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► To cite this version:

C.F. de Angelis, M.P. Soares, D.J. Mckenzie, E.W. Taylor, I.L. Cardoso, et al.. Settleable atmospheric particulate matter affects the swimming performance and aerobic metabolic rate of Nile tilapia (*Oreochromis niloticus*). *Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology*, 2023, 271, pp.109696. 10.1016/j.cbpc.2023.109696 . hal-04238462

HAL Id: hal-04238462

<https://hal.science/hal-04238462v1>

Submitted on 12 Oct 2023

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1 **Settleable atmospheric particulate matter affects the swimming performance and**
2 **aerobic metabolic rate of Nile tilapia (*Oreochromis niloticus*)**

3
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32 **Abstract**

33 Atmospheric particulate matter (APM) produced by the steel industry is a complex
34 mixture of particles that includes a wide variety of metals and metallic nanoparticles.
35 These particles settle out onto areas surrounding the industries. There is evidence that this
36 ‘settleable’ APM (SePM) may cause air-to-water cross-contamination with significant
37 effects on aquatic biota. Recent investigations have reported sublethal impacts on gill
38 structure and blood oxygen-carrying capacity of fishes, which raises the hypothesis that
39 there will be consequences for gas exchange capacity and ability to support aerobic
40 activities. Therefore, we investigated the effects of an environmentally relevant level of
41 SePM contamination on swimming performance and associated aerobic metabolic rates
42 in Nile tilapia, *Oreochromis niloticus*. Short-term exposure (96h) to SePM reduced
43 critical swimming speed, energetic efficiency of aerobic swimming, standard metabolic
44 rate, maximum metabolic rate, and aerobic scope. The compromised swimming
45 performance could have adverse ecological effects by limiting foraging ability, predator
46 evasion, territorial protection, and migration. The impairments to aerobic capacity could
47 also affect overall fish performance by influencing long-term energy balance and
48 allocation to growth and reproduction. Thus, despite being sublethal, SePM
49 contamination is considerably debilitating and, if its limiting effects are not compensated
50 in the longer term, it could reduce the survival and fitness of fish populations.

51
52 **Keywords:** iron processing; industry; metal/metalloid; nanoparticle; swimming
53 challenge; physiological responses.
54

55 **1. Introduction**

56 Small amounts of metals/metalloids occur naturally in the aquatic environment
57 through biogeochemical cycles. However, industrial activity causes a continuous and
58 persistent production of atmospheric particulate matter (APM), affecting land and water
59 bodies in diverse ecosystems (Callén et al., 2009; Park and Kim, 2005; Salgado, 2003;
60 Tsai and Cheng, 2004). APM is usually composed of a complex mixture of particles that
61 vary in shape and size. Its final composition can be variable and contains emerging metal
62 compounds with unpredictable new combinations of metals and metal nanoparticles
63 (Nordberg et al., 2007; Salgado, 2003; Soriano et al., 2012).

64 Although, recent publication has revealed potential risk to fish biota (e.g., Soares
65 et al., 2022 De Angelis et al., 2022), the presence of metallic particles from APM
66 deposition (sedimentable APM - SePM) in water bodies has not been properly monitored.
67 Several environmental regulatory agencies still do not consider air-water cross-
68 contamination in their environmental monitoring programs (e.g., CONAMA, 2018,
69 Environmental Protection Agency – EPA, and European Union and Canada). For
70 example, estuarine waters close to the steel industrial complex in Vitoria Bay in the state
71 of Espírito Santo, Brazil, receive constant deposition, of varying intensity, of metallic
72 particles from SePM such as Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg, and Pb (μg
73 $\cdot \text{L}^{-1}$, Souza et al., 2021b, 2021a).

74 Previous studies in the environs of Vitoria city have found that SePM can affect
75 the aquatic environment and cause bioaccumulation (Souza et al., 2013) and
76 biomagnification processes through the food web (Santos et al., 2017; Souza et al., 2018).
77 Environmentally relevant levels of SePM contamination have adverse effects on fishes,
78 such as deleterious impacts on hematological status and innate immunity, and stress
79 responses (*O. niloticus*, Soares et al., 2022). In particular, SePM had effects that would

80 compromise blood oxygen-carrying capacity, causing a reduction in hematocrit,
81 erythrocyte numbers, and mean corpuscular hemoglobin concentration (Soares et al.,
82 2022). Moreover, exposure to SePM also caused gill damage and ventilatory alterations
83 in the same species (De Angelis et al., 2022). These findings indicate that SePM may
84 cause limitations to physiological performance when aerobic metabolic demand
85 increases, with consequences for activities such as swimming, feeding, reproducing, and
86 diverse other components of population functioning.

