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

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

4

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

26 **Abstract**

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

49

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

52

## 53 **1. Introduction**

54           The steel industry is a significant source of atmospheric particulate matter, a  
55 complex mixture of particles (Callén et al., 2009; Park and Kim, 2005; Tsai and Cheng,  
56 2004) which may vary in composition, shape and size, including metallic nanoparticles  
57 and emergent metallic compounds (Salgado, 2003). APM emission has been  
58 associated with adverse health effects for humans, notably chronic respiratory  
59 diseases, cardiac problems, lung cancer, and other serious health issues (Costa and  
60 Dreher, 1997; Magas et al., 2007; Prieditis and Adamson, 2002; Wild et al., 2009).

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

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

78 important indicator organisms to assess and monitor the presence of water  
79 contaminants and their deleterious ecological consequences (Campos-Garcia et al.,  
80 2015). It is especially valuable to investigate sublethal effects of contaminants, whose  
81 presence and impact are still not well understood.

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

89

## 90 **2. Material and Methods**

91

### 92 *2.1. Atmospheric particulate matter*

93 In order to fully represent the complexity of environmental APM contamination,  
94 SeAPM was collected at Ilha do Boi (20°18'32"S, 40°16'33"O) in the city of Vitória,  
95 state of Espírito Santo (ES), Brazil. The location has major influence of SeAPM emitted  
96 by the steel industry from the Tubarão Complex, which is integrated by an iron pelleting  
97 industry and an iron and steel exportation harbor (Santos et al, 2017). This SeAPM  
98 has a complex composition, and most of it is composed by inorganic metallic  
99 substances (Souza et al, 2021a, Santos et al, 2017, Machado et al, 2018 and Galvão  
100 et al 2019). Studies have shown that there may be seasonal variation in Vitória  
101 estuaries contamination (Souza et al., 2013). Therefore, for repeatability, SePM was  
102 taken from a mixed sample of SeAPM derived from continuous collection over one

103 year, as described by Arrivabene and Souza (Arrivabene et al., 2015; Souza et al.,  
104 2013). The exposure concentration of  $1 \text{ g}\cdot\text{L}^{-1}$  refers to the SeAPM mass and it was  
105 chosen because the environmental relevance, as it is based in the maximum SePM  
106 recorded from the Institute of Environment and Hydric Resources (IEMA, 2021) and  
107 according to previous pilot tests it also produces a level of aquatic metal contamination  
108 similar to what has been reported for the estuarine area close to Vitoria city (Souza et  
109 al., 2013). We have analyzed both nominal and real APM composition.

110

## 111 2.2. Fish

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

119 The same water quality was provided for experimental exposures. The water  
120 was continuously monitored (temperature,  $25.3 \pm 0.91^\circ\text{C}$ ; dissolved oxygen,  $7.1 \pm 0.89$   
121  $\text{mg L}^{-1}$ ; and pH,  $6.3 \pm 0.04$ ), and stated suitable for experiment (Ibrahim and Nagggar,  
122 2010). All procedures were approved by the UFSCar Committee of Ethics in Animal  
123 Experimentation (CEUA-UFSCar protocol nº 8105110718).

124

## 125 2.3. SeAPM exposure and tissue sampling

126 After acclimation, 20 fishes (body mass,  $M_B = 132.65 \pm 26.33\text{g}$ ) were exposed  
127 for 96 h to either SePM (SeAPM group,  $n = 10$ ) or maintained in clean water (control

128 group, n = 10). Contamination was performed by placing individual tilapia in glass  
129 aquaria (200 L) respecting fish:water volume ratio (1:1, OECD 203, 2019) containing  
130 SeAPM concentration of 1 g·L<sup>-1</sup>. After 48 h, 50% of the water was replaced with fresh  
131 water containing SeAPM at the appropriate dose. Control group was treated identically  
132 but without SeAPM addition. After 96h exposure, fish were anesthetized with  
133 benzocaine (100 mg L<sup>-1</sup>) and blood was sampled for analysis.

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

140

#### 141 2.4. SeAPM characterization *quantification*

142 The SeAPM was analyzed (Table1) according to standardized methods. We  
143 used granulometry, muffle and solubilization with HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> in a microwave digester  
144 to access particle size (Gee and Or, 2002), proportion of organic materials (Goldin,  
145 1987; Suguio, 1973), and contents of inorganic material (Environmental Protection  
146 Agency – EPA, 3052 - USEPA, 1996). These analyses were carried out at the Brazilian  
147 Agricultural Research Corporation – EMBRAPA, Brazil and in the Food Science and  
148 Technology Institute of Córdoba (YCITAC - Universidad Nacional de Cordoba,  
149 Argentina).

