THE POTENTIAL TOXIC EFFECTS OF CEFTRIAXONE AND POLYPROPYLENE MICROPLASTICS ON *DANIO RERIO* (HAMILTON, 1822) BEHAVIOUR

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ABSTRACT

The literature contains numerous studies on the individual effects of microplastics and pharmaceuticals that reach aquatic systems through various pathways. However, there are few studies that evaluate their combined effects. Ceftriaxone (CEF) and polypropylene (PP) are emerging pollutants found in the aquatic environment. The aim of the study was to evaluate the effects of ceftriaxone (50 µg L^{-1}) and polypropylene (2 mg L^{-1}) (individually and in mixture) on the behaviour of *Danio rerio*. The exposure to microplastics and antibiotics, alone or combined, differentially affect *Danio rerio* exploratory and social behaviours, with combined exposure potentially exacerbating behavioural alterations.

RÉSUMÉ: Les effets toxiques potentiels de la ceftriaxone et des microplastiques de polypropylène sur le comportement des *Danio rerio*.

La littérature contient de nombreuses études sur les effets individuels des microplastiques et des produits pharmaceutiques qui atteignent les systèmes aquatiques par divers chemins. Cependant, il existe peu d'études qui évaluent leurs effets combinés. La céftriaxone (CEF) et le polypropylène (PP) sont des polluants émergents que l'on trouve dans l'environnement aquatique. L'objectif de l'étude était d'évaluer les effets de la céftriaxone (50 µg L^{-1}) et du polypropylène (2 mg L^{-1}) (individuellement et en mélange) sur le comportement des *Danio rerio*. L'exposition aux microplastiques et aux antibiotiques, seule ou combinée, affecte différemment les comportements exploratoires et sociaux des poissons-zèbres, l'exposition combinée pouvant potentiellement exacerber les altérations comportementales.

REZUMAT: Efectele toxice potențiale ale ceftriaxonei și ale microplasticelor din polipropilenă asupra comportamentului la *Danio rerio*.

În literatura de specialitate există numeroase studii privind efectele individuale ale microplasticelor și ale substanțelor farmaceutice care ajung în sistemele acvatice pe diverse căi. Cu toate acestea, puține studii evaluează efectele combinate ale acestor substanțe. Ceftriaxona (CEF) și polipropilena (PP) sunt poluanți emergenți găsiți în mediul acvatic. Scopul studiului a fost de a evalua efectele ceftriaxonei (50 µg L^{-1}) și polipropilenei (2 mg L^{-1}) (individual și în amestec) asupra comportamentului la *Danio rerio*. Expunerea la microplastice și antibiotice, singure sau în amestec, afectează diferențiat comportamentele de explorare și sociale la *Danio rerio*, expunerea combinată putând exacerba modificările comportamentale.

INTRODUCTION

The widespread use of plastics and pharmaceuticals in everyday life has increased their emission into wastewater (Hejna et al., 2022). Pharmaceuticals are pollutants that threaten global water quality and human health (Burcea et al., 2020). Conventional wastewater treatment systems are insufficient for removing these contaminants (Rout et al., 2021), and studies report wide variations in pharmaceutical use for human and veterinary purposes worldwide (Hughes et al., 2013). Prolonged exposure to these pollutants can induce sublethal effects on organisms, directly disrupt ecological processes by altering the composition and functionality of communities and indirectly influence population and community dynamics through their impact on organismal behaviour (Fekadu et al., 2019). The increasing occurrence of bacterial resistance to antibiotics in freshwater is a major concern due to potential sublethal effects (Michelangeli et al., 2022). Ceftriaxone (CEF) is a third-generation cephalosporin that is found in concentrations ranging from 0.334 mg L^{-1} in wastewater to 0.059 mg L^{-1} in groundwater (Mohamandi et al., 2023). A study conducted in India found that effluents from healthcare facilities and municipal wastewater treatment plants contained CEF concentrations ranging from 1.25 to 29.15 µg mL^{-1} (Karungamye et al., 2022). Another study conducted in India reported higher concentrations of 58.3 and 59.5 µg L^{-1} (Khorsandi et al., 2019). In Romania, CEF was detected in influent wastewater samples at concentrations of 334 µg L^{-1} (Opriș et al., 2013). Zuccato et al. (2000) detected the antibiotic in concentrations of ng L^{-1} in river waters, drinking water and sediments (Khorsandi et al., 2019). Microplastics (MPs < 5 mm), originating from anthropogenic activities, are toxic pollutants that significantly impact both the environment and human health (Kye et al., 2023). Studies have reported that the toxic effects of MPs on cells, organs, and animals were oxidative stress, DNA damage, organ dysfunction, metabolic disorders, immune responses, neurotoxicity, and chronic diseases (Chahardehi et al., 2020). MPs accumulate in the gastrointestinal tract, gills, and liver of *Danio rerio*, causing tissue damage, oxidative stress, inflammatory response, and behavioural changes (Limonta et al., 2019). Polypropylene (PP), a popular synthetic polymer with exceptional properties, is expected to account for 21.65% of global plastic demand by 2050 (Bobori et al., 2022). Its ongoing production and unregulated disposal lead to environmental pollution through fragmentation in aquatic ecosystems and toxicity to fish due to ingestion. In the aquatic environment of the Yellow Sea, PP is the predominant polymer type, accounting for 55.93% of total MPs, with the fragment shape being dominant at 42% (Choi et al., 2024). As the main types of MPs detected in tropical freshwater lakes north of Chennai, India, polyvinyl chloride (PVC) and PP reached an average of 22×10^3 items m^{-3} (Li et al., 2024). The concentration of PP in wastewater treatment plants was 11.821 µg L^{-1} in Germany, and in the effluent was 35.35 µg L^{-1} (Xu et al., 2023). The concentration of PVC and PP in the surface water of the Pearl River Estuary reached 295-545 U m^{-3}, while in the West River it was 2.99×10^3-9.87×10^3 U m^{-3} (Li et al., 2024). Numerous studies have been conducted on the effects of antibiotics entering aquatic systems through various pathways, but there are few studies on their effects in combination with MPs. MPs and antibiotics are common contaminants in aquatic ecosystems, with MPs adsorbing and transporting antibiotics into aquatic environments (Du et al., 2024). *Danio rerio* is widely used in scientific research as a model organism for drug testing and pollutant toxicity screening due to its notable advantages (Chahardehi et al., 2020), including rapid analysis, transparent embryos, short life cycles, high fertility, and 70% genetic similarity to humans (Chahardehi et al., 2020). *Danio rerio* display complex behaviours such as fear, anxiety-like responses, social interaction, learning, memory, and defense (Johnson et al., 2023), using the novel tank and social preference tests.

