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Assessment of photo degraded PVC microplastic in *Oreochromis niloticus* and *Spinacia oleracea* using aquaponic system

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Abstract

Microplastic waste is a pervasive type of contaminant in aquatic ecosystems, being considered a major threat to aquatic biota. One of the problems of microplastics is that they can produce leachates in extremely high concentrations when these leachates are released from the plastic particle; they have the potential to cause toxic effects in the biota. The main objective of the current research is focused on the investigation of microplastic toxicity in controlled aquaponic system. To achieve this natural photo degraded plastics were given to the fish along with economically prepared feed after a period of time, fishes and plants were analyzed for growth parameters. The results showed that in control where no microplastics were added was initially 9.46 ± 0.24 in the first day of experiment and the final weight was 63.71 ± 0.57 on the final day, whereas in the experimental set up, final weight was 61.37 ± 1.03 . The initial length of fishes were 6.38 ± 0.12 in day 1 and the final length was 16.84 ± 0.53 in day 56 in control and 15.25 ± 0.16 in experiment. Length gain of control fishes and experiment fishes were 10.45 ± 0.59 and 8.86 ± 0.16 . These results show that there was a minor reduction in growth of fishes when microplastics were added. The presence of leachates may be the reason for the reduced growth in fishes. Further biochemical, histopathological studies in fishes and hormonal studies in plants are needed to confirm the impact of leachates of microplastics in the aquaponic system.

Keywords: Microplastics, Leachates, Aquaponic system, *Oreochromis niloticus*, *Spinacia oleracea*

Introduction

Plastic materials are of vital use, being non-corrosive, long-lasting, non-reactive, lightweight, easy to handle and its economical manufacturing cost has made it a material of preference. The non-recycled plastic is being dumped to the natural environment in which a major proportion of it is thrown as trash in the water bodies including oceans and rivers. It is estimated that 275 million metric tons of plastic waste is being generated each year (based on reports from 192 coastal countries, 2010). Due to the diversity of physical, chemical and biological conditions of the environment, these non-recycled plastics in the water bodies, break down to form microplastics (MPs). These microplastics are taken in by fishes in a variety of methods and plastic abundance was found between the stomach, gut, and intestine of the fishes (Zhanget al., 2017). They cause adverse effects leading to mortality, neurotoxicity, cytotoxicity, liver stress, behavioral changes, oxidative stress, genotoxicity and many others (Luis et al., 2018) [10]. The potential effect of microplastic leachates is critical as most plastic contamination in the ocean is made of microplastics, which tend to accumulate more persistent pollutants than large debris (Law and Thompson, 2014) [11]. Thus this current study focused on the degraded plastic materials. Aquaponics is an integrated multi-tropic system which is a sustainable and reliable method for global food production that combines elements of recirculating aquaculture and hydroponics (Connolly and Trebic, 2010) [5]. In this study the effect of photo degraded plastic material was analyzed using aquaponics system.

Methodology

Collection and Preparation of micro plastic samples

Plastic pollutants were collected from the polluted area in the ukkadam lake, Coimbatore, Tamil Nadu. Collected PVC pipes were exposed to natural weathering in an exposure site for 180 days. The exposure site is located at the latitude $11^{\circ}06'18.3''N$ and longitude of

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76°56'26.1''E in Coimbatore, and the area characterized by the summer with the average temperature of 31° centigrade and rainy season with the average temperature of 28° centigrade, with the 180 days average direct normal solar irradiance of 5.96 kWh/m²/day and average global horizontal solar irradiance of 6.27 kWh/m²/day (Jan19-May19). Micro plastics of PVC is prepared by scratching surface with using sharp knife and crushed further with mortar and pestle.

Identification of micro plastics size using FESEM image

The samples were identified by their morphological characteristics according to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974)^[1]. In this technique, the sample should be conductive. Surface of non-conductive samples are made conductive usually by coating a thin layer of gold. The beam current and the final spot size determine the depth and resolution of field of the image respectively, which are adjusted with one or more condenser lenses and the probe forming objective lenses.

