Evaluation of the effects of sodium lauryl sulfate on the health status of *Danio rerio*: a study on swimming performance and sociability

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Key words: behaviour; ecotoxicity; detergent; *Danio rerio*; Sodium Lauryl Sulfate; sociability.

Contributions: GP, CF, conceptualization, supervision; MAR, methodology, software; GP, MAR, CF, FI, validation; FI, AC, investigation; FI, MAR, data curation, writing—review and editing; FI, writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

Funding: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest: the authors declare no conflict of interest.

Ethics approval: this experiment was approved by the Ethical Commission from the Faculty of Biology, "Alexandru Ioan Cuza" in the University of Iasi, with registration number 14/2.06.2021. For this study, all animals were kept and handled according to the European Commission Recommendation of 18 June 2007 on the housing and care of animals used for experimental purposes, and according to the European Parliament and Council Guidelines of 22 September 2010 on the protection of animals used for scientific purposes (European Commission, 2010).

Data availability: data are available on request.

Received: 25 August 2024.
Accepted: 26 December 2024.
Early view: 5 November 2025.

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Licensee PAGEPress, Italy
Journal of Biological Research 2025; 98:12967
doi:10.4081/jbr.2025.12967

Abstract

In recent years, the presence of Personal Care Products (PPCs) in the environment has triggered public attention because of their hazardous features in aquatic ecosystems. PPCs arrive in the ecosystem through wastewater, and their continued influx into the aquatic environment supports their pseudo-persistent behavior. One of the most used ingredients in personal care and household cleaning products is Sodium Lauryl Sulfate (SLS). This study aimed to test a 30-hour acute and a 14-day sub-chronic exposure of *Danio rerio* to SLS, observing its main effects on swimming performance and sociability status. The doses ranged from 0.5 to 1.0 µg L^{-1} of SLS for acute exposure and from 0.25, 0.5, 1.0, to 1.5 µg L^{-1} of SLS for subchronic exposure. The video-tracking software EthoVision XT was used to record specific swimming parameters, as well as the time spent in proximity to other zebrafish, to assess social behavior levels. The obtained data did not show many significant changes for the acute exposure compared to the 14-day period. Thus, significant differences (*p<0.05 ANOVA) were recorded for the "active movement" parameter (acute exposure) and the "acceleration" parameter (subchronic exposure). In addition, heat maps were also created to show the social alterations of zebrafish groups.

Introduction

Water is one of the most vital resources worldwide. Currently, its pollution has reached alarming levels, with effects visible not only on various organisms, including humans, but also on entire ecosystems. All water sources can be polluted in various ways, but the most significant pollution is that created by humans through the discharge of chemicals.[1–7] According to the European Environment Agency (EEA) report published in 2021, approximately 22% of Europe's surface water bodies and 28% of groundwaters are affected by pollution caused by agriculture, mainly by the high consumption of pesticides.[8] Besides this, domestic wastewaters contribute to the decrease in water quality and the deterioration of ecosystem balance.[9] For example, Sodium Lauryl Sulfate (SLS), also known as sodium dodecyl sulfate, is an anionic surfactant and is a common ingredient in personal care products (soaps, cosmetics, toothpaste) and household cleaning products (household cleaners for laundry, spray cleaners, dishwasher detergents).[10,11]

The concentration of SLS in consumer products differs by product and manufacturer but typically ranges from 0.01% to 50% in cosmetic products and 1% to 30% in cleaning products.[10,12] Due to the daily use of detergents and shampoos and their use in indus-

trial processes such as textile production, SLS is commonly found in wastewater and sewer networks, becoming a highly concentrated pollutant and a toxic substance for aquatic species.[13,14] Moreover, anionic detergents are characterized by a strong tendency to bind to the lipid component of the membrane, becoming responsible, at high concentrations, for cellular alterations in the organisms.[15]

To date, several studies have provided information about the impact of this detergent on organisms. For instance, a study from 2002 on the fish species *Clarias lazera* revealed that SLS (used in three concentrations: 0.11, 0.22 and 0.44 mg L^{-1} for 9 weeks in total) may change the haematological profile inducing anaemia, leucopenia accompanied with neutrophilia and lymphopenia, increased levels of serum cortisol, Alanine Transaminase (ALT), Aspartate Transaminase (AST), serum urea and creatinine. In addition, histopathological changes were found in the gills, liver, kidneys, and spleen.[16] In the same year, another study confirmed the above data, especially the histopathological lesions (clubbing and fusion of the secondary lamellae, hyperplasia and posterior rupture of the respiratory epithelium, destruction and shortening of gill filaments, and the presence of haemorrhagic foci) and histochemical alterations in the distribution of carbohydrates and proteins in the gills of treated *Scophthalmus maximus* juveniles. These lesions were observed following SLS administration at 3, 5, 7, and 10 mg/L^{-1}, with exposure times ranging from 4 to 384 hours, depending on the concentration[17]. The exposition of *Ctenopharyngodon idella* for 30 days at 2.0, 2.5, and 3.0 mg L^{-1} of SLS induced gills changes (necrosis and oedema), increased levels of Superoxide Dismutase (SOD) and Catalase (CAT) and increased gill availability for the microbial attack.[18] Another work from 2012 highlighted the influence of SLS on the reproductive capacity of *Pseudosida ramosa* with concentrations between 2 and 4 mg L^{-1} that were capable of decreasing the 21-day fecundity and 21-day fertility.[19] The most recent study showed that sublethal concentrations of SLS can impact the growth and the haematological parameters of *Cyprinus carpio*.[20]

Given its frequent use, documented impacts on the development and survival of aquatic fauna, and the concentrations detected in the environment, studies on the influence of this detergent are necessary. To gain more knowledge about this, zebrafish were used as the test organism. Zebrafish (*Danio rerio*), a freshwater teleost from the Cyprinidae family native to South Asia,[21–24] is widely used in vertebrate physiology, developmental genetics, drug production, and ecotoxicology research.[25–31] Known for its experimental advantages, zebrafish's quick responsiveness and sensitivity make it superior to traditional rodent models for evaluating chemical toxicity.[32–35] Its small size (about 3 cm), external fertilization, high reproductive rate (200 to 300 eggs per spawning), and cost-effective maintenance contribute to its popularity in scientific studies.

As a result, zebrafish is regarded as an organism with numerous advantages for toxicological research and is recommended as a low-cost model for studying the toxicity of various substances.[36–43] Therefore, the main purpose of the study was to investigate the possible adverse effects SLS may have on zebrafish in acute and subchronic treatments mimicking real-life scenarios. Consequently, the impact of SLS on locomotor activity parameters and sociability were quantified for both treatments. As far as we know, this is the first study to assess the impact of SLS detergent on the behaviour of zebrafish, as no similar work can be found in the literature.