MATERIAL AND METHODS

Adult *Danio rerio* were exposed to environmentally relevant concentrations of ceftriaxone (CEF), polypropylene (PP), and their mixture. PP fibers (< 5 mm) from the construction industry and CEF powder for injection/infusion solution were used. Before the experiment, the fish were accommodated for two weeks in an 80 L aquarium and for more three days in 10 L aquariums filled with 5 L of tap water, which was changed daily to ensure that it was stale and chlorine-free. The experimental conditions were a water temperature of 26°C ± 1°C, and a light/dark period of 14/10 hours. The water was changed daily, and the fish were fed twice a day. Four experimental batches (n = 5 fish/batch) were tested: 1 – control batch, 2 – CEF batch, 3 – PP batch, and 4 – mixture batch (CEF + PP). The fish were exposed by direct administration (in water) to the two contaminants (50 µg L^{-1} CEF, 2 mg L^{-1} PP, 50 µg L^{-1} CEF + 2 mg L^{-1} PP) for 96 hours. At the end of the exposure period, behavioural tests (novel tank test and social preference test) were performed using EthoVision XT 17 software (Noldus, Netherlands), and the data were statistically analyzed by ANOVA method using Graph Pad Prism v.9.0 (GraphPad Software, San Diego, CA, USA).

Novel tank test

This test uses various 3D video tracking systems to create swimming paths and analyze various behaviours (Jijie et al., 2023). Fish (n = 20) were placed individually in a transparent aquarium containing 6 L of water. A pretreatment test was performed before the administration of the substances. Behavioural activity was analyzed using EthoVision XT 17 software (Noldus, Netherlands) for 5 minutes. 96 hours after the administration of the treatment, tests were performed again. The study analyzes the effects of ceftriaxone and polypropylene on the swimming performance of *Danio rerio*. The aquarium was divided into two zones (upper and bottom) to provide a detailed assessment of activity (Fontana et al., 2022) (Fig. 1). In view of analyzing behaviour, the following parameters were studied: distance moved/total distance, time spent at the top and bottom of the aquarium, clockwise and counterclockwise rotations, velocity mean, mobility, and immobility.

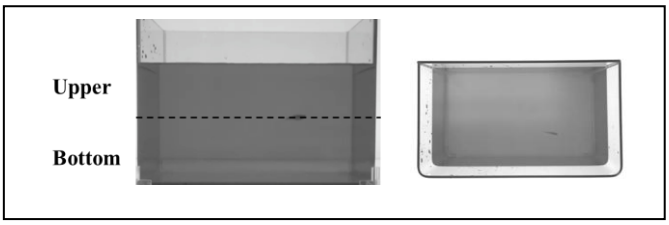

Figure 1: Novel Tank Test - side and top view.

Social preference test

Social preference refers to the preference of fish individuals to live in close proximity to conspecifics. In *Danio rerio*, this behaviour is assessed by social preference test that consist of two operational phases. The first is the habituation phase, in which the test *Danio rerio* is left alone in a test maze to explore the new environment. The second is the interaction phase, which begins with the introduction of the social stimulus consisting of one or usually two conspecifics, or small groups of fish (Ogi et al., 2021).

The social preference test was performed in a modified T-maze, in which *Danio rerio* (n = 20) were placed individually in the starting arm (Fig. 2). The left arm was occupied by other *Danio rerio* (n = 3), separated by a transparent barrier, which served as a social stimulus. The test was performed 96 hours after treatment. Behavioural activity was analyzed using EthoVision XT 17 software (Noldus, Netherlands) for five minutes, and the data were statistically processed using the ANOVA method. In view of analyzing social behaviour, the following parameters were studied: time spent in the starting arm, right arm, and left arm, but also in the decision zone, distance traveled, clockwise and counterclockwise rotations, mean velocity, and movement state.

