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Evaluation of Toxicological Risks and Effects of Microplastics on Nile Tilapia (*Oreochromis niloticus*) under in Vitro Laboratory Conditions

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ABSTRACT

Microplastics have been reported by many literatures as contaminants of environmental water bodies and are ingested by aquatic organisms due to their small sizes. Knowing the effects of microplastics to fresh water fish which are kept in ponds helps in managing fish keeping practice. The objective of research was to determine the toxicity of microplastics to Nile tilapia (*Oreochromis niloticus)*. The Experiment was done in 80 fish samples. Microplastics which were prepared for the batch experiment were introduced in the aquarium followed with observation for 21 days. The digestion of fish gills and intestines involved 10 mL of 10% (w/v) KOH solution and incubation at 65^{o}C for 24 hours. Engulfed microplastics were determined using stereo microscope and At-IR spectrophotometer for confirmation. Engulfed microplastics were observed to be in mean range of 3.37 x 10^{2}±4.04 x 10^{2} to 2.32 x 10^{3} ± 3.57 x 10^{3} particles/kg in gills and 4.68 x 10^{4} ± 3.02 x 10^{4} to4.40 x 10^{4} ± 5.34 x 10^{4} particles/kg in intestines. The observed responses were loss of equilibrium for 35% of fish, abnormal swimming for 49% of fish, abnormal ventilator behavior for 59% of fish, abnormal appearance for 39% of fish and average growth weight increase in control experiment fish was 6.10±2.62 g compared to 1.7 ± 3.62 g in test fish. There was no mortality of Nile tilapia. The responses of fish to the presence of microplastics in aquarium indicated that microplastics had adverse effects to Nile tilapia (*Oreochromis niloticus*). More researches have to be done on fish physiological changes caused by microplastics.

INTRODUCTION

Plastics are produced in large amount for different applications, but have been accompanied with waste deposition in urban areas (Moore, 2008; Zhang, 2017). About 70 to 80% of plastic contaminants originate from anthropological activities (Mvowiec, 2017). Plastic wastes in environments occur in different sizes, the smallest forms are called microplastics (Sadri and Thompson, 2014; Thakur *et al*., 2022). When large proportions

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of plastic wastes are mismanaged, they enter the environments where they evolve into microplastics via progressive fragmentation through the process of bio-degradation, photo-degradation, thermo-oxidative degradation or mechano-chemical degradation (Mvowiec, 2017; Guilhermino, 2021).

Microplastics have been found in marine biota because of their presence in ecosystems (Mistri, 2022). Animals exposed to microplastics under laboratory setting have shown several adverse effects like histological alterations, lesions in the gastrointestinal tract, intestinal inflammation, neurotoxicity, oxidative stress, damage, immuno-regulation, feeding behavioral change, and developmental alterations (Jovanovic, 2017). Oxidative stress and inflammation are caused by generated reactive oxygen species (ROS) (Subaramaniyam et al. 2023;Yao *et al*., 2023).

In urban areas, there are various anthropogenic activities which lead to microplastic accumulation in water bodies in which fish are found living (Khan *et al*., 2018; Mayoma *et al.*, 2020). Fish can be easily stressed due to exposure to toxic substances in water (Reverter, 2018). The reported clinical signs by literatures on fish responses to abnormal water conditions have not been well documented on fresh water fish commonly known as Nile tilapia (*Oreochromis niloticus*) once exposed to microplastics. Fish keeping is highly encouraged and takes place in ponds in various tropical regions in Africa where Tanzania is among of them. With knowledge that fresh water ponds are susceptible to microplastic pollution in urban regions, therefore there was need of finding out how far the microplastic exposure to fresh water fish, especially Nile tilapia could have effect. The research was conducted to find out how fish commonly known as Nile tilapia were affected by microplastics and to evaluate their effects by *in vitro* observation in laboratory**.**

MATERIALS AND METHODS

Materials

An Aquarium was made of glass (30 cm x 30 cm x 60 cm), with a capacity of 40 L. Two aerators, two air and water filters, a wire mesh covering, and an aquarium fish net from the fish equipment business shop were used to support the aquarium. A laboratory thermometer and a multimeter for pH, water conductivity and Total dissolved solids (TDS) were obtained from the laboratory. Plastic buckets, (each 20 L), and a sieve were bought from the domestic shops. Fish feeds were bought from the animal feed shops. Stereo microscope, hand lens, and 100 mL beakers were supplied by the laboratory. Potassium hydroxide (Analar compound) and Standard microplastics: Polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), nylon 6, 6 and polyethylene terephthalate (PET) were supplied by Precur Chem and Equipment Ltd, Tanzania.

Samples of fish for toxicological study

Samples of Nile tilapia for microplastic toxicological study were collected from Kibaha Fish Farming Ponds in Coast Region, Tanzania. The selection of this freshwater fish was based on the fact that it is commonly kept in most fish farmers in tropical regions due to the ability of withstanding extreme conditions like high temperature, change of pH or

changes of total dissolved solids. The fish have also ability to survive during transport and the period of caring and observation. Small fish aged two months old (length 12 mm−14 mm and/ or weight 30 g–50 g) were captured and collected using nets. The fish were captured during the sunset and were kept in ponds within nets until early morning before sunrise where they were collected in two buckets of (20 L) each containing 10 fish. Fish in one bucket was used for test experiment while in another bucket was for the control experiment. All the experiments involving fish samples were performed in batches of 20 fish and a total of 4 batch samples were collected. About 4 L of water from the pond was added in each fish bucket for maintaining fish medium.

Preparation and aquarium maintenance

The test and control aquaria were set near the window where sun rays did not struck directly. Two aerators for each aquarium were connected to the filters, and then placed in the aquarium using aquarium fish net. Clean tap water was half-filled n each aquarium one day before fish introduction. The water in the aquarium was left overnight in order to dechlorinate. In the next day, aeration was performed to increase the amount of oxygen and filtration was done for half an hour to remove some suspended solids. Thereafter, room temperature, pH, conductivity and total dissolved solids (TDS) of the water in the aquaria and the buckets (from the fish ponds) were measured. Each fish was weighed to obtain initial weight (w_1) and length (l_1) prior to placing in the aquarium. The fish were placed in the separate aquaria then were left to acclimatize for 20 min. Then water from fish farm ponds in the buckets was mixed with de-chlorinated aquarium tap water which was already in the aquarium in order to fill the left space. The purpose of retaining the pond water was to keep the association bacteria (symbiosis) used by fish in their life. Lastly, each aquarium was covered with wire mesh in order to ensure fish safety. The fish in both aquaria were starved for one day, then fed with ground fish feed pellets 10% (w/w). The fish were left to acclimatize for nine days before feeding with microplastics. The fish life was maintained by continuous aeration of the aquarium water, and measuring pH, water, room temperature, conductivity and TDS. The nitrate levels were controlled by dilution with 25% of clean water after every two days. The gravel was set as aquarium bed in order to trap suspended solids and feces. The feces were removed after every two days by siphoning.