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

153 inductively coupled plasma (Q-ICPMS, Agilent 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 ( $\times 10^6 \mu\text{L}^{-1}$ ) = fL], mean corpuscular hemoglobin [MCH = (Hb  
174 x 10)/erythrocyte number ( $\times 10^6 \mu\text{L}^{-1}$ ) = pg] and mean corpuscular hemoglobin  
175 concentration [MCHC = (Hb x 100) Ht =  $\text{g dL}^{-1}$ ] were calculated; and also, refractometry  
176 was used to obtain total plasma protein (TPP).



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

188

### 189 *2.7. Statistical analyses*

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

195

## 196 **3. Results**

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

201



205  
206  
207

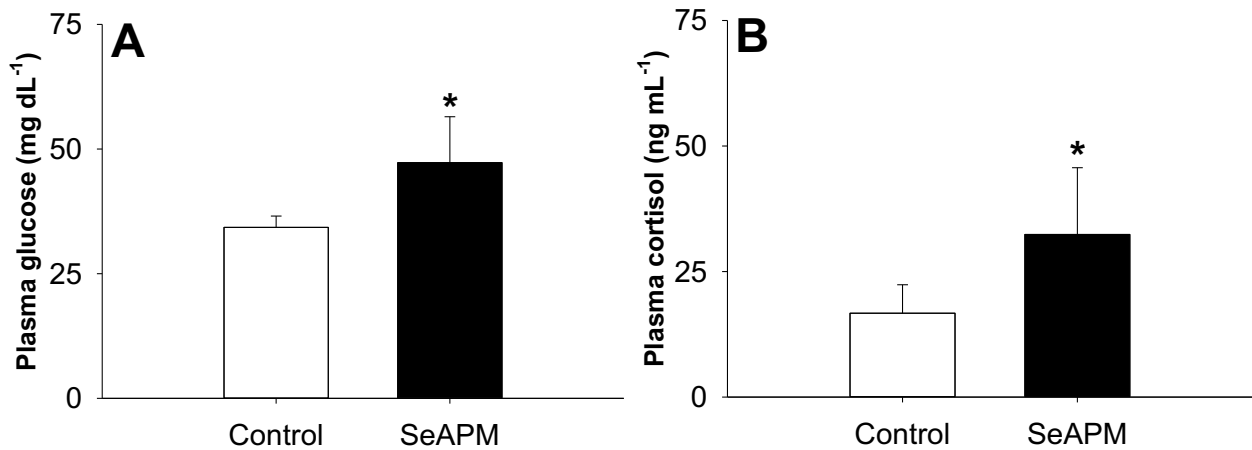
**Table 2. Metal concentration (ug L<sup>-1</sup>) in the experiment water.**

<b>Metal</b>	<b>Control</b>	<b>SeAPM</b>
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  
209  
210

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

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).



213  
 214 **Figure 1. Plasma concentration of glucose (A) and cortisol (B) in Nile tilapia,**  
 215 ***Oreochromis niloticus*, exposed to settleable atmospheric particulate matter**  
 216 **(SeAPM) for 96 h. \* indicates significant difference from the controls ( $p < 0.05$ ).**  
 217

218

219 The SeAPM reduced hematocrit (-12%), hemoglobin (-26%) and red blood cell  
 220 number (-38%) in the tilapia. The MCHC was also reduced (-17%), whereas MCV  
 221 (47%) and MCH (32%) increased (Table 3).

222

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

Parameters	Control	SeAPM
<b>Blood Cells</b>		
Hematocrit (%)	30.77 ± 1.68	27.04 ± 3.13*
Hemoglobin (g dL <sup>-1</sup> )	11.27 ± 1.72	8.32 ± 1.82*
Erythrocytes (×10 <sup>6</sup> μL <sup>-1</sup> )	2.39 ± 0.23	1.48 ± 0.35*
MCV (fL)	130.08 ± 15.78	190.94 ± 39.28*
MCHC (g dL <sup>-1</sup> )	36.87 ± 7.11	30.70 ± 5.32*
MCH (pg)	47.27 ± 6.43	62.18 ± 14.19*
Leukocytes (μL <sup>-1</sup> )	69.96 ± 23.07	39.87 ± 14.35*
Thrombocytes (μL <sup>-1</sup> )	86.81 ± 25.31	30.12 ± 17.23*