Materials and Methods

Animals and housing

A total of 200 wild-type AB zebrafish adults (1:1 sex ratio) were obtained and acclimated for 3 weeks in two 90 L aquaria filled with dechlorinated water. The fish, 6-7 months old, with an average weight of 0.50±0.08 mg, and length of 3.43±0.2 cm, were fed with flakes from Norwin Norvitall (Norwin, Gadstrup, Denmark), twice daily (the feeding rate of zebrafish was 4% of body weight in feed per day). Daily water changes, aeration via air pumps, LED illumination (307.5 lux), and adherence to a 14/10 light/dark cycle were maintained, and the specific parameters for the water quality were measured (Table 1). The study, conducted at the "Ecotoxicology and Animal Behaviour Laboratory" in the University of Iasi "Alexandru Ioan Cuza" (Iasi, Romania), employed Sigma-Aldrich's SLS detergent for acute (0.5 µg L^{-1}, 1.0 µg L^{-1}) and subchronic (0.25 µg L^{-1} to 1.5 µg L^{-1}) exposures on eight groups of 10 zebrafish each.

Chemicals

The SLS detergent selected for this experiment was purchased from Merck KGaA, Darmstadt, Germany ($C_{12}H_{25}O_4SNa$ - FW 288.4) in its salt form. To assess this compound, we prepared a stock solution by dissolving the salt in the system water. Given the dual

Table 1. Water parameters for housing and experimental aquariums.

Parameters	Housing aquarium	Experimental aquarium
pH	7.67±0.10	7.64±0.10
Temperature °C	25±1	24±1
TDS (total dissolved solids) (mg L^{-1})	270	271
Salinity (PSU)	0.26	0.25
Conductivity (µS / cm)	552	553
Oxidation-reduction potential (mV)	370	372
Alkalinity (ppm)	70	65
Hardness (ppm)	98	94
Ammonia (mg L^{-1})	<0.02	<0.02
Nitrite (mg L^{-1})	<1	<1
Nitrate (mg L^{-1})	<50	<50

exposure nature of this study, the test solutions were freshly prepared before the exposure in line with the established protocol: 0.5 µg L^{-1} and 1 µg L^{-1} for the acute treatment and 0.25 µg L^{-1}; 0.50 µg L^{-1}; 1 µg L^{-1} and 1.5 µg L^{-1} for the subchronic treatment. Subsequently, a specific concentration was achieved by transferring and dissolving in the tank treatment filled with 5 L of system water a defined amount of the stock solution in the tank treatment. Despite the existing limitations, this concentration range for SLS testing was chosen based on a review of the limited published studies.[9,39]

Experimental design

This study involved eight experimental groups, categorized into acute (three groups) and subchronic (five groups) exposure types. For the acute exposure, the experiment included three groups: one group exposed to 0.5 µg/L^{-1} SLS, one group exposed to 1.0 µg/L^{-1} SLS, and a control group with no SLS exposure. Each experimental group consisted of 10 animals that were randomly selected and placed in aquaria designated for their specific concentration treatment. This distribution ensured that each aquarium contained a distinct concentration of the detergent for exposure consistency across the groups. The fish were acclimated to the new environment for four days before exposure to the detergent. During this period, each fish was tested in a T-maze, a tool commonly used in behavioral studies that consists of a T-shaped pathway, allowing fish to swim and make a choice between two different arms of the maze. This initial test served as a baseline measure of behavior before treatment. Two concentrations of the detergent, 0.5 µg/L^{-1} and 1.0 µg/L^{-1}, were selected for the study. On the first day of exposure, the detergent was dissolved into the aquarium water at 7 a.m. That day, the fish were tested twice, at 1 p.m. and again at 7 p.m. After each test, the aquarium water was changed, and the detergent reintroduced at the same concentration. A third test was conducted 24 hours after the initial exposure, with a final test at the 30-hour mark. This experiment was conducted in triplicate for consistency (see Figure 1).

For the subchronic exposure, the experiment included five groups: four groups exposed to concentrations of 0.25 µg/L^{-1}, 0.5 µg/L^{-1}, 1.0 µg/L^{-1}, and 1.5 µg/L^{-1} SLS, and a control group with no SLS exposure. This test was conducted once a day for 14 days. At the end of each day, we replaced the water with clean water, fed the fish, and reintroduced the substance at the same concentrations. Upon concluding the experiment, we organized and analyzed the data obtained from video-tracking software using Excel spreadsheets. Over the two weeks, we also measured the sociability of the individuals, quantifying this parameter as the time spent by the tested organism near the group area (the chamber in the left arm). The entire experimental design is presented in Table 2. This test was also done in triplicate.

Experimental tests

Social interaction test

The social interaction test aims to analyze the social behaviour of each tested individual through the time spent in the stimulus zone. This test was chosen because *Danio rerio* is a highly sociable organism and, under normal conditions, prefers to stay with the group. It is evident that, if not subject to behavioural alterations, the tested organism should concentrate its activity where the social stimulus is located. This test was applied with success in other studies.[35]

For this experiment, we opted for a multi-purpose T-maze, along with the video-tracking software. The maze is T-shaped, made of Plexiglas maze 40 × 30 × 10 cm (length × height × width), equipped with three experimental arms in addition to the longest central one, used as a start-point. The structure was filled with clean water daily before the experiment (5 L). Additionally, a transparent plexiglass divider was positioned in the left arm of the T-maze to separate the test fish from the group housed in the stimulus zone. Each test fish was placed at the starting point and allowed to swim freely throughout the T-maze (Figure 2). Recording and analyzing behavioural parameters was conducted

Table 2. Experimental design of the Sodium Lauryl Sulfate (SLS) treatment.

	Acute exposure	Subchronic exposure
Accommodation before the start of the study (days)	4 days	7 days
Pretreatment	Yes	Yes
Treatment concentrations	0, 0.50, and 1.0 µg L^{-1}	0, 0.25, 0.50, 1.0, and 1.5 µg L^{-1}
Number of animals (per group)	10	10
Mode of administration	Dissolved in the medium	Dissolved in the medium
Time of exposure	30 hours	14 days
Experimental tests	Swimming performance test	Social interaction test

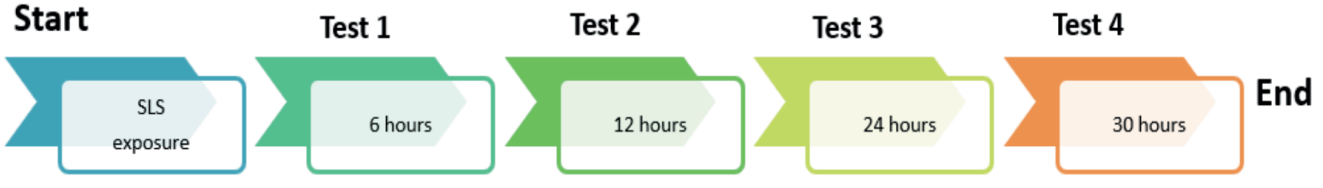

Figure 1. Timeline of the experiment illustrating the acute and subchronic exposure of zebrafish to sodium lauryl sulfate (SLS). The study comprises three groups: a group exposed to 0.5 µg L^{-1} SLS, a group exposed to 1 µg L^{-1} SLS, and a control group with no SLS.

