Settleable atmospheric particulate matter induces stress and affects the oxygen-carrying capacity and innate immunity in Nile tilapia (Oreochromis niloticus)

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Sedimentable atmospheric particulate matter induces stress and affects the oxygen-carrying capacity and innate immunity in Nile tilapia (*Oreochromis niloticus*)

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Abbreviations: APM - atmospheric particulate matter, SeAPM - sedimentable atmospheric particulate matter

Abstract

Steel industry emissions of atmospheric particulate matter (APM) are responsible for air to water cross contamination, which deposits metal/metalloid contaminants in aquatic ecosystems. This source of contamination is not considered in most environmental monitoring protocols being largely neglected. Using sedimentable atmospheric particulate matter (SeAPM) collected in an area with steel industry influence, we analyzed the sublethal effects on the hematological and innate immunological variables in a teleost fish, Nile tilapia *Oreochromis niloticus* after short-term exposure (96 h). After exposure to raw SeAPM (1 $g \cdot L^{-1}$), blood samples were analyzed to evaluate functional indices related to oxygen transport capacity, immune activity and stress. SeAPM exposure reduced blood oxygen carrying capacity by lessening hematocrit, hemoglobin, erythrocyte, and mean corpuscular hemoglobin concentration. Compensatory increments in mean corpuscular volume and mean corpuscular hemoglobin were observed. SeAPM exposure also impacted elements of immune activity by reducing the number of leukocytes, thrombocytes and monocytes; total plasma protein, and leukocyte respiratory activity, and by increasing lysozyme concentrations. Furthermore, SeAPM elicited endocrine stress response, raising plasma cortisol and glucose. The alterations caused by acute exposure to raw SeAPM threatened the capacity to sustain aerobic metabolism and to respond to pathogens which may reduce fitness of fish populations. These results highlight the need to develop proper protocols for monitoring air-water cross-contamination and, also, for further ecotoxicological research to evaluate the dangers of exposure to sublethal SeAPM contamination by steel industries.

Keywords: APM; Steel industry; Metals/Metalloids; nanoparticles; environmental risks; physiological responses.

Abbreviations: APM - atmospheric particulate matter, SeAPM - sedimentable atmospheric particulate matter

1. Introduction

The steel industry is a significant source of atmospheric particulate matter, a complex mixture of particles (Callén et al., 2009; Park and Kim, 2005; Tsai and Cheng, 2004) which may vary in composition, shape and size, including metallic nanoparticles and emergent metallic compounds (Salgado, 2003). APM emission has been associated with adverse health effects for humans, notably chronic respiratory diseases, cardiac problems, lung cancer, and other serious health issues (Costa and Dreher, 1997; Magas et al., 2007; Prieditis and Adamson, 2002; Wild et al., 2009).

In Brazil, sublethal APM contamination has been reported in continental and estuarine waters (Souza et al., 2018b), which could result in important ecological imbalances, reduced fish genetic diversity and even cause extirpation of specific fish population (Bourret et al., 2008; Durrant et al., 2011). Bioaccumulation and biomagnification of APM has been reported in aquatic food chains (Idrus et al., 2018; Souza et al., 2021b and c). However, the worldwide regulatory agencies does not, consider as a rule the air to water cross-contamination in existing environmental monitoring protocols (CONAMA 491/2018 - BRASIL, 2018, Environmental Protection Agency – EPA, European Union and Canada), being case to case studied separately.

Metallic compounds have a number of toxicological impacts on fishes, causing oxidative stress (Cuny et al., 2004; Farombi et al., 2007; Valavanidis et al., 2006); hematological and biochemical disturbances, and immunological depression (Bakshi and Panigrahi, 2018; Hedayati et al., 2016; Hedayati and Darabitabar, 2017; Javed and Usmani, 2015; Khabbazi et al., 2015; Lal Shah, 2010; Lavanya et al., 2011). The susceptibility of fishes to metals and metaloids makes them especially vulnerable to this component of anthropogenic contamination (Saurabh and Sahoo, 2008). Fishes provide essential ecosystem services and are important resources, therefore they are

important indicator organisms to assess and monitor the presence of water contaminants and their deleterious ecological consequences (Campos-Garcia et al., 2015). It is especially valuable to investigate sublethal effects of contaminants, whose presence and impact are still not well understood.

