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

De Angelis, C. F.<sup>1</sup>; Soares, M. P.<sup>1</sup>; McKenzie, D. J.<sup>2</sup>; Taylor, E. W.<sup>3</sup>; Cardoso, I. L.<sup>1</sup>; Souza, I. C.<sup>1</sup>, Wunderlin<sup>4</sup>, D. A., Monferrán<sup>4</sup>, M. V., Fernandes, M.N.<sup>1</sup>; Leite, C.A.C.<sup>1\*</sup>

<sup>1</sup> Department of Physiological Sciences Department, Federal University of São Carlos, Rod Washington Luis km 235,13565-905 São Carlos, SP, Brazil.

<sup>2</sup> MARBEC, Université de Montpellier, CNRS, Ifremer, IRD, Montpellier 34095, France.

<sup>3</sup> School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

<sup>4</sup> ICYTAC: Instituto de Ciencia y Tecnología de Alimentos Córdoba, CONICET and Departamento de Química Orgánica, Universidad Nacional de Córdoba, Bv. Medina Allende s/n, Ciudad Universitaria, 5000- Córdoba, Argentina.

\*Corresponding author: [cleo.leite@ufscar.br](mailto:cleo.leite@ufscar.br)

Department of Physiological Sciences Department, Federal University of São Carlos, Rod Washington Luis km 235,13565-905 São Carlos, SP, Brazil.

Co-authors e-mail address:

Carolina F. De Angelis: [caroldeangelis3@hotmail.com](mailto:caroldeangelis3@hotmail.com)

Michelly P. Soares: [michelly\\_psoares@hotmail.com](mailto:michelly_psoares@hotmail.com)

Israel L. Cardoso: [israelcardoso2008@hotmail.com](mailto:israelcardoso2008@hotmail.com)

Edwin W. Taylor: [tedwtaylor@hotmail.com](mailto:tedwtaylor@hotmail.com)

David J. McKenzie: [david.mckenzie@cnrs.fr](mailto:david.mckenzie@cnrs.fr)

Iara da C. Souza: [iara@ufscar.br](mailto:iara@ufscar.br)

Daniel A. Wunderlin: [danielwunderlin@gmail.com](mailto:danielwunderlin@gmail.com)

Magdalena V. Monferrán: [mmonferran@unc.edu.ar](mailto:mmonferran@unc.edu.ar)

Marisa N. Fernandes: [dmnf@ufscar.br](mailto:dmnf@ufscar.br)

Cleo A. C. Leite: [cleo.leite@ufscar.br](mailto:cleo.leite@ufscar.br)

## Abstract

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

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

## 1. Introduction

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

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

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

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

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

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

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

## **2. Materials and Methods**

### *Settleable Atmospheric Particulate Matter - SePM*

SePM collection and analysis methods have been described previously (Soares et al., 2022). It is composed of a complex mixture of particles, especially emerging metal compounds with unpredictable new combinations of metals and metal nanoparticles; its composition varies in shape and size. (Nordberg et al., 2007; Salgado, 2003; Soriano et al., 2012). The SePM used in this experiment was collected at Ilha do Boi (20°18'32"S, 40°16'33"O), near the industrial complex of Tubarão in Vitória City (5-8 g/m<sup>2</sup> of SePM each month over an area of 5,000 m<sup>2</sup>, Souza et al., 2021a). This location has an intensive influence of SePM emitted by this industrial complex, which is integrated by an iron pelleting industry and an iron and steel exportation harbor (Santos et al., 2017). The SePM presence in the water environment may be related to input from air and water run-off from hard-standing areas (McLusky and Elliott, 2004). Recently, isotopic analyses