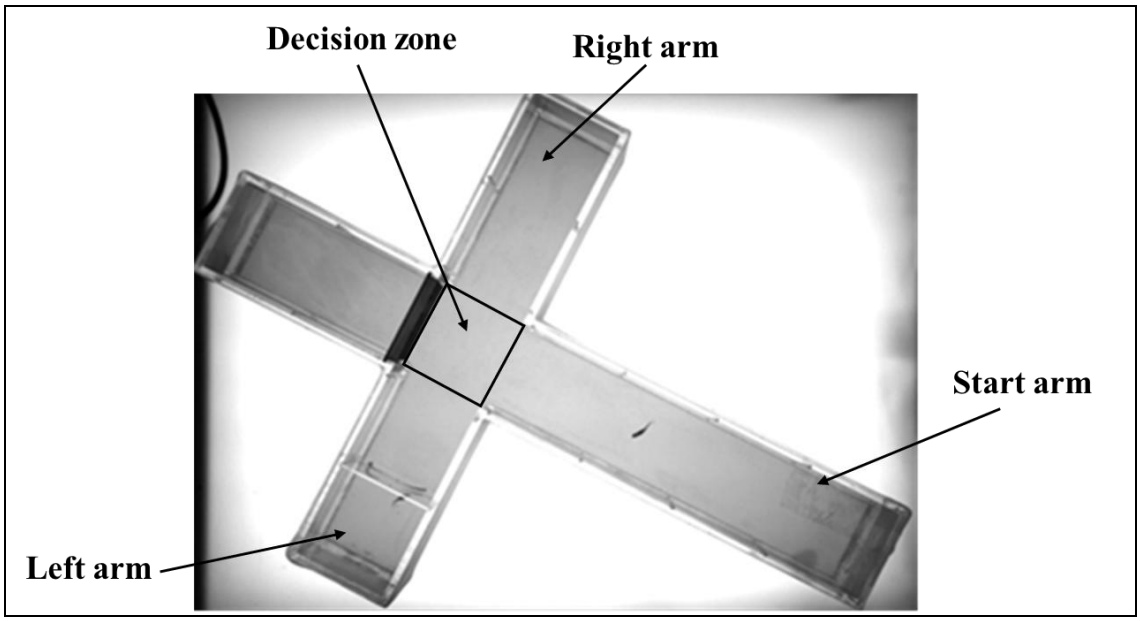

Figure 2: Social preference test – modified T maze.

RESULTS AND DISCUSSION

The results show that exposure to ceftriaxone (50 µg L^{-1}), polypropylene (2 mg L^{-1}), and the mixture (50 µg L^{-1} CEF + 2 mg L^{-1} PP) affected the exploratory behaviour of *Danio rerio*: the CEF and the mixture groups showed an increase in mobility, indicating a possible stress-related behavioural response (Fig. 3).

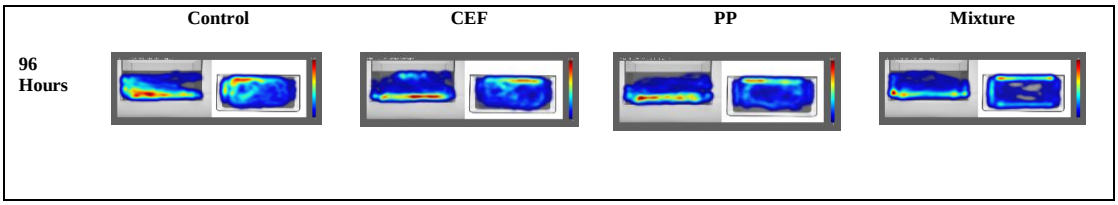

Figure 3: Novel Tank Test:
heatmaps for the control and the groups treated with 50 µg L^{-1} CEF, 2 mg L^{-1} PP,
and mixture (50 µg L^{-1} CEF +2 mg L^{-1} PP)

The novel tank diving test has been widely used and has become one of the two standard tests for anxiety in *Danio rerio* (Haghani et al., 2019). After 96 hours, swimming performance parameters were analyzed (Fig. 4). Regarding average velocity, an increase was recorded for the CEF, the mixture ($p < 0.001$), and the PP – treated group ($p < 0.01$) compared to the control group (Fig. 4A). No substantial variation in distance traveled was observed between the groups after 96 hours ($p > 0.05$), (Fig. 4B). Agostini and co-workers (2020) assert that ceftriaxone can improve exploratory behaviour (impeded by ethanol withdrawal), and enhance glutamate uptake.

In adult *Danio rerio* individuals, reduced mobility is a widely recognized behavioural marker of fear and anxiety – like states (Stewart et al., 2012). Groups treated with CEF ($p < 0.01$) and the mixture ($p < 0.001$) showed a significant increase in mobility, indicating a possible stress-related behavioural response. In contrast, microplastics alone did not significantly alter mobility, suggesting a limited impact on locomotion, at the tested concentration.