Hematological, biochemical and immunological biomarkers are used as physiological early warning signals and are an essential tool to evaluate the general health status of fish species. We used these markers to evaluate the effects of exposure to a sublethal concentration of SeAPM collected from an area influenced mainly by the steel industry, on a representative fish, the Nile tilapia, *Oreochromis niloticus*. It's worldwide distribution and physiological knowledge base make them a good experimental model for general ecotoxicological predictions (Solis et al., 2007).

2. Material and Methods

2.1. Atmospheric particulate matter

In order to fully represent the complexity of environmental APM contamination, SeAPM was collected at Ilha do Boi (20º18'32"S, 40º16'33"O) in the city of Vitoria, state of Espírito Santo (ES), Brazil. The location has major influence of SeAPM emitted by the steel industry from the Tubarão Complex, which is integrated by an iron pelleting industry and an iron and steel exportation harbor (Santos et al, 2017). This SeAPM has a complex composition, and most of it is composed by inorganic metallic substances (Souza et al, 2021a, Santos et al, 2017, Machado et al, 2018 and Galvão et al 2019). Studies have shown that there may be seasonal variation in Vitoria estuaries contamination (Souza et al., 2013). Therefore, for repeatability, SePM was taken from a mixed sample of SeAPM derived from continuous collection over one

year, as described by Arrivabene and Souza (Arrivabene et al., 2015; Souza et al., 2013). The exposure concentration of 1 $g \cdot L^{-1}$ refers to the SeAPM mass and it was chosen because the environmental relevance, as it is based in the maximum SePM recorded from the Institute of Environment and Hydric Resources (IEMA, 2021) and according to previous pilot testsit also produces a level of aquatic metal contamination similar to what has been reported for the estuarine area close to Vitoria city (Souza et al., 2013). We have analized both nominal and real APM composition.

2.2. Fish

Nile tilapia, *Oreochromis niloticus,* were obtained from the Polettini fish farm in Mogi Mirim, state of São Paulo, Brazil, and transported to the Department of Physiological Sciences at the Federal University of São Carlos (UFSCar) in São Carlos (SP). There, they were maintained in 1000L tanks under natural photoperiod (~12 h:12 h), supplied with biofiltered water at 25 ± 1°C and continuous aeration. They were acclimated for 30 days prior to experimentation, and fed *ad libitum* with commercial pellets (Supra® Acqua Nile tilapia feed: 5-8 mm; 12 % moisture; 32 % protein; 6 % fat).

The same water quality was provided for experimental exposures. The water was continuously monitored (temperature, 25.3 ± 0.91 °C; dissolved oxygen, 7.1 ± 0.89 mg L^{-1}; and pH, 6.3 ± 0.04), and stated suitable for experiment (Ibrahim and Naggar, 2010). All procedures were approved by the UFSCar Committee of Ethics in Animal Experimentation (CEUA-UFSCar protocol nº 8105110718).

2.3. SeAPM exposure and tissue sampling

After acclimation, 20 fishes (body mass, M_B = 132.65 ± 26.33g) were exposed for 96 h to either SePM (SeAPM group, n = 10) or maintained in clean water (control

group, n = 10). Contamination was performed by placing individual tilapia in glass aquaria (200 L) respecting fish:water volume ratio (1:1, OECD 203, 2019) containing SeAPM concentration of 1 $g \cdot L^{-1}$. After 48 h, 50% of the water was replaced with fresh water containing SeAPM at the appropriate dose. Control group was treated identically but without SeAPM addition. After 96h exposure, fish were anesthetized with benzocaine (100 mg L^{-1}) and blood was sampled for analysis.

Arterial blood samples were collected by caudal vessel puncture using syringes bathed in anticoagulant (EDTA 3%) for the hematological analyses; in heparin for respiratory burst activity of leukocytes; in GLISTAB (EDTA 6 g dL^{-1} and KF 12 g dL^{-1}, Labtest, Sao Paulo, Brazil; code 29) for plasma collection for analyses of stress variables, and without anticoagulant to obtain serum for lysozyme analyses. With the exception of lysozyme and cortisol, all analyzed were done just after blood collection.

