



Arsenic bioaccumulation and biotransformation in different tissues of Nile tilapia (*Oreochromis niloticus*): A comparative study between As(III) and As(V) exposure and evaluation of antagonistic effects of selenium

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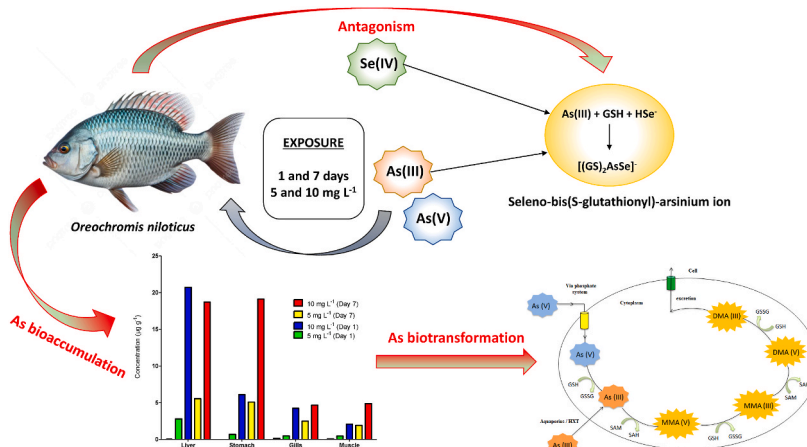
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HIGHLIGHTS

- Bioaccumulation study of inorganic arsenic in tissues of *O. niloticus*.
- Arsenobetaine predominated after As(III) and As(V) exposure.
- Antagonistic effect of selenium in arsenic bioaccumulation.
- Se presence led to a 4-6-fold reduction of arsenic toxicity in Nile tilapia.

GRAPHICAL ABSTRACT



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ABSTRACT

The speciation of arsenic in fish has been widely investigated, but bioaccumulation and biotransformation of inorganic As in different tissues of Nile tilapia (*Oreochromis niloticus*) are not fully understood. The present study

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aimed to investigate the bioaccumulation of As in Nile tilapia, as well as to evaluate the distribution of the main arsenic species (As(III), As(V), MMA, DMA, and AsB) in liver, stomach, gill, and muscle, after controlled exposures to As(III) and As(V) at concentrations of 5.0 and 10.0 mg L⁻¹ during periods of 1 and 7 days. Total As was determined by inductively coupled plasma mass spectroscopy (ICP-MS). For both exposures (As(III) and As(V)), the total As levels after 7-day exposure were highest in the liver and lowest in the muscle. Overall, the Nile tilapia exposed to As(III) showed higher tissue levels of As after the treatments, compared to As(V) exposure. Speciation of arsenic present in the tissues employed liquid chromatography coupled to ICP-MS (LC-ICP-MS), revealing that the biotransformation of As included As(V) reduction to As(III), methylation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), and subsequent conversion to nontoxic arsenobetaine (AsB), which was the predominant arsenic form. Finally, the interactions and antagonistic effects of selenium in the bioaccumulation processes were tested by the combined exposure to As(III), the most toxic species of As, together with tetravalent selenium (Se(IV)). The results indicated a 4–6 times reduction of arsenic toxicity in the tilapia.

1. Introduction

Fish is considered a healthier food than other types of protein, because it provides a variety of vitamins and nutrients, with the added advantage of having a low saturated fat content (Ersoy and Özeren, 2009; Morales and Higuchi, 2018). In addition to its nutritional importance, fish has economic and social roles, since the production chain generates jobs and income for small and large producers, increasing the market for fish in recent years. Tilapia is at the top of the list of the fish species most cultivated and consumed in Brazil. According to the 2023 Yearbook published by the Brazilian Fish Farming Association (Peixe BR), tilapia production in 2022 reached 550,060 tons, representing 63.9% of all Brazilian farmed fish and ranking Brazil in 4th position globally among tilapia producers.

Despite the numerous benefits, tilapias can be exposed to multiple toxic inorganic contaminants present in water, such as arsenic (As), triggering processes of bioaccumulation and biotransformation in aquatic species through the food chain (Azizur Rahman et al., 2012; Chiarelli and Roccheri, 2014). Determination of the total concentration of an element is not enough to provide information about its toxicity, bioavailability, and biochemical function, requiring speciation studies to complement the information. The determination of arsenic in fish is of great analytical interest due to its toxicity, since the toxic effects of arsenic are related to its chemical forms and oxidation states (Martinez et al., 2011; Chen et al., 2020).

Nile tilapia (*Oreochromis niloticus*) has been used as an excellent biological model for toxicological studies of aquatic contamination, as it can accumulate high concentrations of As in its tissues, transferring the metalloid to different trophic levels (Cunningham et al., 2019; Liao et al., 2003; Taweel et al., 2013; Oliveira et al., 2017; Ferreira et al., 2019).

Arsenic is a toxic trace metalloid whose presence in the aquatic environment can be due to its release from natural sources, as a result of soil and rock erosion, or volcanic activity, as well as from anthropogenic activities, such as the combustion of fossil fuels, mining activities, and industrial processes (Fowler et al., 2007; Greani et al., 2017). The toxicity and mobility of As largely depend on the chemical species present. The inorganic forms (iAs), represented by the trivalent (As(III)) and pentavalent (As(V)) species, are considered more toxic than the methylated organic species (monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine (AsB)) that can be produced by physiological processes. The main source of arsenic in the diet is AsB, which is nontoxic and is the principal organic species found in fish, since it is the final product of arsenic metabolism in the aquatic food chain (Barra et al., 2000; Zhang et al., 2022).

Both inorganic and methylated species of As inhibit cellular respiration, leading to the formation of reactive oxygen species (ROS) that cause oxidative stress, which is one of the main hypotheses suggested for the carcinogenicity of As (Ventura-Lima et al., 2011). According to the International Agency for Research on Cancer (IARC), As is a highly toxic and carcinogenic element for humans. However, the effects observed in animal models vary, making it challenging to elucidate the precise

mechanisms by which the toxic effects occur and the level of carcinogenicity (Martinez et al., 2011). Arsenic can undergo several metabolic conversions *in vivo*, with its metabolites interacting with intra- and extracellular macromolecules (Molin et al., 2015). Studies have demonstrated that mechanisms such as enzymatic inhibition, endocrine system disruption, altered DNA repair, cell oxidative stress, and epigenetic modifications in DNA may be related to As toxicity (Yosim et al., 2015; Roy et al., 2020).

However, some chemical elements can reduce the toxic effects caused by As, acting in an antagonistic way, with selenium (Se) being one of these elements. Selenium has great physiological and ecotoxicological importance, as it is an essential micronutrient for most organisms, although it can become toxic at high concentrations (Bodnar et al., 2012; English et al., 2022). It participates in and regulates several biological and biochemical functions, such as protecting membranes against oxidative damage, and is present in the active sites of selenoproteins that have antioxidant functions, such as glutathione peroxidase (GPx) (Toppo et al., 2009; Sharma et al., 2017).

The present study aimed to investigate the bioaccumulation and biotransformation of inorganic arsenic in different tissues of Nile tilapia following As(III) and As(V) exposure. The evaluation was made considering the distributions of the arsenic species As(III), As(V), MMA, DMA, and AsB in liver, stomach, gill, and muscle tissues after controlled exposures of the fish to As(III) and As(V) at different concentrations (5.0 and 10 mg L⁻¹), during periods of 1 and 7 days. Furthermore, another goal was to evaluate the antagonistic effect of selenium on the toxicity of As(III) in Nile tilapia during the bioaccumulation process.

2. Materials and methods

2.1. Test organisms

Juvenile tilapia (*Oreochromis niloticus*), free from any visible deformities, lesions, or diseases, were obtained from a stocked pond at São Paulo State University (UNESP), located in São José do Rio Preto, São Paulo State, Brazil. The fish (n = 75) were distributed in 500-L tanks kept under constant aeration, at controlled temperature (27 ± 2 °C), and were fed artificial diets (purchased from a feed company in São José do Rio Preto) once a day at 2% of their body weight, until the beginning of the assays. Feces and uneaten food were removed once a day. They were acclimated with a 12-h light-dark cycle for 21 days prior to the exposure assays. All experimental procedures involving animals were conducted following the guidelines of the National Institutes of Health (NIH), after approval by the Ethics Committee on the Use of Animals (CEUA) of São Paulo State University (protocol number 204/2019).

2.2. Experimental design

The experimental design was based on the previously published papers (Liao et al. (2003), Tsai and Liao (2006), Chen et al. (2018), and Ferreira et al. (2019)). The assays were conducted as prescribed by test guideline n° 203 published by the Organization for Economic

Co-operation and Development (Organization for Economic Co-operation and Development OECD., 1992). In the *in situ* assays, As(III), As(V), and Se(IV) stock solutions (as sodium arsenite (NaAsO_2), sodium arsenate (Na_2HAsO_4), and sodium selenite (Na_2SeO_3), respectively, 1000 mg L^{-1} , Sigma-Aldrich, St Louis, MO, USA) were spiked into plastic aquariums (containing 10 L of tap water), at constant nominal dissolved As concentrations of 5 and 10 mg L^{-1} for As(III) and As(V), and 1 mg L^{-1} for Se (IV). The assays were carried out statically, with no renewal of the test solution during the exposure period.