87 This hypothesis can be investigated with measurements of swimming capacity and
88 associated aerobic metabolism (McKenzie et al., 2007; Plaut, 2001; Priede, 1985).
89 Swimming capacity is a key physiological variables; that have direct ecological relevance
90 because they define fish capacity to forage, evade predators, protect territory, and migrate,
91 among many other activities that can influence survival and fitness (McKenzie et al.,
92 2007; Plaut, 2001; Priede, 1985). At the same time, the associated aerobic metabolism
93 indicates the resting metabolic rate, the minimum and maximum aerobic capacity, and
94 the respective aerobic scope. Fish activities are energetically located inside its aerobic
95 scope. A scope alteration just changes the overall animal capacity to perform. Therefore,
96 that is directly correlated to its ecology; since aerobic scope is considered a parameter of
97 animal performance in relation to environmental conditions, especially related to
98 instantaneous energy-demanding activities (Domenici et al., 2013; Lucas et al., 2021;
99 Milinkovitch et al., 2012).

100 Swimming performance and elements of aerobic metabolism have long been
101 known to be sensitive to environmental factors and stressors and have been used
102 extensively as biomarkers of the toxicological effects of pollutants (Beamish, 1978; Brett,
103 1967; Sprague, 1971).

104 We assessed the result of short-term (96h) exposure to an environmentally
105 relevant level of SePM contamination on the swimming performance and aerobic
106 metabolism of Nile tilapia. This tropical freshwater species provides a good model for
107 ecotoxicology because its physiology is well-studied (Solis et al., 2007). It has been
108 introduced into many tropical and sub-tropical countries across the globe, where it
109 provides important fisheries (De Silva et al., 2004; Schofield et al., 2011), and it inhabits
110 the SePM-contaminated sites in the Vitória Bay area (Gallão et al., 2019). Our approach
111 allowed us to identify and assess sublethal effects of SePM on functional biomarkers that
112 are relevant to animal performance. This allows projection of consequences for
113 individuals and populations of an important food fish, with implications that are relevant
114 both to environmental quality and human health.

115

116 **2. Materials and Methods**

117 *Settleable Atmospheric Particulate Matter - SePM*

118 SePM collection and analysis methods have been described previously (Soares et
119 al., 2022). It is composed of a complex mixture of particles, especially emerging metal
120 compounds with unpredictable new combinations of metals and metal nanoparticles; its
121 composition varies in shape and size. (Nordberg et al., 2007; Salgado, 2003; Soriano et
122 al., 2012). The SePM used in this experiment was collected at Ilha do Boi (20°18'32"S,
123 40°16'33"O), near the industrial complex of Tubarão in Vitória City (5-8 g/m² of SePM
124 each month over an area of 5,000 m², Souza et al., 2021a). This location has an intensive
125 influence of SePM emitted by this industrial complex, which is integrated by an iron
126 pelleting industry and an iron and steel exportation harbor (Santos et al., 2017). The SePM
127 presence in the water environment may be related to input from air and water run-off from
128 hard-standing areas (McLusky and Elliott, 2004). Recently, isotopic analyses

129 demonstrated that the metals present in that SePM (emitted from the nearby industrial
130 complex) were in fact the main source of aquatic contamination in those water bodies.
131 (Souza et al., 2018). Industrial activity may vary seasonally, influencing SePM deposition
132 (Santos et al., 2017; Souza et al., 2019). Therefore, we sampled the contaminant from a
133 pool of raw SePM collected over a year in order to assure experimental repeatability (De
134 Angelis et al., 2022; Soares et al., 2022).