2.4. SeAPM characterization *quantification*

The SeAPM was analyzed (Table1) according to standardized methods. We used granulometry, muffle and solubilization with HNO_3/H_2O_2 in a microwave digester to access particle size (Gee and Or, 2002), proportion of organic materials (Goldin, 1987; Suguio, 1973), and contents of inorganic material (Environmental Protection Agency – EPA, 3052 - USEPA, 1996). These analyses were carried out at the Brazilian Agricultural Research Corporation – EMBRAPA, Brazil and in the Food Science and Technology Institute of Córdoba (YCITAC - Universidad Nacional de Cordoba, Argentina).

Water from experimental aquaria was sampled (5 mL of water collected in the middle of the water column) for quantitative and qualitative description of SeAPM contamination (Table 2). Quantification was performed in a mass spectrometer with

153 inductively coupled plasma (Q-ICPMS, Aglent 7500 Series CX technology) equipped
154 with an ASX-100 autosampler (CETAC-technologies, Omaha, NE, USA). The analysis
155 was carried out at the Food Science and Technology Institute of Córdoba (YCITAC -
156 Universidad Nacional de Cordoba, Argentina).
157
158 *2.5. Stress variables*
159 Induction of a stress response, by exposure to SeAPM, was evaluated as
160 plasma cortisol and glucose titres. The blood collected in anticoagulant and anti-
161 glycolytic GLISTAB bathed syringes was centrifuged for plasma separation. Glucose
162 was analyzed with a commercial kit (Labtest, Sao Paulo, Brazil; code 84). An aliquot
163 of the plasma was stored at -80°C for cortisol analysis by immunoenzymatic assay
164 using a commercial kit (DRG International, Inc., USA; Cortisol ELISA -EIA - 1887).
165
166 *2.6. Hematological and immunological variables*
167 The hematological analyses aimed to evaluate effects of SeAPM exposure on
168 blood oxygen transport capacity and immune function. The hematocrit was read in
169 heparinized hematocrit tubes after centrifuging at 11200 G-force for 15min.
170 Hemoglobin concentration was analyzed with a commercial kit (Latest, Sao Paulo,
171 Brazil; code 43). Erythrocytes were counted in a Neubauer chamber after blood dilution
172 in formaldehyde citrate solution (1: 200). The mean corpuscular volume [MCV = (Ht x
173 10)/number of erythrocyte (x 10^6 μL^{-1}) = fL], mean corpuscular hemoglobin [MCH = (Hb
174 x 10)/erythrocyte number (x 10^6 μL^{-1}) = pg] and mean corpuscular hemoglobin
175 concentration [MCHC = (Hb x 100) Ht = g dL^{-1}] were calculated; and also, refractometry
176 was used to obtain total plasma protein (TPP).

Total leukocyte, total thrombocyte and differential leukocyte counts were performed on blood smears stained with May-Grunwald-Giemsa-Wright, according to (Paiva et al., 2013). Differential and total cell counts were performed under a microscope using an oil immersion objective (100x). An aliquot of the blood was heparinized and used to analyze leukocyte respiratory activity (RAL) by a colorimetric assay based on the reduction of nitroblue tetrazolium (NBT) dye inside the phagocyte according (Biller-Takahashi et al., 2013). The optical density of the solution was measured by spectrophotometry (Molecular devices SpectraMax® M5) at 540nm. An aliquot of the serum was stored at -80°C to analyze serum lysozyme concentrations (SLC) by a turbidimetric assay, as described by (Demers and Bayne, 1997), with modifications (Zanuzzo et al., 2014).

2.7. Statistical analyses

Analyses were performed with Rstudio (version 3.2.2). Data normality and homogeneity were analyzed using SaphiroWilk and Levene's tests, respectively. Data were normally distributed and therefore unpaired T-test was used to compare the respectively and control groups. Data are expressed as mean values ± standard deviation (SD) and statistical significance was attributed at $P < 0.05$.

3. Results

There was 100% of survival following exposures, confirming that the SeAPM dose was sublethal. Metal composition of SeAPM and the consequent concentrations in contaminated water were dominated by iron ores and aluminium, with the emerging elements titanium and cerium to lesser degrees (Table 1 and 2).