2.2.1. Controlled arsenic assays

The controlled arsenic assays were performed with exposures of 1 and 7 days, using two different concentrations to evaluate the acute toxicity, separately for As(III) and As(V). The As(III) assays employed tilapia aged between 2 and 3 months, with $16.3 \pm 0.03 \text{ cm}$ body length and $120.2 \pm 13.7 \text{ g}$ wet weight. The tilapia used for the As(V) assays had a body length of $16.4 \pm 0.6 \text{ cm}$ and wet weight of $118.3 \pm 18.7 \text{ g}$. The fish were divided into 5 groups: control, 5 mg L^{-1} (1 day), 10 mg L^{-1} (1 day), 5 mg L^{-1} (7 days), and 10 mg L^{-1} (7 days). The As concentrations were 20–50 times higher than those found under field conditions, to produce high As levels in the target tissues. Previous work by our research group showed that for As(III), the LC_{50} values indicated fish mortality at concentrations above 30 mg L^{-1} . The fish showed greater tolerance to exposure to As(V), compared to As(III), with fish mortality after the second day of exposure requiring an As(V) concentration 7-fold higher than for As(III). Hence, the concentrations employed in these controlled assays were below the LC_{50} value (Ferreira et al., 2019).

Water samples were collected from each aquarium at the beginning of the assays, to determine whether the As concentrations were in accordance with the nominal values. Each group was composed of 5 fish exposed individually to the treatment in 10-L plastic aquariums (5 real replicates for each experimental group). After 1 and 7 days of exposure, individuals from each group were collected and anesthetized with benzocaine (28 mg L^{-1} in the water), for removal of the liver, stomach, gill, and muscle tissues. The samples were stored in polypropylene flasks at -80°C to maintain their integrity and avoid the interconversion of As species before subsequent analyses of total As and As speciation.

2.2.2. Antagonism assay

The antagonism assay was performed using As(III) and Se(IV) at predefined concentrations (below the LC_{50} of Se) to evaluate the protective effect of selenium against arsenic toxicity. The assay employed tilapia aged between 2 and 3 months, with $16.7 \pm 0.05 \text{ cm}$ body length and $128.4 \pm 18.2 \text{ g}$ wet weight. The fish were divided into 5 groups: control, 5 mg L^{-1} (1 day), 10 mg L^{-1} (1 day), 5 mg L^{-1} (7 days), and 10 mg L^{-1} (7 days). These groups (except the control group) received a dose of selenium at a concentration of 1 mg L^{-1} . To evaluate the concentrations of As and Se, small aliquots of water were collected from the aquariums at the beginning of the assays. Each group comprised 5 fish exposed individually to the treatments in 10-L plastic aquariums ($n = 5$). After 1 and 7 days of exposure, individuals from each group were collected and anesthetized with benzocaine (28 mg L^{-1} in the water), before removal of the liver, stomach, gill, and muscle tissues. These samples were stored in polypropylene flasks, at -80°C , until the sample preparation step was performed according to the method proposed by Oliveira et al. (2017), followed by total As and Se determination.

2.3. Total arsenic and selenium determinations

The tissue samples from each treatment were freeze-dried and homogenized (as a pool of samples) for total As, selenium, and arsenic speciation analyses. The total As contents were determined following previously validated procedures (Oliveira et al., 2017). About 0.10 g portions of the freeze-dried samples (L101 freeze-dryer, Liobras, São Carlos, Brazil) were weighed out and digested with a mixture of 6 mL of 7 mol L^{-1} sub-distilled (subCLEAN Sub-Boiling Distillation System,

Milestone, Sorisole, Italy) HNO_3 (Sigma-Aldrich, St Louis, MO, USA) and 2 mL of $30\% \text{ v v}^{-1}$ H_2O_2 (Sigma-Aldrich, St Louis, MO, USA), using a microwave digestion system (Multiwave GO, Anton Paar GmbH, Graz, Austria). The digestion procedure consisted of heating to 190°C during 20 min, followed by maintaining at 190°C for 40 min. After cooling, the samples were diluted to 25 mL with ultrapure water ($18.2 \text{ M}\Omega \text{ cm}$, Direct-Q 3, Millipore SAS, France). Blank samples were processed using a similar procedure. The artificial diets were also digested and their As and Se concentrations were simultaneously measured.

The total As and selenium contents were determined by inductively coupled plasma mass spectrometry (ICP-MS) (NexION 300X, PerkinElmer, Shelton, CT, USA). The nebulizer gas flow rate, torch alignment, and quadrupole voltages were adjusted according to the manufacturer's recommendations. The instrumental parameters were optimized before the analyses. The ICP-MS conditions are provided in Table 1. A calibration curve was prepared by serial dilution from a stock solution containing 1000 mg L^{-1} As and Se (TraceCERT® Arsenic Standard for ICP, TraceCERT® Selenium Standard for ICP, Sigma-Aldrich, St Louis, MO, USA) in $1\% \text{ (v v}^{-1})$ HNO_3 (Sigma-Aldrich, St Louis, MO, USA). Total selenium concentrations were determined using a mass/charge ratio (m/z) of 82, in kinetic energy discrimination (KED) mode, with helium (He) as collision gas at a flow rate of 1.5 mL min^{-1} . It was necessary to use the collision cell due to the interference caused by the argon gas dimer with the same m/z ratio as the most abundant isotope of selenium (m/z 80). The accuracy of the method was evaluated by analyzing certified reference materials (CRMs) of fish protein (DORM-3) and dogfish liver (DOLT-5), purchased from the National Research Council of Canada (Ottawa, Ontario, Canada). The data were obtained in triplicate and the As and Se concentrations in the tissues were expressed as $\mu\text{g g}^{-1}$ dry weight.

2.4. Arsenic speciation analysis

The arsenic species in the fish samples were detected using a liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS) system (LC 1200 series and ICP-MS 7800, Agilent Technologies, Tokyo, Japan) equipped with an anion exchange column (G3288-80000, Agilent Technologies, Tokyo, Japan). Integrated method setup and sequence control of the combined LC-ICP-MS system employed the ICP-MS MassHunter (MH) software package (Agilent Technologies, Tokyo, Japan). Briefly, a 5 mL volume of 0.03 mol L^{-1} HNO_3 was added to 0.1 g of the sample, followed by vortexing the mixture for 30 s and heating on a digester block for 40 min at 90°C , according to the method proposed by de Sá et al. (2023). After cooling, the extract was centrifuged at 3200 rpm for 5 min, and 1 mL of the supernatant was transferred to another flask. The extracted solutions were diluted 5-fold with the mobile phase ($10 \text{ mM (NH}_4)_2\text{HPO}_4$; $1\% \text{ (v v}^{-1})$ methanol; pH 8.0), followed by filtration through $0.22 \mu\text{m}$ Millipore filters. The mobile phase was prepared daily by dissolving the $(\text{NH}_4)_2\text{HPO}_4$ salt (Synth, Brazil) in water containing $1\% \text{ (v v}^{-1})$ methanol (HPLC grade, Sigma-Aldrich, St Louis, MO, USA), with the pH adjusted using a saturated aqueous solution of

Table 1
Instrumental and method parameters for ICP-MS analysis.

Instrumental and method parameters	
Radiofrequency power	1600 W
Plasma gas flow rate	18 L min^{-1}
Auxiliary gas flow rate	1.2 L min^{-1}
Nebulizer gas flow rate	1.0 mL min^{-1}
Sample uptake rate	0.7 mL min^{-1}
Sweeps/reading	50
Readings/replicate	1
Replicates	3
Dwell time	25 s
Analytical calibration range	$0.1\text{--}30 \mu\text{g L}^{-1}$
Isotopes monitored	^{75}As , ^{82}Se

NH₃ (Synth, Brazil). Calibration solutions of As(III), As(V), AsB, DMA, and MMA (0.1–20 µg L⁻¹) were prepared daily from 1000 mg L⁻¹ stock solutions previously prepared by dissolution (in water) of the salts NaAsO₂, KH₂AsO₄, C₅H₁₁AsO₂, (CH₃)₂AsO₂Na•3H₂O, and CH₃AsO (ONa)₂•6H₂O, respectively. Elution was performed in isocratic mode, with the mobile phase flow rate kept at 1.0 mL min⁻¹. The instrumental operational parameters are presented in Table 2.

The extraction efficiencies and the analytical methods were evaluated by the analysis of certified reference materials (CRMs) of fish protein (DORM-3), dogfish liver (DOLT-5), and lobster hepatopancreas (TORT-3), all purchased from the National Research Council of Canada (Ottawa, Ontario, Canada).

2.5. Statistical analyses

Statistical analysis was performed using groups with at least three biological replicates, employing Statistica v. 9.0 software (Statsoft Inc., Tulsa, OK, USA). The data were tested for normality and homogeneity of variances, using the Shapiro-Wilk and Levene tests, respectively. All the comparisons were performed using the nonparametric Kruskal-Wallis test, together with Dunn's *post-hoc* test, employing Statistica v. 8.0 software (Statsoft Inc.). Graphs were obtained with GraphPad Prism v. 5.01 for Windows software (GraphPad Software, La Jolla, CA, USA). In the statistical analysis, the results were expressed as mean ± SEM, with statistical significance considered for $p < 0.05$.