135 Similar to previous experiments, we used $1 \text{ g}\cdot\text{L}^{-1}$ of raw SePM mass to produce
136 environmentally realistic contamination in the exposed group (De Angelis et al., 2022;
137 Soares et al., 2022), this level of contamination is similar to that observed in estuarine
138 waters close to Vitória Bay observed in environmental records (aquatic environment) and
139 reported by the Institute of the Environment and Water Resources for the estuarine area
140 near the city of Vitória, ES - Brazil (IEMA, 2021; Santos et al., 2017; Souza et al., 2013).
141 Arrivabene et al. (2015) reported SePM in the estuarine waters of Santa Cruz and Vitória
142 Bay, which is related to air-water cross-contamination from metallurgical activity. Souza
143 et al. (2013) established chemical characterization of metallic contaminants in these
144 estuarine waters and observed significant amounts of metals and metalloids from SePM
145 in surface waters, interstitial water, and sediments (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se,
146 Ag, Cd, Hg, and Pb - $\mu\text{g}\cdot\text{L}^{-1}$). The raw SePM was diluted in the experimental water (see
147 below). A sample of the SePM used for contamination was stored for any further analyses
148 and evaluations. The Control group was not exposed to SePM.

149 We collected water samples from the experimental aquaria for quantitative and
150 qualitative metal analyses. We used mass spectrometry with inductively coupled plasma
151 (Q-ICPMS, Agilent 7500 Series CX technology) equipped with an Awe SX-100
152 autosampler (CETAC-technologies, Omaha, NE, USA) at the Food Science and

153 Technology Institute of Córdoba (YCITAC - Universidad Nacional de Córdoba,
154 Argentina).

155

156 *Fish supply and acclimation*

157 Juveniles of *O. niloticus* were provided by the Polettini fish farm (Mogi-Mirim –
158 SP, Brazil) and transported to the Department of Physiological Sciences at UFSCar (São
159 Carlos – SP), where they were acclimated in 1,000 L holding tanks during 30 d. Each
160 tank had a continuous supply of dechlorinated, well-aerated water ($25 \pm 1^\circ\text{C}$; DO 6.0–7.3
161 mg.L^{-1} ; hardness 48–53 mg.L^{-1} ; pH 6.7–7.3, (as CaCO_3); alkalinity 40–43 mg.L^{-1} as
162 CaCO_3 ; chloride 42 mg.L^{-1} ; ammonium 0.1 mg.L^{-1} ; and conductivity 110 $\mu\text{S.cm}^{-1}$). Fish
163 remained under natural photoperiod (~12 h:12 h) and were fed *ad libitum* with specific
164 commercial pellets: Ø 5-8 mm, 6 % fat, 32 % protein and 12 % moisture (Supra[®] Acqua
165 line) during acclimation period. Food supply was withdrawn 24h before contamination
166 protocol and experiments. The UFSCar Committee of Ethics in Animal Experimentation
167 (CEUA-UFSCar) analyzed and approved all procedures.

168

169 *Experimental Design*

170 After acclimation, 20 fish (body mass, $M_B = 180 \pm 98\text{g}$, length = $17.9 \pm 0.17\text{ cm}$,
171 width = $1.9 \pm 0.02\text{ cm}$, and height = $6.7 \pm 0.09\text{ cm}$) were divided into two groups of
172 similar mass, which underwent the exposure protocol. For 96h, each fish was put in an
173 individual aquarium (200 L) with clean water (Control, n = 10) or water with SePM
174 (SePM, n = 10). Each aquarium was shielded with a black plastic sheet to avoid visual
175 disturbance and respected the recommended OECD fish:water volume ratio (1g:1L,
176 (OECD 203, 2019). After 48h, 50% of aquarium water was replaced with the relevant
177 water. After 96 h, each fish underwent an incremental critical swimming speed (U_{crit})

178 protocol with concomitant respirometry (Brett 1964), in a Steffensen-type acrylic swim-
179 tunnel respirometer as described by McKenzie et al. (2017), see details below.

180 In the present protocol, fish swam in clean, biofiltered, and aerated water, having
181 acclimated to the tunnel for 24h. Therefore, this would allow the SePM group to partially
182 recover from exposure effects, if that were possible. To further investigate the potential
183 for fish to recover from the effects of SePM, a second swimming trial was performed at
184 96 h after the first, with all animals held in uncontaminated water with the same individual
185 aquarium exposure protocol (Rec group). This Rec test was only applied to the SePM
186 group. It has previously been demonstrated that the swimming performance of Nile tilapia
187 is constant over two sequential 96h periods in clean water (McKenzie et al., 2017).