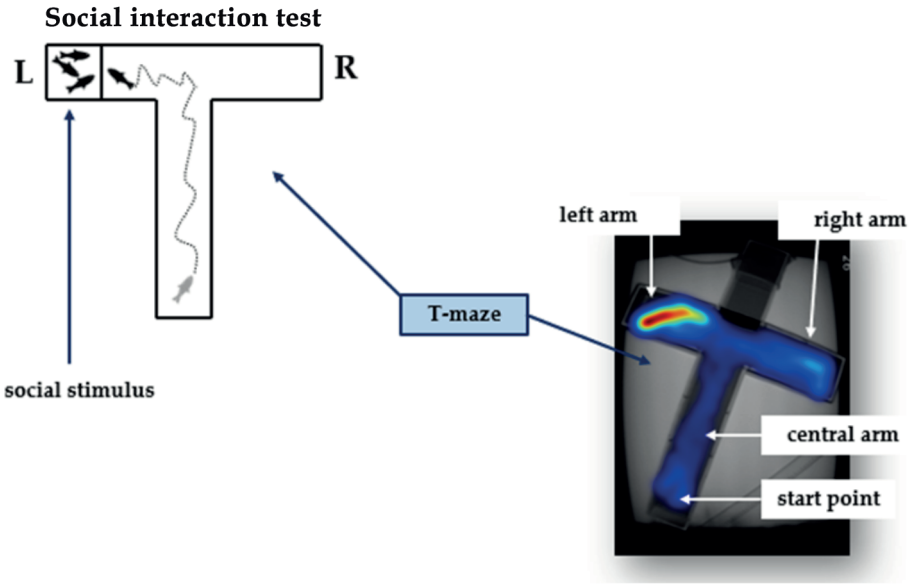

Figure 2. Layout of the T-maze compartments, adaptation for the social interaction test, and corresponding heat map analysis. The black area represents the plexiglass divider, which creates a chamber designated for the social stimulus group.

using the video-tracking software EthoVision XT 11.5 (Noldus, Holland). Each session lasted 4 minutes, with 30 seconds provided for the start point accommodation. The data, captured through a video camera above the maze and connected to the software, were processed to measure the time spent next to the stimulus zone chosen as the primary experimental reference. Thanks to the use of an infrared emission system, the software can find the individual as it moves and follow the individual in all its movements.[24] All recorded data were processed by the software through which the time spent next to the stimulus zone chosen as the main experimental reference was measured.

Swimming performance test

The swimming performance test aims to analyze the locomotor activity of each tested individual through a variety of parameters. In this test, each subject's task was to explore the maze for a 4-minute session, starting from the starting point. For achieving the animal's activity, the same T-maze was used as that from the social interaction test without the plexiglass separator and the social stimulus. The behavioural data was analyzed using the following parameters: total distance moved, active movement, acceleration, and velocity. The total distance moved parameter refers to the total distance swam by the zebrafish during the experimental session. Active movement consists of the time that the individual spends in motion, and acceleration represents how fast the individual can react. The velocity parameter describes how fast/slow the zebrafish swim.

Statistical analysis

The Shapiro-Wilk test was applied to verify the normality and distribution of the data. After, a one-way Analysis of Variance (ANOVA) followed by a post-hoc Tukey's Honestly Significant Difference (HSD) test was performed to assess significant differences in the variance of the behavioural parameters, considering them from the initial condition of the subjects until the end of treatment. The results were presented as mean±Standard Error (SE), and statistical significance was assumed at $p<0.05$. The data was sorted using the Excel files from the Microsoft Office package. Statistical analysis and graphic representation were done with OriginPro 2016 Software v.9.3 (OriginLab Corporation, Northampton, USA). EthoVision XT14 software was used to generate heat maps of the individual's permanence at different points in the T-maze, using heat analysis.

Ethical note

This experiment was approved by the Ethical Commission from the Faculty of Biology, "Alexandru Ioan Cuza" in the University of Iasi, with registration number 14/2.06.2021. Besides this, the animals were also strictly treated according to the Recommendation of the European Commission (2007) on guidelines for the accommodation and care of animals used for experimental and other scientific purposes, Directive 2010/63/EU of the European Parliament, and the Council of September 22, 2010, on the protection of animals used for scientific purposes.

Results

Acute exposure to SLS-induced perturbations in zebrafish locomotor activity

Some swimming performance parameters such as total distance moved, active movement and acceleration status were measured to describe the zebrafish locomotor activity and how SLS presence could modify it. Therefore, for the first parameter quantified, the total distance moved, no significant ($p>0.05$) differences were observed. Both experimental groups showed a similar trend; the distance swam started to increase directly proportional to the increase in exposure time: 0.5 µg L^{-1} pretreatment *vs*. 30 h (516.3±40.6 cm *vs*. 551.3±45.04 cm) and 1 µg L^{-1} pretreatment *vs*. 30 h (454.8±31.7 vs. 566.7±40.3 cm) (Figure 3).

Significant differences were observed (*p <0.05) for the 'active movement' parameter during the acute exposure treatment (Figure 4). In the control group, the animals maintained almost the same activity for all the treatment periods, from 203.62±3.87 s to 200.4±4.42 s at the end of the test. The group with the lowest concentration of SLS (0.5 µg L^{-1}) recorded a decrease in this parameter. Still, it was not statistically significant (p>0.05) as follows: pretreatment: 202.9±3.64 s *vs* 6 h: 194.8±4.24 s vs 12 h: 195.8±5.05 s *vs* 24 h: 195.8±5.77 s *vs* 30 h: 198.2±5.68 s. The group treated with 1 µg L^{-1} SLS showed a significant change in the active movement after 6 h exposure with a value of 192.5±4.22 s (p=0.04) compared to the pretreatment: 203.6±4.41 s. At the end of exposure, fish recorded a similar value as that from the beginning: 203.6±4.41 s. It can therefore be said that this group kept the levels of active movement constant and in line with the pretreatment.

Besides these two parameters, the acceleration status was also assessed (Figure 5). This parameter remained constant in the control group, averaging 58.4±12.1 cm/s². The 0.5 µg L^{-1} SLS group presented in the pretreatment a value of 42.7±5.5 cm/s²; after 6 h the acceleration rose until 68.3±12.9 cm/s² and after 12 h, it slightly decreased to 64.6±12.1 cm/s². After 24 h, the acceleration recorded the highest peak: 86.6±16.7 cm/s² (p=0.02) and again decreased to 37.3±4.1 cm/s² after 30 h of exposure. In the 1 µg L^{-1} group, it can be seen a similar scenario: the acceleration fluctuated, presenting during the pretreatment period a value of 55.7±5.9 cm/s²; after 6 h it decreased to 45.6±5.02 cm/s², after 12 h increased again to 65.2±8.7 cm/s², and then rose sharply after 24 h to 82.6±15.3 cm/s² (p=0.05). After 30 h, the animals showed similar activity to that from the pretreatment period: 52.5±3.8 cm/s².