### Differential leukocytes

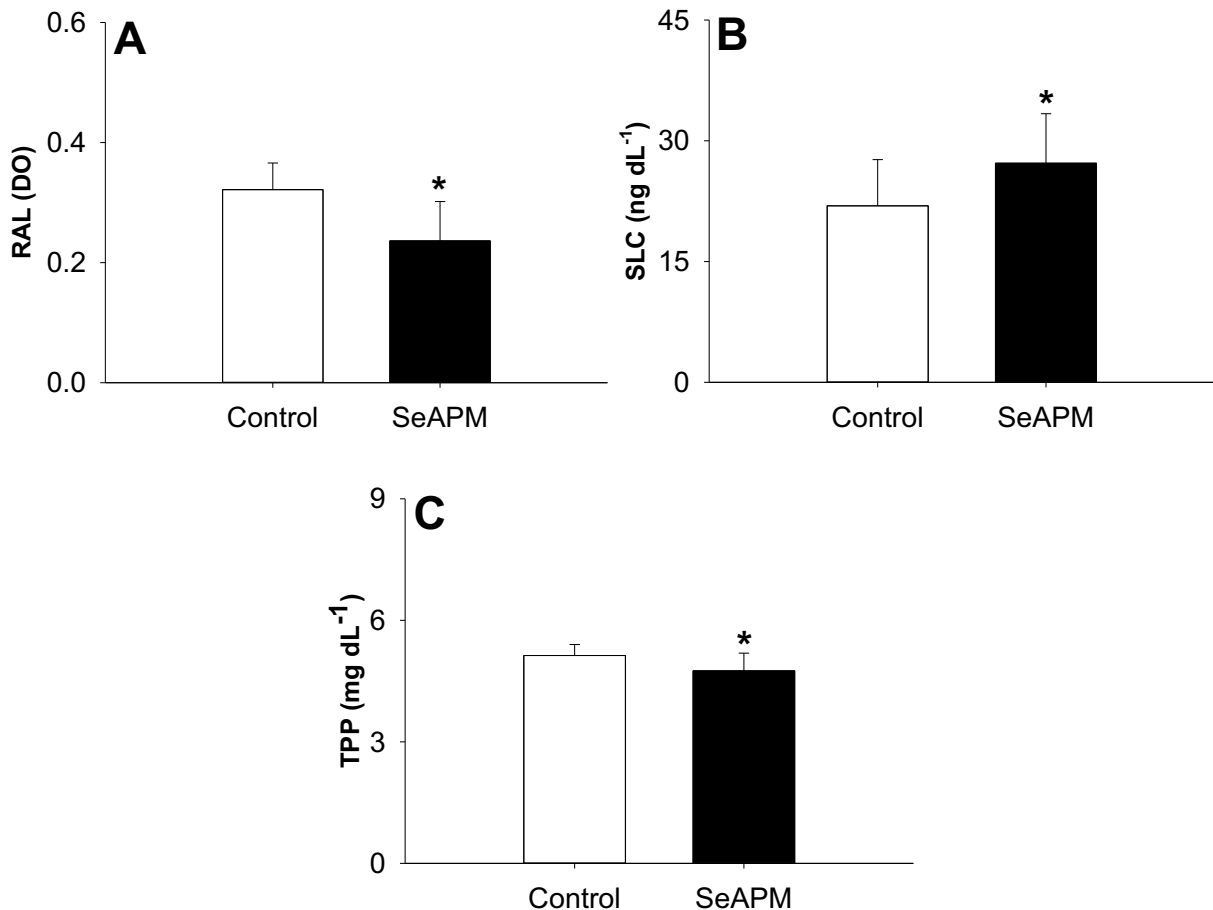
Neutrophils (%)	46.86 ± 18.07	36.92 ± 4.95a
Monocytes (%)	23.42 ± 7.87	15.38 ± 6.02*
Lymphocytes (%)	15.46 ± 5.64	30.83 ± 8.07*

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 leukocytes, 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



238  
239

240

241  
242 **Figure 2. Respiratory activity of leukocytes - RAL (A), serum lysozyme**  
243 **concentrations - SLC (B) and total plasma protein - TPP (C) in Nile tilapia,**  
244 ***Oreochromis niloticus*, exposed to settleable atmospheric particulate matter**  
245 **(SeAPM) for 96 h. \* indicates significant difference from the controls ( $p < 0.05$ ).**  
246

#### 247 **4. Discussion**

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

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

263

#### 264 *SeAPM concentration*

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

269 2017, Machado et al, 2018 and Galvão et al 2019). The inorganic components include  
270 a wide diversity of metals (Arrivabene et al., 2015), and the amount of SeAPM used is  
271 coherent with metal concentrations observed in local estuarine waters (Souza et al.,  
272 2013, 2021b and c). The contamination was dominated by Fe and Al, their nominal  
273 concentrations were in the range reported for Santa Cruz and Vitoria Bay (Fe,  $355 \pm$   
274  $6.0$  and  $511 \pm 5.8 \text{ mg}\cdot\text{L}^{-1}$  and Al,  $138.9 \pm 2.8$  and  $553.9 \pm 3.2 \text{ mg}\cdot\text{L}^{-1}$ , respectively)  
275 (Souza et al. 2013). These concentrations are lower than the LC50 reported for  
276 freshwater fish (Fe,  $1.46\text{-}1.71 \text{ m L}^{-1}$  and Al,  $0.095\text{-}235 \text{ mg L}^{-1}$ ) (Kennedy, 2011). The  
277 values were also below to those established as the maximum limits of the Brazilian  
278 regulatory agency CONAMA 357/2005 (Fe,  $0.3\text{-}5.0 \text{ mg L}^{-1}$  and Al,  $0.1\text{-}0.2 \text{ mg L}^{-1}$ ) for  
279 freshwater bodies of classes 1 to 3, referred as protected for aquatic communities,  
280 aquaculture, fishing, and supply.