Exposure of Nile tilapia to microplastics

Microplastics with size of 30 µm for PVC, and 1000 µm for PET, 2000 µm for PE, 2000 µm for PP and 600 µm nylon 6,6 were mixed together in a 250 mL beaker followed with mixing with water to form a suspension. The microplastics were prepared in different concentration dose for exposure to fish; 2.7 x 10^7, 9.0 x 10^8, 1.3 x 10^8 and 6.7 x 10^7 particles/m^3. The prepared micro plastic suspension was introduced in the test aquarium on the tenth day. The aquarium contained fish of average weight 40 g. Then the observation was performed for 21 days according to the Organization for Economic Co-operation and Development (OECD, 2019). The behaviours (responses) of fish in the test aquarium were compared with the fish in the control aquarium. The toxic effects of

micro plastics on fish were observed by noting and recording the number of individuals affected using some established clinical signs (Table 1) as stipulated in the OECD (2019). Furthermore, the physical characteristics of the aquarium media (pH and conductivity), weight of fish, and concentration of microplastics were observed.

Table 1. Clinical signs used in observation of responses of Nile tilapia to microplastics exposure

Observation	Clinical Sign
Loss of Equilibrium	Abnormal horizontal orientation
	Abnormal vertical orientation
	Loss of buoyance control (floating at surface or sinking to the bottom)
Abnormal swimming behavior	Hypo activity (Decrease in spontaneous activity)
	Hyperactivity (Increase in spontaneous activity)
	Corkscrew swimming (Rotation around long axis, erratic movement)
	Abnormal surface distribution (abnormal depth selection, close to water air interface
	Abnormal bottom distribution/behaviour abnormal depth selection, bottom of tank)
	Over-reactive to stimulus (flight, or avoidance response to: visual, tactile touch) or vibration stimulus
	Loss of schooling/shoaling behaviour (individual fish show, loss of aggregation and social interaction)
	Dense schooling/shoaling behaviour (increase in clumped association of fish)
Abnormal ventilator (Respiratory) function	Hyperventilation (increase in frequency of opercula ventilator movements with possible open mouth and extended opercula)
	Hypoventilation (decreased frequency of opercula ventilator movements).
	Coughing (fast reflex expansion of mouth and opercula not at water surface-assume to clear ventilator channels)
	Gulping (mouth movements at water surface resulting in intake of water and air)
	Head shaking (rapid lateral head movements)
Abnormal skin pigmentation	Darkened (malefic markings)
	Lightened (weak pigmentation)
	Mottled (discoloration)
Appearance and Behaviour Abnormalities	Oedema (abnormal swelling due to accumulation of fluids)
	Haemorrhage (sub-mucus bleeding)
	Faecal (anal) casts (string of faeces hanging from anus or on tank floor)

Source: OECD (2019).

Analysis of engulfed microplastics

After 21 days, all fish from test and experiment aquaria were weighed for final length and weight, and then were dissected to collect intestinal parts and gills. The gills and intestines were separately digested using 10% KOH solution (10% (w/v) following with incubation at 65^{o}C for 24 hours. Later, distilled water was added to dilute the unused KOH before filtration. The particles on the filter paper were washed thoroughly with water during filtration. A stereo microscope (10 x magnifications) was used to count the microplastics which was performed first by placing all microplastics in the petri-dish which had four partitions prepared by lines of

pen-ink. Secondly, the petri-dish was placed on the microscope stage for visualization and counting of microplastics in all petri-dish compartments (Masura *et al*., 2015). Further confirmation of the microplastics was done using Attenuated Fourier Transform Infrared Spectrophotometer instrument (At-FT-IR, Bruker, Massachusetts, USA).

Data analysis

Excel descriptive statistics in analysis ToolPak was used to analyse row data for mean, range and standard deviation of microplastics engulfed by Nile tilapia. Pearson correlation was used to determine the relationship between microplastics which were engulfed and responses of Nile tilapia. Student t-test (n = 20)was used to determine the significant difference of effects of engulfed microplastics on Nile tilapia and significant difference in growth among the microplastic fed fish (tested fish) and fish which were not fed with microplastics (non-tested fish).

RESULTS

Water temperature, pH, total dissolved solids (TDS) and electric conductivity

Water temperature, electric conductivity, pH and total dissolved solids were monitored as part of water quality assurance for fish growth in both test and control experiments. Ammonia content in aquarium which was caused by defecation and food remains was controlled by diluting water and siphoning out after every two days of experiments. Dilution and siphoning were also means of regulation of pH, TDS and conductivity. The room temperature was controlled by air circulation using fans and opening windows. The room temperature therefore, ranged from 24.8 to 27^{o}C and water temperature ranged from 24.00 to 26.00 ^{o}C. The temperature range was within the water quality requirement for fish farming, which is 24^{o}C to 30^{o}C (Ezeanya *et al*., 2015). The water conductivity (mean ± standard deviation) in tested fish had changed by 62% (465 ±117.15 µs/cm) from the pond water conductivity (750±254 µs/cm), the pH had changed by 0.9% (6.8 ± 0.4) from the pond pH (6.7 ± 0.2) and TDS had changed by 63.9% (236.5 ±32.4 ppm) from the pond TDS (370.0 ± 127.3 ppm). The water conductivity in non-tested fish had changed by 64% (480 ± 80.1 µs/cm) the pond water measurements were 750±254 µs/cm (electric conductivity), pH had changed by 0.9% (6.8 ± 0.2) from the pond pH, 6.7 ± 0.2, and total dissolved solids had changed by 72% (269.75 ±104.85 ppm) from the pond TDS, 370.0 ± 127.3 ppm. There was no significant difference between water conductivity (t-test, p = 0.7, df = 40), total dissolved solids (t-test, p = 0.09, df = 40), and pH (t-test, p = 0.05, df = 40) in test and control experiments. The recommended pH range for fish growth is 6−9, electric conductivity is 100−2000 µs/cm (Ezeanya *et al*., 2015) while total dissolved solids is 500 ppm−1500 mg/L (Scannell and Jacobs, 2001).