The last analyzed parameter, velocity, presented a similar trend as the total distance moved parameter (Figure 6) with no significant changes across the experimental groups (p > 0.05).

The heat maps presented in Figure 7 show the activity of the experiment groups before and after the treatment with SLS. The ani-

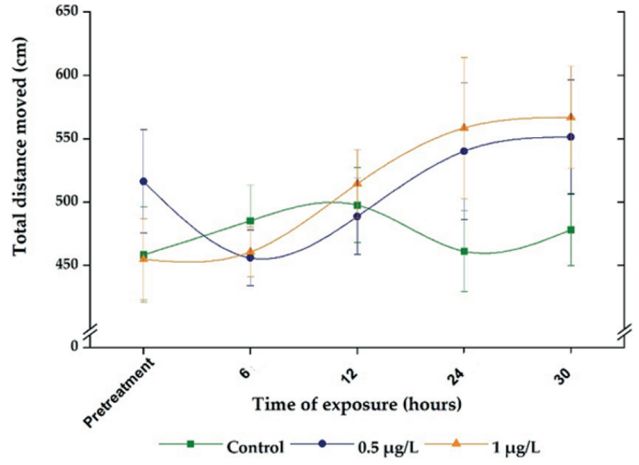

Figure 3. Total distance moved parameter in the acute treatment (n=10 per group). Data is represented as average±standard error (SE).

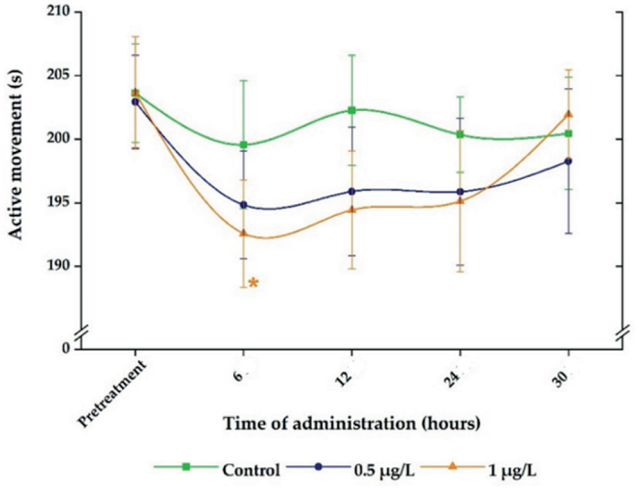

Figure 4. Active movement parameter in the acute treatment (n=10 per group). Data is represented as average±standard error (SE). A single asterisk (*) indicates statistically significant differences at p<0.05.

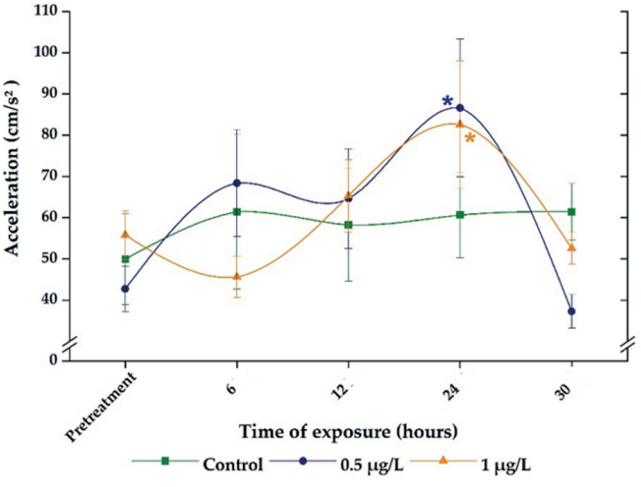

Figure 5. Acceleration parameter in the acute treatment (n=10 per group). Data is represented as average±standard error (SE). A single asterisk (*) indicates statistically significant differences at p<0.05.

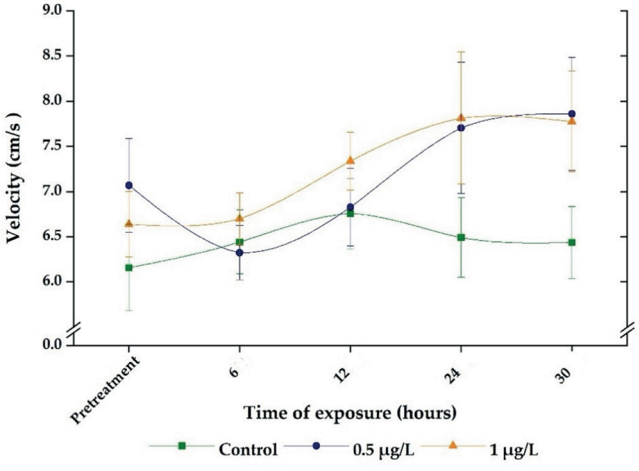

Figure 6. Velocity parameter in the acute treatment (n=10 per group). Data is represented as average±standard error (SE).