The electrons interact with the sample from few nanometers to several microns of the surface of the sample depending on the beam parameters and the sample type. Primarily, electrons are emitted from the sample as either backscattered or as secondary electrons, and are detected by scintillation photomultiplier detector. Secondary electrons have the energy of 3–5 eV and are generally used for the visualization of surface morphology and roughness, as they can spot the exact position of the beam on the sample surface and give morphological information with high resolution. FESEM produces clearer and less distorted images with spatial resolution down to 1.5 nm, which is 3 to 6 times better than conventional scanning electron microscopy (SEM) (Stein *et al.*, 2003).

Energy Dispersive X-ray Spectroscopy

Energy Dispersive X-ray Spectroscopy (EDX or EDS) is a relatively simple yet powerful technique used to identify the elemental composition of as little as a cubic micron of material. The equipment is attached to the SEM to allow for elemental information to be gathered about the specimen under investigation. The technique is non-destructive and has a sensitivity of >0.1% for elements heavier than C. EDS works by detecting X-rays that are produced by a sample placed in an electron beam. The electron beam excites the atoms in the sample that subsequently produce X-rays to discharge the excess energy. The energy of X-rays is characteristic of the atoms that produced them, forming peaks in the spectrum. Please note individual elements may have more than one peak associated with them and some peaks from different elements may overlap to a certain degree. As the electron beam can be precisely controlled, EDX spectra can be collected from a specific point/particle on the sample, giving an analysis of a few cubic microns of material. Alternatively the beam can sweep over a selected area of the sample to identify the elements in that region. In addition, line profiles and X-ray maps can be acquired which depict the elemental distribution across the specimen.

Aquaponics construction

Aquaponic system was designed based on the Bernstein, (2011) model. The Aquaponic system was comprised of the following 3 experimental setup tanks. The tank was with height, breadth and length of 2.5×2×5. Water capacity was of

the tank 318 liters. Each tank is connected to the sub-merged motor with the capacity of 20 watts for the water flow from fish tank to the hydroponic system, and each tank holds aerator with 10 watts. Hydroponic system was constructed using 120mm thickness PVC pipes, where plant trays are placed in the holes of water flow chamber. These holes are made in the PVC pipes using driller with the distance of 10cm. The water from the hydroponic grow bed was flowed by the gravity and enters the fish tank. Each hydroponic grow bed is filled layers of gravel, soil and coco peat.

Healthy Freshwater fish *Oreochromis niloticus* was collected from Tamil Nadu fisheries Development Corporation, Bhavanisagar dam in Erode district Tamil Nadu, India. It was transported to the laboratory in well aerated polythene bags. They were allowed to acclimatize in the laboratory condition for 2 – 3 weeks and then used for further experimental studies. Experiments were carried out in the constructed aquaponic setup. The fishes were stocked at the rate of 15 Tilapia fingerlings of length 6cm and weight 9.5gms. Each fish tank was filled with 318 liters of water. Spinach (*Spinacia oleracea*) seed was collected from the Seed Centre, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Hydroponic seed trays were used for planting in aquaponic system. Seed germination was carried out separately using coco peat with the environmental factor dark, day/night 18-20 °C, 80% humidity. After the appearance of two leaves, plant saplings were transferred to hydroponic trays of constructed aquaponic system. Plant saplings were observed daily for the pest attack, nutrient deficiency and growth throughout the experimental period.

Introducing micro plastic to fish

The collected PVC plastic samples were given to *Oreochromis niloticus* along with the prepared fish feed (1gm/l) for 30 days. Two experiments were setup one was maintained as control.

Fish growth parameter analysis

Length and weight of the fishes are measured in the interval of 7 days for 2 months. Length was measured using centigrade scale and weight using digital weighing machine. The water temperature ranged between (28 to 30 °C ± 2 °C) during experimental period). The fishes were given prepared fish feed with the protein content of 32%, totally sixty (*Oreochromis niloticus*) fishes with average initial body weight of 9.5 gm/fish and average initial body length 6.5 gm/fish were equally divided into 2 treatments.

Plant growth parameter analysis

Plant growth parameters like measurements of height number of leaves, wet and dry weight was recorded on weekly basis.