202 **Table 1. Particle size analysis and major compounds present**
203 **in settable atmospheric particulate matter (SeAPM).**

Metals (µg/g)		Particle size (%)	
P	<LOD	1000 µm	0.81
Ca	19048.08	500 µm	14.17
Mg	7907.11	250 µm	17.56
Na	148.70	106 µm	25.91
K	844.82	53 µm	11.80
B	20.03	<53µm	29.75
Al	5522.80		
Ti	1108.94		
V	28.22	**Compounds (%)**	
Cr	34.53	Organic matter	20.14
Mn	554.99	Inorganic matter	79.86 %
Fe-56	69199.04		
Ni	14.74		
Cu	84.79		
Zn	274.86		
As	2.93		
Se	2.25		
Rb	4.92		
Sr	56.63		
Y	17.40		
Zr	43.79		
Nb	2.56		
Ag	0.10		
Cd	1.47		
Sn	15.22		
Ba	1.33		
La	29.90		
Ce	65.49		
Ta	0.00		
W	1.63		
Hg 201	0.32		
Hg 202	0.35		
Pb	50.86		
Bi	1.08		

204 LOD: limit of detection.

205
206 **Table 2. Metal concentration (ug L^{-1}) in the**
207 **experiment water.**

Metal	Control	SeAPM
B	<LOD	<LOD
Al	69.73±0.25	553.99±3.23
Ti	<LOD	96.39±1.77
V	1.08±0.008	3.62±0.03
Cr	<LOD	1.45±0.01
Mn	<LOD	37.58±0.17
Fe-56	<LOD	511.05±5.82
Fe-57	<LOD	545.30±2.66
Ni	<LOD	<LOD
Cu	4.97±0.05	15.39±0.17
Zn	9.82±0.22	29.32±0.18
As	<LOD	<LOD
Se	<LOD	<LOD
Rb	10.36±0.04	10.04±0.001
Sr	22.65±0.09	26.85±0.11
Y	<LOD	1.17±0.016
Zr	<LOD	1.84±0.065
Nb	<LOD	<LOD
Ag	0.33±0.004	0.80±0.009
Cd	<LOD	<LOD
Sn	<LOD	<LOD
Ba	0.58±0.001	0.29±0.002
La	<LOD	1.31±0.03
Ce	<LOD	2.93±0.03
Ta	<LOD	<LOD
W	<LOD	<LOD
Hg-201	<LOD	<LOD
H-202	<LOD	<LOD
Pb	0.89±0.001	3.23±0.04
Bi	<LOD	<LOD

208 Values are mean ± SEM. LOD: limit of detection. Atmospheric
209 particulate matter (APM)
210

211 Exposure to SeAPM triggered a clear stress response, with an almost doubling
212 of plasma cortisol (94%) associated with an increase in plasma glucose (38%) (Fig 1).

