the FAMEs.

This extract was analyzed by CGMS, SIM mode, using DB-23 column (30 m × 0.250 mm × 0.25 μm), He as carrier (1.5 mL min$^{-1}$), split mode injection (270 °C, 50:1 ratio), temperature 130 °C 1 min, 6.5 °C min$^{-1}$ to 170 °C, 2.75 °C min$^{-1}$ to 215 °C, 40 °C min$^{-1}$ to 230 °C 3 min. The fatty acids analyzed are shown in Table 2.

For the development of analysis of FAMES, recovery tests were carried out (triplicate), adding a known amount of fatty acids studied to 100 μL of physiological solution, and proceeding to the same treatment and analysis that show them, obtaining recovery values ($r$) between 0.875 and 0.965 (Table 2).

Dry eye

It is important to note that the diagnosis of dry eye requires several clinical trials, due to its different etiologies. For this reason, objective evaluation criteria were adopted. Thus, the necessary tests for the trial included Schirmer test, fluorescein-tear breakup time, vital staining with fluorescein and lissamine green, and lid parallel conjunctival folds (LIPCOF). During this study, it was considered as condition for dry eye diagnostic having at least three of these tests with values out of the normal range (Schirmer I $\leq$ 10 mm/5 min, Fluorescein-tear breakup time (FBUT) $< 10$ s, vital staining with fluorescein and lissamine green $> grade 3$, and LIPCOF $> grade 3$).
Statistical analysis

Data was tested for normality and heterogeneity of variance using Chi² analysis and Bartlett’s test, respectively. Mann-Whitney test determined statistically significant differences between two means when normally distributed data was analyzed. The differences were considered significant when $p < 0.05$. All calculations were performed with Infostat software. Multiple Correspondence Analysis was used to detect association between the presence of certain FA in a differential form between the urban and industrial areas, using STATISTICA 7.1 software (Fig. 2).

Results

Fatty acid methyl esters (FAMES) were analyzed by GC-MS, which allowed us to detect 35 peaks with valid quantification, corresponding to fatty acids (FA), in the range of C10 to C24. The following table shows the descriptive statistics of the different concentrations of FA found in each of the urban and industrial areas.

| FAMES | $t_R$(min) | $m/z$ | $r$ |
|-------|-----------|-------|-----|
| Caproic | C₆ | 2.02 | 74,87,99 | 0.965 |
| Caprilic | C₈ | 2.76 | 74,87,127 | 0.964 |
| Capric | C₁₀ | 4.31 | 74,87,143 | 0.897 |
| Undecanoic | C₁₁ | 5.46 | 74,87,169 | 0.899 |
| Lauric | C₁₂ | 6.79 | 74,87,143,171 | 0.931 |
| Tridecanoic | C₁₃ | 8.25 | 74,87,143,185 | 0.912 |
| Myristic | C₁₄ | 9.78 | 74,87,143,199 | 0.875 |
| Myristoleic | C₁₄,₁ | 10.39 | 74,87,208 | 0.895 |
| Pentadecanoic | C₁₅ | 11.32 | 74,87,143,217 | 0.960 |
| Cis-10-Pentadecanoic acid | C₁₅,₁ | 11.95 | 74,87,202 | 0.905 |
| Palmitic | C₁₆ | 12.88 | 74,87,143,217 | 0.941 |
| Palmitoleic | C₁₆,₁ | 13.31 | 74,96,236 | 0.879 |
| Heptadecanoic | C₁₇ | 14.36 | 74,87,143,241 | 0.919 |
| Cis-10-Heptadecanoic acid | C₁₇,₁ | 14.81 | 74,87,98 | 0.921 |
| Stearic | C₁₈ | 15.83 | 74,87,143,255 | 0.946 |
| Elaidic | C₁₈,₁₁ | 16.03 | 74,87,264 | 0.913 |
| Oleic | C₁₈,₁є | 16.18 | 74,87,264 | 0.920 |
| Linotyope | C₁₈,₂₁ | 16.53 | 67,8195,137 | 0.883 |
| Linoleic | C₁₈,₂₂є | 16.85 | 67,81,195,137 | 0.892 |
| γ-Linolenic | γ-C₁₈,₃ | 17.24 | 79,93,121 | 0.934 |
| Linolenic | C₁₈,₃ | 17.72 | 79,93,108 | 0.925 |
| Arachidic | C₂₀ | 18.58 | 74,87,143,283 | 0.901 |
| Cis-11-Eicosenoic | C₂₀,₁ | 18.92 | 69,97,292 | 0.886 |
| Cis-11,14-Eicosadienoic acid | C₂₀,₂ | 19.6 | 81,95,109 | 0.921 |
| Methyl-cis-8,11,14-eicosatrienoic acid | C₂₀,₃ | 19.89 | 74,87,143,292 | 0.886 |
| Heneicosonic | C₂₁ | 19.98 | 79,91,108 | 0.946 |
| Arachidononic | C₂₀,₄ | 20.21 | 79,95,108 | 0.920 |
| Cis-11,14,17-Eicosatrienoic | C₂₀,₃ | 20.42 | 79,91,119 | 0.908 |
| Cis-5,8,11,14,17-Eicosapentaenoic acid | C₂₀,₅ | 21.03 | 74,87,143 | 0.895 |
| Behenic | C₂₂ | 21.15 | 69,97,320 | 0.906 |
| Erucic | C₂₂,₁ | 21.48 | 81,95,313 | 0.912 |
| Cis-13,16-Docosadienoic | C₂₂,₂ | 22.14 | 74,87,143 | 0.936 |
| Tricosanoic | C₂₃ | 22.34 | 74,87,143,382 | 0.901 |
| Lignoceric | C₂₄ | 23.53 | 79,91,313 | 0.889 |
| Cis-4,7,10,13,16,19-Docosahexanoic acid | C₂₂,₆ | 23.81 | 83,97,313 | 0.895 |
| Nervon | C₂₄,₁ | 23.87 | 74,87,272,313 | 0.900 |