281

### 282 *Effects of SeAPM on fish physiology*

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

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

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

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

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

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

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

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

379

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395 **References**

- 396 Abdel-Khalek, A.A., Badran, S.R., Marie, M.A.S., 2016. Toxicity evaluation of copper  
397 oxide bulk and nanoparticles in Nile tilapia, *Oreochromis niloticus*, using  
398 hematological, bioaccumulation and histological biomarkers. *Fish Physiol.*  
399 *Biochem.* 42, 1225–1236. <https://doi.org/10.1007/s10695-016-0212-8>
- 400 Arrivabene, H.P., Souza, I. da C., C3, W.L.O., Conti, M.M., Wunderlin, D.A., Milanez,  
401 C.R.D., 2015. Effect of pollution by particulate iron on the morphoanatomy,  
402 histochemistry, and bioaccumulation of three mangrove plant species in Brazil.  
403 *Chemosphere* 127, 27–34. <https://doi.org/10.1016/j.chemosphere.2015.01.011>
- 404 Bakshi, A., Panigrahi, A.K., 2018. A comprehensive review on chromium induced  
405 alterations in fresh water fishes. *Toxicol. Reports* 5, 440–447.  
406 <https://doi.org/10.1016/j.toxrep.2018.03.007>
- 407 Biller-Takahashi, J., Takahashi, L., Saita, M., Gimbo, R., Urbinati, E., 2013. Leukocytes  
408 respiratory burst activity as indicator of innate immunity of pacu *Piaractus*  
409 *mesopotamicus*. *Brazilian J. Biol.* 73, 425–429.  
410 <https://doi.org/http://dx.doi.org/10.1590/S1519-69842013000200026>
- 411 Bourret, V., Couture, P., Campbell, P.G.C., Bernatchez, L., 2008. Evolutionary  
412 ecotoxicology of wild yellow perch (*Perca flavescens*) populations chronically  
413 exposed to a polymetallic gradient. *Aquat. Toxicol.* 86, 76–90.  
414 <https://doi.org/10.1016/j.aquatox.2007.10.003>
- 415 Call3n, M.S., de la Cruz, M.T., L3pez, J.M., Navarro, M. V., Mastral, A.M., 2009.  
416 Comparison of receptor models for source apportionment of the PM10 in  
417 Zaragoza (Spain). *Chemosphere* 76, 1120–1129.  
418 <https://doi.org/10.1016/j.chemosphere.2009.04.015>
- 419 Campos-Garcia, J., Martinez, D.S.T., Alves, O.L., Leonardo, A.F.G., Barbieri, E., 2015.  
420 Ecotoxicological effects of carbofuran and oxidised multiwalled carbon nanotubes  
421 on the freshwater fish Nile tilapia: Nanotubes enhance pesticide ecotoxicity.  
422 *Ecotoxicol. Environ. Saf.* 111, 131–137.  
423 <https://doi.org/10.1016/j.ecoenv.2014.10.005>
- 424 Canesi, L., Ciacci, C., Vallotto, D., Gallo, G., Marcomini, A., Pojana, G., 2010. In vitro  
425 effects of suspensions of selected nanoparticles (C60 fullerene, TiO2, SiO2) on  
426 *Mytilus* hemocytes. *Aquat. Toxicol.* 96, 151–158.  
427 <https://doi.org/10.1016/j.aquatox.2009.10.017>
- 428 Carvalho, C.S., Fernandes, M.N., 2006. Effect of temperature on copper toxicity and  
429 hematological responses in the neotropical fish *Prochilodus scrofa* at low and high  
430 pH. *Aquaculture* 251, 109–117. <https://doi.org/10.1016/j.aquaculture.2005.05.018>
- 431 Costa, D.L., Dreher, K.L., 1997. Bioavailable transition metals in particulate matter  
432 mediate cardiopulmonary injury in healthy and compromised animal models.  
433 *Environ. Health Perspect.* 105 Suppl, 1053–1060.  
434 <https://doi.org/10.2307/3433509>
- 435 Cuny, D., Van Haluwyn, C., Shirali, P., Zerimech, F., J3r3me, L., Haguenoer, J.M.,  
436 2004. Cellular impact of metal trace elements in terricolous lichen *Diploschistes*  
437 *muscorum* (Scop.) R. Sant. - Identification of oxidative stress biomarkers. *Water.*  
438 *Air. Soil Pollut.* 152, 55–69. <https://doi.org/10.1023/B:WATE.0000015332.94219.ff>
- 439 Demers, N.E., Bayne, C.J., 1997. The immediate effects of stress on hormones and