Microplastic abundance and wet weight change of Nile tilapia

Microplastics were found in both intestines and gills of the tested fish in great abundance. Polyvinyl chloride microplastics were the most abundant because of the size (30 µm) which was smaller than other microplastic sizes. Other microplastics with size 600 µm to 2000 µm were not determined in both gills

and intestines. The microplastic concentrations in intestines were in concentration range of 4.68 x 10^3 ± 3.02 x 10^3particles/kg to 4.40 x 10^4±5.34 x 10^4 particles/kg (mean ± standard deviation) and in gills with concentration range of 3.37 x 10^2 ± 4.04 x 10^2particles/kg to 2.32 x 10^3 ± 3.57 x 10^3 particles/kg (Table 3).

The wet weight concentration (mean ± standard deviation) increase for test fish was 1.7± 0.66–3.66±0.74g and for non-test fish was 5.2 ± 1.85–6.54 ± 2.11 g. The results indicated that the non-test fish had great increase in wet weight compared to test fish (Table 2) although the statistical comparison (ONE way-ANOVA) indicated that there was no significant difference in growth changes (df = 79, p = 0.9) between test fish and non-test fish. The change in growth length was high in non-test fish. There was significant difference ($p<0.05$, p = 0.00, t-test) in the effects of microplastics among test fish, also there was medium correlation (r = 0.3, p = 0.00) between microplastic effects on fish weight changes. The change in growth weight of test fish gave scattered cycles which were underlying straight line to show linear relationship between the two variables. The relationship between growth weight change and microplastics did not depend on concentration of microplastics, which means even low concentration could affect negatively the fish growth (Figure 1).

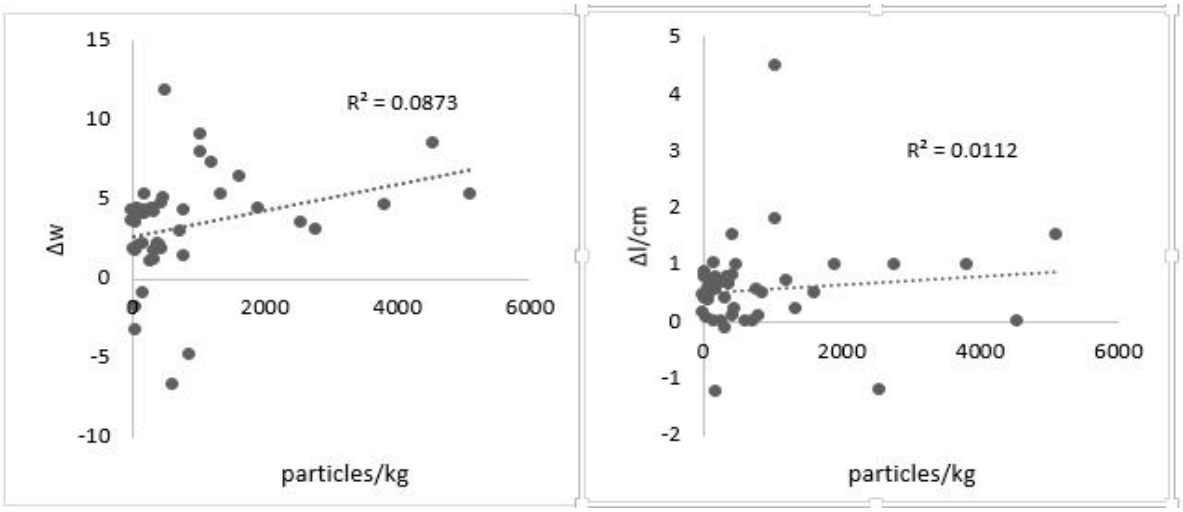


Figure 1. Relationship between (a) Growth change in weight and microplastic concentration (b) Growth length change and microplastic concentration

The change in growth length of test fish had a range of 0.1 ± 0.06 to 1.21 ± 1.16 cm (mean ± standard deviation) compared to the non-test which had range of 0.62±0.12 to 1.7±1.0 cm (Table 2). The mean length growth change in test fish was smaller compared to non-tested fish because of some fish which had fin rot which resulted to loss of tails. The results indicated that there was small correlation (r= 0.1, p = 0.00) between microplastic effects on fish length changes. The change in growth length of tested fish gave scattered cycles which were underlying straight line to show linear relationship between the two variables. The relationship between growth length

change and microplastics did not depend on concentration of microplastics.

Table 2. Engulfed microplastics in gills and intestines and fed microplastics and Comparison of mean change in fish weight (g), change in fish length (cm) between tested and non-tested fish in relation to the engulfed MPs (particles/kg)

Engulfed microplastics (mean ± standard deviation) in gills and intestines together with fed microplastics			
Exp	**Exposed Ps/m^3**	**Gills, Particles/kg**	**Intestines**
1	2.7×10^7	$3.37 \times 10^2 \pm 4.04 \times 10^2$	$4.68 \times 10^3 \pm 3.02 \times 10^3$
2	9.0×10^7	$9.95 \times 10^2 \pm 9.70 \times 10^2$	$5.50 \times 10^3 \pm 9.31 \times 10^3$
3	1.3×10^8	$1.79 \times 10^3 \pm 1.88 \times 10^3$	$4.40 \times 10^4 \pm 5.34 \times 10^4$
4	6.7×10^8	$2.32 \times 10^3 \pm 3.57 \times 10^3$	$1.08 \times 10^4 \pm 1.00 \times 10^4$