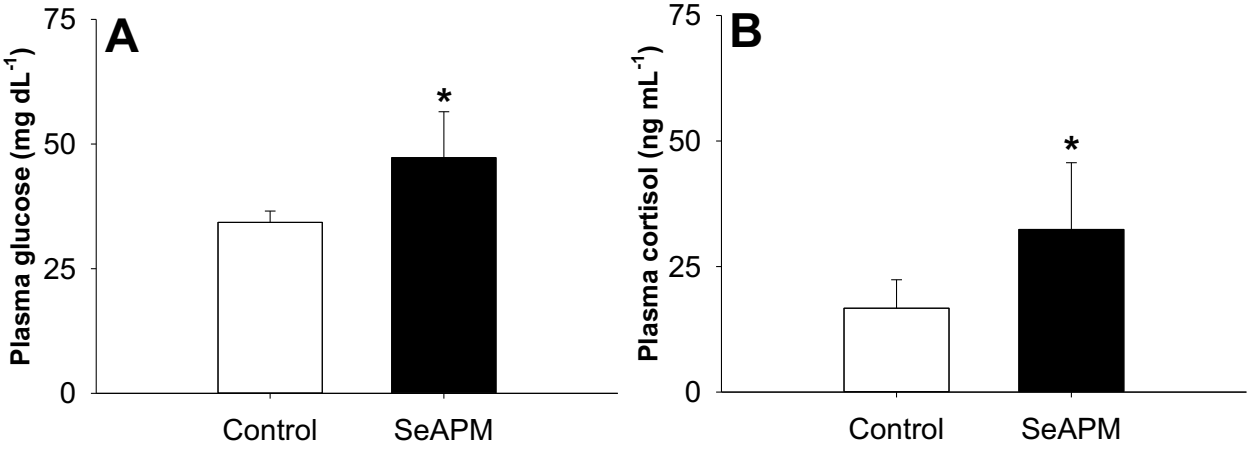

Figure 1. Plasma concentration of glucose (A) and cortisol (B) in Nile tilapia, *Oreochromis niloticus*, exposed to settleable atmospheric particulate matter (SeAPM) for 96 h. * indicates significant difference from the controls ($p < 0.05$).

The SeAPM reduced hematocrit (-12%), hemoglobin (-26%) and red blood cell number (-38%) in the tilapia. The MCHC was also reduced (-17%), whereas MCV (47%) and MCH (32%) increased (Table 3).

Table 3. Hematologic variables in Nile tilapia, *Oreochromis niloticus,* exposed to settleable atmospheric particulate matter (SeAPM) for 96 h: hematocrit; hemoglobin; erythrocyte; mean corpuscular volume (MCV); mean corpuscular hemoglobin concentration (MCHC); mean corpuscular hemoglobin (MCH); total and differential leukocytes counts and thrombocytes.

Parameters	Control	SeAPM
Blood Cells		
Hematocrit (%)	30.77 ± 1.68	27.04 ± 3.13*
Hemoglobin (g dL^{-1})	11.27 ± 1.72	8.32 ± 1.82*
Erythrocytes (×106 μL^{-1})	2.39 ± 0.23	1.48 ± 0.35*
MCV (fL)	130.08 ± 15.78	190.94 ± 39.28*
MCHC (g dL^{-1})	36.87 ± 7.11	30.70 ± 5.32*
MCH (pg)	47.27 ± 6.43	62.18 ± 14.19*
Leukocytes (μL^{-1})	69.96 ± 23.07	39.87 ± 14.35*
Thrombocytes (μL^{-1})	86.81 ± 25.31	30.12 ± 17.23*

Differential leukocytes		
Neutrophils (%)	46.86 ± 18.07	36.92 ± 4.95a
Monocytes (%)	23.42 ± 7.87	15.38 ± 6.02*
Lymphocytes (%)	15.46 ± 5.64	30.83 ± 8.07*

228 Values are mean ± SEM. * indicates significant difference from the controls ($p < 0.05$).
229

230 Immune cell were also affected, fish exposed to SeAPM had lower number of
231 leukocytes (-43%) and thrombocytes (-65%). Considering differential leokocytes, the
232 monocytes were reduced (-34%); in contrast, the lymphocytes increased by almost
233 double (99%) and the neutrophil percentage were similar in SeAPM and control groups
234 (Table 3).

235 After exposure to SeAPM, the tilapia also exhibited significant decrease in TPP
236 (-7%) and RAL (-27%) however, SLC increased (24%) (Fig 2).