- 440 plasma lysozyme in rainbow trout. *Dev. Comp. Immunol.* 21, 363–373.  
441 [https://doi.org/10.1016/S0145-305X\(97\)00009-8](https://doi.org/10.1016/S0145-305X(97)00009-8)
- 442 Durrant, C.J., Stevens, J.R., Hogstrand, C., Bury, N.R., 2011. The effect of metal  
443 pollution on the population genetic structure of brown trout (*Salmo trutta* L.)  
444 residing in the River Hayle, Cornwall, UK. *Environ. Pollut.* 159, 3595–3603.  
445 <https://doi.org/10.1016/j.envpol.2011.08.005>
- 446 El-Sayed, Y.S., Saad, T.T., El-Bahr, S.M., 2007. Acute intoxication of deltamethrin in  
447 monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical,  
448 biochemical and haematological effects. *Environ. Toxicol. Pharmacol.* 24, 212–  
449 217. <https://doi.org/10.1016/j.etap.2007.05.006>
- 450 Farmen, E., Mikkelsen, H.N., Evensen, Einset, J., Heier, L.S., Rosseland, B.O., Salbu,  
451 B., Tollefsen, K.E., Oughton, D.H., 2012. Acute and sub-lethal effects in juvenile  
452 Atlantic salmon exposed to low µg/L concentrations of Ag nanoparticles. *Aquat.*  
453 *Toxicol.* 108, 78–84. <https://doi.org/10.1016/j.aquatox.2011.07.007>
- 454 Farombi, E.O., Adelowo, O.A., Ajimoko, Y.R., 2007. Biomarkers of oxidative stress and  
455 heavy metal levels as indicators of environmental pollution in African cat fish  
456 (*Clarias gariepinus*) from Nigeria Ogun River. *Int. J. Environ. Res. Public Health*  
457 4, 158–165. <https://doi.org/10.3390/ijerph2007040011>
- 458 Ghiasi, F., Mirzargar, S.S., Badakhshan, H., Shamsi, S., 2010. Fish Phagocytosis. Pdf.  
459 *J. Fish. Aquat. Sci.* 5, 113–119. <https://doi.org/10.3923/jfas.2010.113.119>
- 460 Goldin, A., 1987. Communications in Soil Science and Plant Analysis Reassessing the  
461 use of loss - on - ignition for estimating organic matter content in noncalcareous  
462 soils. *Commun. Soil Sci. Plant Anal.* 18, 1111–1116.  
463 <https://doi.org/doi.org/10.1080/00103628709367886>
- 464 Gordon, L.I., Douglas, S.D., Kay, N.E., Yamada, O., Osserman, E.F., Jacob, H.S.,  
465 1979. Modulation of neutrophil function by lysozyme. Potential negative feedback  
466 system of inflammation. *J. Clin. Invest.* 64, 226–232.  
467 <https://doi.org/10.1172/JCI109443>
- 468 Hedayati, A., Darabitar, F., 2017. Lethal and sub-lethal impacts of lead on some  
469 hematological, biochemical and immunological indices in Caspian roach ( *Rutilus*  
470 *rutilus* ). *Pollution* 3, 21–27. <https://doi.org/10.7508/pj.2017.01.003>
- 471 Hedayati, A., Rezaei, H., Darabitar, F., 2016. Iranica Journal of Energy &  
472 Environment Environmental Impact of Zinc on hematological , biochemical and  
473 immunological parameters in Caspian Roach ( *Rutilus caspicus* ). *Iran. J. Energy*  
474 *Environ.* 7, 382–386.
- 475 Hoffman R, Benz EJ, Furie B, S.S., 2009. ) Hematology: basic principles and practice.  
476 Churchill Livingstone, Philadelphia.
- 477 Houston, A.H., 1997. Are the classical hematological variables acceptable indicators  
478 of fish health? *Trans. Am. Fish. Soc.* 126, 879–894.
- 479 Ibrahim, N., Naggar, G. El, 2010. Water quality, fish production and economics of Nile  
480 tilapia, *Oreochromis niloticus*, and African catfish, *Clarias gariepinus*, monoculture  
481 and polycultures. *J. World Aquac. Soc.* 41, 574–582.  
482 <https://doi.org/10.1111/j.1749-7345.2010.00397.x>
- 483 Idrus, F.A., Basri, M.M., Rahim, K.A.A., Rahim, N.S.A., Chong, M.D., 2018.  
484 Concentrations of Cadmium, Copper, and Zinc in *Macrobrachium rosenbergii*