Comparison of (mean ±standard deviation) change in fish weight (g), change in fish length (cm) between tested and non-tested fish in relation to the (mean ±standard deviation) engulfed microplastics (particles/kg)					
Exp	**Change weight**		**Change in Length**		**Engulfed particles**
	Tested fish	**Non-Test fish**	**Tested fish**	**Non-Test fish**	
1	1.75 ±0.66	5.2 ±1.88	0.46±0.42	0.65±0.34	$5.03 \times 10^3 \pm 3.07 \times 10^3$
2	3.66 ±0.74	6.54 ±2.11	0.6±0.18	0.62±0.12	$3.58 \times 10^3 \pm 9.94 \times 10^3$
3	2.53 ±2.9	6.52 ±2.32	1.21±1.16	0.8±0.5	$4.59 \times 10^4 \pm 5.36 \times 10^4$
4	1.7 ±3.62	6.10 ±2.62	0.1±0.06	1.7±1.0	$2.33 \times 10^4 \pm 3.85 \times 10^4$
Mean	2.41±0.92	6.09±0.54	0.57±0.49	0.95±0.53	$1.94 \times 10^4 \pm 1.71 \times 10^4$

Biological Effects of Microplastics on Nile tilapia

The microplastic effect was found in the fourth day from the test initiation. The fish which were tested with microplastics and indicated loss of equilibrium were 4±1 fish (mean ± standard deviation), abnormal swimming behavior were 4±2 fish, abnormal ventilatory function were 6 ± 4 fish and abnormal external appearance were 3±2 fish (Table 3).

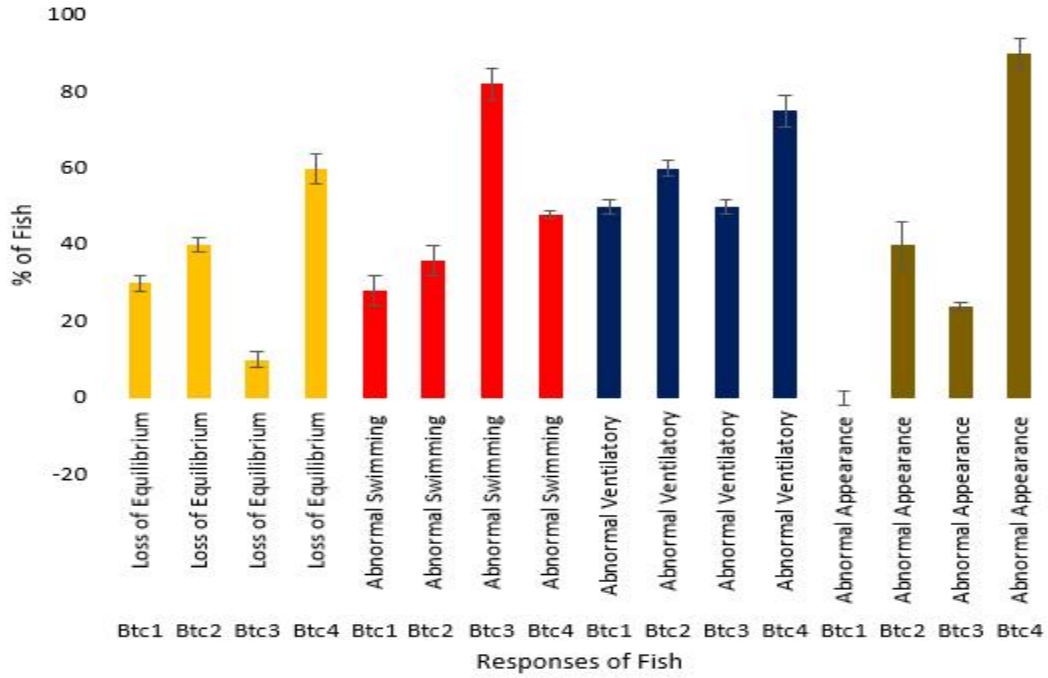
Table 3. The number (mean ± standard deviation) of Nile tilapia which had positive responses on microplastic exposure and the observed clinical signs.

Observation	**Abnormal clinical sign**	**Number of fish**
Loss of Equilibrium	Horizontal Orientation	4 ± 2
	vertical orientation	4 ± 2
	Loss of buoyancy control	4 ± 2
Swimming Behavior	Hypo activity	4 ± 4
	Hyperactivity	5 ± 4
	Surface distribution	6 ± 4
	Bottom distribution	2 ± 4
	Dense schooling	8 ± 1
Ventilatory/Respiration Function	Hyperventilation	9 ± 2
	Hypoventilation	1 ± 2
	Irregular ventilation	6 ± 2
	Gulping	9 ± 4
Abnormal skin pigmentation	Lightened	1 ± 2
External Appearance	Excess mucus secretion	5 ± 6
	Faecal casts (anal)	1 ± 1
	Fin rot	3 ± 4

Some tested fish with difficulty in ventilation by gulping behaviour and selection of space aggregated in one space near the aquarium wall, and some fish had fin rot compared to the non-tested fish without microplastics. However, results did not involve any mortality cases of fish because they were not observed in experiments. The No Observable Effect Concentration (NOEL) was assumed to correspond to the highest concentration tested which was 4.40 x 10^4 particles/kg similar to the report by Hasselerharm (2022).

Evaluation of microplastic toxicity on Nile tilapia

The percentage of fish which had abnormal external appearance was high, followed by abnormal swimming behaviour, abnormal ventilatory function and lastly loss of equilibrium (Figure 2).



Key: Btc is for butch number

Figure 2. Percentage of Fish with their response to Microplastics

The loss of equilibrium was determined by observing fish which had shown clinical signs, namely abnormal horizontal orientation, abnormal vertical orientation and loss of buoyance which was in 35% of fish. The percentage of fish which had shown loss of equilibrium gave scattered cycles which were underlying straight line to show linear relationship between the two variables. The relation to engulfed microplastics had a large negative correlation coefficient, r = -0.7, p = 0.14. Therefore, there was a large relationship between percentage of fish which had loss of equilibrium and the engulfed microplastics (Figure 3).