Results

The nature of plastic used in our experimental study and their characteristic features like color, thickness, specific gravity are given in Table (1).

Table 1: Characterizations of microplastics used in experimental study

S. No.	Parameters	Sample
1	Colour	Grey
2	Thickness	3mm
3	Solar irradiance (jan19-jun19)	5.96 kwh/m ² /day
4	Aging of plastics	180 days
5	Specific gravity	1.4

Color of the PVC pipe was milky white and polypropylene is

transparent, Thickness of PVC pipe was 3mm Specific gravity of sample was 1.4 solar irradiance mentioned in the table 1 is the average value of 6 months (January 2019 – June 2019), aging of the plastic mentions the photo degradation period of plastics used. In the current study photo degradation results in the discoloration of PVC from gray to chalky white as showed complete destruction results in the formation of microplastic (Fig. 1).



Fig 1: Photo degraded PVC

FESEM of the Microplastics analyzed

Figure 2 shows the field emission scanning electron microscopic image of PVC plastics the size ranged less than 5µm.

EDX of the Microplastics analyzed

The result of elemental composition of photo degraded PVC microplastics is given in the Table 2 and Figure 3. There were 7 different types of elements, oxides, silica, calcium, aluminum, iron, chlorine and magnesium in the sample.

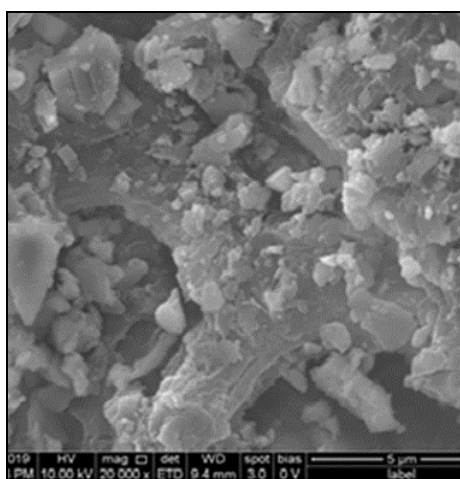


Fig 2: FESEM of PVC

Table 2: Elemental composition of photo degraded PVC Microplastics through FESEM-EDX

31 AN	Series	Unn. C norm.	C Atam.	C Error (1 Sigma)
	[wt.%]	[wt.%]	[at.%]	[wt.%]
8 K-series	26.04	40.26	57.62	3.59
3i 14 K-series	13.89	21.48	17.51	0.63
:a 20 K-series	7.19	11.11	6.35	0.25
Al 13 K-series	6.49	10.03	8.51	0.35
Fe 26 K-series	5.86	9.06	3.71	0.20
7.1 17 K-series	2.84	4.39	2.83	0.13
Ag 12 K-series	2.38	3.68	3.47	0.17
Total:	64.70	100.00	100.00	

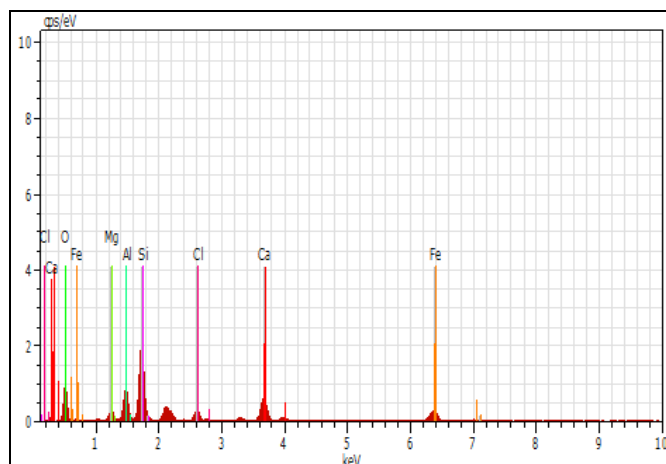


Fig 3: Elemental composition through FESEM-EDS peak formation.

