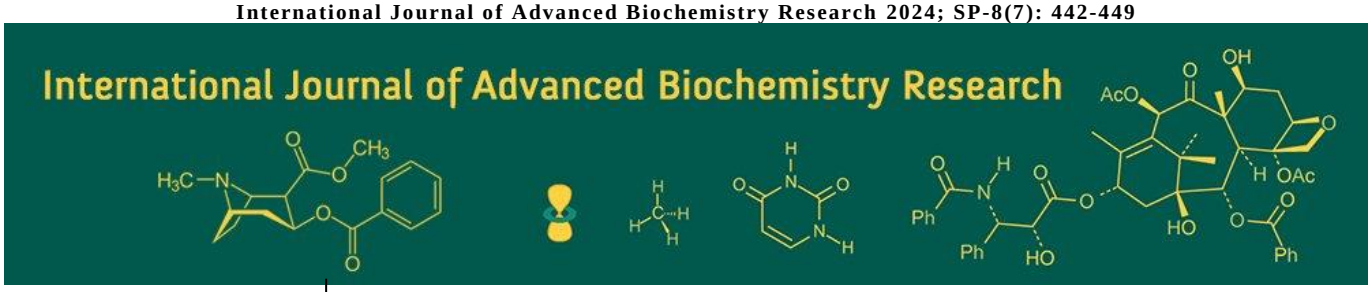

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Srianga Tilak Patnaik
Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Arpita Kullu
Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Sushree Sangita Panigrahi
Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Corresponding Author:
Srianga Tilak Patnaik
Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Impact of secondary microplastics high-density polyethylene in fish feed and its possible adsorbent monocrotophos in aquatic media on tissue protein and catalase activity in *Anabas testudineus*

Srianga Tilak Patnaik, Arpita Kullu and Sushree Sangita Panigrahi

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Abstract
The aquatic pollution has increased manifold in recent years due to various sources like pesticides from agricultural runoff and the disintegration of plastics into various types of microplastics (MPs). In the current study, the Climbing perch (*Anabas testudineus*) is exposed to Monocrotophos (MCP) (36% S.L.) and fish feed contaminated with secondary MPs high-density polyethylene or HDPE (>10%) for 3 days (72 hours). The biochemical parameters like the levels of protein and the enzymatic activity of catalase (CAT) in the liver and muscle tissues of the fishare evaluated. The results of the treatment as compared to the control reveal that there is a decreasing trend in the protein content and increased CAT activity. The results statistically suggest HDPE as an adsorbent of MCP and that tissue protein level and CAT activity can be used as potential biomarkers to examine the toxicity level of MPs in water and its effect in combination with contaminants like pesticides on fishes.

Keywords: Microplastics, high-density polyethylene, adsorbent monocrotophos, *Anabas testudineus*

1. Introduction
Plastic (Greek 'plastikos' meaning 'moldable') is a synthetic organic polymer having boundless utilization due to its prolonged half-life. As a result of their unsystematic waste management, there is an undesired assemblage of these substances in the surroundings that acts as its limitation (Wagner *et al.*, 2014, Ivleva *et al.*, 2017) [49, 17]. Nearly 14 million tonnes of micro-plastics partitioned from the heaps of garbage is discharged to the ocean annually according to Australia's National Science Agency. Researchers at CSIRO used a robotic submarine to gather samples 3,000 metres (9,850 feet) deep, off the South Australian coast. The outcome revealed microplastics going down to the ocean floor (Barrett *et al.*, 2020) [4]. Plastics Are Commonly Categorized As Mega – debris (100 mm), macro-debris (20 mm), meso-debris (20–5 mm), and micro-debris (<5 mm) (Barnes *et al.*, 2009) [3]. Since 2004 the term microplastics (MPs) has been extensively applied to mention anthropogenic detritus which is a collective term for explaining a heterogeneous combination of substances differing in size from a few microns to several millimetres (Bergmann *et al.*, 2015) [6]. MPs originate from diversified sources, but 4 major methods of emergence are: decaying of larger fragments, straight discharge into waterbodies, coincidental destruction of industrial raw material, and release of macerated or squashed waste (Faure *et al.*, 2012) [12]. According to the above considerations, MPs fall into primary and secondary groups.
Plastic disintegration takes place due to different factors and leads to plastic debris of size <5mm (Betts, 2008; Hidalgo-Ruz *et al.*, 2012) [6, 16] termed microplastics (MPs) which are unsafe for aquatic life. After the consumption of MPs by fish critical health conditions occur causing the death of fish (Carlos de Sa *et al.*, 2018) [7]. Microplastics (MPs) are the main sources of plastic contamination in marine (Lusher *et al.*, 2015) [28], freshwater (Eerkes-Medrano *et al.*, 2015) [11], and terrestrial ecosystems. Due to their small size, and fascinating coloration, these substances are bioavailable to several groups of organisms all over the food web, comprising zooplankton, cetaceans, and fish, owing to these organisms' incapacity to distinguish between MPs and prey. Besides, MPs can behave as very productive vectors for adsorbing harmful contaminants present in water, involving waterborne persistent organic