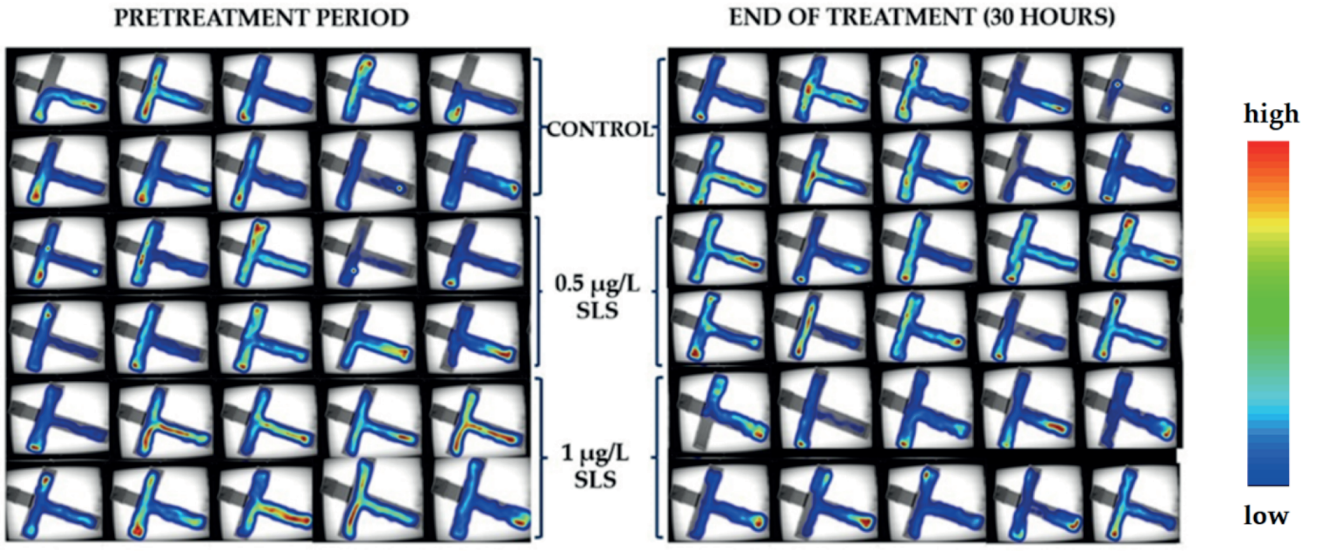

Figure 7. Heat maps of the zebrafish activity during the trials of the swimming performance test. SLS, Sodium Lauryl Sulfate.

mals did not exhibit any preferences for any zone of the T-maze being highly active.

Subchronic exposure to SLS-induced social changes in zebrafish

During the subchronic treatment, besides the social interaction test, several parameters specific to locomotor activity were analyzed, such as total distance moved, active movement, and acceleration. The data obtained showed significant changes in the animals' activity, recording ups and downs, in other words, an irregular trend.

For instance, the total distance parameter for the 0.25 µg L^{-1} SLS group showed significantly higher activity in D_1 (1577.6±151.1 cm, $p < 0.05$), D_3 (1514.3±123.1 cm, $p \leq 0.0405$) and D_6 (1574.7±148.1 cm, $p \leq 0.0305$) compared to the initial behaviour (1206.5±79.6 cm). The 0.50 µg L^{-1} and 1 µg L^{-1} SLS groups showed no significant differences ($p > 0.05$). By contrast, the 1.5 µg L^{-1} SLS group exhibited a significant decrease in total distance at D_3 (815.7±61.8 cm, ($p \leq 0.0201$), D_4 (842.8±63.7 cm, ($p < 0.05$), D_7 (784.5±86.8 cm, $p=0.03$), D_8 (684.8±59.8 cm, D_9 (733.9±49.1, $p=0.001$), D_{10} (744.3±58.1 cm, $p < 0.05$) compared to the pretreatment value (1025.1±58.7 cm). These data are shown in Figure 8.

Regarding the active movement of the control group, no significant differences were noted before and after treatment simulation (227.4±2.9 s vs. 227.0±3.7 s; $p > 0.05$) (Figure 9). The active movement of the 0.25 µg L^{-1} SLS group recorded a slight but non-significant decrease at D_2–D_7 ($p > 0.05$), stabilizing after one week (224.3±3.4 s vs. 232.1±1.5 s). The 0.50 µg L^{-1} group maintained a

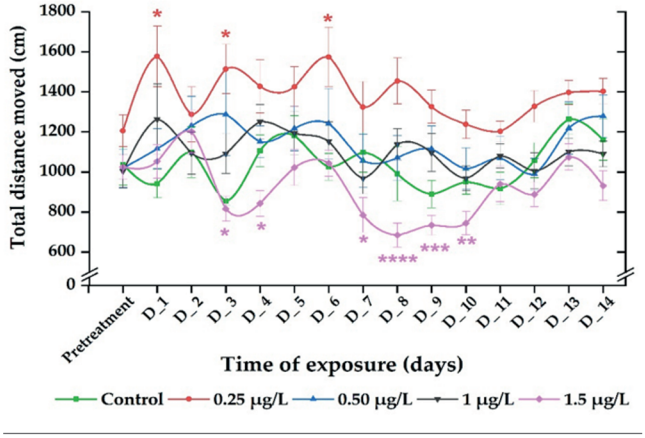

Figure 8. Results of total distance moved in the subchronic treatment (n=10 per group). Data is represented as average±standard error (SE). A single asterisk (*) indicates statistically significant differences at $p<0.05$, while two asterisks (**) indicate statistically significant differences at $p<0.01$, and three asterisks (***) indicate $p < 0.001$. Four asterisks (****) indicate $p<0.0001$.

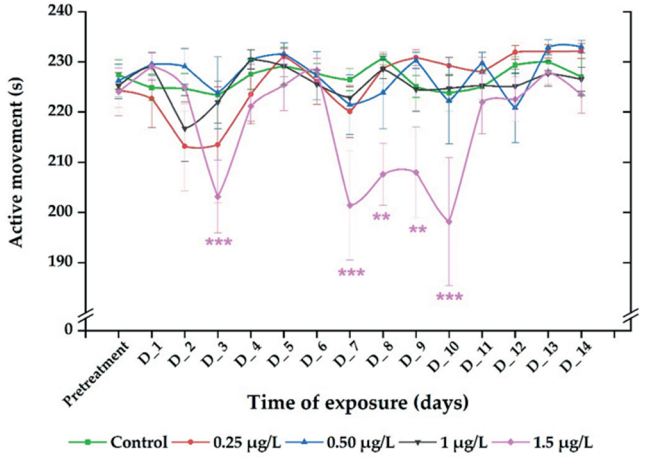

Figure 9. Active movement results in subchronic treatment (n=10 per group). Data is represented as average±standard error (SE). Two asterisks (**) indicate $p < 0.01$, and three asterisks (***) indicate $p < 0.001$, as determined by ANOVA compared to the pretreatment period.

similar trend ($p > 0.05$), while the 1 µg L^{-1} group showed temporary variations without statistical significance ($p > 0.05$). Conversely, the 1.5 µg L^{-1} group exhibited a significant decrease in active movement at D_3, D_7–D_{10} ($p < 0.05$).

Regarding acceleration, the animals' activity showed high variability following SLS administration (Figure 10). There was no significant difference among control animals ($p > 0.05$). At the lowest concentration (0.25 µg L^{-1} SLS), a significant increase was observed on the first day of exposure (585.7±6 cm s^{-2}, $p = 0.01$) compared to the pretreatment value (553.6±11.4 cm s^{-2}). The 0.50 µg L^{-1} group exhibited significant peaks at D_3 ($p < 0.05$) and D_6 ($p < 0.05$), followed by significant decreases at D_7 and D_{11} ($p < 0.05$). The 1 µg L^{-1} SLS group showed significant increases at D_2, D_6, and D_{14} ($p < 0.05$). The 1.5 µg L^{-1} SLS group displayed the most irregular pattern, with a significant decrease at D_4 ($p < 0.05$).