In terms of immobility, a statistically significant decrease was noticed for the CEF ($p < 0.01$) and the mixture groups ($p < 0.001$) compared to the control group. Also, no substantial variation in immobility was recorded for the PP – treated group ($p > 0.05$), (Fig. 4D). The study conducted by Shishis et al. (2022) states that immobility is often used as an index of fear or anxiety. However, recent studies have revealed the transmission of fear, where the alarm response of erratic movements and freezing spreads from stressed individuals to others (Burbano et al., 2023).

These results indicate that antibiotics, particularly when combined with microplastics (Du et al., 2024), elicit a pronounced locomotor response in adult *Danio rerio*, but microplastics (Chen et al., 2020) alone exhibit minimal behavioural effects at the tested concentration.

In order to evaluate anxiety indicators, the study also involved assessing the thigmotaxis of *Danio rerio*, an important parameter, which showed significant differences for the anxiety indicators (Fontana et al., 2019). Thigmotaxis represents swimming along the walls of the tank (Jijie et al., 2023). However, in our study a decrease was observed for the PP – treated group ($p < 0.01$) compared to the control group indicating a possible decrease in anxiety – like behaviour or altered risk assessment (Fig. 4E). Moreover, no substantial alterations were noticed for the CEF and mixture treated groups ($p > 0.05$) suggesting that these treatments did not affect swimming along the wall's behaviour despite changes in overall locomotion.

Clockwise and counterclockwise rotations are described in the specific literature as very important markers, suggestive of fish anxious behaviour (Buzenchi et al., 2024), describing *Danio rerio* swimming or turning behaviour (Stewart et al., 2014). A significant increase in the clockwise rotations was recorded for the mixture group ($p < 0.05$) compared to the control group ($p < 0.01$). No substantial variations in the frequency of clockwise rotations were recorded for the CEF and the PP – treated group ($p > 0.05$), (Fig. 4F). Some studies suggest that antibiotic exposure in *Danio rerio* may result in enhanced rotation or turning movements (Suryanto et al., 2022). Concerning the counterclockwise rotations, in our research a decrease was observed for the PP treated group ($p < 0.001$) compared to the control group. No significant increase was identified in the mixture group ($p > 0.05$), while the CEF – treated group showed a no significant decrease ($p > 0.05$) compared to the control group (Fig. 4G).

In this research, clockwise and counterclockwise rotations showed distinct patterns in response to different treatments. This may be due to erratic movements induced by the treatments (Petersen et al., 2022). The mixture group exhibited a significant increase in clockwise rotations, suggesting neurological disruption (Audira et al., 2018), potentially resulting from combined effects of the antibiotic and the microplastic. In contrast, the PP – treated fish group showed a significant decrease in counterclockwise rotations. The CEF group did not demonstrate significant changes in either direction, although trends suggested slight effects.

Danio rerio when are positioned in a new environment (a new tank), instinctively dive imediatelly to the bottom part of the new tank then progressively begin to explore upright levels as perceived anxiety diminishes (Egan et al., 2009). Adult *Danio rerio* who stay at the bottom and slowly habituate to the rest of the tank, was interpreted in the appropriate literature as a precautionary antipredatory response followed by alleviation of anxiety (Haghani et al., 2019).

Regarding the duration of fish swimming in the upper part of the tank, an increase was recorded for the CEF group ($p < 0.001$) compared to the control group. No substantial variation in the duration of fish swimming in the upper part of the tank was noticed for the PP and the mixture groups ($p > 0.05$), (Fig. 4H). In the case of the time spent in the lower part of the tank, a decrease was recorded for the CEF group ($p < 0.001$) compared to the control group. However, no substantial variation was recorded for the PP and the mixture – treated groups ($p > 0.05$), (Fig. 4I).

The significant increase in time spent of fish swimming in the upper part of the tank for the CEF – treated group, along with the corresponding decrease in time in the lower part of the tank, suggests a reduction in anxiety-like behaviour. In *Danio rerio*, increased exploration of the upper tank area is commonly interpreted as a sign of reduced anxiety or increased boldness, since the upper region of the tank is characteristically perceived as much more exposed (Egan et al., 2009). This reduction in anxiety-like behaviour is sometimes abnormal, as the fish may lose the ability to recognize and be familiar with their aquatic environment (Kotova et al., 2023) due to the CEF exposure. In some studies antibiotics like trimethoprim (Suryanto et al., 2022), enrofloxacin (Hillman et al., 2024), oxytetracycline, and ciprofloxacin (Kotova et al., 2023) reduce exploration in the upper part of the tank signifying increased anxiety or reduced exploratory behaviour (Haghani et al., 2019). In *Danio rerio*, increased exploration of the upper tank area is commonly interpreted as a sign of reduced anxiety or increased boldness, since the upper region is typically perceived as more exposed (Egan et al., 2009).

The lack of significant changes in the PP and mixture researched groups indicates specifically that, at the tested concentrations, these treatments did not remarkably disturb anxiety-related vertical fish swimming behaviour. Some other scientific studies suggest that *Danio rerio* individuals subjected to nanoplastics (Sun et al., 2024) exhibited a pronounced inclination towards the bottom regions of the tank, characterized by diminished entry into the higher zones and an extended latency to access the upper sections (Sarasamma et al., 2020).