pollutants (POPs) within the environment, such as organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), solvents, and toxic m*et al.*s commonly used in colouring. Hence, the association between MPs and these chemicals is connected to a wide range of sub-lethal effects on fish upon intake, including intestinal modifications, oxidative stress, etc. Removing and identifying microplastics in biological samples is a critical step. Quite a few techniques have been recruited to obtain microplastics from deposits and biological tissues.

Fish being among the vertebrates dominating the aquatic habitats of the earthplay a considerable role in certain regions of the world's food supply. Fishes are used as biological assets for disease control (Legner*et al.*, 1984) [25]. The minnows *Gambusia* and *Poecilia*are used worldwide in the biological control of mosquitoes (Legner and Sjogren, 1984) [25]. *Tilapia zilli*is an example that performs both as a habitat reducer (aquatic weed feeder) and as an insect predator (Legner and Bellows, 1999) [24]. Fishes do contribute to various fields of research by being model research organisms in various fields. The flexibility of fishes to acclimatize to relatively created natural environment and easy observation backs them as model animals. Fishes are also used as model systems for the study of vertebrate apoptosis or the so-called programmed cell death (Krumschnabel and Podrabsky, 2009) [20]. *Anabas*, a hardy fish of considerable fisheries interest, has tremendous market demand as food owing to its fine flavour, restorative values, and prolonged freshness out of water. Commonly called climbing perch for its ability to walk on land by spines.

The Food and Agriculture Organization (FAO) has defined pesticide as any substance or mixture of substances intended for destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm or interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. Pesticides that run off from agricultural farms in to inland aquaculture systems strongly activate the outburst of diseases, as they worsen the ecosystem, as well as affect the fish's immune system. Organophosphate (OP) compounds are a group of chemical agents used for protecting crops, livestock, and human health. Monocrotophos (MCP) is an organophosphate insecticide that is used to keeps away various crops such as cotton, coconut, coffee, maize, sugarcane, etc., from several pests. In India, MCP is prohibited for use on vegetables and is under the "restricted use" category. It inhibits acetylcholinesterase (AchE) activity and arrests the breakdown of the neurotransmitter acetylcholine (Ach), resulting in the accumulation of Ach in the synapses and inducing neurotoxicity. Due to its easy availability, they are used widely.

Exposure to pollutants or other stress conditions increases oxidative stress in animals. Severe oxidative stress can cause cellular lesions and, finally, cell death. Thus, we have tried to not only study the effects of the Microplastic and HDPE but also evaluate the combined effects of HDPE (adsorbent of the pesticide) and Monocrotophos (pesticide) on *Anabas*.