3. Results and discussion

3.1. Arsenic bioaccumulation

The total arsenic concentrations in different tissues of *O. niloticus* after the As(III) and As(V) exposures are presented in Table 3. The descriptive analysis clearly showed an increasing pattern of As bioaccumulation in the groups exposed to As(III) and As(V) at 5 and 10 mg L⁻¹, compared to the control group. For the groups exposed to As(III), the As concentrations were in the ranges 2.80–20.7 µg g⁻¹ in the liver, 0.69–19.1 µg g⁻¹ in the stomach, 0.15–4.66 µg g⁻¹ in the gills, and 0.08–4.88 µg g⁻¹ in muscle. For the *O. niloticus* groups exposed to As(V), the As concentrations were in the ranges 0.09–15.7 µg g⁻¹ in the liver, 0.10–6.48 µg g⁻¹ in the stomach, 0.13–3.55 µg g⁻¹ in the gills, and 0.27–2.28 µg g⁻¹ in muscle. The concentration of As in the artificial diets was below the limit of quantification for determination by ICP-MS (2.2 × 10⁻⁴ mg L⁻¹, the diet used during the acclimatization period did not influence the accumulation of As in the tissues studied).

Monitoring and determination of As concentrations were carried out by collecting small aliquots of water from the aquariums at the beginning of the assay, which were diluted for analysis by ICP-MS. In the assay carried out with As(III), the average concentrations in water were 5.09 ± 0.19 mg L⁻¹ and 10.1 ± 0.16 mg L⁻¹, while in the assay carried out

Table 2
Instrumental parameters for As speciation by LC-ICP-MS.

As speciation analysis (LC)	
Mobile phase	10 mM (NH ₄) ₂ HPO ₄ + 1% (v v ⁻¹) methanol; pH 8.0
Mobile phase flow rate	1.0 mL min ⁻¹
Elution mode	Isocratic
Injection volume	50 µL
Analysis time	720 s
ICP-MS	
Radiofrequency power	1550 W
Sampling depth	8 mm
Nebulizer gas flow rate	1.1 L min ⁻¹
He gas flow rate	4.5 mL min ⁻¹
Nebulizer	Micromist
Spray chamber	Scott-type, double pass
Isotope monitored	⁷⁵ As

Table 3

Total arsenic concentrations (µg g⁻¹) in different tissues of *O. niloticus* after exposure to As(III) and As(V) (n = 3; mean ± SD).

As bioaccumulation in fish tissue (µg g ⁻¹)				
As(III) exposure				
Treatment	Liver	Stomach	Gills	Muscle
Control	0.04 ± 0.002	< LOQ	0.15 ± 0.01	0.08 ± 0.001
5 mg L ⁻¹ (D1)	2.80 ± 0.14	0.69 ± 0.005	0.47 ± 0.01	0.46 ± 0.01
10 mg L ⁻¹ (D1)	20.7 ± 0.14	6.10 ± 0.10	4.23 ± 0.001	2.08 ± 0.01
5 mg L ⁻¹ (D7)	5.55 ± 0.03	5.09 ± 0.04	2.49 ± 0.01	1.91 ± 0.01
10 mg L ⁻¹ (D7)	18.7 ± 0.15	19.1 ± 0.17	4.66 ± 0.12	4.88 ± 0.03
As(V) exposure				
Treatment	Liver	Stomach	Gills	Muscle
Control	0.09 ± 0.01	0.10 ± 0.01	0.13 ± 0.004	0.27 ± 0.01
5 mg L ⁻¹ (D1)	1.27 ± 0.06	1.19 ± 0.10	0.61 ± 0.01	0.52 ± 0.01
10 mg L ⁻¹ (D1)	7.15 ± 0.56	6.48 ± 0.09	3.55 ± 0.16	1.74 ± 0.02
5 mg L ⁻¹ (D7)	15.7 ± 1.08	4.53 ± 0.17	1.47 ± 0.49	1.55 ± 0.06
10 mg L ⁻¹ (D7)	8.66 ± 0.66	4.32 ± 0.24	3.52 ± 0.02	2.28 ± 0.02

D1: 1 day of exposure. D7: 7 days of exposure.

with As(V), the average concentrations were 5.19 ± 0.12 mg L⁻¹ and 10.2 ± 0.15 mg L⁻¹. For both assays, the concentration in the control group water was below the limit of quantification for determination by ICP-MS (2.2 × 10⁻⁴ mg L⁻¹).

After 1 day (D1), there was a significantly higher concentration of As (III) in the liver after exposure to As(III) at 10 mg L⁻¹, compared to exposure to 5 mg L⁻¹ (Fig. 1b). Although not statistically significant, the same pattern was observed for all experimental situations. The groups exposed to As(III) at 10 mg L⁻¹ presented the highest levels of bioaccumulation for all tissues (Fig. 1). The lowest and highest levels of As (III) bioaccumulation for all tissues were observed for 5 mg L⁻¹ on D1 and 10 mg L⁻¹ on D7, respectively, except for the liver, in which the highest concentration was observed for 10 mg L⁻¹ on D1.

As: Arsenic. D1: 1 day of exposure. D7: 7 days of exposure. * Statistically significant difference between the indicated groups. The representative values of the three replicates were expressed as mean with standard error (±SEM). Tests performed: Kruskal-Wallis complemented by Dunn's *post-hoc* test; $p < 0.05$ was statistically significant.

The statistical analysis for As(V) revealed a significant increase on D1 between As(III) at 10 mg L⁻¹ and 5 mg L⁻¹ in the stomach and gills (Fig. 2a and c). The same pattern was observed in the liver and muscle on D1, although it was not statistically significant. Furthermore, similar to As(III), the lowest levels of As(V) bioaccumulation for all tissues were observed for 5 mg L⁻¹ on D1. On the other hand, the highest As concentration varied between the evaluated tissues; for the stomach and gills, the highest values were for 10 mg L⁻¹ on D1 (Fig. 2a and c), whereas for the muscle and liver, the highest values were for 10 mg L⁻¹ on D7 (Fig. 2d) and for 5 mg L⁻¹ on D1 (Fig. 2b), respectively.

In the descriptive analysis, for both the As(III) and As(V) exposure treatments, the As bioaccumulation levels were in the following order: liver > stomach > gills > muscle. This trend was also observed in previous work by the same group (Ferreira et al., 2019). As reported by Tsai et al. (2013), the liver and stomach have the greatest capacity to absorb this contaminant, with these organs acting as sites for long-term storage of As, under conditions of chronic exposure.

The liver is the tissue most recommended for studying pollution of aquatic environments. It presents the highest contaminant concentrations and is the main organ responsible for the metabolization and toxicity of As (Licata et al., 2005). Due to its anatomical and physiological characteristics, such as lipid composition, high metabolic activity, and high amount of blood received, the liver is an organ that can accumulate large amounts of As, suggesting its importance in possible biotransformation processes (Squadrone et al., 2013; Ferreira et al., 2019).

The descriptive results obtained for both bioaccumulation assays

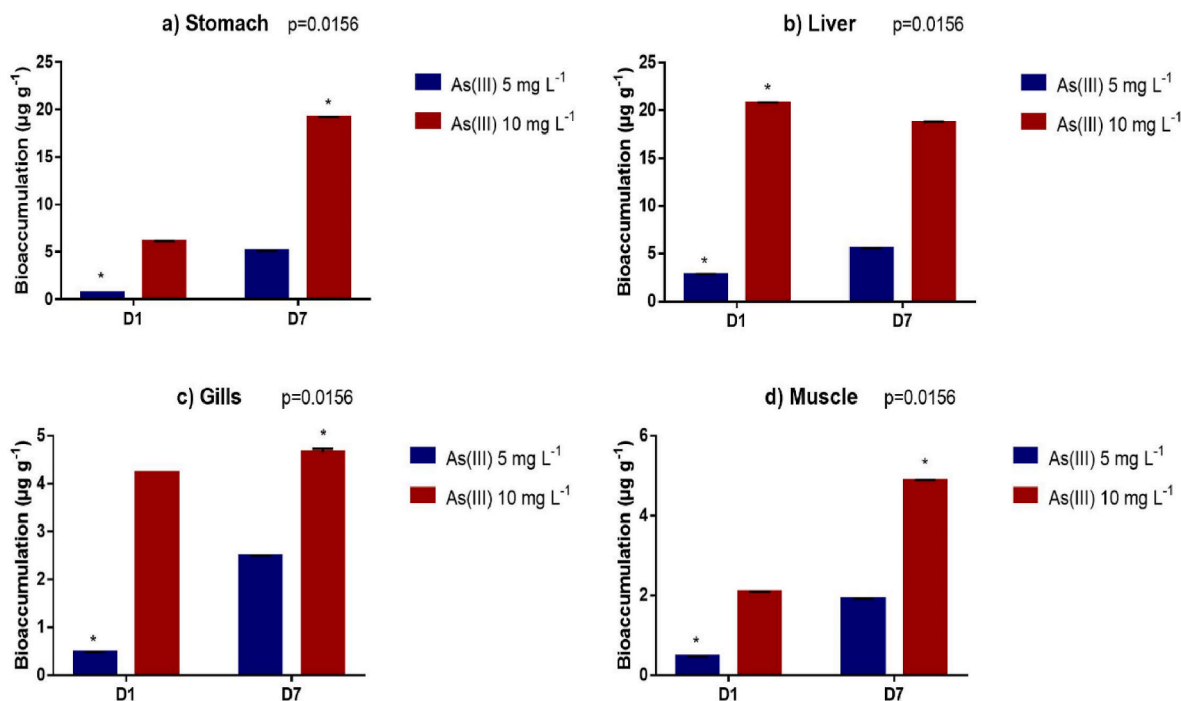


Fig. 1. Arsenic bioaccumulation ($\mu\text{g g}^{-1}$) in different tissues of *O. niloticus* after exposure to As(III).