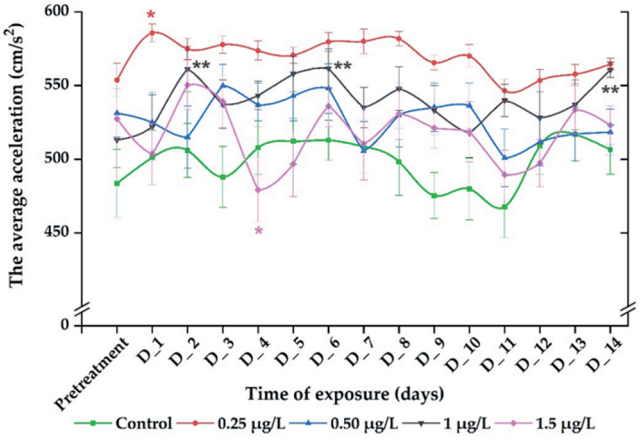

Figure 10. Average acceleration of experimental groups during subchronic treatment (n=10 per group). Data isare represented as mean±SE. A single asterisk (*) indicates statistically significant differences at $p<0.05$, while two asterisks (**) indicate statistically significant differences at $p<0.01$, as determined by ANOVA compared to the pretreatment period.

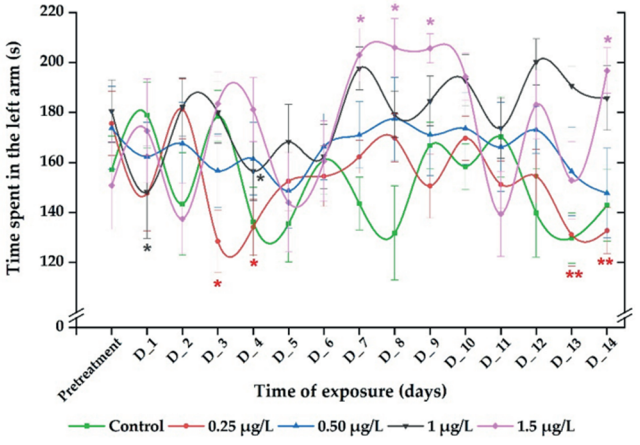

Figure 11. Time spent in the left arm by experimental groups during subchronic treatment (n=10 per group). Data is represented as average±SE. A single asterisk (*) indicates statistically significant differences at $p<0.05$, while two asterisks (**) indicate statistically significant differences at $p<0.01$, as determined by ANOVA compared to the pretreatment period.

The time spent in the left arm, where the social stimulus was added, varied among experimental groups (Figure 11). No significant differences were found in the control and 0.50 µg L^{-1} groups ($p > 0.05$). The 0.25 µg L^{-1} group showed significant decreases at D_3 ($p < 0.05$), D_4 ($p < 0.05$), D_{13} ($p < 0.05$), and D_{14} ($p < 0.05$) compared with the initial behavior (175.5±12.8 s). The 1 µg L^{-1} group also exhibited significant decreases at D_1 ($p < 0.05$) and D_4 ($p < 0.05$) compared with pretreatment (180.4±12.4 s). In contrast, the 1.5 µg L^{-1} group showed significant increases at D_7–D_9 and D_{14} ($p < 0.05$) relative to pretreatment (150.7±17.5 s).

Discussion

The extensive use of SLS in daily life has led to its accumulation in sewage systems and wastewater, making it a prominent emerging pollutant. Consequently, the environmental impact of this compound requires further evaluation. This study aims to contribute new data on the effects of SLS on zebrafish as an aquatic model.

Drawing on existing literature and our results, alterations in zebrafish were observed following exposure to SLS, manifested as significant changes in behavior and metabolic processes. In ecotoxicological studies, organism behavior is a key indicator, reflecting environmental adaptation.[44] Within the broader context of animal biology, behaviors play a pivotal role in ensuring survival, and responding to internal and external cues that influence an organism's fitness. Deviations from normal behaviors, as seen in this study, raise concerns about the potential survival implications associated with the accumulation of SLS in aquatic environments. Including behavioral assessments in studies investigating disease and neurotoxin exposure is crucial, especially when dealing with non-lethal impairments. Behavioral endpoints not only provide insights into the immediate effects of the exposure but can also serve as rapid indicators of molecular to systemic-level changes.[45] This aligns with the observed alterations in zebrafish locomotor and sociability parameters, suggesting a sensitive response to SLS.

During the acute treatment, the most significant changes were noted in active movement and acceleration parameters. The time spent moving showed decreased activity after 6, 12, and 24 hours, returning to nearly pretreatment levels, implying the zebrafish's ability to detect and adapt to the disruptive agent in their environment. Acceleration, another parameter that measures the reaction of an animal, showed a similar trend to the previous parameter. Usually, a higher acceleration is linked to better performance.[43,46,47] In this case, the detergent generated a certain level of stress, which could be seen in the elevated values of the acceleration parameter, but for a short period. At the end of the exposure (30 h), zebrafish returned to the initial behaviour recorded in the first stage of the experimental design. This observation could suggest that fish were able to recognise the SLS presence in the medium and their capacity to adapt to the new factor.

With prolonged exposure, SLS induced irregular activities in active movement and acceleration parameters. This imbalance in locomotor activity can also be attributed to the appearance of oxidative stress as a consequence of the administration of SLS. This has also been mentioned in experimental studies with other fish species and molluscs. For instance, SOD and CAT levels were increased in

grass carp (*Ctenopharyngodon idella)* after 30 days of 2, 2.5, and 3 mg L^{-1} SLS treatment.[18] Similar results were obtained by Freitas et al. after exposure of *Mytilus galloprovincialis* to 0.5, 1, 2, and 4 mg L^{-1} of SLS.[39]

It is interesting to note that the fish in the aquarium with the highest concentration of SLS, immediately after water replacement and the reintroduction of the detergent, started to stay at the bottom of the tank and maintain a motionless position. Most of the animals also continued to move their opercula insistently and to open and close their mouths. These signs of stress were absent in the control group. According to a study conducted in 2023,[47] these behaviours are the result of an evident state of stress and/or anxiety. Moreover, during this behaviour, which is referred to as freezing by the above-mentioned study, opercular movements (the rhythmic opening and closing of the gill cover, indicative of respiration) are usually very common. In contrast, immobility not associated with stress does not typically involve increased opercular movements.

In addition, a stress condition can be associated with increased oxidative stress. For example, Meseli *et al*.[48] proved that lipid peroxidation can be an early parameter for this condition. According to their study, zebrafish embryos exposed to 0.01 and 0.04 mg/10 mL expressed high levels in the lipid peroxidation process after 72 h.[48] A similar behavioral change linked with the presence of oxidative stress was seen in mussels (*Mytilus galloprovincialis)* when both females and males were exposed to 4 mg/L of SLS for 28 days. Mussels closed their valves for longer periods, had the tendency to accumulate the compound, and increased their metabolism in order to adjust to the SLS presence.[49] In another work, the impact of 4 mg/L SLS on mussels in certain salinities conditions for 28 days proved to be more pronounced on reproductive behavior and on the metabolic capacity.[50]

Regarding the social behavior of the treated zebrafish, it exhibited significant changes for the 0.25, 1, and 1.5 µg L^{-1} SLS groups. The time spent in the left arm, close to the stimulus zone, decreased in the 0.25 and 1 µg L^{-1} SLS groups compared to the initial behaviour. As opposed to this activity, the 1 µg L^{-1} SLS group presented high values of this parameter when compared with the initial one. A possible explanation for this type of behaviour may be an increase in the aggressiveness of the tested animal and not a sign of sociability.