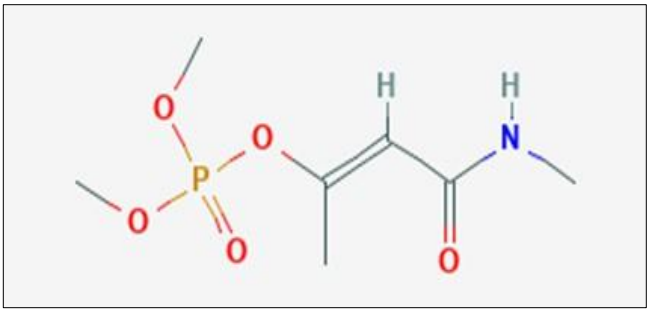

Fig 1: Molecular structure of Monocrotophos

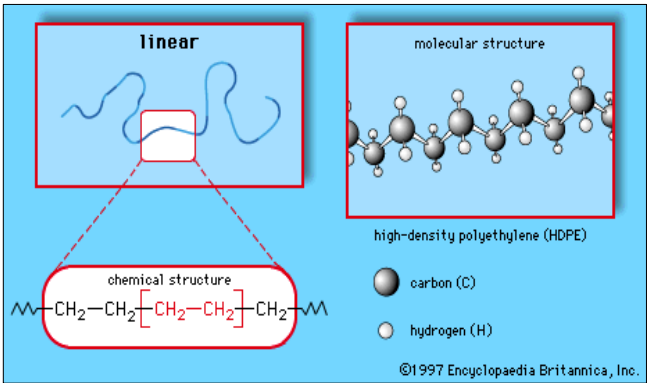

Fig 2: Molecular structure of high-density polyethylene

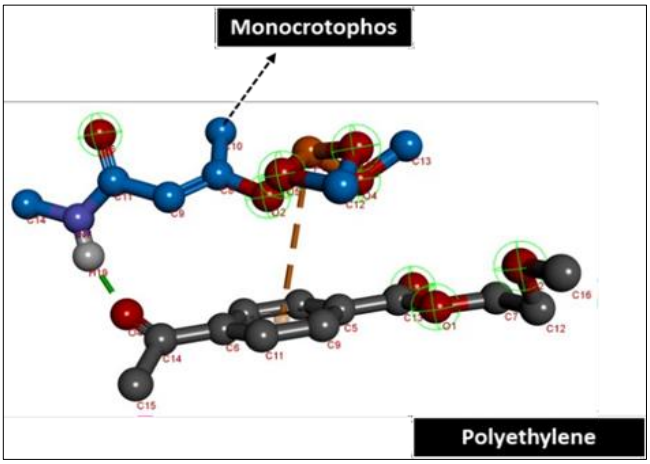

Fig 3: Molecular docking result showing the interaction between Monocrotophos and Polyethylene

2. Materials and Methods

2.1 Experimental setup

In the laboratory, for the experimental setup, similar-sized fish tanks of optimum dimensions were used in triplicate. Fishes were grouped as control (C), Monocrotophos treated (T_1), HDPE microplastic treated (T_2) and HDPE micro-plastic + Monocrotophos treated (T_3).Each group comprised of 5 fish. Aerators were used for a continuous supply of dissolved oxygen in the aquatic media. The fish were regularly monitored from time to time. The water quality was evaluated periodically according to standard methods. The physicochemical parameters of the water were determined daily, using a water and soil analysis kit (Systronics Water Analyser 371), and mean values were maintained at temperature 25 °C ± 2.50 °C, pH 7.2±0.05, conductivity 34.8±4.22 µS, dissolved oxygen (D.O.) measured as per Winkler, 1888 that was found to be 8.8±2.0 mg/L, and total hardness ($CaCO_3$) measured by EDTA titration method was 136.0±3.4 mg/L.

2.2 Experimental animal
Air-breathing fish, (*Anabas testudineus*) used as a sample for the experiment was procured of uniform size (26±4 grams weight, 15±3 cm length) from the local market in Bhubaneswar, Odisha. All the fishes were collected from the same stocking fish pond which was artificially constructed for their inhabitation. The disease-free, healthy fish were collected having no prior record of biochemical treatment, i.e., they were completely untreated. In the lab, fish were allowed to acclimate for 3 days before exposure to the desired treatment. They were fed with commercial floating fish feed during this period at 5% body. The commercially formulated pesticide, Monocrotophos 36% S.L. AALPHOS (Agastya Agro Limited) was purchased from the local market (M/S Bharat seed and nursery store, Bhubaneswar) to introduce into the media.

2.3 Preparation of fish feed and micro-plastic
Formulation of two types of fish feed- normal feed (100%), and 100% normal feed with 10% HDPE MPs in addition- was done as per the recommendation of ICAR-CIFA Bhubaneswar and experimental need, respectively.For *Anabas testudineus*, LC_{50} of MCP was calculated as per OECD guidelines (OECD, 1992) and Probit analysis (Finney, 1971) [13]. 48 h LC_{50} was found to be 106 mg/L or ppm (Mohapatra *et al.*, 2020) [32]. The collected pristine HDPE was refrigerated at – 20 °C for 96 hours followed by grinding and homogenization for the preparation of HDPE secondary MPs. A scanning electron microscope (SEM) was used to confirm the size of the generated MP.