188

189 *Swimming Performance*

190 After exposure protocol, each fish was put into a bucket which was flushed with
191 clean biofiltered water for 5 min. After that, the fish was transferred to the respirometry
192 chamber of the swim tunnel (volume 13.4 L) and left to recover for 24h in aerated clean
193 water at $25 \pm 1^\circ\text{C}$. During recovery, they were kept swimming at 1 body length $\cdot \text{s}^{-1}$ (BL $\cdot\text{s}^{-1}$)
194 ¹). In that gentle speed, fish could maintain body position using some pectoral sculling
195 and gentle tail beats (see McKenzie et al., 2003). The anterior portion of the swim
196 chamber was covered with opaque black plastic sheeting to avoid visual disturbance of
197 the fish, which spontaneously occupied the covered area.

198 The swimming trial was based on the protocol of McKenzie et al. (2017). Each
199 fish underwent increasing levels of swimming speed. Each level increased 1 BL $\cdot\text{s}^{-1}$ in
200 swimming speed and had 30 min duration. After reaching 4 BL $\cdot\text{s}^{-1}$, increments were
201 reduced to 0.5 BL $\cdot\text{s}^{-1}$ until exhaustion (collapsing against the back of the swim chamber,
202 McKenzie et al., 2017).

203 Swimming speeds were corrected for the blocking effect of each fish (Bell and
204 Terhune, 1970). The second swimming trial (Rec group) was held with the same
205 characteristics as the first. The U_{crit} ($BL \cdot s^{-1}$) was calculated by adding the velocity of the
206 most recently completed swimming step to the product of the velocity incremental
207 increase and the time proportion of swimming until fatigue at the last current velocity, as
208 described by Brett (1964):

$$209 \quad (1) U_{crit} = U_s + (U_{ss} \cdot T_{ss})$$

210 where U_s is the last completed velocity of the swimming step, U_{ss} is the incremental
211 increase in velocity, and T_{ss} is the time proportion of swimming until fatigue at the last
212 current velocity.

213

214 *Swimming respirometry*

215 During swimming, oxygen uptake was measured by intermittent closed
216 respirometry in two cycles of 15 min at each speed (measuring time – 9 minutes, waiting
217 time – 1 minute, and flushing time – 5 minutes). An optical oxygen probe and the
218 associated software (Fibox, GmbH and Oxyview, Pre-sens) allowed for continuous
219 recordings of the oxygen tension in the respirometry chamber. The oxygen uptake rate
220 was calculated at each swimming speed based on the gradient of decline in O_2 during the
221 closure period, at a sampling rate of once each 15 s. The rate of oxygen uptake ($\dot{M}O_2$ -
222 $mMolO_2 \cdot kg^{-1} \cdot h^{-1}$) was then calculated as

$$223 \quad (2) \dot{M}O_2 = [\beta_{O_2} \cdot V_{RE} \cdot W_0^{-1} \cdot (\frac{\delta p_{O_2}}{\delta t})]$$

224 where β_{O_2} denotes the coefficient for O_2 solubility in water (mg of $O_2 \cdot L^{-1}$); V_{RE} , the
225 chamber volume (L); W_0^{-1} , fish body mass (Kg); and $(\frac{\delta p_{O_2}}{\delta t})$ indicates the O_2 reduction
226 over time (% of $O_2 \cdot h^{-1}$). After each swimming protocol, blank tests were performed to

227 measure rates of background microbial oxygen uptake, then the respirometer was
228 thoroughly washed with clean water.

229 For each swim test, a least-squares exponential regression was applied to the
230 relationship between swimming speed and the $\dot{M}O_2$ measurements. Extrapolation back
231 to the y-intercept, a notional swimming speed of zero, was employed to estimate standard
232 metabolic rate (SMR), the minimum rate of ATP use required to sustain life, in the
233 absence of voluntary muscular movements and digestion/absorption of nutrients (Chabot
234 et al., 2016). Maximum metabolic rate (MMR) was measured as the highest
235 $\dot{M}O_2$ achieved during the swimming trail, which is assumed to represent the maximum
236 rate of oxygen uptake that an animal can achieve to create ATP aerobically (Norin and
237 Clark, 2016). Aerobic metabolic scope (AS) was measured as:

$$238 \quad (3) \text{ AS} = \text{MMR} - \text{SMR}$$

239 which estimates the maximum capacity to supply oxygen to sustain metabolic activities
240 beyond SMR, activities such as digestion, locomotion, growth, reproduction, and so on
241 (Fry, 1971). We also assessed the net metabolic cost of swimming (COS - $\text{mMolO}_2 \cdot \text{kg}^{-1}$
242 $\cdot \text{h}^{-1}$ at a sustained speed of $2 \text{ BL} \cdot \text{s}^{-1}$ as:

$$243 \quad (4) \text{ COS} = \dot{M}O_2 (\text{at } 2 \text{ BL} \cdot \text{s}^{-1}) - \text{SMR}$$

244 an estimate of the metabolic cost of activity at low velocity (Allen et al., 2021).