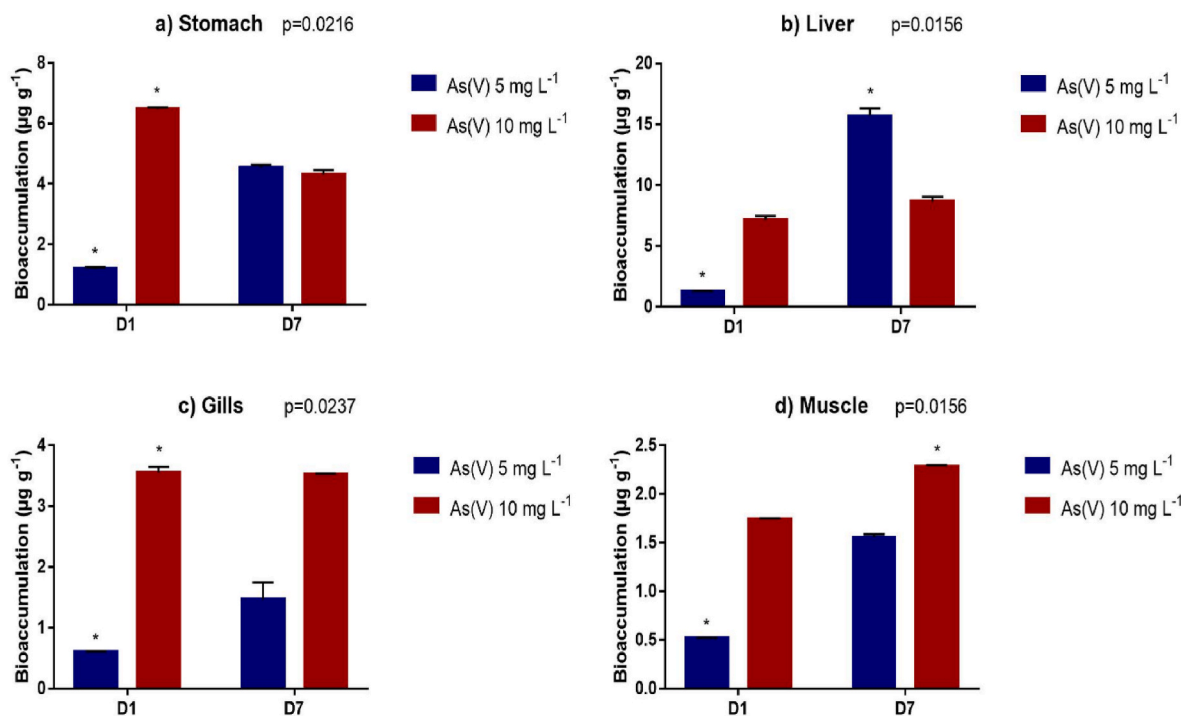


Fig. 2. Arsenic bioaccumulation ($\mu\text{g g}^{-1}$) in different tissues of *O. niloticus* after exposure to As(V). As: Arsenic. D1: 1 day of exposure. D7: 7 days of exposure. * Statistically significant difference between the indicated groups. The representative values of the three replicates were expressed as mean with standard error (\pm SEM). Tests performed: Kruskal-Wallis complemented by Dunn's *post-hoc* test; $p < 0.05$ was statistically significant.

showed that the gill and muscle tissues presented the lowest concentrations of As, in agreement with several studies reported in the literature. In the work by Tsai and Liao (2006), the lowest concentrations of As were also found in the gills and muscle of tilapia (*Oreochromis mossambicus*) exposed to As at a concentration of 1 mg L^{-1} during a period of 7 days. Ferreira et al. (2019) performed bioaccumulation assays using Nile tilapia (*Oreochromis niloticus*), with As(III) and As(V) at fixed

concentrations of 1 mg L^{-1} for 1, 4, and 7 days. In all cases, the lowest As concentrations were also observed in the gills and muscles. Cui et al. (2020) carried out tests using the fish *Carassius auratus*, with dietary exposure to As (at concentrations of 50 and $100 \mu\text{g g}^{-1}$, dry weight basis) during periods of 10 and 20 days, where the lowest concentrations were found in muscle, compared to liver, kidney, spleen, and intestine tissues. Schleichtriem et al. (2012) observed a significant positive correlation

between the accumulation of contaminants and the lipid content of the tissues. Therefore, the fat content of the liver could explain the higher concentration of As, compared to muscle tissue, where the low fat content results in lower bioaccumulation of As.

The bioaccumulation of As in the different *O. niloticus* tissues was greater when the fish were exposed to As(III), compared to As(V), which could be attributed to the different absorption processes of As(III) and As(V). The results suggested that As(III) permeated more easily through the epithelium and/or was more readily metabolized in the tissues, compared to As(V), when the fish were exposed to high concentrations of As. Similar findings were reported by Zhang et al. (2015), where As(III) was more bioavailable than As(V) in Bombay oyster (*Saccostrea cucullata*) after waterborne exposures.

3.2. Arsenic speciation

Certified reference materials were used to confirm the accuracy of the proposed methods for sample preparation and analysis. It is important to mention that the speciation of As in solid matrices is a challenge, since the integrity of the species must be maintained at all stages of the analysis, so the choice of extraction method is one of the most important steps in the speciation analysis. The stability of As species in solution after sample preparation must be ensured to avoid under- or overestimation of target species (Batista et al., 2012; Schmidt et al., 2018).

Fig. 3 shows a chromatogram of the five As species standards, including AsB, As(III), DMA, MMA, and As(V), obtained using the LC-ICP-MS method. The LC and ICP-MS operating conditions enabled the different As species to be satisfactorily separated in a run time of 12 min. The results of speciation analysis using the CRMs are presented and compared with certified values in Table 4.

The accuracy of the analysis method was evaluated by analysis of the CRMs (DOLT-5, DORM-4, and TORT-3). A mass balance of As was performed by comparing the sum of the mass fractions of the extracted species with the total As mass fractions determined in the extracts by ICP-MS. The results (Table 4) were consistent with the certified As values, for a confidence interval of 95%. The quantitation limits for AsB, As(III), DMA, MMA, and As(V) were 14.6, 76.4, 98.4, 68.5, and 23.7 ng g⁻¹, respectively. Chromatograms for all the CRMs and a real sample after extraction are shown in Fig. 4.

The chromatograms (Fig. 4) obtained for speciation analysis of arsenic in the CRM samples revealed the predominance of the AsB species in DOLT-5, DORM-4, and TORT-3. The presence of DMA was

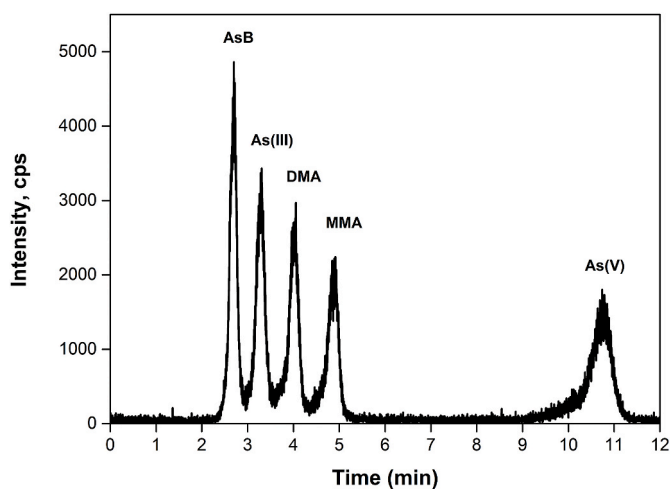


Fig. 3. Chromatographic separation of As species using 10 mmol L⁻¹ (NH₄)₂HPO₄ diluted in 1% (v v⁻¹) methanol as mobile phase in the LC-ICP-MS method. AsB: arsenobetaine. As(III): arsenite. DMA: dimethylarsinic acid. MMA: monomethylarsonic acid. As(V): arsenate.

observed, while As(III), MMA, and As(V) were below the limits of quantification (LOQ). The mean concentration values obtained using the CRMs showed that the sample preparation method was acceptable and could be used for the analysis of real samples, as shown in Fig. 4d. Extraction using diluted HNO₃ (0.03 mol L⁻¹) at 90 °C for 40 min provided average recovery values of between 89 and 94% from the CRMs, so the method was employed for preparation of the biological tissue samples.

