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BIOACCUMULATION OF NAPHTHALENE IN A FEW TISSUES OF FRESH WATER FISH *Rasbora daniconius*

ADVAIT BHAGADE[1*]

[1]Department of Zoology, St. Francis De Sales College, Seminary Hills, Nagpur - 440006, India.

AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Chronic exposure of the freshwater fish Rasbora daniconius to Naphthalene showed bioaccumulation in varying concentrations in the gill, liver, intestine and kidney tissues. The extent of bioaccumulation of Naphthalene was assessed with the help of GCMS in terms of wet weight of tissue. Maximum bioaccumulation of Naphthalene was found in intestine tissue, to the extent of 0.33 µg g-1 wet weight of tissue, followed by kidney, gills and liver. Bioaccumulation found in the tissues of fish indicates the possibility of biomagnifications across various trophic levels of food chain and a possible ultimate effect upon human health.

Keywords: *Rasbora daniconius*; Naphthalene; Chronic exposure; bioaccumulation; GCMS.

1. INTRODUCTION

Naphthalene is a polyaromatic hydrocarbon (PAH). Its common use has rendered its presence in air, water and soil almost ubiquitous. Naphthalene is a white crystalline solid having the chemical formula $C_{10}H_8$, is obtained from distillation of coal tar and other hydrocarbon mixtures, has wide applications, primarily used as mothballs. Naphthalene, used in any manner, finds its way into air, water and soil media, thereby polluting them, and tends to bioaccumulate and bioconcentrate in tissues of organisms, leading to acute as well as chronic deleterious effects on their tissues, and may prove to be fatal at higher doses. A large quantity of work has been carried out to analyze the effects of various pollutants such as pesticides, heavy metals, etc. on fish. Various workers have used diverse methods to study such effect of pollutants. Their work includes study of the toxic and bioaccumulative effects of PAHs (polycyclic Aromatic Hydrocarbons) [1–9]. PAHs have been seen to accumulate in liver and muscle of eel *Anguilla anguilla*, and also to cause gill lesions and liver and spleen tumors [10]. PAHs have been seen to accumulate in gill, liver, kidney and intestine tissues of freshwater fish, *Rasbora daniconius* and *Puntius ticto* [11-14].

The present work deals with the study of the extent of bioaccumulation of the PAH Naphthalene in kidney, intestine, liver and gill tissues of the freshwater fish *Rasbora daniconius* after chronic exposure to a sub lethal dose of Naphthalene at intervals of every 5 days, for 30 days.

The bioaccumulation may ultimately affect human health and life through bioaccumulation and biomagnifications through various trophic levels of food chains.

2. EXPERIMENTAL

2.1 Materials and Reagents

Freshwater fish *Rasbora daniconius* were obtained from local freshwater bodies. Factors such as availability, size, weight, ease of stocking and

**Corresponding author: Email: bhagade.sfscollege@gmail.com;*

handling, etc. were considered for the choice of fish species for the present study. The fish were acclimatized in dechlorinated tap water for about 10 days at room temperature. Characteristics of dilution water [15] were analyzed and have been described in Table 1.

Naphthalene is not soluble in water. A stock of the test solution was prepared using 1 mg /ml of Naphthalene with Dichloromethane (MERCK) as an organic solvent and then diluted for use as per the requirement. Acute bioassays, at 24, 48, 72 and 96 hour intervals by exposing the test organisms to varying concentrations of such solutions of Naphthalene in Dichloromethane. Naphthalene of MERCK make was used for the present work. A sub lethal concentration, based on the LC_{50} values obtained during acute bioassay, was arrived at.

Table 1. Characteristics of dilution water

Parameters	Values *
Temperature °C	25-27
pH	7.5-8.2
Total Alkalinity as $CaCO_3$	156-190
Total Hardness as $CaCO_3$	142-172
Ca Hardness as $CaCO_3$	80-94
Mg Hardness as $CaCO_3$	62-78
Dissolved Oxygen	6.9-7.3
Calcium as Ca	32-38
Magnesium as Mg	14-18
Sodium as Na	36-38
Potassium as K	2-4
Chloride	126

The incipient LC_{50} is recommended as the most useful single criterion of toxicity evaluation [16]. Based on these values, a sub lethal concentration of the substance under study was determined, and further chronic toxicity tests were carried out on the test fish over a period of 30 days, with samples taken at intervals of 5, 10, 15, 20, 25 and 30 days. Many references are available in literature where such a method has been used for toxicity evaluation. Test fish have been exposed to pollutants for 24, 48, 72 and 96 hours [17,18]. Percent mortality was noted for these different concentrations every 24, 48, 72 and 96 hours.

Based on these observations, the LC_{50} values, NOEC and 95% confidence interval were calculated using probability graph paper using standard methods in literature [19] and used for determining sub lethal concentrations of Naphthalene for chronic toxicity testing. Test fish were exposed to such a safe, sub lethal 0.5 µg l^{-1} concentration of Naphthalene, repeated every 24 hours, using a continuous flow through method using an indigenously designed dosing apparatus (Fig. 1). Appropriate controls were maintained at all stages of the experiment.

The dosing apparatus consists of two Perspex cylindrical reservoirs kept one above the other. The upper reservoir is closed at the top with a rubber bung and is airtight. At the bottom, it has an inlet on one side for flow of solution and a glass tube is fitted in the central hole. An annular Perspex ring of smaller diameter is fixed to the bottom of the upper reservoir on the lower side. The lower reservoir has a stop cock acting as an outlet near the bottom and an air vent at the top. Capacity of the dosing unit is 10 litres.