245 The swimming efficiency (Eswim - $\mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$) was evaluated as
246 described by McKenzie et al. (2017):

$$247 \quad (5) \text{ Eswim} = \text{COS} \cdot 2^{-1} (\text{speed of } 2 \text{ BL} \cdot \text{s}^{-1})$$

248 Finally, we analyzed the maximum energetic efficiency of swimming
249 performance (E_{max} - $\mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$), a variable of significant ecological relevance
250 (Ohlberger et al., 2006), was calculated based on Kunz et al. (2018):

$$251 \quad (6) \text{ Emax} = \text{MMR} \cdot \text{Ucrit}^{-1}$$

252

253 *Statistical analyses*

254 All data were analyzed for normality and homogeneity (Shapiro-Wilk and
255 Levene). Differences between experimental groups (Control and SePM) were analyzed
256 by Student's T-test. Recovery was tested by comparing the mean values of SePM (first
257 swimming test) and Rec (second swimming test) with paired Student's T-test. All data
258 were analyzed with R v.4.1.1 (R Core Team 2021). We assumed the significance level of
259 95% ($P < 0.05$). The relevant data are presented as means \pm SE.

260

261 **3. Results**

262 *Settleable Atmospheric Particulate Matter*

263 SePM contamination introduced several metal elements into the water. The main
264 bioavailable components were iron and aluminum, followed by titanium and cerium
265 (Table 1). Fe and Al represented composed approximately 97% of the total metals
266 available in the contaminated water. However, despite their high concentration, both
267 metals were below the reference LC50 for fishes (Fe - 1.46-1.71 $\text{mg} \cdot \text{L}^{-1}$ and Al - 0.095-
268 235 $\text{mg} \cdot \text{L}^{-1}$ -Kennedy, 2011). Additionally, these metals were below the upper limit (Fe,
269 0.3-5.0; Al, 0.1-0.2 $\text{mg} \cdot \text{L}^{-1}$) indicated for protected freshwater bodies (for drinking
270 supply, aquatic communities, aquaculture, and fishing, CONAMA, 2005).

271

272 *SePM affected swimming performance and aerobic metabolic rates*

273 The Control group had a mean Ucrit of $5.68 \pm 0.21 \text{ BL} \cdot \text{s}^{-1}$ at 25°C. This
274 swimming performance was associated with maintenance of significant respiratory
275 support for activity (Figure 1). SMR was $4.49 \pm 0.32 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the Control
276 group. Activity increased metabolic rate so that MMR was 3.2 times higher at $14.58 \pm$

277 $0.82 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, such that Control AS was $10.09 \pm 0.61 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$
278 (Figure 2). The Control group had COS at $3.21 \pm 0.51 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, Eswim of 1.58
279 $\pm 0.25 \text{ } \mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$, while Emax was $2.75 \pm 0.12 \text{ } \mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$ (Figure
280 3).

281 The SePM group showed impaired performance with a Ucrit of $4.15 \pm 0.26 \text{ BL} \cdot$
282 s^{-1} , 27% lower than the Control fish ($p < 0.001$ - Figure 1). This reduction in performance
283 was associated with effects of SePM on respiratory metabolism. SePM contamination
284 reduced SMR by 26 % ($3.32 \pm 0.17 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, $p = 0.001$ - Figure 2A) and MMR
285 by 27 % ($10.51 \pm 0.78 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, $p = 0.002$ - Figure 2B) when compared to the
286 Control group. Additionally, SePM contamination decreased AS by 29 % compared to
287 the control group ($7.19 \pm 0.77 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, $p = 0.003$ - Figure 2C).

288 SePM did not change the energetics of sustained swimming at low velocity, with
289 values of COS of $3.72 \pm 0.31 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ($p = 0.333$ - Figure 3A) and Eswim of
290 $2.09 \pm 0.23 \text{ } \mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$ ($p = 0.102$ - Figure 3B) that were similar to the Control
291 group. However, SePM contamination did alter Emax, which was 11% lower in the SePM
292 group compared to the Control, at $2.46 \pm 0.07 \text{ } \mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$ ($p = 0.011$ - Figure
293 3C).