- 485 (Giant Freshwater Prawn) from Natural Environment. Bull. Environ. Contam.  
486 Toxicol. 100, 350–355. <https://doi.org/10.1007/s00128-018-2270-3>
- 487 Javed, M., Ahmad, I., Ahmad, A., Usmani, N., Ahmad, M., 2016. Studies on the  
488 alterations in haematological indices, micronuclei induction and pathological  
489 marker enzyme activities in *Channa punctatus* (spotted snakehead) perciformes,  
490 channidae exposed to thermal power plant effluent. Springerplus 5.  
491 <https://doi.org/10.1186/s40064-016-2478-9>
- 492 Javed, M., Usmani, N., 2015. Impact of Heavy Metal Toxicity on Hematology and  
493 Glycogen Status of Fish: A Review. Proc. Natl. Acad. Sci. India Sect. B - Biol. Sci.  
494 85, 889–900. <https://doi.org/10.1007/s40011-014-0404-x>
- 495 Jovanović, B., Palić, D.Š., 2012. Immunotoxicology of non-functionalized engineered  
496 nanoparticles in aquatic organisms with special emphasis on fish-Review of  
497 current knowledge, gap identification, and call for further research. Aquat. Toxicol.  
498 118–119, 141–151. <https://doi.org/10.1016/j.aquatox.2012.04.005>
- 499 Katuli, K.K., Massarsky, A., Hadadi, A., Pourmehran, Z., 2014. Silver nanoparticles  
500 inhibit the gill Na<sup>+</sup>/K<sup>+</sup>-ATPase and erythrocyte AChE activities and induce the  
501 stress response in adult zebrafish (*Danio rerio*). Ecotoxicol. Environ. Saf. 106,  
502 173–180. <https://doi.org/10.1016/j.ecoenv.2014.04.001>
- 503 Kennedy, C.J., 2011. Toxicology | The Toxicology of Metals in Fishes, Encyclopedia of  
504 Fish Physiology. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-374553-8.00236-7>  
505 7
- 506 Khabbazi, M., Harsij, M., Ali, S., Hedayati, A., Gholipour, H., 2015. Effect of CuO  
507 nanoparticles on some hematological indices of rainbow trout *Oncorhynchus*  
508 *mykiss* and their potential toxicity. Nanomedicine J. 2, 67–73.
- 509 Lal Shah, S., 2010. Hematological changes in *Tinca tinca* after exposure to lethal and  
510 sublethal doses of Mercury, Cadmium and Lead. Iran. J. Fish. Sci. 9, 434–443.
- 511 Lavanya, S., Ramesh, M., Kavitha, C., Malarvizhi, A., 2011. Hematological,  
512 biochemical and ionoregulatory responses of Indian major carp *Catla catla* during  
513 chronic sublethal exposure to inorganic arsenic. Chemosphere 82, 977–985.  
514 <https://doi.org/10.1016/j.chemosphere.2010.10.071>
- 515 Magas, O.K., Gunter, J.T., Regens, J.L., 2007. Ambient air pollution and daily pediatric  
516 hospitalizations for asthma. Environ. Sci. Pollut. Res. 14, 19–22.  
517 <https://doi.org/10.1065/espr2006.08.333>
- 518 McCarthy, J.F., Shugart, L.R., 1990. Biological markers of environmental  
519 contamination., in: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of  
520 Environmental Contamination. USA, pp. 353–360.
- 521 Mir, A., 1977. Lysozyme : a brief review. Postgrad. Med. J. 53, 257–259.
- 522 Paiva, M.J.T.R. De, Pádua, S.B. De, M. Tavares-Dias, M.I., 2013. Métodos para análise  
523 hematológica em peixes, ditora da. ed. Maringá.
- 524 Park, S.S., Kim, Y.J., 2005. Source contributions to fine particulate matter in an urban  
525 atmosphere. Chemosphere 59, 217–226.  
526 <https://doi.org/10.1016/j.chemosphere.2004.11.001>
- 527 Prado, L.R.G.B., Felix, C., Abessa, D.M.S., Buruaem, L.M., Abujamara, L.D.,  
528 Kirschbaum, A.A., Turatti, G.C.R., Ranzani-Paiva, M.J.T., Correia, A.T., Seriani,