2.4 Fish sampling and chemical treatment
20 healthy disease-free fishes (*Anabas testudineus*) of the grow-out stage (age group 7 weeks±2 weeks) were acclimatized to the laboratory conditions for 3 days (72 hours). Commercially available, untreated feed was given to all the fish during this acclimatization period. This was followed by a treatment period of 24 h, 48 h and 72 h. The inception of each treatment period of the experiment included specified doses of formulated fish feed and 5.3 ppm sublethal concentration of MCP (36%) for respective experimental pots. Firstly, MCP doses were directly administered to the media of group T_1 and group T_3 with the help of a micropipette. Secondly, fish feed was given after a short while just after MCP had mixed with media.

2.5 Processing of fish for preparation of tissue sample
Fish from each of the experimental sets (control and treated), collected at an interval of 24 hours, 48 hours, and 72 hours, was dissected for the collection of liver and muscle tissues.

500 mL phosphate buffer of pH 7.4 was prepared beforehand by adding 1.22 g KH2PO4and 2.8 g K2 hPO4to 400 mL distilled water and then the volume and pH were adjusted to 500 ml and 7.4, respectively. The prepared buffer was stored in a refrigerated condition. The fish tissue was homogenized with0.05 M Phosphate Buffer of pH 7.41:4 (w/v) in a porcelain mortar and pestle under chilled conditions. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4 °C using tabletop cooling centrifuge (REMI CM- 12 PLUS). The supernatant was collected in an Eppendorf tube and stored at -26 °C in a deep freezer (Celfrost) for further biochemical analysis.

2.6 Statistical analysis
ANOVA was done to test the significance of the difference in the mean values of the data for various parameters between control and treatment. All statistical analyses and graphs were done by XL-STAT.

3. Results
3.1 Tissue protein
A time-specific significant change was observed in the total tissue protein sample collected from *Anabas testudineus* which was exposed to HDPE, alone and HDPE in the presence of MCP.

3.1.1 Liver tissue
The level of protein in C was found to be 218.99±4.78 mg/g tissue after 24 hours. The amounts of protein in treatments T_1, T_2, and T_3 after 24 h were 215.61±2.85 mg/g tissue, 217.30±14.50 mg/g tissue, and 177.21±7.93 mg/g tissue, respectively. When compared with control the result seemed to decrease which indicates a decline in protein level in liver tissue.

Similarly, after 48 h of exposure period the value in C was 246.29±6.49 mg/g tissue. The values of treatments T_1, T_2, and T_3 were obtained as 241.03±5.02 mg/g tissue, 234.70±2.53 mg/g tissue and 169.41±3.49 mg/g tissue. The protein level decreased in all the 3 treatments as compared to control.

The 72 h exposure period revealed the values of C, T_1, T_2, and T_3 as 277.004±2.56 mg/g tissue,

271.94±0.97 mg/g tissue, 266.24±14.001 mg/g tissue and 159.92±2.03 mg/g tissue, respectively. After comparison with C, there was a decreasing trend in the tissue protein level in the treatments.

ANOVA results indicated a significant ($p<0.05$) variation in total tissue protein between control and treatments.

Table 1: Mean ± Standard deviation (SD) of protein estimation of Liver tissue (mg/g tissue)

Experimental group	Protein (mg/g tissue) Day 1 (24 h)	Protein (mg/g tissue) Day 2 (48 h)	Protein (mg/g tissue) Day 3 (72 h)
C	218.99±4.78	246.29±6.49	277.004±2.56
T_1	215.61±2.85	241.03±5.02	271.94±0.97
T_2	217.30±14.50	234.70±2.53	266.24±14.001
T_3	177.21±7.93	169.41±3.49	159.92±2.03

3.1.2 Muscle tissue
The protein levels in C after 24 h, 48 h and 72 h exposure periods were found to be 95.15±5.31 mg/g tissue, 109.49±2.90 mg/g tissue and 121.73±2.99 mg/g tissue. The level of protein in T_1

after 24 h was 92.83±9.56 mg/g tissue, after 48 h was 106.75±1.59 mg/g tissue and after 72 h was 116.67±4.12 mg/g tissue. After comparing with the C set with corresponding exposure periods it was seen that the protein level declined.