294

295 *Second swimming trial*

296 The second swimming trial provided no evidence of recovery in swimming
297 performance or metabolic rates (Table 2). Swimming performance was 18% further
298 reduced in the Rec group, with a Ucrit of 3.41 ± 0.17 (Table 2). All other variables
299 remained stable when compared to the first swimming trial. Although all 10 fish in the
300 SePM group survived the 96h contamination and the first swim test, 3 of these fish did

301 not survive the additional 96h recovery period before the second swim test. All fish that
302 underwent the second swim test survived to the end of the experimental protocol.

303

304 **Discussion**

305 Our experimental approach used a functional test to address the effects of
306 sublethal levels of contamination on a widely-studied fish species. An environmentally
307 relevant concentration of SePM limited the swimming performance of *O. niloticus*, an
308 effect that was associated with substantial alterations in respiratory metabolism. This is
309 clear evidence of effects on functional integrity, an impairment that indicates significant
310 limitations on respiratory metabolism that may reduce fish fitness due to direct and
311 indirect impacts on mobility and energy supply.

312

313 *SePM and general metal toxicology*

314 Our exposure protocol used an environmentally relevant level of SePM
315 contamination and was able to replicate the presence and concentration of bioavailable
316 metals as reported in Santa Cruz and Vitoria Bay (IEMA, 2021; Souza et al., 2021a; Souza
317 et al., 2021; Soares et al., 2022; De Angelis et al., 2022). The analysis of SePM confirmed
318 that the contamination is highly complex in metal composition. The observed range of
319 metals corroborates the results of previous studies (Galvão et al., 2022; Soares et al.,
320 2022; Souza et al., 2021a; Souza et al., 2021). Metals have a broad range of impacts on
321 fish physiology, including oxidative stress, genotoxicity, hematological alterations,
322 histopathological alterations in many organs, and problems in diverse physiological
323 systems (Lushchak, 2011; Pyle et al., 2002; Taylor et al., 1996; Giles, 1984). Moreover,
324 previous analyses also described the presence of different-sized particles, which may vary

325 in shape and composition. Furthermore, part of these are in nanometric scale (Salgado,
326 2003; Santos et al., 2017; Souza et al., 2021b).

327 Therefore, the bioavailable metals in experimental water comprise a complex and
328 unpredictable contaminant due to the range of different elements, possible combinations
329 of metallic/metalloid components, and the presence of metallic nanoparticles (Salgado,
330 2003; Souza et al., 2018; 2021a; 2021b; Soares et al., 2022). This makes it difficult to
331 identify the main components responsible for functional alterations observed in tilapia.
332 Presently, using raw SePM in experimental protocols is the only way to replicate such
333 environmental contamination and study its impacts (Soares et al., 2022; De Angelis et al.,
334 2022). Although the precise mechanisms underlying the SePM effect have yet to be
335 revealed, the observed limits to performance are of obvious interest since the level of
336 individual metals was below their lethal concentration (Kennedy, 2011) and under the
337 maximum limits set by the Brazilian regulatory agency (CONAMA, 2005). It seems that
338 we are dealing with an emerging problem that might be highly ecologically relevant.

339

340 *SePM exposure limits swimming performance*

341 Short-term swimming performance tests have generated valuable data to evaluate
342 fish health after physiological challenges, such as infections (Tierney and Farrell, 2004)
343 and sublethal toxicant exposures (Beaumont et al., 1995; McKenzie et al., 2007).
344 Swimming performance has been used to identify physiological damage caused by
345 diverse dissolved metals in salmonids, such as copper (Waiwood and Beamish, 1978),
346 zinc (Alsop et al., 1999), and nickel (Pane et al., 2004). This was the first investigation
347 that used fish swimming performance to evaluate the effect of SePM.

348 Previous experiments using a similar swimming protocol at the same temperature
349 in Nile tilapia reported Ucrit values similar to our Control group (McKenzie et al., 2017),

350 confirming the consistent quality of the fish population used and the utility of the
351 swimming trials. Consequently, the reduced values of Ucrit and Emax in SePM-
352 contaminated fish identify clear impairment of swimming performance and metabolic
353 effectiveness.