The observed alterations in zebrafish behavior following exposure to SLS prompt a deeper exploration into potential neurotoxic effects. While the current study sheds light on the behavioral changes, it is crucial to consider the neurological implications of SLS exposure, especially in the absence of existing literature on the subject. Neurological effects are a plausible avenue for investigation, considering the well-established link between environmental pollutants and neurological disruption. Unfortunately, the available literature on the neurotoxic effects of SLS remains sparse.

However, a study by Basak *et al*. investigated the early effects of SDS on α-Synuclein, a protein implicated in neurodegenerative diseases.[51] The study revealed that SDS induced conformational changes and aggregation of α-Synuclein, providing a molecular perspective on the potential neurotoxic effects of surfactants. The observed correlation between conformational changes and aggregation in the context of α-Synuclein may offer parallels to our findings on the behavioral alterations in zebrafish exposed to SLS. While the study by Basak *et al.* specifically focuses on α-Synuclein, it underscores the importance of understanding the intricate relationships between surfactants and neurological processes. These insights prompt further exploration into the potential neurotoxic effects of SLS and related detergents, particularly considering their structural similarities.

Conclusions

Both acute and subchronic treatments revealed important changes in the zebrafish behavior triggering fluctuations after coming into contact with the detergent. The changes in the active movement parameter may suggest anxious behaviour. However, a specific test would be more appropriate to validate this statement, which can be considered a limitation of the current work. Also, alterations were seen in the sociability of the individuals: they tended to isolate themselves, spending more time alone than in a group. Due to these results and knowing the toxicity of detergents on aquatic organisms, it is clear and necessary to further investigate in this direction, with more studies on the detergent effects to assess the behavioural effects of this compound and the perturbation of fish communities' interactions. The observed behavioral changes in zebrafish following exposure to SLS prompt considerations about the potential ecological consequences of detergent pollutants. As pollutants continue to pose a threat to aquatic ecosystems, a more comprehensive understanding of the impact on fish behavior and community dynamics becomes crucial for informed environmental management and conservation efforts.

References

1. Ravi R, Athisuyambulingam M, Kanagaraj S, et al. Impact of chlorpyrifos on cytopathological indices in mangrove crab, *Episesarma tetragonum* (Fabricius). Vet Sci 2023;10:53.
2. Shiry N, Alavinia SJ, Impellitteri F, et al. Beyond the surface: consequences of methyl tert-butyl ether (MTBE) exposure on oxidative stress, haematology, genotoxicity, and histopathology in rainbow trout. Sci Total Environ 2023;900:165784.
3. Aliko V, Vasjari L, Ibrahimi E, et al. "From shadows to shores" - quantitative analysis of CuO nanoparticle-induced apoptosis and DNA damage in fish erythrocytes: A multimodal approach combining experimental, image-based quantification, docking and molecular dynamics. Sci Total Environ 2024;906:167698.
4. Impellitteri F, Riolo K, Multisanti CR, et al. Evaluating quaternium-15 effects on *Mytilus galloprovincialis*: new insights on physiological and cellular responses. Sci Total Environ 2024;170568.
5. Impellitteri F, Briglia M, Porcino C, et al. The odd couple: caffeine and microplastics. Morphological and physiological changes in *Mytilus galloprovincialis*. Microsc Res Tech 2024;87:1092-110.
6. Impellitteri F, Yunko K, Calabrese G, et al. Chlorpromazine's impact on *Mytilus galloprovincialis*: A multi-faceted investigation. Chemosphere 2024;350:141079.
7. Banaee M, Beitsayah A, Zeidi A, et al. Toxicity of cigarette butts (CBs) leachate on Nile tilapia (*Oreochromis niloticus*): blood biochemical parameters, oxidative stress biomarkers, and metabolic profile. Ecotoxicol Environ Saf 2024;279:116514.
8. European Environment Agency. Consolidated annual activity report. 2021; No 04/2022.
9. Mousavi SA, Khodadoost F. Effects of detergents on natural ecosystems and wastewater treatment processes: a review. Environ Sci Pollut Res Int 2019;26:26439–48.
10. Messina CM, Faggio C, Laudicella VA, et al. Effect of sodium dodecyl sulfate (SDS) on stress response in the Mediterranean mussel (*Mytilus galloprovincialis*): regulatory volume decrease (RVD) and modulation of biochemical markers related to oxidative stress. Aquat Toxicol 2014;157:94–100.