237

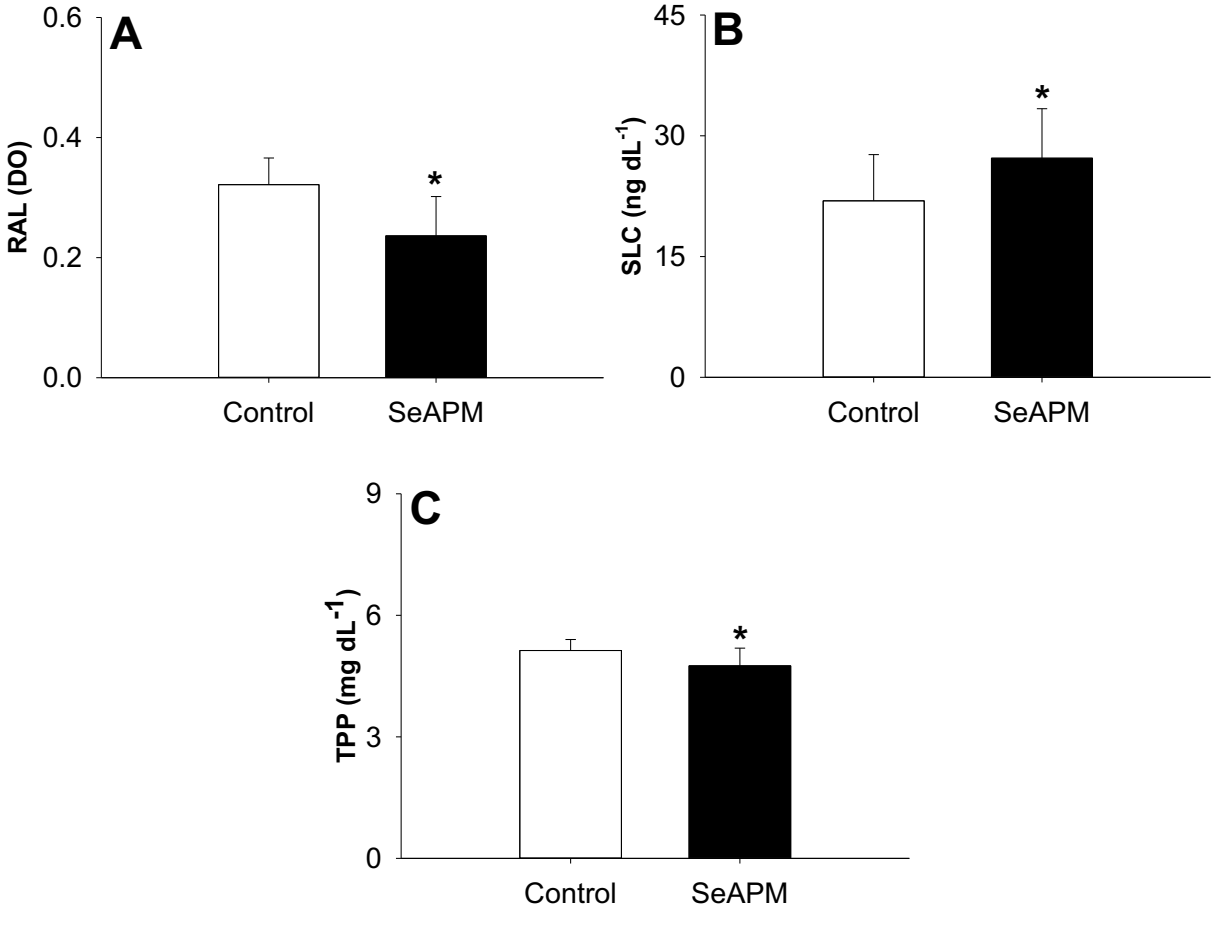

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Figure 2. Respiratory activity of leukocytes - RAL (A), serum lysozyme concentrations - SLC (B) and total plasma protein - TPP (C) in Nile tilapia, *Oreochromis niloticus*, exposed to settleable atmospheric particulate matter (SeAPM) for 96 h. * indicates significant difference from the controls ($p < 0.05$).

4. Discussion

Exposure to SeAPM caused significant alterations to hematological variables related to oxygen carrying capacity, immune activity and stress, indicating major impacts on fish physiology under routine conditions. Although the SeAPM is sublethal, it involved sensitive physiological systems crucial for maintaining the homeostasis and performance of fish in their natural environment. Unless these short exposure impacts can be properly compensated for, during long term contamination, they could have significant ecological consequences, threatening species survival and fitness.

All measured variables were strongly affected by the short-term contamination with SeAPM. Hence, their combined response, including studies of the effect of different levels of contamination, could become useful for monitoring SeAPM presence and toxicity levels in the environment as fish provide potentially valuable tool to evaluate the existence and extent of environmental impacts. There is a need for further dedicated studies, to understand the progression of the contamination and associated damage to fauna. Such research could then support the design of environmental monitoring protocols and risk analyses.