354 It is parsimonious to attribute the Ucrit reduction to the associated changes in
355 aerobic metabolism. Functional cardiorespiratory integrity is necessary to support a high
356 level of aerobic swimming (McKenzie, 2011). Therefore, a break in the integrity of
357 mechanisms for oxygen uptake and delivery may account for the reduction in swimming
358 capacity. The observed decrease in Emax supports such an argument.

359 Our experimental approach allowed us to reveal that SePM limited the ability to
360 swim, which has obvious ecological relevance. Swimming relates to the capacity to
361 perform essential behavioral tasks for interactions with the environment, such as
362 maintaining a position against currents, foraging, migrating, reproducing, and many
363 others. The evidence that swimming capacity was limited by impaired metabolic support
364 is also relevant because it indicates that other aerobically demanding activities would be
365 similarly impacted.

366

367 *SePM exposure affected aerobic metabolism*

368 Metabolic measures provide a significant description of energy mobilization in
369 individual animals (Rosewarne et al., 2016), which is considered a direct reflection of the
370 functional integrity of an individual and its potential to operate in nature (Rice, 1990).
371 The assessment of aerobic profile has been reported as ecologically relevant as a potential
372 bioindicator of metabolic performance, growth rate, and survival (Killen et al., 2010) as
373 they bring a unifying currency of animal performance (Wikelski and Ricklefs, 2001). The
374 swimming performance protocol allowed for calculating robust metabolic indexes

375 regarding the fish metabolic profile and revealed aspects of the energy used for basal
376 maintenance (SMR) and the relative cost of the activity under low (COS, Eswim) and
377 high demand reaching up its limit and the related increase scope (MMR, AS, and Emax).

378 Control tilapia had a metabolic profile very similar to previous reports for the
379 species. For instance, using a similar swimming technique at the same temperature,
380 McKenzie et al. (2017) reported similar mean values for SMR, MMR, and AS
381 (respectively, 4.1 ± 0.5 , 16.8 ± 1.3 , 12.6 ± 0.1 mMolO₂ · Kg⁻¹ · h⁻¹) in the same strain of
382 Nile tilapia.

383 The 96h exposure to SePM clearly limited both the total energy used and the
384 efficiency of the use of this energy. The exposed fish had reduced standard metabolic
385 rates. Although they were capable of matching the aerobic demands of low levels of
386 sustained swimming (COS and Eswim), SePM-contaminated fish could not achieve the
387 same metabolic rate to support high swimming speeds. Therefore, the overall aerobic
388 metabolic phenotype was affected, with a reduction in basal metabolic processes and an
389 impaired ability to meet the increased metabolic rate required to support intense activity
390 such as swimming at high speeds.

391 Xenobiotics are reported to cause apparently conflicting metabolic effects, such
392 as: metabolic limitations and metabolic masking (Hammer, 1995). The first condition
393 would impair both metabolite supply and removal. Therefore, it would limit the SMR and
394 would have an additional contribution to the reduction in the energetic efficiency of
395 swimming and the critical speed. Additionally, it would reduce the maximal energy
396 production capacity and aerobic scope. Even at sublethal level, metals have been reported
397 to induce alterations with an ultimate metabolic consequence and long-term effects such
398 as reduction in development, growth, and reproduction (Lucas et al., 2021). For instance,
399 a low level of Cu and Cd was observed to reduce the metabolic rate in the freshwater fish

400 species *Pimephales promelas* (Pistole et al., 2008). Zn also reduced the metabolic activity
401 in *Rhamdia quelen* (Leitemperger et al., 2019).

402 Previous experiments reported that SePM contamination has a deleterious effect
403 on both capacities for oxygen uptake and delivery in the tilapia (De Angelis et al., 2022;
404 Soares et al., 2022). That was also associated with a compensatory ventilatory increase
405 and ended up reducing the tolerance to hypoxia (De Angelis et al., 2022). Such effects
406 are coherent with the current findings of reduced swimming performance and alterations
407 in aerobic metabolism. The same authors reported that tilapia could sustain its routine
408 metabolic rate (RMR) after a similar SePM contamination (De Angelis et al., 2022). We
409 suggest that the combination of these factors (maintenance of RMR while reducing SMR
410 with high ventilation cost) indicates problems with energy allocation. A larger part of the
411 total energy would be used for ventilation, while other processes potentially have limited
412 energy support. That interpretation corroborates the observed energetic limitation of
413 swimming. A higher ventilatory effort is expected to increase the energy used for active
414 ventilation at each swimming level (Freadman, 1981). Therefore, such an energy
415 reallocation was revealed as inefficient energy use (i.e., low E_{max}), lower MMR and AS
416 when the fish were forced to increase their metabolism for swimming.