The use of dilute acid solutions for the extraction of As species has been reported in previous works, where good extraction efficiency was observed, without compromising the stability of the species. Goessler and Pavkov (2003) studied the chemical stability of As species when exposed to nitric acid, high temperatures, and different pressures. It was found that dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), and the tetramethylarsonium ion (TETRA) were all stable at 200 °C; methylarsonic acid (MA) began to degrade into arsenate (As(V)) at around 140 °C; arsenobetaine (AsB) was stable at around 100 °C; and arsenocholine (AsC) began to degrade into TMAO and TETRA at lower temperatures, with 60% of AsC not degraded at 100 °C. Hence, when using relatively aggressive extractants such as nitric acid, the major forms of As, with the exception of As(III), which would be expected to oxidize to As(V), will not change during extractions using acid conditions and relatively low temperatures (<100 °C). Foster et al. (2007) carried out quantitative extraction of As from plant and marine animal tissues using different methods (water, orthophosphoric acid, methanol-water, and dilute nitric acid), where the best recoveries were obtained using 2% (v/v) HNO₃ and heating in a microwave oven at 95 °C for 6 min. Schmidt et al. (2018) proposed a simple method using 0.03 mol L⁻¹ HNO₃ solution at 100 °C for 30 min in a heating block for the extraction of As species from seafood samples, with recoveries from CRMs of between 94 and 103% and good accuracy for AsB, As(III), DMA, MMA, and As(V).

Therefore, the results described above demonstrated that the extraction and LC-ICP-MS methodology developed in this study has broad applicability, excellent accuracy, and resilience for complex sample matrices, making it suitable for accurately determining arsenic species in fish used for human consumption.

3.3. Arsenic biotransformation

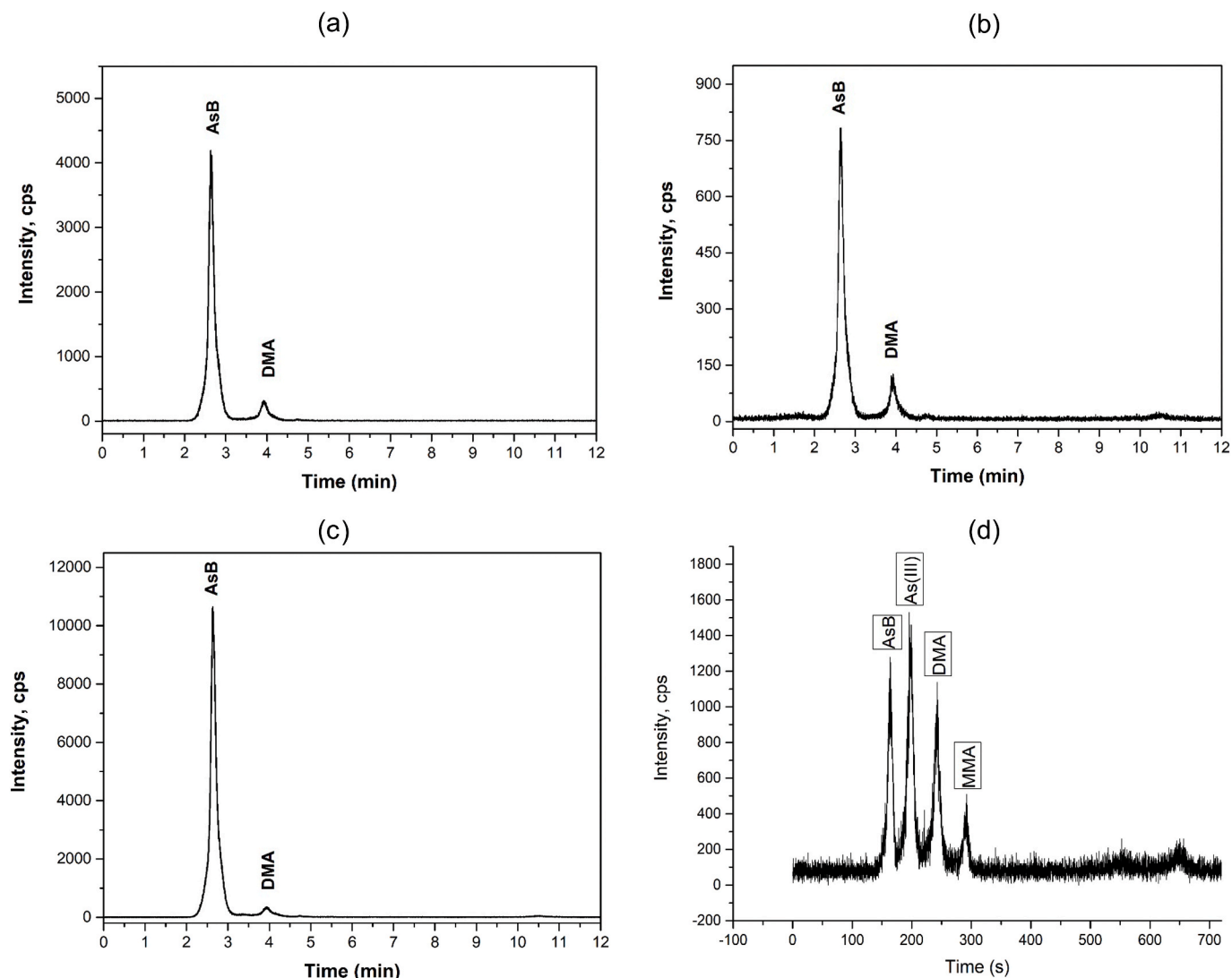
Biotransformation is a process whereby a substance within an organism undergoes chemical reactions, usually mediated by enzymes, which convert it into a compound different from the original one and with lower toxicity. Arsenic can be biotransformed in three ways: (1) redox transformation between As(III) and As(V); (2) reduction of As(V) to As(III); and (3) methylation of As, with formation of the metabolites monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), followed by subsequent conversion to arsenobetaine (AsB) (Kumari et al., 2017). The present study provides some new insights into the biotransformation of As in different tilapia tissues. Tables 5 and 6 show the mass fractions of the five As species in the tissues of the fish exposed to As(III) and As(V), respectively.

After exposure of the fish to As(III) (Table 5), the presence of As(V) was detected in all the tissues, indicating that As(III) could be oxidized to As(V) in Nile tilapia. Oxidation of As(III) to As(V) has been observed previously in microorganisms, but rarely in fish. Rahman and Hassler (2014) reported that mechanisms of As(III) oxidation by microorganisms include chemolithoautotrophic metabolism, where As(III) is used as an energy source, and extracellular oxidation of As(III) mediated by arsenite oxidase. However, the mechanisms of As(III) oxidation in fish are unclear and need further exploration.

When the fish were exposed to As(V) (Table 6), the As(III) species was subsequently found in all the tissues, suggesting that the reduction of As(V) to As(III) occurred in the fish body. Due to physicochemical similarities between As(V) and phosphate, As(V) enters cells by means of the phosphate system and is then enzymatically reduced to As(III), with

Table 4Mass fractions ($\mu\text{g g}^{-1}$) of total arsenic and arsenic species in the certified reference materials (DOLT-5, DORM-4, and TORT-3) ($n = 3$; mean \pm SD).

CRM	AsB	As(III)	DMA	MMA	As(V)	\sum As species	Total As	R (%)
DOLT-5	30.03 ± 2.52	< LOQ	0.03 ± 0.02	< LOQ	< LOQ	30.06 ± 2.54	32.02 ± 2.75	94
DORM-4	5.63 ± 0.43	< LOQ	0.01 ± 0.01	< LOQ	< LOQ	5.64 ± 0.44	6.34 ± 0.54	89
TORT-3	55.4 ± 1.9	< LOQ	0.05 ± 0.01	< LOQ	< LOQ	55.4 ± 1.9	58.75 ± 3.03	94

Certified values: DOLT-5 = $34.6 \pm 2.4 \mu\text{g g}^{-1}$; DORM-4 = $6.80 \pm 0.64 \mu\text{g g}^{-1}$; TORT-3 = $59.5 \pm 3.8 \mu\text{g g}^{-1}$.**Fig. 4.** Chromatograms for (a) DOLT-5, (b) DORM-4, (c) TORT-3, and (d) real sample. AsB: arsenobetaine. As(III): arsenite. DMA: dimethylarsinic acid. MMA: monomethylarsonic acid. As(V): arsenate.

the concomitant oxidation of glutathione (GSH) to glutathione disulfide (GSSG) in a reaction catalyzed by arsenate reductase (Allevato et al., 2019; Sodhi et al., 2019). The As(V) reduction has been recognized as a detoxification process, because although As(III) is more toxic than As(V), various processes act against the toxicity of As(III) in organisms, including the expulsion of As from cells and its transformation into organic As (Rahman and Hassler, 2014).

The biotransformation and distribution of As in fish can be influenced by the metabolic roles of tissues and the different As assimilation/elimination relationships between them. The liver is considered the most important organ responsible for As biotransformation. It is probably the first site involved in this process, before the As species are translocated to other tissues or are excreted in bile (Cui et al., 2020). Among all the tissues, the largest proportion of methylated As (MMA and DMA) was

found in the liver. Compared to MMA, DMA represented a higher proportion of total As, suggesting that DMA was a more stable form in the tissues.

In the descriptive analysis, the highest fractions of AsB in the liver were observed for the assay using As(III). Comparison of the results obtained using different concentrations (5 and 10 mg L^{-1}), for the same exposure time, revealed a pattern of dose-response effect, with the AsB value increasing 2-fold when the exposure concentration was also doubled. These results suggested that AsB was probably an end product of As biotransformation and could be accumulated in the body instead of being excreted.