The protein level in T_2 after 24 h was 84.81±0.97 mg/g tissue, after 48 h was 103.80±3.49mg/g tissue and after 72 h was 114.98±6.85 mg/g tissue. A declining trend in the protein level was observed after the values of treatments were compared to corresponding exposure periods of C.

In T_3, the protein level after 24 h, 48 h, and 72 h were obtained as 64.13±5.53mg/g tissue, 93.25

±23.46 mg/g tissue and 67.30±6.97 mg/g tissue, respectively. Here also, it was seen a decrease in protein levels after the comparison.
ANOVA results indicated a significant (p<0.05) variation in total tissue protein between control and treatments.

Table 2: Mean ± Standard deviation (SD) of protein estimation of Muscle tissue (mg/g tissue)

	Day 1 (24 h)	Day 2 (48 h)	Day 3 (72 h)
C	95.15±5.31	109.49±2.90	121.73±2.99
T_1	92.83±9.56	106.75±1.59	116.67±4.12
T_2	84.81±0.97	103.80±3.49	114.98±6.85
T_3	64.13±5.53	93.25±23.46	67.30±6.97

3.2 CAT activity
3.2.1 Liver tissue
The catalase activity in C was found to be (0.267±0.004 U/mg protein) after 24 hours. The activities of catalase in treatments T_1, T_2, and T_3 after 24 h were 0.288±0.017U/mg protein, 0.295±0.004U/mg protein, and 0.355±0.021U/m g protein, respectively. When compared with the control set the result seemed to increase which indicates elevation in catalase activity in liver tissue.
Similarly, after 48 h of exposure period the value in C was 0.247±0.008 U/g protein. The values of treatments T_1, T_2, and T_3 were obtained as 0.266±0.010 U/mg protein, 0.284±0.013 U/mg protein and 0.431±0.016 U/mg protein. On comparing with C, the result showed that catalase activity increased in all of the 3 treatments.
The 72 h exposure period revealed the values of C, T_1, T_2, and T_3 as 0.248±0.011 U/mg protein, 0.267±0.005 U/mg protein, 0.279±0.006 U/mg protein and 0.491±0.005 U/mg protein, respectively. After comparison with C, there was an increasing trend in the tissue catalase activity in the treatments.
ANOVA results indicated a significant (p<0.05) variation in total tissue protein between control and treatments.

Table 3: Mean ± Standard deviation (SD) of CAT activity of Liver tissue (U/mg protein)

	Day 1 (24 h)	Day 2 (48 h)	Day 3 (72 h)
C	0.267±0.004	0.247±0.008	0.248±0.011
T_1	0.288±0.017	0.266±0.010	0.267±0.005
T_2	0.295±0.004	0.284±0.013	0.279±0.006
T_3	0.355±0.021	0.431±0.016	0.491±0.005

3.2.2 Muscle tissue
The catalase activities in Cafter 24 h, 48 h and 72 h exposure periods were found to be 0.011±0.005U/mg protein, 0.027±0.005 U/mg protein and 0.030±0.003 U/mg protein.
The activity of catalase in T_1 after 24 h was 0.015±0.007 U/mg protein, after 48 h was 0.033±0.012 U/mg protein and after 72 h was 0.037±0.009 U/mg protein. After comparing with the C set with corresponding exposure periods it was seen that the catalase activity got inclined.
The catalase activity in T_2 after 24 h was 0.026±0.001U/mg protein, after 48 h was0.039±0.004 U/mg protein and after 72 h was 0.040±0.007 U/mg protein. An increasing trend in the catalase activity was observed after the values of treatments being compared to corresponding exposure periods of C.

In T_3, the catalase activities after 24 h, 48 h, and 72 h were obtained as 0.025±0.001 U/mg protein, 0.041±0.011 U/mg protein and 0.060±0.006 U/mg protein, respectively. Here also, it was seen an increase in catalase activity after the comparison.
ANOVA results indicated a significant (p<0.05) variation in total tissue protein between control and treatment.