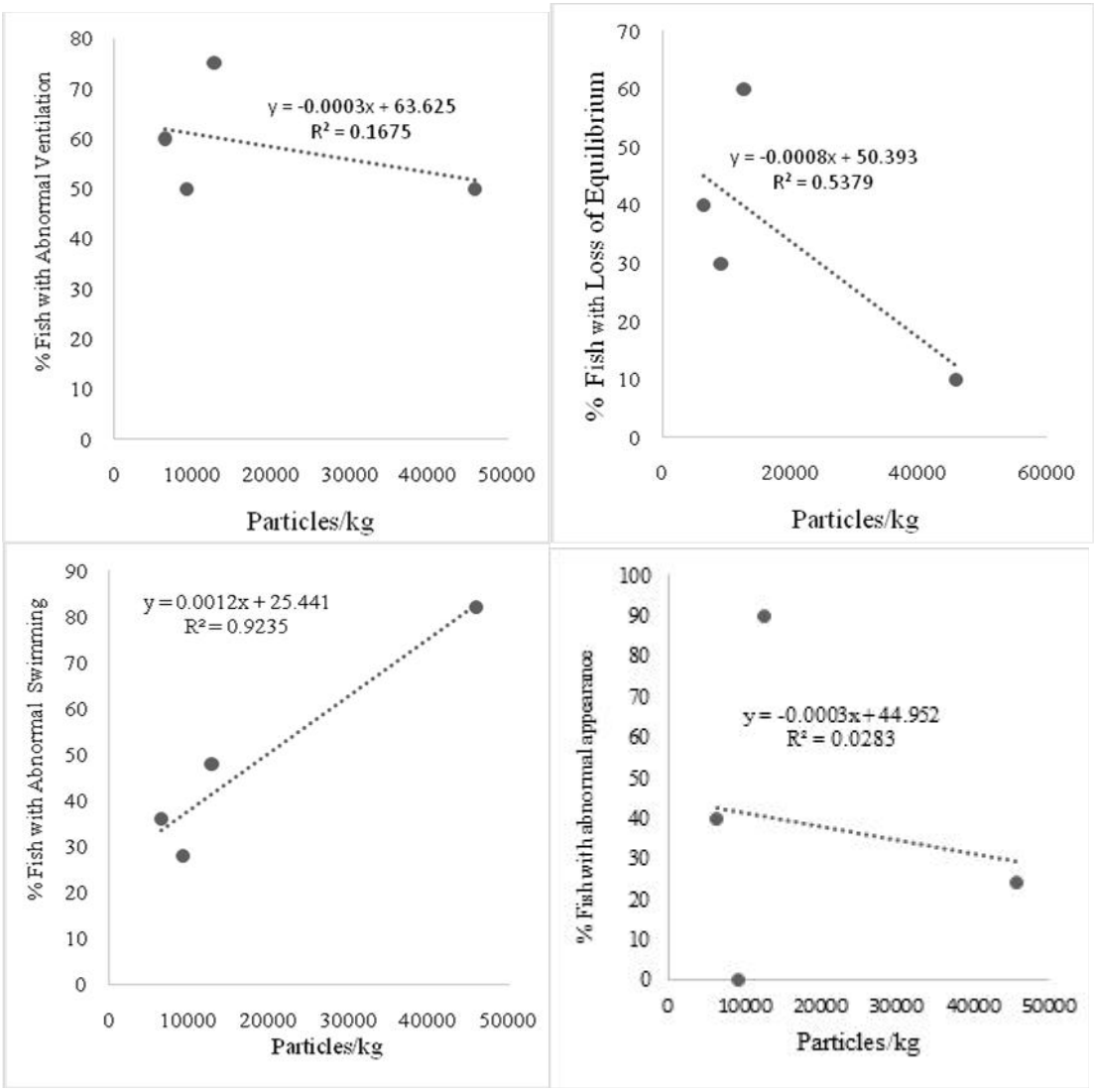


Figure 3. Relationship between fish with abnormal appearance and concentration of engulfed microplastics

The abnormal swimming in fish was observed in 48.1% of fish. The clinical signs which were observed in fish for abnormal swimming were: Hypo activity, abnormal bottom distribution and dense schooling or shoaling. The percentage of fish which had abnormal swimming indicated relationship with engulfed microplastics (Figure 3), where the clustered cycles were close to the straight line. The correlation coefficient, r = 0.9, p = 0.14 indicated presence of large relationship between the percentage of fish with abnormal swimming and engulfed microplastics.

Abnormal ventilation in test fish was observed in 58.8% of fish. The clinical signs for abnormal ventilation observation were: hyper ventilation and hypoventilation. The percentage of fish which had abnormal ventilation indicated a relationship with number of engulfed microplastics (Figure 3)

where the clustered cycles were close to the straight line. The correlation coefficient, r = -0.4, p = 0.13 indicated the presence of medium correlation between percentage of fish with abnormal ventilation and engulfed microplastics.

Abnormal appearance of test fish was not observed in 38.5%. The clinical signs were; oedema, fin rot, and excessive mucus on epidermal part. The clustered cycles were close to the straight line to show close relationship between percentage of fish with abnormal appearance and engulfed microplastics (Figure 3). The correlation coefficient, r = -0.17, p=0.13 indicated small relationship between percentage fish with abnormal appearance and engulfed microplastics.