After methylation, the body has different strategies for As detoxification. In this study, after exposure to As(III) and As(V) at 5 and 10 mg L^{-1} for 1 and 7 days, AsB was the predominant species in all the tissues

Table 5Arsenic species mass fractions ($\mu\text{g g}^{-1}$) in different tissues after exposure of Nile tilapia to inorganic As(III) (n = 3, mean \pm SD).

	Treatment	Tissue	Arsenic species					Σ As species
			AsB	As(III)	DMA	MMA	As(V)	
As(III) exposure	Control	Liver	0.04 \pm 0.002	< LOQ	< LOQ	< LOQ	< LOQ	0.04 \pm 0.002
		Stomach	0.003 \pm 0.002	0.05 \pm 0.002	< LOQ	< LOQ	< LOQ	0.053 \pm 0.004
		Gill	0.075 \pm 0.0004	< LOQ	< LOQ	< LOQ	< LOQ	0.075 \pm 0.0004
		Muscle	0.07 \pm 0.001	< LOQ	< LOQ	< LOQ	< LOQ	0.07 \pm 0.001
	5 mg L ⁻¹ (D1)	Liver	2.28 \pm 0.12	0.20 \pm 0.03	0.04 \pm 0.02	0.05 \pm 0.003	0.26 \pm 0.01	2.84 \pm 0.18
		Stomach	0.24 \pm 0.01	0.24 \pm 0.01	0.08 \pm 0.01	< LOQ	< LOQ	0.57 \pm 0.03
		Gill	0.21 \pm 0.02	0.09 \pm 0.001	0.01 \pm 0.001	0.03 \pm 0.001	0.12 \pm 0.0001	0.46 \pm 0.02
		Muscle	0.25 \pm 0.002	0.11 \pm 0.02	0.08 \pm 0.02	< LOQ	< LOQ	0.44 \pm 0.04
	10 mg L ⁻¹ (D1)	Liver	5.36 \pm 0.08	2.41 \pm 0.002	3.13 \pm 0.16	7.01 \pm 0.04	1.40 \pm 0.01	19.3 \pm 0.29
		Stomach	2.35 \pm 0.05	1.12 \pm 0.11	1.05 \pm 0.05	1.26 \pm 0.13	0.25 \pm 0.01	6.03 \pm 0.36
		Gill	0.83 \pm 0.003	0.76 \pm 0.02	2.08 \pm 0.02	0.28 \pm 0.01	0.27 \pm 0.006	4.21 \pm 0.07
		Muscle	0.27 \pm 0.16	0.14 \pm 0.003	1.17 \pm 0.003	0.16 \pm 0.01	0.23 \pm 0.008	1.97 \pm 0.19
	5 mg L ⁻¹ (D7)	Liver	4.82 \pm 0.69	0.34 \pm 0.02	0.06 \pm 0.02	0.34 \pm 0.04	0.45 \pm 0.01	6.01 \pm 0.76
		Stomach	1.02 \pm 0.008	1.51 \pm 0.08	1.60 \pm 0.14	0.66 \pm 0.004	0.39 \pm 0.01	5.17 \pm 0.24
		Gill	0.46 \pm 0.10	0.28 \pm 0.06	0.70 \pm 0.001	0.41 \pm 0.05	0.27 \pm 0.02	2.12 \pm 0.24
		Muscle	1.11 \pm 0.02	0.10 \pm 0.001	0.54 \pm 0.06	0.01 \pm 0.01	0.09 \pm 0.0002	1.86 \pm 0.09
	10 mg L ⁻¹ (D7)	Liver	10.8 \pm 0.17	1.80 \pm 0.07	1.26 \pm 0.06	1.13 \pm 0.01	1.79 \pm 0.29	16.8 \pm 0.60
		Stomach	2.46 \pm 0.14	7.03 \pm 0.05	4.82 \pm 0.13	2.13 \pm 0.14	1.34 \pm 0.02	17.8 \pm 0.48
		Gill	1.08 \pm 0.06	0.73 \pm 0.02	1.31 \pm 0.01	0.93 \pm 0.03	0.84 \pm 0.02	4.89 \pm 0.14
		Muscle	1.12 \pm 0.04	0.39 \pm 0.01	0.88 \pm 0.01	1.24 \pm 0.04	1.21 \pm 0.02	4.84 \pm 0.12

D1: 1 day of exposure. D7: 7 days of exposure. AsB: arsenobetaine. As(III): arsenite. DMA: dimethylarsinic acid. MMA: monomethylarsonic acid. As(V): arsenate..

Table 6Arsenic species mass fractions ($\mu\text{g g}^{-1}$) in different tissues after exposure of Nile tilapia to inorganic As(V) (n = 3, mean \pm SD).

	Group	Tissue	As speciation					Σ As species
			AsB	As(III)	DMA	MMA	As(V)	
As(V) exposure	Control	Liver	< LOQ	< LOQ	< LOQ	0.001 \pm 0.001	0.09 \pm 0.002	0.09 \pm 0.003
		Stomach	< LOQ	< LOQ	< LOQ	0.004 \pm 0.0005	0.09 \pm 0.004	0.09 \pm 0.009
		Gill	< LOQ	< LOQ	< LOQ	0.001 \pm 0.001	0.11 \pm 0.001	0.11 \pm 0.001
		Muscle	< LOQ	< LOQ	< LOQ	0.003 \pm 0.002	0.23 \pm 0.01	0.23 \pm 0.014
	5 mg L ⁻¹ (D1)	Liver	0.51 \pm 0.07	0.08 \pm 0.02	0.48 \pm 0.12	0.01 \pm 0.001	0.07 \pm 0.004	1.15 \pm 0.21
		Stomach	0.21 \pm 0.07	0.60 \pm 0.21	0.01 \pm 0.004	0.04 \pm 0.01	0.17 \pm 0.02	1.04 \pm 0.31
		Gill	0.12 \pm 0.07	< LOQ	0.15 \pm 0.08	0.01 \pm 0.003	0.37 \pm 0.02	0.65 \pm 0.18
		Muscle	0.25 \pm 0.05	< LOQ	0.09 \pm 0.003	0.01 \pm 0.002	0.19 \pm 0.003	0.53 \pm 0.06
	10 mg L ⁻¹ (D1)	Liver	1.38 \pm 0.03	1.03 \pm 0.02	3.56 \pm 0.16	0.37 \pm 0.02	0.39 \pm 0.004	6.71 \pm 0.23
		Stomach	3.20 \pm 0.001	1.16 \pm 0.07	1.94 \pm 0.24	0.70 \pm 0.15	0.34 \pm 0.004	7.34 \pm 0.47
		Gill	0.35 \pm 0.06	0.73 \pm 0.20	1.77 \pm 0.40	0.23 \pm 0.05	0.18 \pm 0.001	3.25 \pm 0.71
		Muscle	0.49 \pm 0.09	0.30 \pm 0.14	0.84 \pm 0.12	0.07 \pm 0.02	0.26 \pm 0.15	1.96 \pm 0.51
	5 mg L ⁻¹ (D7)	Liver	10.8 \pm 0.18	1.11 \pm 0.12	2.15 \pm 0.14	0.77 \pm 0.17	0.35 \pm 0.005	15.2 \pm 0.61
		Stomach	0.69 \pm 0.11	1.97 \pm 0.23	0.68 \pm 0.01	0.63 \pm 0.02	0.43 \pm 0.18	4.40 \pm 0.55
		Gill	0.78 \pm 0.02	< LOQ	0.52 \pm 0.001	0.07 \pm 0.03	0.18 \pm 0.001	1.55 \pm 0.05
		Muscle	1.13 \pm 0.19	< LOQ	0.38 \pm 0.01	0.02 \pm 0.01	0.18 \pm 0.003	1.71 \pm 0.21
	10 mg L ⁻¹ (D7)	Liver	4.02 \pm 0.35	2.48 \pm 0.26	1.27 \pm 0.07	0.87 \pm 0.13	0.35 \pm 0.006	8.99 \pm 0.82
		Stomach	1.32 \pm 0.006	0.65 \pm 0.001	1.21 \pm 0.06	0.32 \pm 0.02	0.65 \pm 0.02	4.15 \pm 0.11
		Gill	1.28 \pm 0.20	0.22 \pm 0.01	1.48 \pm 0.02	0.19 \pm 0.06	0.29 \pm 0.006	3.46 \pm 0.30
		Muscle	0.88 \pm 0.04	0.18 \pm 0.01	0.49 \pm 0.006	0.61 \pm 0.01	0.16 \pm 0.01	2.30 \pm 0.08

D1: 1 day of exposure. D7: 7 days of exposure. AsB: arsenobetaine. As(III): arsenite. DMA: dimethylarsinic acid. MMA: monomethylarsonic acid. As(V): arsenate.

and for all experimental conditions, with fractions ranging from 13% to 80%, indicating that Nile tilapia could convert MMA and DMA into AsB, which is one of the least reactive and toxic As species. The mechanisms of AsB synthesis are not completely understood, with the current models for its formation being mainly based on the potential biosynthetic precursors and the detection of intermediates. As reported by Zhang et al. (2022), several possible pathways exist for AsB synthesis. The primary hypothesis is the degradation of dimethylated/trimethylated arsenosugars, followed by the formation of AsB via methylation and oxidation. Other pathways that have been described are the oxidation of arsenocholine (AsC) to AsB and the methylation of DMA. It has been reported that microorganisms and organisms at low trophic levels are involved in the formation of AsB, and that AsB detected in organisms at higher trophic levels is mainly due to trophic accumulation along the food chain (Popowich et al., 2016).