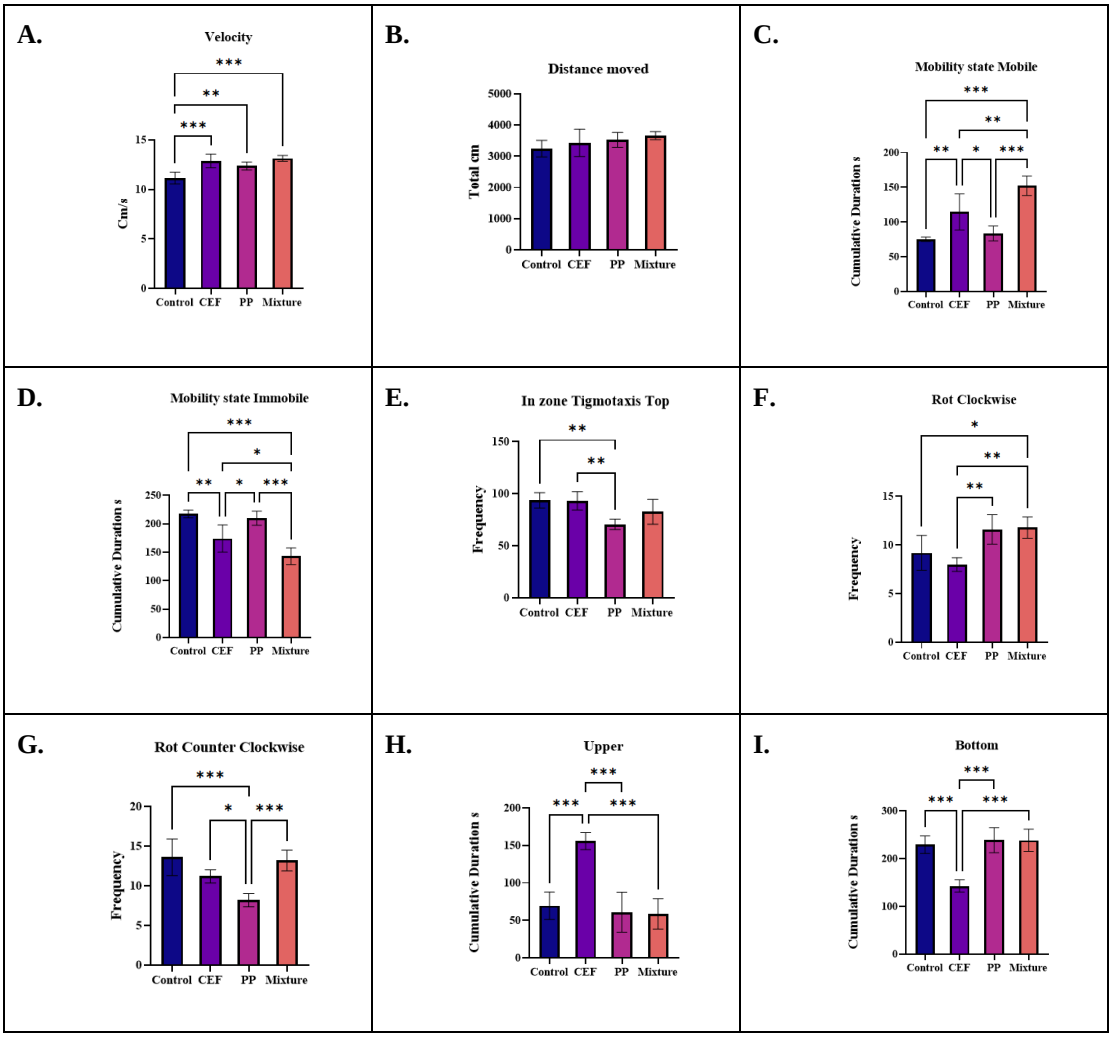

Figure 4: Behavioural parameters of adult *Danio rerio* from the control group and the groups treated with 50 µg L^{-1} CEF, 2 mg L^{-1} PP, and mixture (50 µg L^{-1} CEF +2 mg L^{-1} PP); A – mean velocity, B – distance moved, C – mobility duration, D – immobility duration, E – thigmotaxis, F – clockwise rotations, G – counterclockwise rotations, H – cumulative duration in the upper part of the tank, I – cumulative duration in the lower part of the tank. Data are expressed as mean ± SD and were processed by a one-way ANOVA followed by a Tukey's test. Statistically significant differences are denoted by * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

The results show that exposure to ceftriaxone (50 µg L^{-1}), polypropylene (2 mg L^{-1}), and the mixture (50 µg L^{-1} CEF +2 mg L^{-1} PP) affected the social behaviour of *Danio rerio* (Fig. 5).

The CEF group chose to spend more time in the right arm than in the left arm, compared to the control group, indicating a potential sign of anxiety. The PP group presented social avoidance or impaired sociability.