FAMEs with their retention times ($t_R$) and the ions used for their quantification, where $m/z$ is the mass ratio load.
populations. Mann-Whitney test was performed to determine significant differences between the different FA and areas.

In order to detect if there is an association between the presence of certain FA in a differential form between the urban and industrial areas, a Contingency Table Analysis was carried out; using this data, a Multiple Correspondence Analysis was also performed to discover these associations, which are shown in the following figure.

The graph represents the Chi$^2$ distance between the FA and study area. Each FA is represented like a point, so points near each other are more closely associated than points far apart. From what it can be seen, there is a connection between the FAs that are circumscribed in the red box for area I, and the blue set for U area. We consider fatty acids as long chain FA (FA-lc) beyond C18, while short chain (FA-sc) between C4-C17. Almost all FA-lc are entirely in area I, except for $\gamma$-C18:3; C18:t; C20:2; and C23, which are more associated with the U area. By contrast, only three associations to short chain FA are present in area I, such as C10; C17; and C17:1.

At the same time, from Table 3, we discover that the concentrations of 15 of the 35 FA determined in the tear present statistically significant differences between both areas, when analyzed via the Mann-Whitney test.

Additionally, Table 4 also shows saturated fatty acids (SFA), long chain saturated fatty acids (SFA-lc) and short chain saturated fatty acids (SFA-sc), unsaturated fatty acids (UFA), monounsaturated fatty acids (MFA) and polyunsaturated fatty acids (PFA), and the ratio between the unsaturated/saturated fatty acids (UFA/SFA).

Furthermore, Table 4 displays the FA composition in the study populations as well as how it is distributed inversely, with a higher percentage of SFA in area I and a higher percentage of UFA in area U.

Regarding dry eye, Table 5 shows the cases found in each area, according to the clinical criteria: Schirmer I $\leq$ 10 mm/5 min, Fluorescein-tear breakup time (FBUT) $<$ 10 s, lacrimal pattern, vital staining with fluorescein and lissamine green $>$ grade 3, and LIPCOF $>$ grade 3.

The results exhibit significant differences between the evaluated areas when the incidence of dry eye was determined by the clinical criteria ($p < 0.001$).

When performing a multiple linear regression (Fig. 3) between the content of total fatty acids (FA) and PM$_{10}$ and PM$_{2.5}$ levels for each zone, $p$ values $< 0.05$ for PM$_{10}$ is obtained in both, so there is a significant linear relationship between PM$_{10}$ levels and FA levels found in our study.

In the case of dry eye, since it is a clinical entity that involves different tests for its diagnosis, a multiple linear regression analysis cannot be applied, since the dry eye variable is not quantitative.