- 529 R., 2014. Hematological parameters and nuclear abnormalities in peripheral  
530 erythrocytes of *Achirus lineatus* (Pleuronectiformes: Achiridae). *Comp. Clin. Path.*  
531 24, 169–175. <https://doi.org/10.1007/s00580-014-1880-3>
- 532 Prieditis, H., Adamson, I.Y.R., 2002. Comparative pulmonary toxicity of various soluble  
533 metals found in urban particulate dusts. *Exp. Lung Res.* 28, 563–576.  
534 <https://doi.org/10.1080/01902140290096782>
- 535 Rauta, P., Samanta, M., Dash, H., Nayak, B., Das, S., 2014. Toll-like receptors (TLRs)  
536 in aquatic animals: signaling pathways, expressions and immune responses.  
537 *Immunol. Lett.* 158, 14–24.  
538 <https://doi.org/https://doi.org/10.1016/j.imlet.2013.11.013>
- 539 Robertson, L., Thomas, P., Arnold, C.R., Trant, J.M., 1987. Plasma cortisol and  
540 secondary stress responses of red drum to handling, transport, rearing density,  
541 and a disease outbreak. *Progress. Fish-Culturist* 41, 1–12.
- 542 Sadauskas-Henrique, H., Sakuragui, M.M., Paulino, M.G., Fernandes, M.N., 2011.  
543 Using condition factor and blood variable biomarkers in fish to assess water  
544 quality. *Environ. Monit. Assess.* 181, 29–42. [https://doi.org/10.1007/s10661-010-](https://doi.org/10.1007/s10661-010-1810-z)  
545 [1810-z](https://doi.org/10.1007/s10661-010-1810-z)
- 546 Salgado, P.E.T., 2003. Informações gerais e ecotoxicológicas de material particulado,  
547 Série Cadernos de Referência Ambiental v. 14, Centro de. ed. Salvador.
- 548 Santos, J.M., Reis, N.C., Galvão, E.S., Silveira, A., Goulart, E.V., Lima, A.T., 2017.  
549 Source apportionment of settleable particles in an impacted urban and  
550 industrialized region in Brazil. *Environ. Sci. Pollut. Res.* 24, 22026–22039.  
551 <https://doi.org/10.1007/s11356-017-9677-y>
- 552 Saurabh, S., Sahoo, P.K., 2008. Lysozyme: An important defence molecule of fish  
553 innate immune system. *Aquac. Res.* 39, 223–239. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2109.2007.01883.x)  
554 [2109.2007.01883.x](https://doi.org/10.1111/j.1365-2109.2007.01883.x)
- 555 Seriani, R., Abessa, D.M. de S., Pereira, C.D.S., Kirschbaum, A.A., Assunção, A.,  
556 Ranzani-Paiva, M.J.T., 2013. Influence of seasonality and pollution on the  
557 hematological parameters of the estuarine fish *Centropomus parallelus*. *Brazilian*  
558 *J. Oceanogr.* 61, 105–111. <https://doi.org/10.1590/S1679-87592013000200003>
- 559 Seriani, R., Abessa, D.M.S., Moreira, L.B., Cabrera, J.P.G., Sanches, J.Q., Silva,  
560 C.L.S., Amorim, F.A., Rivero, D.H.R.F., Silva, F.L., Fitorra, L.S., Carvalho-Oliveira,  
561 R., Macchione, M., Ranzani-Paiva, M.J.T., 2015. In vitro mucus transportability,  
562 cytogenotoxicity, and hematological changes as non-destructive physiological  
563 biomarkers in fish chronically exposed to metals. *Ecotoxicol. Environ. Saf.* 112,  
564 162–168. <https://doi.org/10.1016/j.ecoenv.2014.11.003>
- 565 Singh, D., Nath, K., Trivedi, S.P., Sharma, Y.K., 2008. Impact of copper on  
566 haematological profile of freshwater fish, *Channa punctatus*. *J. Environ. Biol.* 29,  
567 253–257.
- 568 Solis, J.C., Santerre, A., Pérez, M.I.G., Orozco, R.R., Zaitseva, G., 2007. A  
569 comparative study of phagocytic activity and lymphoproliferative response in five  
570 varieties of tilapia *Oreochromis* spp. *J. Fish Biol.* 71, 1541–1545.  
571 <https://doi.org/10.1111/j.1095-8649.2007.01601.x>
- 572 Sopinka, N.M., Donaldson, M.R., O'Connor, C.M., Suski, C.D., Cooke, S.J., 2016.  
573 Stress Indicators in Fish, *Fish Physiology*. Elsevier Inc.