11. Bondi CA, Marks JL, Wroblewski LB, et al. Human and environmental toxicity of sodium lauryl sulfate (SLS): evidence for safe use in household cleaning products. Environ Health Insights 2015;9:27–32.
12. Cosmetic Ingredient Review. "Final report on the safety assessment of sodium lauryl sulphate and ammonium lauryl sulphate." JACT 1983;2.7:127–9.
13. Singer MM, Tjeerdema RS. Fate and effects of the surfactant sodium dodecyl sulfate. Rev Environ Contam Toxicol 1993;133:95–149.
14. Cserháti T, Forgács E, Oros G. Biological activity and environmental impact of anionic surfactants. Environ Int 2002;28: 337–48.
15. Lawrence C. The husbandry of Zebrafish (*Danio rerio*): a review. Aquaculture 2007;269:1–20.
16. Makhbatly A, Selim A, El-Sheikh K. Clinicopathological studies on the effect of sodium lauryl sulphate in *Clarias lazera*. Zag Vet J 2002;30:117–24.
17. Rosety-Rodríguez M, Ordoñez FJ, Rosety M, et al. Morpho-histochemical changes in the gills of turbot, *Scophthalmus maximus* L., induced by sodium dodecyl sulfate. Ecotoxicol Environ Saf 2002;51:223–8.
18. Susmi TS, Rebello S, Jisha MS, Sherief PM. Toxic effects of sodium dodecyl sulphate on grass carp *Ctenopharyngodon idella*. Fishery Technology 2011;47:145.
19. Freitas EC, Rocha O. Acute and chronic effects of atrazine and sodium dodecyl sulfate on the tropical freshwater cladoceran *Pseudosida ramosa*. Ecotoxicology 2012;21:1347–57.
20. Bhattacharya R, Daoud I, Chatterjee A, et al. An integrated *in silico* and *in vivo* approach to determine the effects of three commonly used surfactants sodium dodecyl sulphate, cetylpyridinium chloride and sodium laureth sulphate on growth rate and hematology in *Cyprinus carpio* L. Toxicol Mech Methods 2022;32:132–44.
21. Brunelli E, Talarico E, Corapi B, et al. Effects of a sublethal concentration of sodium lauryl sulphate on the morphology and Na+/K+ ATPase activity in the gill of the ornate wrasse (*Thalassoma pavo*). Ecotoxicol Environ Saf 2008;71:436–445.
22. Boxall ABA, Rudd MA, Brooks BW, et al. Pharmaceuticals and personal care products in the environment: what are the big questions? Environ Health Perspect 2012;120:1221–29.
23. Barman R. A taxonomic revision of the indo-burmese species of *Danio rerio*. Rec Zool Surv India Occ Papers 1991;137:1–91.
24. Pritchard VL. Behaviour and morphology of the Zebrafish, *Danio rerio*. PhD dissertation, University of Leeds, 2001.
25. Briggs JP. The Zebrafish: A new model organism for integrative physiology. Am J Physiol Regul Integr Comp Physiol 2002;282.
26. Ahmadifar E, Sheikhzadeh N, Roshanaei K, et al. Can dietary ginger (*Zingiber officinale*) alter biochemical and immunological parameters and gene expression related to growth, immunity and antioxidant system in zebrafish (*Danio rerio*)? Aquaculture 2019;507:341–8.
27. Faria M, Prats E, Rosas Ramírez JR, et al. Androgenic activation, impairment of the monoaminergic system and altered behavior in zebrafish larvae exposed to environmental concentrations of fenitrothion. Sci Total Environ 2021;775:145671.
28. Hoseinifar SH, Yousefi S, Capillo G, et al. Mucosal immune parameters, immune and antioxidant defence related genes expression and growth performance of zebrafish (*Danio rerio*) fed on *Gracilaria gracilis* powder. Fish Shellfish Immunol 2018;83:232–7.
28. Rashidian G, Boldaji JT, Rainis S, et al. Oregano (*Origanum vulgare*) extract enhances zebrafish (*Danio rerio*) growth performance, serum and mucus innate immune responses and resistance against *Aeromonas hydrophila* challenge. Animals 2021;11:299.
30. of age, time of day, and experiment on the basal locomotor activity and light-off visual motor response assays in zebrafish larvae. Toxics 2024;12:381.
31. Jijie R, Mihalache G, Balmus IM, et al. The evaluation of the antioxidant activity of methanol extract of *Schisandra chinensis* on *Danio rerio* exposed to oxidative stress. Arch Biol Sci 2017;69:143.
32. Merola C, Fabrello J, Matozzo V, et al. Dinitroaniline herbicide pendimethalin affects development and induces biochemical and histological alterations in zebrafish early-life stages. Sci Total Environ 2022;828:154414.
33. Plhalova L, Sehonova P, Blahova J, et al. Evaluation of tramadol hydrochloride toxicity to juvenile zebrafish—morphological, antioxidant and histological responses. Appl Sci 2020;10:2349.
34. Vaz RL, Outeiro TF, Ferreira JJ. Zebrafish as an animal model for drug discovery in Parkinson's disease and other movement disorders: a systematic review. Front Neurol 2018;9:347.
35. Ilie OD, Duta R, Jijie R, et al. Assessing anti-social and aggressive behavior in a Zebrafish (*Danio rerio*) model of Parkinson's disease chronically exposed to rotenone. Brain Sci 2022;12:898.
36. Maradona A, Marshall G, Mehrvar M, et al. Utilization of multiple organisms in a proposed early-warning biomonitoring system for real-time detection of contaminants: preliminary results and modeling. J Hazard Mater 2012;219-220:95–102.
37. Nabinger DD, Altenhofen S, Bitencourt PER, et al. Nickel exposure alters behavioral parameters in larval and adult zebrafish. Sci Total Environ 2018;624:1623–33.
38. Rashidian G, Mohammadi-Aloucheh R, Hosseinzadeh-Otaghvari F, et al. Long-term exposure to small-sized silica nanoparticles (SiO2-NPs) induces oxidative stress and impairs reproductive performance in adult zebrafish (*Danio rerio*). Comp Biochem Physiol C Toxicol Pharmacol 2023;273:109715.
39. Freitas R, Silvestro S, Coppola F, et al. Toxic impacts induced by sodium lauryl sulfate in *Mytilus galloprovincialis*. Comp Biochem Physiol 2020;242:110656.
40. Yalsuyi AM, Hajimoradloo A, Ghorbani R, et al. Behavior evaluation of rainbow trout (*Oncorhynchus mykiss*) following temperature and ammonia alterations. Env Toxicol Pharmacol 2021;86:103648.
41. Streisinger G, Walker C, Dower N, et al. Production of clones of homozygous diploid Zebrafish (*Brachydanio rerio*). Nature 1981;291:293–6.
42. Curpan AS, Impellitteri F, Plavan G, et al. Review: *Mytilus galloprovincialis*: An essential, low-cost model organism for the impact of xenobiotics on oxidative stress and public health. Comp Biochem Physiol 2022;256:109302.
43. Merola C, Fabrello J, Matozzo V, et al. Dinitroaniline herbicide pendimethalin affects development and induces biochemical and histological alterations in zebrafish early-life stages. Sci Total Environ 2022;828:154414.
44. Paduraru E, Flocea EI, Lazado CC, et al. Vitamin C mitigates oxidative stress and behavioral impairments induced by deltamethrin and lead toxicity in zebrafish. Int J Mol Sci 2021;22:12714.
45. Melvin SD, Wilson SP. The utility of behavioral studies for aquatic toxicology testing: A meta-analysis. Chemosphere 2013; 93:2217–23.
46. Kalueff AV, Gebhardt M, Stewart AM, et al. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish 2013;10:70–86.

47. Impellitteri F, Multisanti CR, Rusanova P, et al. Exploring the impact of contaminants of emerging concern on fish and invertebrates physiology in the Mediterranean Sea. Biology 2023; 12:767.
48. Meşeli S, Kaplan G, Cansız D, et al. The biocompatibility of sodium lauryl sulphate on developing zebrafish embryos. Experimed 2021;11:67-72.
49. Hodkovicova N, Hollerova A, Svobodova Z, et al. Effects of plastic particles on aquatic invertebrates and fish – A review. Environ Toxicol Pharmacol 2022;96:104013.
50. Freitas R, Coppola F, Meucci V, et al. The influence of salinity on sodium lauryl sulfate toxicity in *Mytilus galloprovincialis*. Environ Toxicol Pharmacol 2021;87:103715.
51. Basak S, Prasad GRK, Varkey J, Chattopadhyay K. Early sodium dodecyl sulfate-induced collapse of α-synuclein correlates with its amyloid formation. ACS Chem Neurosci 2015;6:239–46.