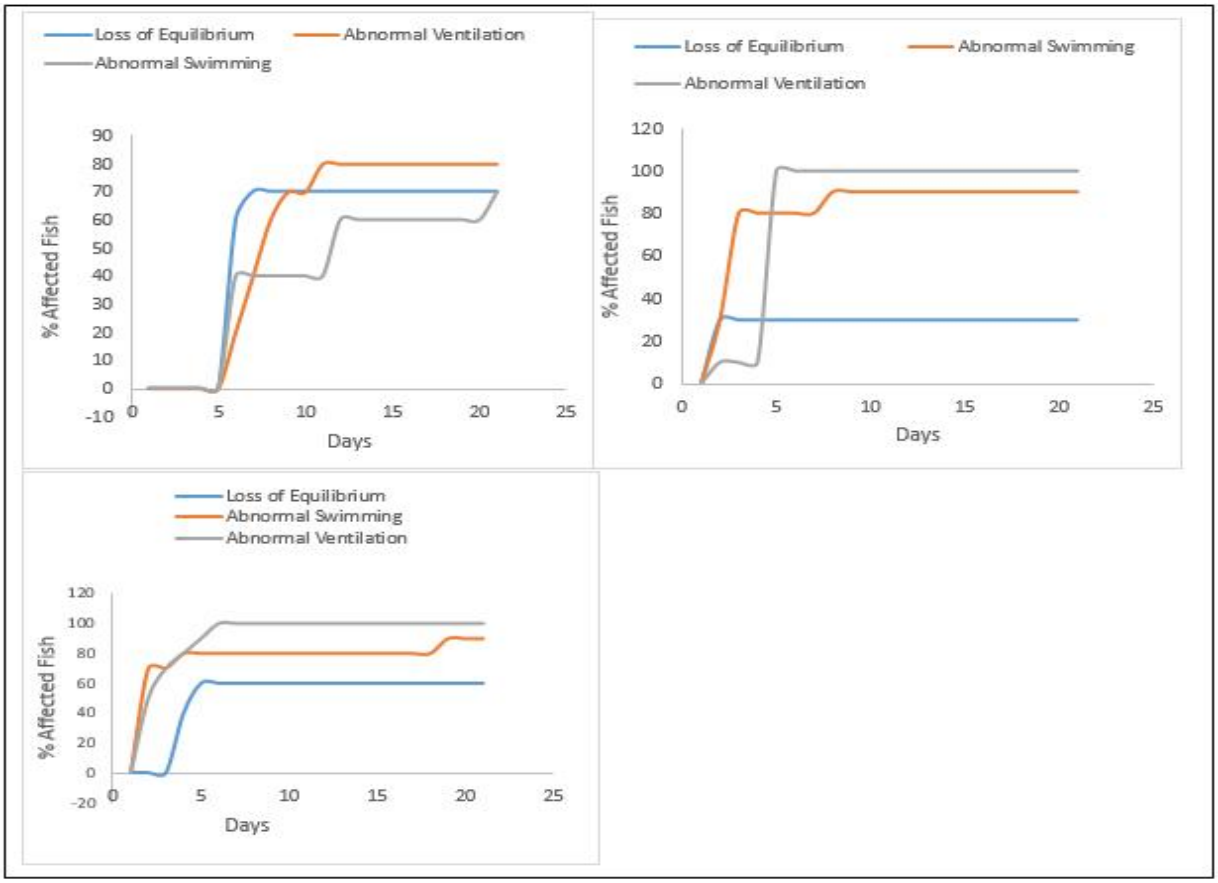


Figure 4. Responses of fish to microplastic exposure in four different experiments

The responses of fish on exposure to microplastics was monitored daily and results indicated that the percentage of tested fish which had positive responses started within day 3 to day 5 from the day microplastics were introduced, then increased to the maximum in 10 days (Figure 4). Among the fish which were exposed to microplastics, 72.5% of them had small weight and 75% had smaller length growth compared to fish which were not exposed to microplastics. The observation was made for daily increase of wet weight in both tested fish and non-tested fish.

The daily wet weight increase in tested fish was low compared to the non-tested fish (Figure 5).

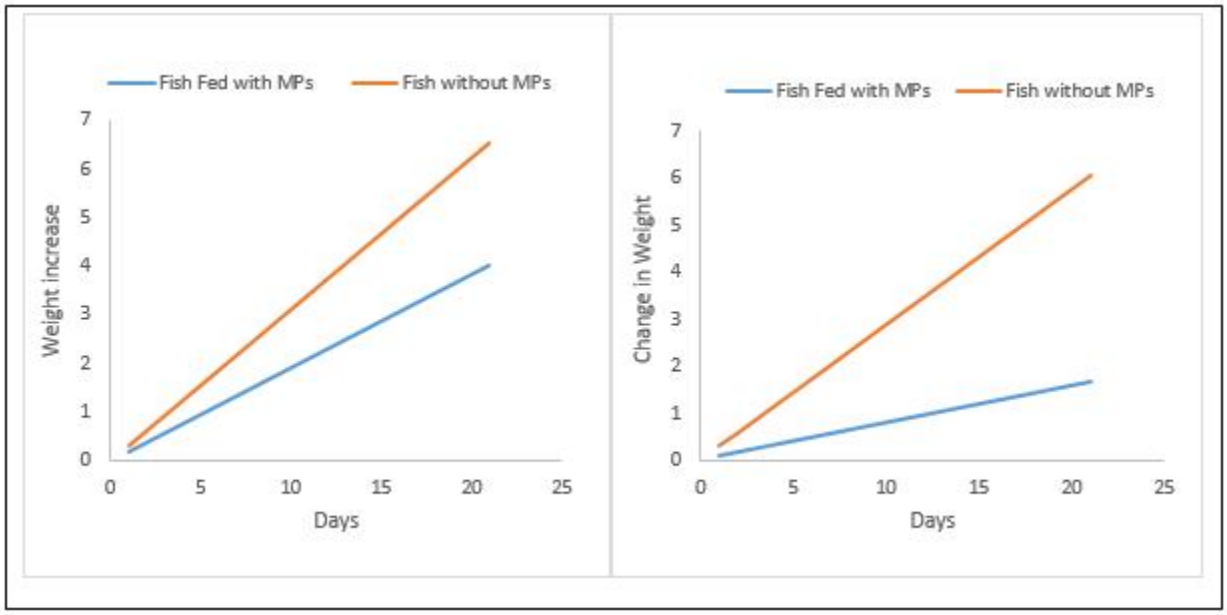


Figure 5. Change in Wet Weight of *Oreochromis niloticus* with days for tested fish and non-tested fish in two different experiments.

DISCUSSION

The temperature conditions which were used in keeping fish for study were in the normal range which is required for fish farming and did not differ much from the temperature 17.7ºC to 27.7ºC used by Chaudhary and Sharma (2018) in the experiment for tilapia in fish tank. Water electric conductivity, total dissolved solids, and pH were within the limit of water quality requirements where pH is 6.0-9.0 for freshwater fish, TDS upper limit is 1500 ppm, although the one that causes effect is 5000−10,000 ppm (Scannel and Jacobs, 2001). One report by Chaudhary and Sharma (2018) for fresh water fish kept in the tank, indicated that water was kept alkaline with pH range of 8.3−8.50 in both control and test tanks and the water electric conductivity was 1730µs/cm–1980 µs/cm, the conditions which were different from what used in this study but both were within the limits of freshwater fish growth. In this study, it was found that microplastics concentrated in gills and intestines the scenario which indicates that the test fish in aquarium could not manage eliminating particles of microplastics during taking in water through gills, and eating food. Events like these have been reported also in other studies for microplastics in marine biota because of presence in ecosystems (Thompson *et al*., 2009).