SeAPM *concentration*

SeAPM produced by the industries in the Tubarão Complex (Souza et al., 2018), settles and accumulates over surfaces throughout the city of Vitoria. The SePM comprises a complex mixture of different sized particles that vary in shape and composition. It includes a diversity of organic and inorganic substances (Santos et al,

2017, Machado et al, 2018 and Galvão et al 2019). The inorganic components include a wide diversity of metals (Arrivabene et al., 2015), and the amount of SeAPM used is coherent with metal concentrations observed in local estuarine waters (Souza et al., 2013, 2021b and c). The contamination was dominated by Fe and Al, their nominal concentrations were in the range reported for Santa Cruz and Vitoria Bay (Fe, -355 ± 6.0 and 511 ± 5.8 $mg \cdot L^{-1}$ and Al, 138.9 ± 2.8 and 553.9 ± 3.2 $mg \cdot L^{-1}$, respectively) (Souza et al. 2013). These concentrations are lower than the LC50 reported for freshwater fish (Fe, 1.46-1.71 m L^{-1} and Al, 0.095-235 mg L^{-1}) (Kennedy, 2011). The values were also below to those established as the maximum limits of the Brazilian regulatory agency CONAMA 357/2005 (Fe, 0.3-5.0 mg L^{-1} and Al, 0.1-0.2 mg L^{-1}) for freshwater bodies of classes 1 to 3, referred as protected for aquatic communities, aquaculture, fishing, and supply.

Effects of SeAPM *on fish physiology*

Several different biomarkers have been identified as sensitive indicators of toxic effects in target organisms. They indicate deviation from normal status, function impairment and/or pathological symptomatology (McCarthy and Shugart, 1990). Therefore, they can be important for monitoring the quality of aquatic ecosystems. In the current study, the control data are in accordance with the reference values for these biomarkers in farmed *O. niloticus* (Tavares-Dias and Mariano, 2015) validating the fish group used and the protocol.

Based on the observed reductions in erythrocyte count, hemoglobin content and hematocrit, SeAPM exposure has a fast and significant effect on erythrocytes. Erythrocyte destruction decreases hemoglobin content and hematocrit values. Metals and nanoparticles also reduced RBC, hemoglobin content and hematocrit in Nile tilapia

(Abdel-Khalek et al., 2016). The authors suggested that such effects could be due to a series of factors: hemolysis of erythrocytes in the blood vessels of the liver and kidneys; rupture of hematopoietic tissues; osmoregulatory dysfunction that increases the erythrocyte destruction rate in hematopoietic organs, or even damage from perfusion though injured gill tissue.

The high MCV and MCH, and reduced MCHC, reflected erythrocyte swelling in tilapia exposed to SeAPM, which is related to intracellular osmotic disorders (macrocytic anemia). In studies evaluating the effect of metals on fishes, enlarged red blood cells have been associated with hypoxia that can be caused by gill damage, leading to compromised gas exchange (Abdel-Khalek et al., 2016). The increased MCV with concomitant decreased in RBC, Hb concentrations and MCHC indicated cellular swelling as a mechanism to compensate oxygen transport (Carvalho and Fernandes, 2006). Increases in MCV and MCH and decreases in MCHC levels have also been reported in fish exposed to industrial effluents (Javed et al., 2016; Zutshi et al., 2010). In addition, the changes regarding MCH and MCHC can be attributed to red blood cell hemolysis and a reduction in red blood cell production in hemopoietic tissues. Low MCHC indicates hypochromic anemia, which may relate to malfunction in DNA synthesis (Hoffman R, Benz EJ, Furie B, 2009; Wang et al., 2009). These results are in agreement with general effects of metal contamination reported in various studies on fishes (Cu in *Cyprinus carpio*, *Channa punctatus* and *O. niloticus*; Cd in *C. carpio* and *Scyliorhinus canícula*; Hg in *O. niloticus*, *Clarias gariepinus* and *C. punctatus*; Pb in *O. niloticus*, *Anguilla anguilla* and *O. mossambicus*; Mn in *C. punctatus*, Tilapia sparmanii and *O. mossambicus*; Zn in *C. carpio* and *O. mossambicus*; Cr in *Saccobranchus fossilis*, *Labeo rohita* and *C. carpio*; Ni in *Colisa*

318 *fasciatus* and *C. gariepinus*) (apud Abdel-Khalek et al., 2016; Javed and Usmani, 2015;
319 Singh et al., 2008).