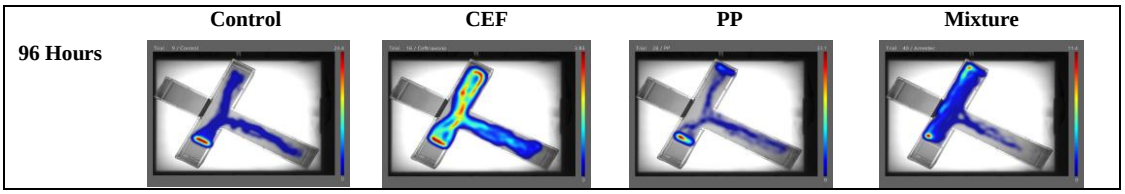

Figure 5: Social Preference Test: heatmaps for the control and the groups treated with 50 µg L^{-1} CEF, 2 mg L^{-1} PP, and mixture (50 µg L^{-1} CEF +2 mg L^{-1} PP).

Danio rerio exhibits clear social behaviours, including interaction with conspecific fish (Kalueff et al., 2013). Some studies suggest that social behaviours in *Danio rerio* can only be evaluated at early juvenile stages (Petersen et al., 2023). From the perspective of behavioural analysis, the social preference test was conducted, but swimming performance parameters were also analyzed (velocity mean, distance, movement), (Swain et al., 2004) to assess whether the presence of other fish influenced swimming behaviour or avoidance responses.

After 96 hours, swimming performance parameters were analyzed (Fig. 6). In the case of mean velocity, a decrease was recorded for PP ($p < 0.05$) compared to the control group. No substantial variations in mean velocity were recorded for the CEF and the mixture groups ($p > 0.05$), (Fig. 6A). The noticed decrease in mean velocity in the PP – treated group suggests that microplastic exposure impairs overall locomotor activity in adult *Danio rerio* (Kalueff et al., 2013). Regarding the distance, an increase was observed for the PP ($p < 0.01$) compared to the control group. The significant increase by the PP – treated group may indicate hyperactivity or increased exploratory behaviour. No substantial variations in the distance were recorded for the CEF and the mixture groups ($p > 0.05$), (Fig. 6B). Chen et al. (2020) found that adult *Danio rerio* exposed to 5 µm polystyrene (PS) microplastics (0.001-20 mg/L) exhibited an increase in swimming distance showing hyperactivity. In contrast, the absence of significant changes in the CEF and the mixture groups suggests that, under the conditions tested, antibiotics alone or combined with microplastics do not markedly affect general swimming performance in the social preference test.

In the case of movement, an increase was noticed for the mixture ($p < 0.001$) compared to the control group. No substantial variations in the case of movement were recorded for the CEF and the PP – treated groups ($p > 0.05$), (Fig. 6C). Regarding the time spent without moving, a decrease was noticed for the mixture ($p < 0.01$) compared to the control group. Also, no substantial variations in the case of time spent without moving were recorded for the CEF and the PP – treated groups ($p > 0.05$), (Fig. 6D). The significant increase in overall movement and corresponding decrease in the time spent without moving observed in the mixture group suggest heightened locomotor activity, which may reflect increased anxiety-like behaviour in adult *Danio rerio* (Kalueff et al. 2013). In contrast, the lack of significant changes in the CEF and PP – treated groups alone suggests that neither treatment individually alters spontaneous movement or immobility under the tested conditions.

The frequency of the fish visits to the decision point area was analyzed after a period of 96 hours, a decrease was noticed for the CEF and PP – treated groups ($p < 0.001$) and an increase was observed for the mixture group ($p < 0.001$) compared to the control group (Fig. 6E). For the frequency of entries to the right arm an increase was observed for the CEF group ($p < 0.01$) and the mixture group ($p > 0.05$) compared to the control group. No substantial variations in the frequency of entries in the right arm for the PP group was recorded ($p > 0.05$), (Fig. 6F).

The frequency of entries in the left arm represents an important parameter in the sociability test. An increase was noticed for the mixture group ($p < 0.001$) and a decrease for the PP group ($p < 0.01$) compared to the control group. No substantial variations in the frequency of entries in the left arm for the CEF group was recorded ($p > 0.05$), (Fig. 6G).