There are studies that have indicated a correlation between the gut microbiota and the accumulation of AsB. Foster and Maher (2016)

suggested that bacteria present in the intestines of herbivores can synthesize AsB by pathways that remain uncharacterized. Zhang et al. (2020) demonstrated that AsB synthesis was mediated by the gut microbiota in marine medaka (*Oryzias melastigma*), and that it contributed to high bioaccumulation of As. Song et al. (2022) reported that gut microbiota promoted As bioaccumulation and biotransformation in tilapia (*Oreochromis mossambicus*), and could transform As(V) into organoarsenicals.

Since the descriptive data indicated that tilapia could convert MMA and DMA into AsB, a statistical analysis of these data was performed to better understand the influence of AsB for each tissue. Statistically, the AsB values obtained after exposure of the tilapia to inorganic As(III) presented the same pattern for all the tissues, except the muscle (Fig. 5d), with the lowest and highest levels of AsB being observed for 5 mg L⁻¹ exposure on D1 and 10 mg L⁻¹ exposure on D7, respectively (Fig. 5a–c). In the muscle, the highest concentration was observed for 5 mg L⁻¹ exposure on D7, although this was not statistically significant. It

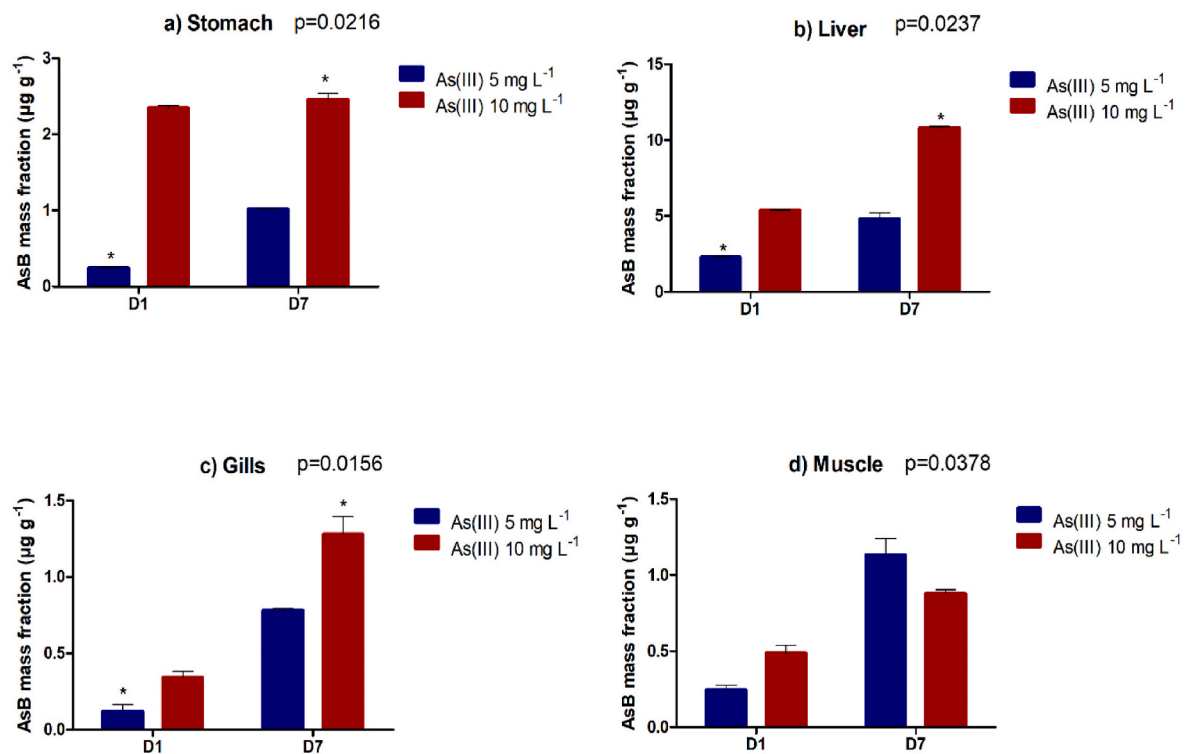


Fig. 5. AsB mass fractions ($\mu\text{g g}^{-1}$) in different tissues after exposure of Nile tilapia to inorganic As(III). AsB: arsenobetaine. D1: 1 day of exposure. D7: 7 days of exposure. * Statistically significant difference between the indicated groups. The representative values of the three replicates were expressed as mean with standard error (\pm SEM). Tests performed: Kruskal-Wallis complemented by Dunn's *post-hoc* test; $p < 0.05$ was statistically significant.

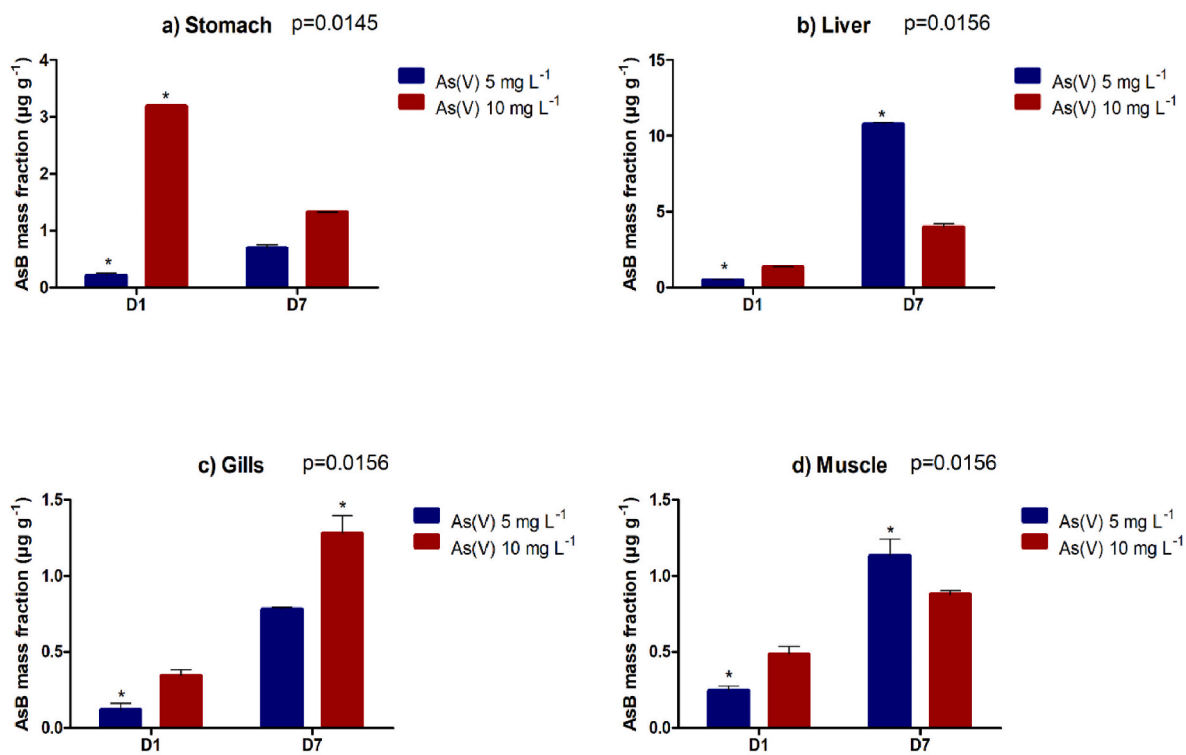


Fig. 6. AsB mass fractions ($\mu\text{g g}^{-1}$) in different tissues after exposure of Nile tilapia to inorganic As(V). AsB: arsenobetaine. D1: 1 day of exposure. D7: 7 days of exposure. * Statistically significant difference between the indicated groups. The representative values of the three replicates were expressed as mean with standard error (\pm SEM). Tests performed: Kruskal-Wallis complemented by Dunn's *post-hoc* test; $p < 0.05$ was statistically significant.

is noteworthy that the liver presented the highest levels of biotransformation to AsB (Fig. 5b), as also shown in the descriptive analysis, with these levels being highest for As(III) at 10 mg L⁻¹.

However, considering the AsB values after exposure of the tilapia to inorganic As(V), only the gill tissue (Fig. 6c) presented the pattern described above. Among the other tissues, for both liver and muscle (Fig. 6b and d), exposure to 5 mg L⁻¹ led to the lowest (D1) and highest (D7) values, despite only the liver showing a drastic increase (~10-fold) between these experimental situations. Unlike the other tissues, which presented the highest biotransformation rates on D7, the stomach (Fig. 6a) presented the highest level on D1, for exposure to 10 mg L⁻¹.