Table 4: Mean ± Standard deviation (SD) of CAT activity of Muscle tissue (U/mg protein)

	Day 1 (24 h)	Day 2 (48 h)	Day 3 (72 h)
C	0.011±0.005	0.027±0.005	0.030±0.003
T_1	0.015±0.007	0.033±0.012	0.037±0.009
T_2	0.026±0.001	0.039±0.004	0.040±0.007
T_3	0.025±0.001	0.041±0.011	0.060±0.006

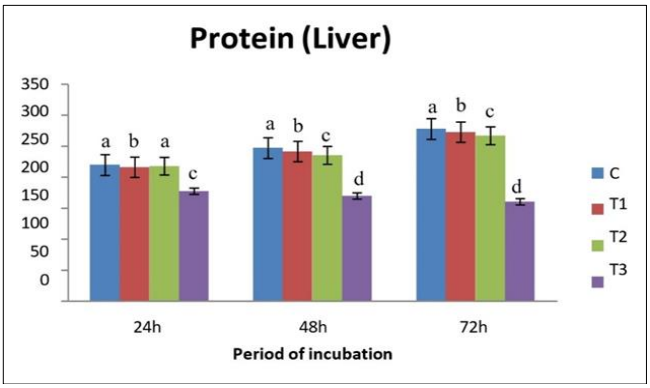

Fig 1: Plotting of graph depicting changes in protein content of liver tissue

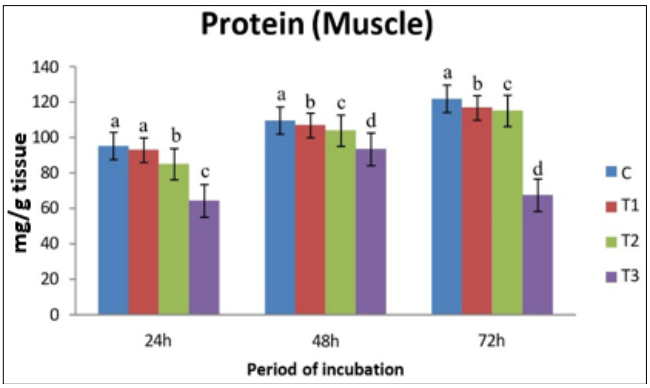

Fig 2: Plotting of graph depicting changes in protein content of muscle tissue

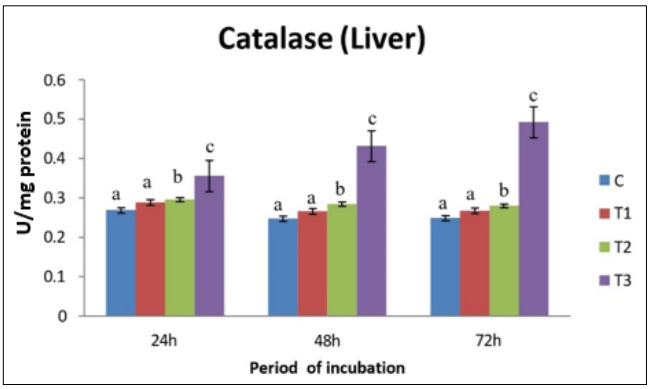

Fig 3: Plotting of graph depicting changes in CAT activity of liver tissue

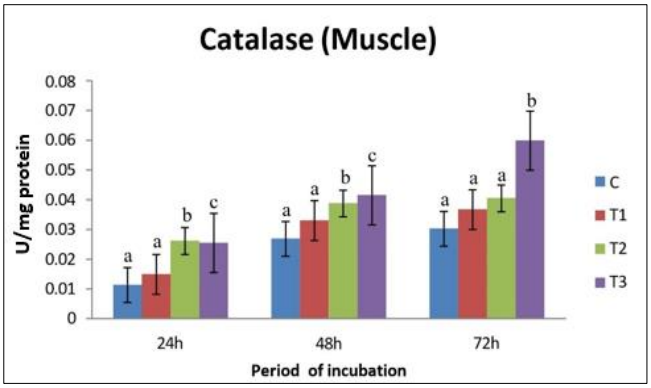

Fig 4: Plotting of graph depicting changes in CAT activity of muscle tissue

Discussion

Proteins are important organic substances essential for organisms in tissue formation and also have an important function in energy metabolism (Yeragi *et al.*, 2003) [53]. Protein in the homogenates was determined by the method of Lowry *et al.*, (1951) [27]. The protein level was seen to be decreased in the muscle tissue of *Tilapia mossambica* when exposed to the median lethal concentration (LC_{50}) of monocrotophos (Remia *et al.*, 2008) [42]. In *Mystusvittatus*, depletion in protein content was reported which might have occurred because of impaired protein synthesis due to liver disorders when exposed to monocrotophos (Lynch *et al.*, 1969; Dalela *et al.*, 1981) [29, 8]. According to (Dubhat and Bapat, 1984; Patel and Parmar, 1993) [10, 40], there was a significant depletion in protein levels in the liver of *Channa orientalis*and *Baleopthalmus dussumieri*. Das *et al.*, (2000) [22] reported on the effects of exposure to sublethal concentrations of the organophosphate pesticide, quinalphos on the biochemical parameters of muscle such as the protein level of the Indian major carp, *Labeorohita.* The effect of pesticides on muscle protein has been studied by many workers (Wild, 1975; Ganesan *et al.*, 1980; Murty and Devi, 1982) [50, 14, 34]. Exposure of fingerlings of *L. rohita*to sublethal concentrations of quinalphos showed changes in the muscle protein level. A decline in muscle protein was indicative of depleted protein synthesis and low assimilation of food and low amino acid intake for protein synthesis. Organophosphates are known to methylate and phosphorylate cellular proteins directly. Murty and Devi worked on the effect of endosulfan on tissue protein in the fish *Channa punctata.* An inclined concentration of the pesticide caused a remarkable decline of protein in the liver. Similarly, a decrease in the protein content of the liver caused by cython was studied by (Narayan Ramand Satyanesan, 1986) [35]. Klassan, (1991) [19] reported that depletion of protein content suggested increased proteolysis and possible utilization of the products of their degradation for metabolic purposes in malathion-induced *L. rohita.