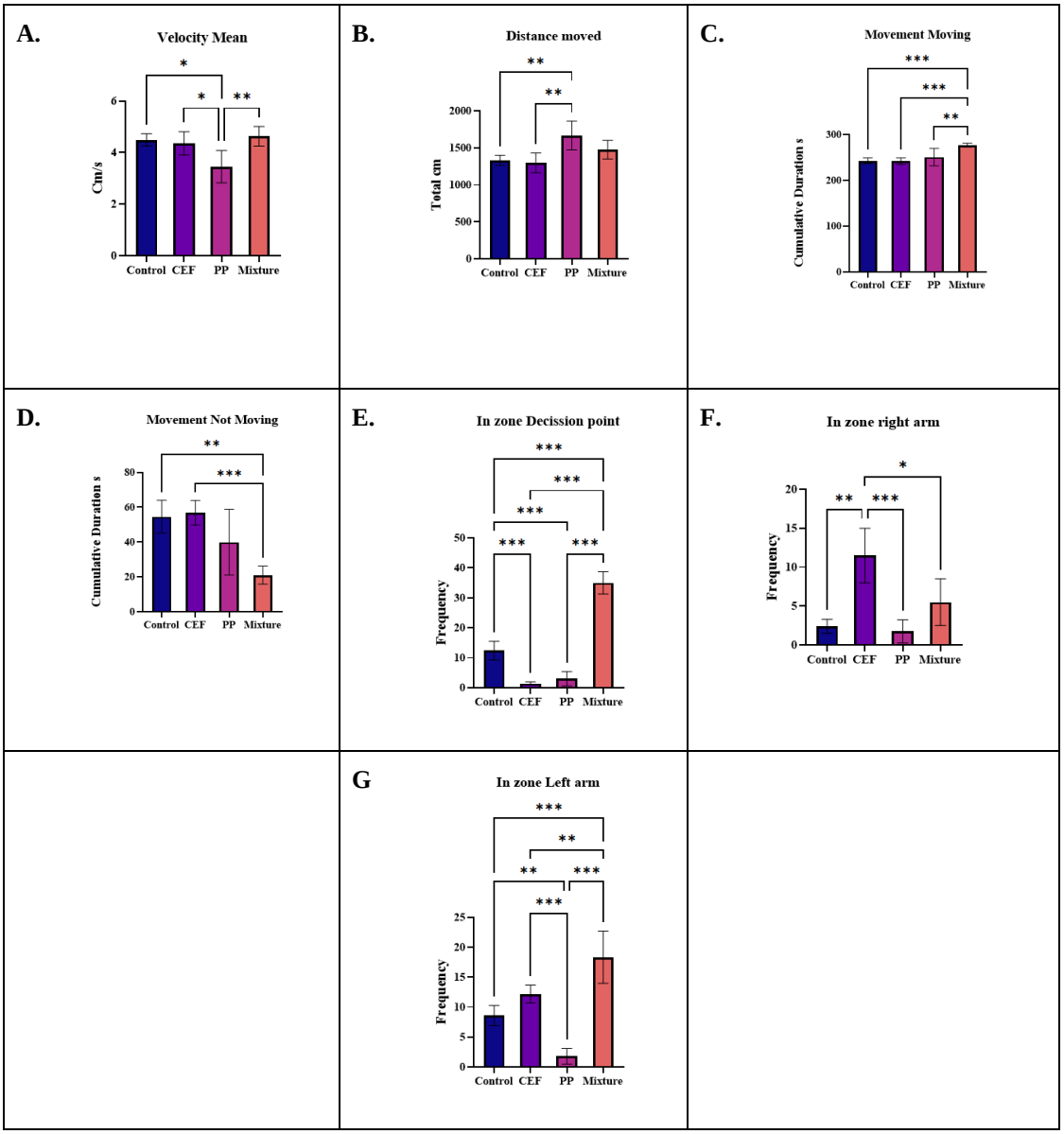

Figure 6: Social behaviour parameters of adult *Danio rerio* from the control group and the groups treated with 50 µg L^{-1} CEF, 2 mg L^{-1} PP, and mixture (50 µg L^{-1} CEF + 2 mg L^{-1} PP). A – Mean velocity, B – Distance moved, C – Mobility duration, D – Immobility duration, E – Decision zone frequency, F – Right arm frequency, G – Left arm frequency. Data are expressed as mean ± SD (n = 5) and were analyzed by one-way ANOVA followed by a Tukey's test. Statistically significant differences are denoted by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

The decreased frequency of visits in the decision point for both CEF and PP – treated groups suggests a reduction in exploratory behaviour or impaired decision-making, possibly reflecting reduced curiosity or increased anxiety (Yang et al., 2025). Furthermore, the mixture group's increased frequency of visits may indicate heightened activity or altered cognitive processing (Kalueff et al. 2013), potentially due to the effects of the combined contaminants. The significant increase in right-arm entries in the CEF group, and a trend toward increase in the mixture group, suggests a possible lateralized preference (Ogi et al., 2021) or altered behaviour, while the PP group showed no significant change.

Importantly, in the sociability test, the mixture group's significantly increased frequency in the left arm suggests enhanced social interaction, whereas the PP group's decrease indicates social avoidance or impaired sociability (Shams et al., 2018). The CEF group's absence of change suggests no substantial effect on social behaviour under the tested conditions.

Overall, these findings imply that exposure to microplastics and antibiotics, alone or combined, differentially affect *Danio rerio* exploratory and social behaviours, with combined exposure potentially exacerbating behavioural alterations.

There are few studies on the effects of ceftriaxone on adult *Danio rerio* behaviour. However, there is one study conducted by Agostini et al., 2020 on the influence of ceftriaxone (100 µM) on behavioural patterns, glutamate transporter activity, and oxidative stress in the brain of *Danio rerio* subjected to ethanol (0.3%) withdrawal. After exposure to 100 µM CEF, ethanol withdrawal reduced anxiety-like behaviour and time spent in the tank, CEF treatment restored exploratory behaviour and reduced glutamate uptake, indicating that the treatment combined with ethanol withdrawal induces anxiolytic-like effects (Agostini et al., 2020).