Under the experimental conditions, tilapia was exposed to inorganic As, so the AsB detected in the tissues was due to biotransformation by methylation of inorganic As and subsequently conversion to AsB. In the present experiment, the descriptive analysis indicated that the AsB fraction was higher for the As(III) treatment, compared to the As(V) treatment, during the entire exposure period. Thomas et al. (2004) demonstrated that arsenic metabolism involves a series of reduction and oxidation reactions, where pentavalent species are reduced to trivalent species and oxidative methylation produces methylated tri- and pentavalent metabolites. However, in addition to being a detoxification mechanism, it has been proposed that methylation might activate the toxic and carcinogenic potential of As. The intermediates and products formed in this pathway may be more reactive and toxic than the inorganic As species, affecting the transcription of genes and acting as more potent cytotoxic enzymatic inhibitors, compared to non-methylated species (Martinez et al., 2011; Roy et al., 2020). The DMA and AsB species are the final products of the process, with the greater presence of these species being indicative of the progression of the metabolic process. In contrast, the accumulation of the monomethylated species (MMA) and intermediate metabolites of inorganic As indicates a lower rate of metabolism, affecting the distribution and excretion of As (Rahman et al., 2019).

3.4. Antagonistic effects between arsenic and selenium

Before carrying out the antagonism assay between As(III) and Se(IV), it was necessary to perform an acute toxicity test with Se to determine the LC₅₀ value, which is the lethal dose concentration capable of causing the death of half of the study population. The LC₅₀ value obtained was 2.49 mg L⁻¹, so a dose of 1 mg L⁻¹ of Se(IV) was used in the antagonism assay, which would not cause the death of the Nile tilapia population during the assay. There are few studies in the literature that have reported the acute toxicity of Se in fish, including in Nile tilapia. Most studies have used supplementation to investigate the protective effect of this element when fish are exposed to contaminants. Ranzani-Paiva et al. (2011) investigated the median LC_{50-96h} lethal concentration of tetravalent selenium in Nile tilapia (*Oreochromis niloticus*) fingerlings, obtaining a value of 4.42 mg L⁻¹.

The total arsenic and selenium concentrations in the different tissues after the antagonism assay are presented in Tables 7 and 8, respectively. The As accumulation in the fish tissues varied from 0.02 to 5.55 µg g⁻¹ in the liver, from 0.10 to 11.7 µg g⁻¹ in the stomach, from 0.11 to 3.43 µg

Table 7

Total As concentrations (µg g⁻¹) in the different tissues of *O. niloticus* after exposure to As(III) and Se(IV) (n = 3, mean ± SD).

Group	Concentration (µg g ⁻¹)			
	Liver	Stomach	Gills	Muscle
Control	0.02 ± 0.005	0.10 ± 0.035	0.11 ± 0.007	0.05 ± 0.003
5 mg L ⁻¹ (D1)	2.12 ± 0.03	2.50 ± 0.002	0.83 ± 0.03	0.73 ± 0.02
10 mg L ⁻¹ (D1)	3.14 ± 0.007	2.64 ± 0.001	0.95 ± 0.1	0.89 ± 0.007
5 mg L ⁻¹ (D7)	3.22 ± 0.001	3.01 ± 0.24	1.34 ± 0.008	0.95 ± 0.02
10 mg L ⁻¹ (D7)	5.55 ± 0.48	11.7 ± 0.004	3.43 ± 0.45	3.03 ± 0.05

D1: 1 day of exposure. D7: 7 days of exposure..

Table 8

Se concentrations (µg g⁻¹) in the different tissues of *O. niloticus* after exposure to As(III) and Se(IV) (n = 3, mean ± SD).

Group	Concentration (µg g ⁻¹)			
	Liver	Stomach	Gills	Muscle
Control	0.02 ± 0.0004	0.11 ± 0.03	0.06 ± 0.007	0.02 ± 0.005
5 mg L ⁻¹ (D1)	1.79 ± 0.003	3.07 ± 0.001	2.10 ± 0.104	0.35 ± 0.002
10 mg L ⁻¹ (D1)	1.53 ± 0.03	2.17 ± 0.001	1.97 ± 0.180	0.28 ± 0.12
5 mg L ⁻¹ (D7)	4.22 ± 0.01	3.90 ± 0.08	3.00 ± 0.004	1.36 ± 0.02
10 mg L ⁻¹ (D7)	3.55 ± 0.16	7.39 ± 0.001	3.70 ± 0.295	1.57 ± 0.03

D1: 1 day of exposure. D7: 7 days of exposure.

g⁻¹ in the gills, and from 0.05 to 3.03 µg g⁻¹ in the muscle, while the Se accumulation varied from 0.02 to 4.22 µg g⁻¹ in the liver, from 0.11 to 7.39 µg g⁻¹ in the stomach, from 0.06 to 3.70 µg g⁻¹ in the gills, and from 0.02 to 1.57 µg g⁻¹ in the muscle of *O. niloticus* exposed to the combination of As(III) and Se(IV).

The concentrations of As and Se at the beginning of the assay were determined by collection of small aliquots of water for analysis by ICP-MS. The average As concentrations were 5.20 ± 0.07 mg L⁻¹ and 10.1 ± 0.03 mg L⁻¹, while the average Se concentration was 1.11 ± 0.15 mg L⁻¹. The concentrations of As and Se in the water of the control group were below the limits of quantification for determination by ICP-MS (1.6 × 10⁻⁴ mg L⁻¹ and 1.2 × 10⁻⁴ mg L⁻¹, respectively).

Comparing the results obtained in the As(III) controlled assay (Table 3) with the data obtained in the antagonism assay with Se(IV) and As(III), it was evident that the As values decreased in all the tissues studied, for the different concentrations and exposure periods. The greatest effect was in the liver, the main As metabolizing organ, for exposure to 10 mg L⁻¹ during 1 and 7 days, with concentration reductions of 6 and 4 times, respectively, indicating the protective effect of selenium against arsenic toxicity.

Reports in the literature indicate that Se has a protective effect against several contaminants in the aquatic environment. Atencio et al. (2009) investigated the effect of Se(IV) supplementation in Nile tilapia (*Oreochromis niloticus*) on the toxicity induced by cyanobacterial cells containing microcystins. The results showed that Se had a protective effect, since the tissue changes induced by cyanobacterial infections in the liver, kidney, heart, and gastrointestinal tract were improved by the highest dose of Se tested. Cabezas-Sanchez et al. (2019) evaluated the protective effect of Se(IV) co-administration against the bioaccumulation and toxicity of methyl mercury (MeHg) in zebrafish (*Danio rerio*), observing significant decreases in the bioaccumulation factor and the oxidative stress caused by MeHg, with the activation of different defense mechanisms induced by the co-administration of Se (IV).

Arsenic and selenium present a mutual toxicity antagonism, despite their similarities in terms of chemical properties and metabolic pathways. Arsenic inhibits cellular respiration, which causes the formation of reactive oxygen species (ROS), consequently generating oxidative stress (Ventura-Lima et al., 2011). Selenium is an element that has great physiological and ecotoxicological importance. It is an essential micronutrient for most organisms, but depending on the chemical form, in excess Se can be toxic and can increase the production of ROS by reaction with thiol groups (Bodnar et al., 2012). Selenium regulates several biological and biochemical functions, such as the protection of membranes against oxidative damage, and is present in the active sites of selenoproteins that have antioxidant functions, such as GPx, which acts to reduce cellular ROS levels (Toppo et al., 2009).

Several mechanisms responsible for the interactions between Se and As have been suggested. A direct mechanism for their mutual

detoxification is by formation of the arsenic-glutathione complex. Se(IV) is rapidly absorbed and converted into hydrogen selenide (HSe^-), while in the presence of As(III) and reduced GSH, the seleno-bis(S-glutathionyl) arsinium ion ($[(\text{GS})_2\text{AsSe}]^-$) is formed. Studies have identified this complex in the bile of laboratory animals co-treated with As(III) and Se(IV), as confirmed by X-ray spectroscopy, providing a molecular basis for the antagonistic interaction between these two species (Gailer et al., 2000; Gailer, 2009; George et al., 2016).

4. Conclusions

This study investigated the bioaccumulation and biotransformation of inorganic As in different tissues of Nile tilapia (*Oreochromis niloticus*) exposed to As(III) and As(V) at different concentrations (5.0 and 10.0 mg L^{-1}) and for different exposure periods (1 and 7 days). The ability of Nile tilapia to bioaccumulate inorganic As varied depending on the As species. The fish exposed to As(III) showed higher levels of As in all the tissues studied, compared to As(V) exposure. For both treatments, the highest concentrations of As were found in the liver and the stomach, followed by the gills and muscles. In this study, the inorganic As species were biotransformed into organic methylated forms (MMA and DMA), with subsequent conversion to nontoxic AsB, the predominant species. Finally, the antagonism assay between As(III) and Se(IV) revealed that selenium can reduce As toxicity in the bioaccumulation process in the different tissues of Nile tilapia, with the selenium acting as a chemical antagonist.

CRedit authorship contribution statement

Nathalia dos Santos Ferreira: Writing – original draft, Validation, Investigation, Formal analysis, Conceptualization. **Pedro Henrique da Costa:** Writing – review & editing, Investigation. **Ívero Pita de Sá:** Writing – review & editing, Investigation, Formal analysis. **Victoria Simões Bernardo:** Writing – review & editing, Formal analysis. **Flaviane Felix Torres:** Writing – review & editing, Formal analysis. **Jozi Godoy Figueiredo:** Writing – review & editing. **Clarice Dias Britto do Amaral:** Writing – review & editing. **Ana Rita Araujo Nogueira:** Writing – review & editing. **Danilo Grünig Humberto da Silva:** Writing – review & editing, Methodology, Formal analysis. **Mario Henrique Gonzalez:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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