Fish growth analysis

The parameters analyzed in the fishes are listed in the Table 3. There were minor changes between the Control and the experimental fishes, in control were no microplastics were added was Initially 9.46 ± 0.24 in day 1 and the final weight was 63.71 ± 0.57 in day 56, whereas in the experiment, final weight was 61.37 ± 1.03 . Initial length of control fishes were 6.38 ± 0.12 in day 1 and the final length was 16.84 ± 0.53 in day 56, whereas final length of experimental setup was 15.25 ± 0.16 . Length gain of control fishes and experiment fishes were 10.45 ± 0.59 and 8.86 ± 0.16 . These results show that the microplastics influence the fish growth.

Table 3: Growth parameters of *Oreochromis niloticus*

Parameter	Control	Experiment
Initial weight	9.46 ± 0.24	9.46 ± 0.24
Final weight	63.71 ± 0.57	61.37 ± 1.03
Initial length	6.38 ± 0.12	6.38 ± 0.12
Final length	16.84 ± 0.53	15.25 ± 0.16
Length gain	10.45 ± 0.59	8.86 ± 0.16
Weight gain	54.25 ± 0.46	49.90 ± 1.01
Specific_growth_rate	1.3 ± 0.016	1.01 ± 0.018

Plant growth analysis

The growth parameters analyzed for the plants in the experiments are given in the Table 4. There was no significant difference in the control and experiment this shows that the microplastics did not affect the growth of the plant in aquaponic system.

Table 4: Growth parameters of *Spinacia oleracea*

S. No	Parameters	Control	Experiment
1	Initial leaves	2 ± 0.0	2 ± 0.0
2	Final leaves	13.5 ± 1.37	15.83 ± 0.75
3	Initial height	3.37 ± 0.13	3.37 ± 0.13
4	Final height	13.58 ± 0.91	16.83 ± 0.68
5	Initial wet weight	0.46 ± 0.01	0.46 ± 0.01
6	Final wet weight	1.17 ± 0.11	1.69 ± 0.03
7	Initial dry weight	0.11 ± 0.01	0.12 ± 0.01
8	Final dry weight	0.09 ± 0.01	0.19 ± 0.01
9	Moisture content	75.62 ± 2.62	74.51 ± 2.67

Discussion

These aquaponic models do not require the addition of synthetic, chemical fertilizer as the fish waste from the rearing tank and mineralization of bacteria provides sufficient amount of ammonia, nitrate, nitrite, phosphorus, potassium and

micronutrients (Diver 2006; Connolly and Trebic, 2010) [5]. Hence no fertilizers are used in the current study.

PVC, weakens sooner by releasing hydrogen chloride (HCl), forming conjugated double bonds which give a yellow-brownish color and a 'burned' surface appearance. At the second stage, the exposure results also in mechanical deterioration (Bernstein, 2011) [2]. In the present study the photo degradation was done for 180 days.

Leachates released into the water from the plastics are a mixture of chemical substances used to increase the life, durability, and need of products. During weathering only such chemicals seep into the water bodies whereas there no evidence of polymer backbone degradation. (Sofia Bejgarn *et al.*, 2014).

According to the studies of Cole *et al.*, 2011 [4]; Gall and Thompson, 2015 [7]; Gregory, 2009 [9] It was known that the direct interaction of marine organisms with plastic debris through ingestion and entanglement is harmful. Polyethylene was the most common type of MP studied in fish, Lusher *et al.*, (2013) [12] showed that over one third of fish examined in their study had ingested MPs, with pelagic and benthic fish displaying similar gut contents, resulted in lower weight gain. Browne *et al.* (2013) [3] documented increased mortality of the annelid worm *A. marina* exposed to PVC MPs and the antibiotic triclosan.

Conclusion

The present study implies on toxicological implications of micro plastic pollution in the controlled aquatic environment. Plastic particles are abundant in natural environment, which undergoes various degradation processes like photo oxidation, mechanical degradation, biodegradation and releases microplastics at the water surface. These microplastics are accidentally entered into aquatic food web and enter into the human resulting in several disorders. Along with the plastics, its associated chemical pollutants like its additives, fillers, carcinogenic metals release leachates which can cause major issues in the environment.

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