417 Additionally, AS has been proposed as an index of animal performance in relation
418 to environmental conditions, since it is related to instantaneous energy-demanding
419 activities and may demonstrate the physiological consequences of several environmental
420 constraints such as temperature increase, hypoxia, and pollutants (Domenici et al., 2013;
421 Lucas et al., 2021; Milinkovitch et al., 2012). Therefore, the observed reduction in AS on
422 exposure to SePM may restrain a range of relevant energetically demanding activities
423 such as growth, reproduction, and offspring size (Claireaux and Lefrançois, 2007).

424 Furthermore, the capacity to support increased energy demand levels is also
425 supposed to impact recovery after exhausting activity. Part of the SePM group (30%) died
426 during the additional recovery period after the first swimming trial. This may be related
427 to SePM negative effects previously reported; SePM caused significant damage to the gill
428 structure, aerobic capacity, oxygen-carrying capacity of the blood, and energy allocation
429 in Nile tilapia (De Angelis et al., 2022; Soares et al., 2022), impairing O₂ uptake from the
430 water, the metabolism and swimming performance as observed in the present experiment.
431 MMR can be associated with intrinsic and extrinsic factors (Norin and Clark, 2016). Thus,
432 the maximum swimming activity may cause a transitory debilitation in every vertebrate
433 since it depletes cellular energy reserves and pH alteration due to lactic acid produced,
434 and others. It also causes ionic unbalance in fish associated with the increased ventilatory
435 effort. Post-activity recovery might cause long-term demands for an aerobic effort to
436 sustain the biochemical effort to recover osmolarity, pH, and energetic balance.
437 Apparently, SePM-contaminated fish was unable to recover as fast as control fish.
438 Therefore, these casualties reinforce the apparent severity of this type of pollution and
439 suggest that if such limitations are not compensated in the long term, SePM will impact
440 a fish's overall use of energy. Consequently, it may have effects on development, growth,
441 reproduction, post-prandial metabolic increment, and any other metabolically demanding
442 activities (McKenzie et al., 2007; Killen et al., 2010; Plaut, 2001; Priede, 1985; Rice,
443 1990) with potential consequent reductions in the overall fitness of the fish population
444 and ecological impact.

445

446 *Final Conclusions*

447 An environmentally relevant concentration level of SePM significantly limited the
448 swimming performance of *O. niloticus* and caused significant reductions in metabolic

449 indexes, SMR, MMR, Emax, and AS. Although exposure to environmentally relevant
450 levels of SePM did not lead to immediate death, it did induce significant limitations on
451 physiological performance. Such a combination between swimming and metabolic
452 limitations may affect activities that support fish population dynamics. SePM has the
453 potential to affect multiple levels of fish performance, and therefore, it is still a rather
454 unexplored toxic component that may lead to a growing and silent threat to aquatic biota.
455 It does require further study to understand toxicity thresholds, the mechanisms of toxicity
456 to several organisms, and forms of risk assessment. Future work should also address the
457 effect of long-term contamination by SePM over a range of natural physicochemical
458 variations such as salinity and temperature.

459

460 **Acknowledgments**

461 The authors thank Piscicultura Poletini for donating the fish for the present study,
462 Gustavo Marega Oda (DCF) for his valuable assistance in animal care and experiment
463 performing, the NGO “JUNTOS SOS – ES” for collecting the SePM, and the Brazilian
464 Agricultural Research Corporation (EMBRAPA) for the analysis provided.

465

466 **Financial support**

467 This study was supported by the São Paulo Research Foundation (FAPESP,
468 2019/08491-0) and Agencia Nacional de Promoción Científica y Técnica (FONCYT
469 PICT-2015-2819), Secretaría de Ciencia y Tecnología Universidad Nacional de Córdoba
470 (SECyT-UNC 411/18). I.C. Souza was a Postdoc supported by the São Paulo Research
471 Foundation (FAPESP, 2016/025257-2). C.F. De Angelis, M.P. Soares, and I.L. Cardoso
472 were a doctorate supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível
473 Superior - Brasil (CAPES) - Finance Code 001.

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