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

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

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

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

#### *Fish supply and acclimation*

Juveniles of *O. niloticus* were provided by the Poletini fish farm (Mogi-Mirim – SP, Brazil) and transported to the Department of Physiological Sciences at UFSCar (São Carlos – SP), where they were acclimated in 1,000 L holding tanks during 30 d. Each tank had a continuous supply of dechlorinated, well-aerated water ( $25 \pm 1^\circ\text{C}$ ; DO 6.0–7.3 mg.L<sup>-1</sup>; hardness 48–53 mg.L<sup>-1</sup>; pH 6.7–7.3, (as CaCO<sub>3</sub>); alkalinity 40–43 mg.L<sup>-1</sup> as CaCO<sub>3</sub>; chloride 42 mg.L<sup>-1</sup>; ammonium 0.1 mg.L<sup>-1</sup>; and conductivity 110 µS.cm<sup>-1</sup>). Fish remained under natural photoperiod (~12 h:12 h) and were fed *ad libitum* with specific commercial pellets: Ø 5-8 mm, 6 % fat, 32 % protein and 12 % moisture (Supra<sup>®</sup> Acqua line) during acclimation period. Food supply was withdrawn 24h before contamination protocol and experiments. The UFSCar Committee of Ethics in Animal Experimentation (CEUA-UFSCar) analyzed and approved all procedures.

#### *Experimental Design*

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

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

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

#### *Swimming Performance*

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

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

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

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

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

#### *Swimming respirometry*

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

$$(2) \dot{M}O_2 = [\beta O_2 \cdot V_{RE} \cdot W_0^{-1} \cdot (\frac{\delta pO_2}{\delta t})]$$

where  $\beta O_2$  denotes the coefficient for  $O_2$  solubility in water ( $mg$  of  $O_2 \cdot L^{-1}$ );  $V_{RE}$ , the chamber volume (L);  $W_0^{-1}$ , fish body mass (Kg); and  $(\frac{\delta pO_2}{\delta t})$  indicates the  $O_2$  reduction over time ( $\%$  of  $O_2 \cdot h^{-1}$ ). After each swimming protocol, blank tests were performed to

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

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

$$(3) AS = MMR - SMR$$

which estimates the maximum capacity to supply oxygen to sustain metabolic activities beyond SMR, activities such as digestion, locomotion, growth, reproduction, and so on (Fry, 1971). We also assessed the net metabolic cost of swimming ( $COS - m\text{Mol}O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) at a sustained speed of  $2 \text{ BL} \cdot \text{s}^{-1}$  as:

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

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

The swimming efficiency ( $E_{\text{swim}} - \mu\text{mol}O_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$ ) was evaluated as described by McKenzie et al. (2017):

$$(5) E_{\text{swim}} = COS \cdot 2^{-1} (\text{speed of } 2 \text{ BL} \cdot \text{s}^{-1})$$

Finally, we analyzed the maximum energetic efficiency of swimming performance ( $E_{\text{max}} - \mu\text{mol}O_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$ ), a variable of significant ecological relevance (Ohlberger et al., 2006), was calculated based on Kunz et al. (2018):

$$(6) E_{\text{max}} = MMR \cdot U_{\text{crit}}^{-1}$$

### Statistical analyses

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

## 3. Results

### *Settleable Atmospheric Particulate Matter*

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

### *SePM affected swimming performance and aerobic metabolic rates*

The Control group had a mean Ucrit of  $5.68 \pm 0.21$  BL  $\cdot$  s<sup>-1</sup> at 25°C. This swimming performance was associated with maintenance of significant respiratory support for activity (Figure 1). SMR was  $4.49 \pm 0.32$  mMolO<sub>2</sub>  $\cdot$  kg<sup>-1</sup>  $\cdot$  h<sup>-1</sup> in the Control group. Activity increased metabolic rate so that MMR was 3.2 times higher at  $14.58 \pm$

0.82 mMolO<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup>, such that Control AS was 10.09 ± 0.61 mMolO<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup> (Figure 2). The Control group had COS at 3.21 ± 0.51 mMolO<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup>, Eswim of 1.58 ± 0.25 μmolO<sub>2</sub> · Kg<sup>-1</sup> · BL<sup>-1</sup>, while Emax was 2.75 ± 0.12 μmolO<sub>2</sub> · Kg<sup>-1</sup> · BL<sup>-1</sup> (Figure 3).