The responses of Nile tilapia on exposure to microplastics in this study have been reported as fish stress caused by toxic contaminants of water in many literatures (Coyle, 2004; Davidson *et al*., 2011; Reverter, 2018; Sridhar, 2021). Cocci *et al*. (2022) reported that microplastic occurrence in fish was found to be correlated with antioxidant enzymes (catalase and superoxide dismutase) and cytokinases (interleukin 1 ß, 10 and

interferon) levels, causing reactive oxygen species (ROS) generation and immune cell infiltration in the gut. Moreover the study by Alimba and Faggio (2019) indicated that microplastic presence in marine vertebrates produces oxidative stress in proteins, lipids and deoxyribonucleic Acid (DNA) by altering the antioxidant defuse mechanisms, i.e. enzyme catalase (CAT), superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione peroxidase (GP_x) and reduced glutathione (GSH) genes at the catalytic and transcriptional levels. They regulate the gene expression that controls oxidative stress by acting as pro-oxidant stimuli activating antioxidant gene expression through nuclear factor erythroid 2–related factor 2(N_rf2)-dependent mechanism. The clinical signs like abnormal swimming which was observed in Nile tilapia in this study has been reported also by Wright *et al*. (2020) who had observed that the polyvinyl chloride which were exposed to brown trout had reduced swimming activity and there was change in their circadian rhythms. Furthermore, the report by Tang *et al*. (2018) indicated that microplastic exposure in sea bream heads altered the JNK (c-Jun N-terminal kinase) and ERK (extracellular signal-regulated kinase) signalling pathways which are involved in the detoxification process of fish. Even other abnormal behaviours observed in Nile tilapia in this study might be attributed to the similar situation as Yao *et al*. (2023) reported for the exposed high concentration of polystyrene microplastics in golden pompano (*Trachinotus ovatus*) which had caused oxidative damage and up-regulation of genes (GrPn78, X6p-1, and Elf-2α), resulting in endoplasmic reticulum stress (ERS) and severe oxidative stress which raised the BAX/BcL-2 ratios and induced death. More studies report similar cases of microplastic exposure effects although they do not mension directly the clinical signs which have been considered in our observation in Nile tilapia but the oxidative stress must be the reason. For instance; the study by Cao *et al*. (2023) reported the effects of polyethylene microplastics in carp and found that they elevated the expression of P53, NF-KB, P6S, 1KKα, 1KKß, caspase-3 genes in the gills. Also the extended exposure to polystyrene microplastics in coach juveniles (*Paramisgurnus dabbyanus*) was studied by Wang *et al*. (2022) and found to inhibit the expression of keep 1-Nrf2 signalling pathway genes inducing aptosis by upgrading proteins (P53, gadd 45 ba and caspase 3b) expressions, also were found to up-regulate TNF-α and PTGS2A which are gene markers in the inflammatory mechanism in zebra fish (Umamaheswari *et al*., 2021). Lastly, the study by Chen *et al*. (2021) on tilapia had found that polyethylene microplastics caused oxidative stress in the liver by damaging cell membrane increasing lipid peroxidation (PLO) levels, in the brain, dorsal muscles and gills, higher brain activity where 32% of the fish had microplastics in the dorsal muscle (Barboza *et al*., 2020). In addition poor quality of water that was contributed by presence of microplastic contamination might have lead also to diseases that rise from bacteria and fungi. Because fouling organisms like fungi and bacteria can easily attach to microplastics causing them to be agents of diseases (Dekiff *et al*., 2014). Furthermore, responses indicated by clinical signs in this study have also been observed in other studies for effects of toxin

contamination where 30% to 35% of individual fish responded positively for example some had frequent surface movement with gulping of air (Islam *et al*., 2021), the abnormal alterations were associated with physiological responses which were inductive of stress.

CONCLUSION AND RECOMMENDATION

The test fish engulfed microplastics which were found in intestines and gills. The engulfed microplastics resulted to stress in Nile tilapia which was observed from different responses according to clinical signs and the lag in proper growth. There were no mortality of Nile tilapia but the responses to presence of microplastics in aquarium indicated that microplastics had adverse effects to Nile tilapia. The evaluation of toxicity of microplastics to Nile tilapia indicated that fish in contaminated ponds have high susceptibility to the effect of microplastics which might lead to death if the conditions have not been controlled. Although there have been reports on the effects of microplastics as a cause of oxidative stress to fish, more studies need to be done to give clear description for some observations like fin rot and abnormal mucus secretion responses which might be associated to microplastic harbouring microorganism in large amount.

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References

Alimba C.G. and Faggio C. 2019. Microplastics in the marine environment: Current trends in environmental pollution and mechanisms of toxicological profile. *Environ. Toxicol. Pharmacol.* **68**: 61–74. doi:10.1016/j.etap.2019.03.001

Barboza L.G.A., Lopes C., Oliveira P., Bessa F., Otero V. and Henriques B. 2020. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. *Sci. Total Environ*. **717**, 134625. doi:10.1016/j.scitotenv.2019.134625

Cao J., Xu R., Wang F., Geng Y., Xu T. and Zhu M. 2023. Polyethylene microplastics trigger cell apoptosis and inflammation via inducing oxidative stress and activation of the NLRP3 inflammasome in carp gills. *Fish Shellfish Immunol*. **132**, 108470. doi:10.1016/j.fsi.2022.108470

Chen Q., Li M., Li L., Li J., Li Y. and Luo Y. 2021. Polyethylene microplastics cause oxidative stress and DNA damage in the liver of tilapia (Oreochromis niloticus). *Environ. Pollut.* **272**, 115964. doi:10.1016/j.fsi.2022.09.022

Cocci P., Gabrielli S., Pastore G., Minicucci M., Mosconi G. and Palermo F.A. 2022. Microplastics accumulation in gastrointestinal tracts of Mullus barbatus and Merluccius is associated with increased cytokine production and signaling. *Chemosphere* **307**, 135813. doi:10.1016/j.chemosphere.2022.135813

Coyle S.D, Durborow R.M. and Tidwell J.H. 2004. Anaesthetics in aquaculture. Southern Regional Agricultural Centre. *SRAC Publication* No. 3900.

Davidson J., Good C., Welsh C. and Summerfelt S.T. 2011. Abnormal swimming behaviour and increased deformities in rainbow trout *Oncorhynchus mykiss* cultured in low exchange water recirculating aquaculture systems. *Aquac. Eng*. **45**: 109–117

Dekiff J.H., Remy D., Klasmeier J. and Fries E. 2014. Occurrence and Spatial Distribution of Microplastics in Sediments from Norderney. *Environ. Pollut.* **186**, 248e256.