- 574 <https://doi.org/10.1016/B978-0-12-802728-8.00011-4>
- 575 Souza, I. da C., Arrivabene, H.P., Craig, C.A., Midwood, A.J., Thornton, B., Matsumoto,  
576 S.T., Elliott, M., Wunderlin, D.A., Monferrán, M. V., Fernandes, M.N., 2018.  
577 Interrogating pollution sources in a mangrove food web using multiple stable  
578 isotopes. *Sci. Total Environ.* 640–641, 501–511.  
579 <https://doi.org/10.1016/j.scitotenv.2018.05.302>
- 580 Souza, I. da C., Mendes, V.A.S., Duarte, I.D., Rocha, L.D., Azevedo, V.C., Matsumoto,  
581 S.T., Elliott, M., Wunderlin, D.A., Monferrán, M. V., Fernandes, M.N., 2019.  
582 Nanoparticle transport and sequestration: Intracellular titanium dioxide  
583 nanoparticles in a neotropical fish. *Sci. Total Environ.* 658, 798–808.  
584 <https://doi.org/10.1016/j.scitotenv.2018.12.142>
- 585 Souza, I.C., Duarte, I.D., Pimentel, N.Q., Rocha, L.D., Morozesk, M., Bonomo, M.M.,  
586 Azevedo, V.C., Pereira, C.D.S., Monferrán, M. V., Milanez, C.R.D., Matsumoto,  
587 S.T., Wunderlin, D.A., Fernandes, M.N., 2013. Matching metal pollution with  
588 bioavailability, bioaccumulation and biomarkers response in fish (*Centropomus*  
589 *parallelus*) resident in neotropical estuaries. *Environ. Pollut.* 180, 136–144.  
590 <https://doi.org/10.1016/j.envpol.2013.05.017>
- 591 Suguio, K., 1973. *Introdução à sedimentologia*. São Paulo, SP: USP.
- 592 Tavares-Dias, M., Mariano, W.S., 2015. Parâmetros sanguíneos de referência para  
593 espécies de peixes cultivados, in: Editores, P. e J. (Ed.), *Aquicultura No Brasil:*  
594 *Novas Perspectivas*. São Carlos, pp. 10–30.
- 595 Tsai, Y.I., Cheng, M.T., 2004. Characterization of chemical species in atmospheric  
596 aerosols in a metropolitan basin. *Chemosphere* 54, 1171–1181.  
597 <https://doi.org/10.1016/j.chemosphere.2003.09.021>
- 598 USEPA, 1996. Method 3052: microwave assisted acid digestion of siliceous and  
599 organically based matrices. USEPA (United States Environ. Prot. Agency)  
600 Washingt.
- 601 Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullou, M., 2006. Molecular  
602 biomarkers of oxidative stress in aquatic organisms in relation to toxic  
603 environmental pollutants. *Ecotoxicol. Environ. Saf.* 64, 178–189.  
604 <https://doi.org/10.1016/j.ecoenv.2005.03.013>
- 605 Wang, Y.H., Yan, F., Zhang, W.B., Ye, G., Zheng, Y.Y., Zhang, X.H., Shao, F.Y., 2009.  
606 An investigation of vitamin B12 deficiency in elderly inpatients in neurology  
607 department. *Neurosci. Bull.* 25, 209–215. [https://doi.org/10.1007/s12264-009-](https://doi.org/10.1007/s12264-009-0224-9)  
608 [0224-9](https://doi.org/10.1007/s12264-009-0224-9)
- 609 Wild, P., Bourgard, E., Paris, C., 2009. Lung cancer and exposure to metals: the  
610 epidemiological evidence. *Methods Mol. Biol.* 472, 467–475.  
611 <https://doi.org/10.1007/978-1-60327-492-0>
- 612 Witeska, M., Wakulska, M., 2007. The effects of heavy metals on common carp white  
613 blood cells in vitro. *ATLA Altern. to Lab. Anim.* 35, 87–92.  
614 <https://doi.org/10.1177/026119290703500123>
- 615 Wood, C.M., Hogstrandl, C., Munger, R.S., 1996. The physiology of waterborne silver  
616 toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 35, 93–  
617 109.
- 618 Yildiz, H.Y., 2006. Plasma lysozyme levels and secondary stress response in rainbow

619 trout, *Oncorhynchus mykiss* (Walbaum) after exposure to Leteux-Meyer mixture.  
620 Turkish J. Vet. Anim. Sci. 30, 265–269.

621 Zanuzzo, F.S., Urbinati, E.C., Rise, M.L., Hall, J.R., Nash, G.W., Gamperl, A.K., 2014.  
622 *Aeromonas salmonicida* induced immune gene expression in aloe vera fed  
623 steelhead trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture* 435, 1–9.  
624 <https://doi.org/10.1016/j.aquaculture.2014.09.010>

625 Zutshi, B., Prasad, S.G.R., Nagaraja, R., 2010. Alteration in hematology of *Labeo*  
626 *rohita* under stress of pollution from Lakes of Bangalore, Karnataka, India.  
627 *Environ. Monit. Assess.* 168, 11–19. <https://doi.org/10.1007/s10661-009-1087-2>

628