**Discussion**

In this paper, tear lipid profiles that could be associated with particulate matter levels of exposure were analyzed in the city of La Plata and Ensenada.

The industrial zone where the study subjects are exposed is mainly related to the petrochemical industry (YPF) and the generation of petroleum coke (COPETRO). Both are important sources of particulate matter emission, with the petrochemical industry also being an important source of VOC emissions. Previous studies in the region show that although...
the levels of VOCs are higher in the industrial zone than in the urban area, these levels have decreased substantially in the last decade, with traffic becoming an important source of COVs emission in both regions (Massolo et al. 2010, Colman Lerner et al. 2014a, b). In contrast, PM levels have increased in the industrial area (mainly PM10), remaining constant in the urban area (Wichmann et al. 2009; Colman Lerner 2013). When taking into account, SO2 levels in the industrial zone are less than 16 ppb according to previous studies (Rato et al. 2010) being lower than the guide levels of Argentina (27 ppb for 30 days) and the WHO (19 ppb for one year).

That is why when focusing on the levels of particulate material we observe an increase in the levels of PM10 that increase in the area I with respect to the area U, showing significant differences in the concentrations of PM10. The obtained levels for annual averages in both sampling sites exceed guideline values of 20 \( \mu \text{g m}^{-3} \) (WHO 2006), although they were almost doubled in the industrial area. This can be associated with the influence of the petrochemical pole (Colman Lerner 2013; Colman Lerner et al. 2014a, b; Wichmann et al. 2009).

In this regard, PM10 determination allows us to point out the difference concerning air quality in the study areas. Previous studies show how the exposure to PM affects the level of eye inflammatory response, consequently reducing viability and cell proliferation, and increasing apoptosis (Fu et al. 2017a; Novaes et al. 2007; Tau et al. 2013; Torricelli et al. 2014). According to different studies of dry eye disease (DED) incidence, the inflammatory process induces tear film instability, stromal immune cells infiltration, and interruption of the volume and composition of the lacrimal film mediated by lacrimal glands (Alves et al. 2014).

When we evaluate the lacrimal film lipid composition through the characterization of fatty acids, it was

Table 3  Concentrations of FA (\( \mu \text{g mL}^{-1} \)) found in each of the populations

| Variables | Industrial area | Urbana area |
|-----------|----------------|-------------|
|           | n  \( \mu \)  m SD | n  \( \mu \)  m SD |
| C10       | 36 0.06 0.06 0.03 | 14 0.05 0.03 0.03 |
| C11       | 8 0.04 0.02 0.04 | 32 0.05 0.05 0.02 |
| C12       | 64 0.14 0.12 0.08 | 88 0.18 0.12 0.36 |
| C13       | 46 0.03 0.03 0.001 | 46 0.06 0.06 0.03 |
| C14       | 66 0.75 0.6 0.71 | 88 0.72 0.19 2.35 |
| C14-1     | 48 0.24 0.16 0.18 | 52 0.19 0.18 1.12 |
| C15       | 64 0.12 0.09 0.14 | 84 0.13 0.05 0.36 |
| C15-1     | 46 0.15 0.09 0.23 | 62 0.33 0.15 0.62 |
| C16       | 66 4.19 2.59 4.85 | 88 4.98 0.96 18.12 |
| C16-1     | 50 0.45 0.19 0.9 | 70 0.12 0.08 0.16 |
| C17       | 58 0.22 0.18 0.18 | 28 0.45 0.05 1.35 |
| C17-1     | 20 1.09 0.08 3.12 | 2 0.02 0.02 0.067 |
| C18       | 64 2.34 1.72 2.03 | 14 3.29 3.21 1.66 |
| C18-1     | 62 5.64 1.29 23.36 | 80 18.36 2.1 80 |
| C18-1c    | 64 2.11 1.29 27.7 | 28 0.53 0.18 0.72 |
| C18-2     | 44 0.12 0.08 0.16 | 8 0.66 0.5 0.71 |
| C18-2c    | 56 0.48 0.47 0.41 | 16 1.39 0.27 0.7 |
| C18-3     | 58 0.22 0.18 0.18 | 6 0.1 0.04 0.11 |
| C19       | 58 0.1 0.06 0.16 | 16 0.05 0.03 0.04 |
| C20       | 52 0.21 0.17 0.16 | 22 0.43 0.06 0.87 |
| C20-1     | 40 0.22 0.19 0.17 | 34 0.38 0.17 0.7 |
| C20-2     | 52 0.35 0.12 0.37 | 12 0.1 0.1 0.06 |
| C20-3     | 12 0.67 0.58 0.37 | 2 0.58 0.58 0 |
| C20-4     | 38 0.3 0.08 0.76 | 16 0.57 0.15 0.94 |
| C20-5     | 56 0.16 0.13 0.17 | 16 0.44 0.13 0.52 |
| C20-6     | 60 0.25 0.23 0.19 | 24 0.6 0.14 1.58 |
| C22       | 64 0.15 0.16 0.08 | 12 0.12 0.11 0.09 |
| C22-1     | 60 0.23 0.11 0.21 | 10 0.16 0.12 0.09 |
| C22-2     | 58 0.93 0.89 0.12 | 6 0.7 0.54 0.54 |
| C23       | 58 1.85 1.35 2.25 | 12 2.76 0.29 3.9 |
| C24       | 58 10.34 8.64 7.22 | 8 9.06 3.85 11.15 |
| C22-6     | 64 3.35 2.15 2.84 | 6 4.13 4.24 1.84 |
| C24-1     | 8 1.89 1.18 2.15 | 2 4.72 4.72 0 |