* Kutty *et al.* (2013) [22] reported that the, Ekalux (an organophosphorus pesticide) had a direct influence on biochemical parameters in *A. testudineus.* Results displayed that the level of total amino acid after exposure to Ekalux was higher than compared with the control group which implied a decrease in the protein content.

The activity of CAT increases with an increase in the physiological stress of the animal. It helps in scavenging the free radicals. Varga *et al.*, (1997) [47] studied the effect of the organophosphate insecticide Dichlorvos on the catfish (*Ictalurus nebulosus*). The catalase activity was evaluated by the spectrometric method of Beers and Sizer, from the extinction decrease characteristic of peroxide at 240nm. The catalase activity in the liver pesticide increased dose-dependently. Patil and David (2013) [41] studied CAT activity in malathion-induced *L. rohita.* Catalase activity increased during experimental periods in the liver tissue. Kuppuswamy and Seetharaman, (2020) [21] gave an account of the brain of adult zebrafish (*Danio rerio*) when exposed to sublethal concentration of monocrotophos displayed modification in antioxidant enzymes such as CAT. Induction of the enzyme provides the first line of defense against the ROS generated by xenobiotics (Pandey *et al.* 2001; Pandit *et al.* 2014) [38, 39] as CAT converts the peroxide top water and molecular oxygen (Shao *et al.*, 2012) [45].

Similar results i.e., a decrease in protein level and increments in CAT activity were obtained in the T_1 batch which consisted of the pesticide, Monocrotophos in the media, after comparison with the control group.

Zitouni *et al.*, (2020) [54] reported on the presence and accumulation of microplastics in the gastrointestinal tract and muscle of the teleost *Serranus scriba.* Determination of microplastic accumulation in the tissues was quantified using Raman spectroscopic analysis. The estimation of MP loads in fish gives imperative insights into the characteristics of its potential impacts. Several studies have indicated that plastic may carry persistent organic pollutants (POPs) and m*et al.*s from the environment that adsorb or adhere to their surface (Ashton *et al.*, 2010; Rochman *et al.*, 2013) [1, 43]. The results of the work also reported an association between small MP particles and the induction of oxidative stress. Perhaps there was obstruction of the digestive organs and intervention in feeding in ichthyoplankton in their later developmental stages due to unintentional ingestion of microplastics (Mazurais *et al.*, 2015) [30]. It results in starvation of ichthyoplankton, decrease in rate of growth, general fitness, etc.