Ezeanya N.C., Chukwuma G.O, Naigwe K.N. and Egwuonu C.C. 2015. Standard water quality management strategies for fish farming (a case study of Otamiri River). *Int Res J Eng Technol.*. eISSN: 2319-1163 pISSN: 2321-7308

Guilhermino A.B, Martins A., Lopes C., Raimundo J., Vieira L., Gabriel A., Barboza, Costa J., Antunes C., Caetano M. and Vale C. 2021. Microplastics in

fishes from an estuary (Minho River) ending into the NE Atlantic Ocean Lúcia. *Mar. Pollut. Bull*. **173** 113008

Hasselerharm P.E, Rico A., Albert A. and Koelmans A.A 2022. Risk assessment of microplastics in freshwater sediments guided by strict quality criteria and data alignment methods. *J. Hazard. Mater*. **441** 129814

Islam A., Amin S.M.N., Brown C.L, Juraimi A.S. Uddin M.K. and Arshad A. 2021. Determination of median lethal concentration (LC50) for endosulfan, heptachlor and dieldrin pesticides to African catfish, *Clarias gariepinus* and their impact on its behavioural patterns and histopathological responses. *Toxics* **9**, 340. https://doi.org/10.3390/toxics9120340

Jovanovic B. 2017. Ingestion of microplastics by fish and its potential consequences from aphysical perspective: Potential consequences of fish ingestion of microplastics. *Integr Environ Assess Manag* **13**

Khan F.R., Mayoma B.S., Biginagwa F.J. and Syberg K. 2018. Micro plastics in inland African matters: Presence, sources, and fate. Fresh water microplastics, *Handbook of Env Chem* 58, DOI: 1007/978-3-319-61615-5_6

Masura J., Baker J., Foster G. and Arthur C. 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48.

Mayoma B.S., Sørensen C., Shashoua Y. and Khan F.R 2020. Microplastics in beach sediments and cockles (*Anadara antiquata*) along the Tanzanian coastline. *Bull Environ Contam Toxicol* https://doi.org/10.1007/s00128-020-02991-x

Mistri M., Sfriso A.A, Casoni E., Nicoli M., Vaccaro C. and Munari C. 2022. Microplastic accumulation in commercial fish from the Adriatic sea. *Marine Pollution Bulletin* **174** 113279

Moore C.J. 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environ. Res*. **108**: 131–139.

Mvowiec B. 2017. Plastic pollutants in water environment. *Environmental protection and natural resources*. DoI 10. 1515/OSZN-2017-0030,Vol 28No 4(74):51-55

OECD (Organization for Economic Co-operation and Development) 2019. Guidelines for the testing of chemicals: Test Guideline No. 203 Fish, Acute Toxicity Testing. http://www.oecd.org/termsandconditions/.

Reverter M., Bontemps N.T, Lecchini D., Banaigs B. and Sasal P. 2018. Biological and ecological roles of external fish mucus: A Review. *Fishes* **3**(**4**), 41; doi: 10.3390/fishes3040041

Sadri S.S. and Thompson R.C. 2014. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. *Mar. Pollut. Bull*. **81**: 55−60

Scannell P.W. and Jacobs L.L 2001. Effects of total dissolved solids on aquatic organisms. Technical Report NO. 01-06. Alaska department of fish and game division of habitat and restoration

Sridhar A., Manikandan D.B., Palaniyappan S., Sekar R.K. and Ramasamy T. 2021. Correlation between three freshwater fish skin mucus antiproliferative effect and its elemental composition role in bacterial growth. *Turkish J. Fish. Aquat. Sci*. **21**: 233−244. http://doi.org/10.4194/1303-2712-v21_5_03

Subaramaniyam U., Allimuthu R.S., Vappu S., Ramalingam D., Balan R., Paital B., Panda N., Rath P.K., Ramalingam N. and Sahoo D.K. 2023. Effects of microplastics, and pesticides and nano-materials on fish health, oxidative stress and antioxidant defense mechanism. *Front. Physiol*. 14:1217666, 1-24. doi: 10.3389/fphys.

Tang J., Ni X., Zhou Z., Wang L. and Lin S. 2018. Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral Pocillopora damicornis. *Environ. Pollut.* **243**: 66–74. doi:10.1016/j. envpol. 2018.08.045

Thakur S., Mathur S., Patel S. and Paital B. 2022. Microplastic accumulation and degradation in environment via biotechnological approaches. *Water*. **14**(**24**): 4053. .https://doi.org/10.3390/w14244053

Thompson R.C., Moore C.J., vomSaal F.S. and Swan S.H. 2009. Plastics, the environment and human health: Current consensus and future trends. Philosophical transactions of the Royal Society B: *Biological Sciences* **364**: 2153–2166

Umamaheswari S., Priyadarshinee S., Bhattacharjee M., Kadirvelu K. and Ramesh M. 2021. Exposure to polystyrene microplastics induced gene modulated biological responses in zebrafish (*Danio rerio*). *Chemosphere* **281**, 128592. doi:10.1016/j. chemosphere. 2020.128592

Wang X., Jian S., Zhang S., Wu D., Wang J. and Gao M. 2022. Enrichment of polystyrene microplastics induces histological damage, oxidative stress, Keap1-Nrf2 signalling pathway-related gene expression in loach juveniles (*Paramisgurnus dabryanus*). Ecotoxicol. *Environ. Saf.* **237**, 113540. doi:10.1016/j.ecoenv.2022.113540

Wright S.L., Ulke J., Font A., Chan K.L.A. and Kelly F.J. 2020. Atmospheric microplastic deposition in an urban environment and an evaluation of

transport. *Environ. Int.* **136**, 105411. doi:10.1016/j.envint.2019.105411

Yao F.C., Gu Y., Jiang T., Wang P.F., Song F.B. and Zhou Z. 2023. The involvement of oxidative stress mediated endoplasmic reticulum pathway in apoptosis of Golden Pompano (*Trachinotus blochii*) liver under PS-MPs stress. Ecotoxicol. *Environ. Saf.* **249**, 114440. doi:10.1016/j.ecoenv.2022.114440

Zhang H. 2017. Transport of Micropalstics in coastal Seas. *Estuarine, Coastal and Shelf Science* **199**:77-86.