The values of the number of the sample (n) are detailed, the mean (\( \mu \)), the median (m), and the standard deviation (SD), for each area. The \( p \) value is also reported according to Non-Parametric Comparisons (Mann-Whitney test). The variables that presented significant differences between populations (\( p \) value < 0.05) are presented in italics.

Table 4  Different groups of FA in both areas

| Groups of FA | I\% | U\% |
|-------------|-----|-----|
| SFA         | 54.50 | 29.20 |
| SFA-sc      | 17.0 | 22.8 |
| SFA-c       | 37.4 | 6.5 |
| UFA         | 45.5 | 70.8 |
| MFA         | 27.8 | 64.2 |
| PFA         | 17.8 | 6.6 |
| UFA/SFA     | 0.8  | 2.4  |

The results are expressed as percentage of the different groups of FA

%I and %U refers to the percentage composition of each group of fatty acid (FA) for each population (industrial and urban).

Table 5  Data from dry and normal eye cases

| Area | n  | Cases |
|------|----|-------|
|      |    | Normal eye | Dry eye |
| Industrial | 34 | 13 | 21 |
| Urban   | 44 | 33 | 11 |
| Total   | 78 | 78 |

Dry and normal eye cases against objective criteria in both areas (Chi\(^2\) = 9.94, \( p \) value < 0.001), performed with Infostat software.
When we analyze each area of study, in which we examined volunteers with dry and normal eyes, by comparing the values of AF that have an influence on the inflammatory response, such as FA ω-6 (Linoleic acid, γ-Linolenic acid, Linolenic acid, and arachidonic acid), we observed that both areas exhibit increased mean concentration values of FA ω-6 in volunteers with dry eye (1.40 μg mL$^{-1}$ U zone and 0.83 μg mL$^{-1}$ I zone), whereas they remained constant in volunteers with normal eyes (0.78 μg mL$^{-1}$ U zone and 0.61 μg mL$^{-1}$ I zone). This coincides with Walter et al. (2016), who asseverates that FA ω-6 have pro-inflammatory...
capacity, evidencing their higher concentration in patients with dry eye.

Conclusions

This study associates PM (PM_{2.5} and PM_{10}) levels with the manifestation of ocular diseases, generated by the instability of the lipid layer. To this end, the lipid profile in populations exposed to different levels of particulate matter was evaluated. It is noteworthy to mention that the population exposed to a higher level of PM_{10} (\(p < 0.05\)) had significantly increased presence of long chain FA, a higher proportion of SFA, and lower UFA, which evidences a differentiated profile that may be associated with PM level. It was also found that there is a significant linear relationship between PM levels and AF levels in both regions.

According to the investigation, the incidence of dry eye was greater in the industrial area (\(p < 0.001\)), plus it showed higher FA \(\alpha-6\) levels in both populations with this pathology, which are responsible for the inflammation process.

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Compliance with ethical standards

The research protocol was approved by the Comité Consultivo Central de Bioética (Central Advisory Committee on Bioethics) of the Universidad Nacional de La Plata (UNLP), the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and the Centro de Investigaciones Científicas (CIC).

Conflict of interest The authors declare that they have no conflict of interest.

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