320 The reduction in oxygen carrying capacity lead to obvious limitations for
321 homeostasis impairing the necessary adjustment to deal with common environmental
322 challenges such as hypoxia, swimming, feeding and reproduction.
323 Therefore, despite sublethal, such damage can lead to imminent ecological problems
324 threaten a long-term population survival and/or exertion of its ecological role. Designed
325 protocols to experimentally investigate SeAPM damage on the resistance to hypoxia,
326 swimming capacity, reproductive process, and specific dynamic action (SDA) should
327 properly address such questions.

328 Stress indicators can reveal whether environmental changes are perceived as
329 harmful, which can influence the capacity of the organism to cope. Our data clearly
330 indicate that SeAPM triggered a stress response in tilapia. Plasma levels of cortisol
331 and glucose are the most widely used stress indicators in fishes (Robertson et al.,
332 1987; Sopinka et al., 2016), and their alteration has been reported after metal
333 contamination in other species, such as zebrafish, *Danio rerio* (Katuli et al., 2014);
334 Atlantic salmon, *Salmo salar* (Farmen et al., 2012); and rainbow trout, *Oncorhynchus*
335 *mykiss* (Wood et al., 1996). They are complementary to functional indices, for example
336 the erythrocyte counts, hemoglobin content and hematocrit measurement (Houston,
337 1997).

338 Furthemore, in our study, the decrease in white blood cell count, monocytes
339 PPT and RAL suggest an immunological suppression. It is well established that the
340 immune system of fishes can be severely affected by various stresses, such as metal
341 pollutants (Ghiasi et al., 2010; Jovanović and Palić, 2012; Witeska and Wakulska,
342 2007). It is possible that a significant decline in leukocyte count may affect RAL.

Besides, nanoparticles (especially metallic nanoparticles) can interact with free proteins in plasma resulting in protein conformational changes and decreased activity (Jovanović and Palić, 2012). Adicionally, the higher number of lymphocytes may be associated with defense mechanisms after tissue damage. As a relevant window to environment, the gills are likely to be an affected organ reinforcing the relevance of the previous described damage to oxygen cascade. Lymphocytes are particularly involved in the production of antibodies and inflammatory processes (El-Sayed et al., 2007; Sadauskas-Henrique et al., 2011) and consequently, stimulation of lymphopoiesis and increased release of lymphocytes from lymphomyeloid tissue occurs under toxic stress (Seriani et al., 2015). That alteration is directly related to SeAPM stress and reinforces the reported immune umbalance. Similar tendence were observed in *Labeo rohita*, *Achirus lineatus* and *Centropomus parallelus* exposed to metal contamination (Prado et al., 2014; Seriani et al., 2013; Zutshi et al., 2010).

Furthermore, the SLC increase further supports the SeAPM impact on immune activity. The level of lysozyme in fish serum is an important parameter to monitor the potential impact of environmental alterations on innate immunity, it is the first line of defense mechanisms (Saurabh and Sahoo, 2008). Exposure to heavy metals affects lysozyme levels, and the nature in complex ways dependent on the stress degree, duration and type (Saurabh and Sahoo, 2008; Yildiz, 2006). It has been suggested that high levels of lysozyme after acute exposure to nanoparticles, such as the reported SeAPM effect, can relate to an inflammatory response (Canesi et al., 2010; Gordon et al., 1979; Jovanović and Palić, 2012; Mir, 1977). Despite that is hard to interpret, that is a major alteration since fish protect themselves from pathogens mainly with the help of innate or nonspecific immunity (Rauta et al., 2014). Thus, the SeAPM related

reduction in the innate immunological parameters decreases fish's ability to defend itself against pathogens and infectious diseases.

Therefore, using a range of biomarkers, this study demonstrated that exposure to sublethal concentrations of a real SeAPM mixture from the steel industry, causes severe impairments to physiological and immunological performance in a representative fish species. This has important implications for the damage that SeAPM may cause to aquatic ecosystems and the fishery resources that they support. The Nile tilapia provides an good indicator species to monitor levels and impacts of SeAPM contamination in water bodies. After developing a database on this contamination, wild fish monitoring could alert the threat level before the big bounces of animal mortality. Due to the obvious damage, we caution the need for more in-depth analyses involving different species under a range of contamination levels.

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