Oliveira et al. (2023) evaluated the effects of CEF on the development of *Danio rerio* embryos and larvae. However, the results showed that the tested concentrations (0.05 mg/L, 0.5 mg/L, 5.0 mg/L, 50 mg/L, and 100 mg/L) did not cause lethal or sublethal effects. Another study on *Danio rerio* embryos and larvae found that 1, 2, and 5 mmol L^{-1} of CEF and its impurities can increase liver fat, showing potential hepatotoxicity (Rui et al., 2024).

There are studies on the effects of MPs and pharmaceuticals on *Danio rerio* behaviour. A study found no significant differences between the toxic effects of methionine and polypropylene (PP) on social isolation. The data suggests that PP blocks the toxic effects of methionine, reducing anxiety levels in both groups. However, all groups showed an increase in anxiety and aggression (Săvuca et al., 2022). Another study was conducted on the effects of the individual and combined effects of polyethylene (PE), polypropylene (PP), and valproic acid (VPA). Exposure to the PP shows a more agitated behaviour and the mixture decreases the aggressiveness (Săvuca et al., 2024).

In addition to studies on *Danio rerio* behaviour, biochemical analyses were also performed related to oxidative stress markers and enzyme activities. A study examined the effects of exposing juvenile *Danio rerio* to different concentrations of PP microplastics (11.86 ± 44.62 µm) for 28 days, chronic exposure. The study concluded that microplastics bioaccumulate in organisms, leading to oxidative stress and liver tissue damage (Priyadharshini et al., 2024). *Danio rerio* embryos exposed to varying concentrations of PP exhibited increased body weight, reduced heart rate, and impaired behaviour. Transcriptomic analysis indicated potential inhibition of glycolysis/gluconeogenesis and oxidative phosphorylation pathways. Abnormal mitochondrial ultrastructures were observed in the intestine and liver, and oxidative stress was induced, with increased levels of reactive oxygen species (ROS) and reduced activities of catalase (CAT), superoxide dismutase (SOD), and glutathione GSH (Hua et al., 2024)

In another study, the toxic effects of MPs (polyethylene-PE, polyethylene terephthalate-PET, polypropylene-PP, and polystyrene-PS) on *Danio rerio* embryos and larvae were evaluated. The results showed that MPs had no impact on embryonic development at low concentrations, but increased heart rate and death when exposed to SiO_2, PE, and PS. However, SiO_2 and microplastics inhibited larval locomotion and acetylcholinesterase (AChE) activity (Chen et al., 2023). *Danio rerio* were exposed to polyvinyl chloride (PVC) or polypropylene and cadmium (Cd) for 28 days, which exacerbated the effects of Cd on their intestines. The synergistic toxicity of PP and Cd is higher than that of PVC (Li et al., 2024). UV-weathered polypropylene induced substantial biological consequences, encompassing intestinal damage, heightened oxidative stress, elevation of inflammatory gene expression, and alterations in gut microbial diversity (Choi et al., 2024).

Some specific studies have evaluated the adverse effects of polystyrene and polypropylene fragments on the microalgae *Raphidocelis subcapitata* (72-hour growth inhibition assay) and on *Danio rerio* embryos (96-hour exposure) at environmentally relevant concentrations of 2000-200000 microplastics/L^{-1} and 12.5-100 mg L^{-1}. In algae, the high concentrations tested promoted growth, while the environmentally relevant concentration induced either inhibition or promotion of growth, and in the case of *Danio rerio* embryos, environmentally relevant concentrations decreased body length and heart rate (Prata et al., 2022).

The toxic effects of five types of MPs were analyzed: polyamide (PA), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS) particles. MPs caused intestinal damage, and exposure to 5.0 mg m^{-2} of MPs for 2 days significantly inhibited survival rates, body length, and reproduction in *Caenorhabditis elegans* tissues (Lei et al., 2018).

According to a study using *Danio rerio* as a model organism to investigate the toxic effects of PP MPs on the intestines and liver, an increase in Rhizobiales order and a decrease in *Cetobacterium* spp. in the intestine were demonstrated, as well as changes in hepatic pathways (Tian et al., 2024). Another research evaluated the cytotoxicity of 8-10 μm PP MPs in the liver and gill tissues of *Danio rerio* and *Perca fluviatilis* at concentrations of 1 mg/g (low dose) and 10 mg/g dry food (high dose). The results showed that PP impaired cellular function through lipid peroxidation, DNA damage, protein ubiquitination, apoptosis, autophagy, and changes in metabolite concentrations (Bobori et al., 2022).

CONCLUSIONS

This preliminary study provides insight into the behaviour of adult *Danio rerio* individuals exposed to environmentally relevant concentrations of ceftriaxone and polypropylene. These results show that antibiotics, particularly when combined with MPs, elicit a pronounced locomotor response in adult *Danio rerio*, but MPs alone exhibit minimal behavioural effects at the tested concentration, in the novel tank test.

In social preference test, MPs and antibiotics, alone or combined, differentially affect *Danio rerio* exploratory and social behaviours, with combined exposure potentially exacerbating behavioural alterations.

When combined, these results suggest that antibiotics alone may reduce anxiety-like behaviour, MPs impair motor and social functions, and their combination alters multiple behavioural parameters, highlighting the importance of studying mixture effects in environmental toxicology.

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