Videira *et al.*, (2001) [48] reported that organophosphorus pesticides due to their low cumulative ability, rapid disintegration in water and short-term persistence in the environment, have completely replaced the persistent chlorinated pesticides at the beginning of the 1980s.Methylparathion in water bodies gets easily absorbed through gills and accumulates in the organs like brain, liver and gut (De la Vega Salazar *et al.*, 1997) [9]. Methyl parathion has a high oxidative- stress-inducing potential in *Catla catla* about which the concerned work was conducted. The observed increase in catalase level in the liver in the MP-treated fish indicated a detoxifying mechanism against toxicity. A related observation was also seen in tissues of *Bryconcephalus* exposed to MP (Monteiro *et al.*, 2009; Modesto and Martinez, 2010) [33, 31]. The highest CAT activity in the liver of *Acipenser naccarii*and trout *Oncorhynchus mykiss* implied that the enzyme has an important function in opposing the production of superoxide radical (O2-) and H2O2 from the potent metabolic activity characteristic of the liver (Trenzado *et al.*, 2006) [46]. In general, the increased antioxidant actions indicated adaptive reactions of organisms to counteract the oxidative effect of generated ROS (Hegazi *et al.*, 2010) [15].

The results in T_2 batch (after comparison with the control) which consisted of HDPE (10% over and above) given to fishes via the prepared feed showed similar results due to the effect of MP. The depletion of protein level might have

taken place due to accumulation in the gut which subsequently reduces food intake resulting in low energy availability to the body tissue. The CAT activity increases due to more oxidative stress generated in the body due tothe presence of MP.

Barboza *et al.*, (2018) [2] reported that the concentration of mercury in the liver of *Dicentrarchus labrax* juveniles was notably higher in the presence of microplastics than in their absence which implied that MPs had an impact on the bioaccumulation of the m*et al.* in the liver tissue. MPs may have absorbed mercury from the water body and function as another exposure passage to the m*et al.* The concentrations of MPs and mercury examined (3.4 + 5.2 folds = 8.6 folds), alone and in mixtures (10.8 folds), caused oxidative stress in the liver tissue of *D. labrax*. A few works have suggested that MPs act as vectors, both for persistent organic pollutants (POPs) and silver to freshwater fish (Oliveira *et al.*, 2013; Rochman *et al.*, 2014; Khan *et al.*, 2015) [37, 44, 18]. The partition coefficient of phenanthrene on polyethylene was much higher than any other type of MPs which shows good adsorption capacity of the PE. Lan *et al.*, (2021) [23] reported that some aged polyethylene adsorbed pesticides more easily as compared to pristine or original polyethylene as the aged ones had rougher surfaces, more cracks, and some oxygen-containing functional groups.

TheT_3batch (MCP +HDPE) showed significant changes, as in the decrease in protein level and increase in CAT activity after being compared to the control set. Such variation syndicated that more molecules of MCP might have been adsorbed on the surface of HDPE impacting greatly on the biochemical parameters of the fish.

The results obtained from the study thus corroborate the results of various investigators discussed above.

Conclusion

The persistence of MPs, pesticides, and several other contaminants in the aquatic ecosystem has become a global menace and a great challenge to the scientific community and researchers all over the world for their elimination. In the conducted experiment, the impacts of the MCP and HDPE, individually or in mixtures, were detrimental to the muscle and liver tissues of the experimental animal. The affected biochemical parameters *viz*. depletion in the protein level and increase in the CAT activity were examined in the present study that corroborates previous similar investigations. CYGNSS (Cyclone Global Navigation Satellite System) and Micro-robots are two of the most recent findings to curb micro-plastics. Further experimental studies on the adsorption capacity and residential time of the MPs in the gastrointestinal tract need utmost importance for the estimation of the MPs load to which a fish is exposed in its lifetime as well as the protection and management, using a combination of various techniques of the contaminated aquatic biome.

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