The SePM group showed impaired performance with a Ucrit of 4.15 ± 0.26 BL · s<sup>-1</sup>, 27% lower than the Control fish (p < 0.001 - Figure 1). This reduction in performance was associated with effects of SePM on respiratory metabolism. SePM contamination reduced SMR by 26 % (3.32 ± 0.17 mMolO<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup>, p = 0.001 - Figure 2A) and MMR by 27 % (10.51 ± 0.78 mMolO<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup>, p = 0.002 - Figure 2B) when compared to the Control group. Additionally, SePM contamination decreased AS by 29 % compared to the control group (7.19 ± 0.77 mMolO<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup>, p = 0.003 - Figure 2C).

SePM did not change the energetics of sustained swimming at low velocity, with values of COS of 3.72 ± 0.31 mMolO<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup> (p = 0.333 - Figure 3A) and Eswim of 2.09 ± 0.23 μmolO<sub>2</sub> · Kg<sup>-1</sup> · BL<sup>-1</sup> (p = 0.102 - Figure 3B) that were similar to the Control group. However, SePM contamination did alter Emax, which was 11% lower in the SePM group compared to the Control, at 2.46 ± 0.07 μmolO<sub>2</sub> · Kg<sup>-1</sup> · BL<sup>-1</sup> (p = 0.011 - Figure 3C).

### *Second swimming trial*

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

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

## **Discussion**

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

### *SePM and general metal toxicology*

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

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

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

#### *SePM exposure limits swimming performance*

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

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

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

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

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

#### *SePM exposure affected aerobic metabolism*

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

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

Control tilapia had a metabolic profile very similar to previous reports for the species. For instance, using a similar swimming technique at the same temperature, McKenzie et al. (2017) reported similar mean values for SMR, MMR, and AS (respectively,  $4.1 \pm 0.5$ ,  $16.8 \pm 1.3$ ,  $12.6 \pm 0.1 \text{ mMolO}_2 \cdot \text{Kg}^{-1} \cdot \text{h}^{-1}$ ) in the same strain of Nile tilapia.

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

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

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

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

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

Furthermore, the capacity to support increased energy demand levels is also supposed to impact recovery after exhausting activity. Part of the SePM group (30%) died during the additional recovery period after the first swimming trial. This may be related to SePM negative effects previously reported; SePM caused significant damage to the gill structure, aerobic capacity, oxygen-carrying capacity of the blood, and energy allocation in Nile tilapia (De Angelis et al., 2022; Soares et al., 2022), impairing O<sub>2</sub> uptake from the water, the metabolism and swimming performance as observed in the present experiment. MMR can be associated with intrinsic and extrinsic factors (Norin and Clark, 2016). Thus, the maximum swimming activity may cause a transitory debilitation in every vertebrate since it depletes cellular energy reserves and pH alteration due to lactic acid produced, and others. It also causes ionic unbalance in fish associated with the increased ventilatory effort. Post-activity recovery might cause long-term demands for an aerobic effort to sustain the biochemical effort to recover osmolarity, pH, and energetic balance. Apparently, SePM-contaminated fish was unable to recover as fast as control fish. Therefore, these casualties reinforce the apparent severity of this type of pollution and suggest that if such limitations are not compensated in the long term, SePM will impact a fish's overall use of energy. Consequently, it may have effects on development, growth, reproduction, post-prandial metabolic increment, and any other metabolically demanding activities (McKenzie et al., 2007; Killen et al., 2010; Plaut, 2001; Priede, 1985; Rice, 1990) with potential consequent reductions in the overall fitness of the fish population and ecological impact.

#### *Final Conclusions*

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

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

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