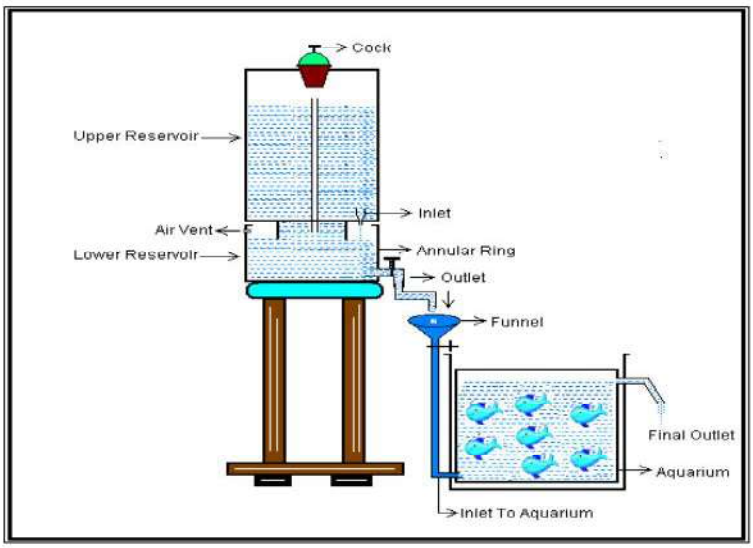

Fig. 1. Schematic diagram of the experimental set-up for chronic bioassay studies

The upper reservoir is filled with test solution having known concentration of Naphthalene and the upper hole is closed with the rubber bung. Liquid starts flowing into the lower reservoir through the inlet tube. When liquid in the lower reservoir rises to the rim of the annular ring and into the glass tubing, tension is created, resulting in break of the flow. A continued discharge through the outlet brings down the liquid level and the flow starts again, when the liquid level falls below the rim. This creates an automatic make and break of flow in the lower reservoir. The stop cock was adjusted to discharge the required flow of 14 ml/ min.

As soon as the liquid level in the lower reservoir drops below the rim of the annular ring, air enters through the 'air vent' and instant flow from the upper reservoir commences. This cycle repeats as the liquid level touches and breaks from the annular ring continuously, keeping the level of liquid in the lower reservoir at almost constant height.

Experiments were carried out using a 20 litre rectangular glass aquarium. Experiments were continued for a time interval of 5, 10, 15, 20, 25 and 30 days only. The dosing unit was normally filled up with dilution water and required concentration of Naphthalene solution was added to it. The same concentration of Naphthalene solution was also added to the aquarium at the beginning of the experiment. The flow rate was adjusted so as to allow a flow of 20 litres of solution through the aquaria in 24 hours. The incoming solution was delivered at the bottom of the aquarium through a funnel so as to avoid short circuiting. An overflow arrangement at the top of the aquarium was provided to maintain a constant volume in the aquarium. 20 numbers of fish were introduced in the aquarium. Feeding and replacements of fresh solutions were followed as per details reported in literature [20].

Test organisms, *Rasbora*, under study were sacrificed at intervals of every 5 days up to 30 days, and their tissues were collected for bioaccumulation study. These were fixed in Buoin's fixative prior to further processing. Fixed tissues were thoroughly washed, weighed, macerated and then extracted in Dichloromethane by clean up using Celite 545 filter aid medium, as indicated in literature [21].

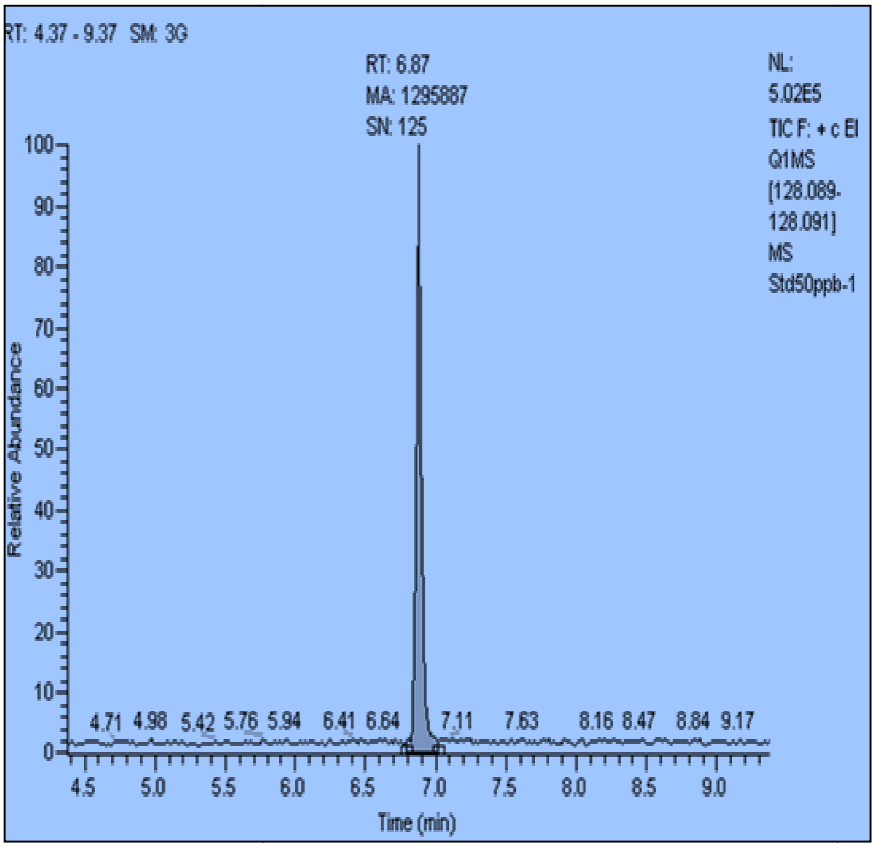

Fig. 2. Standard chromatogram of naphthalene

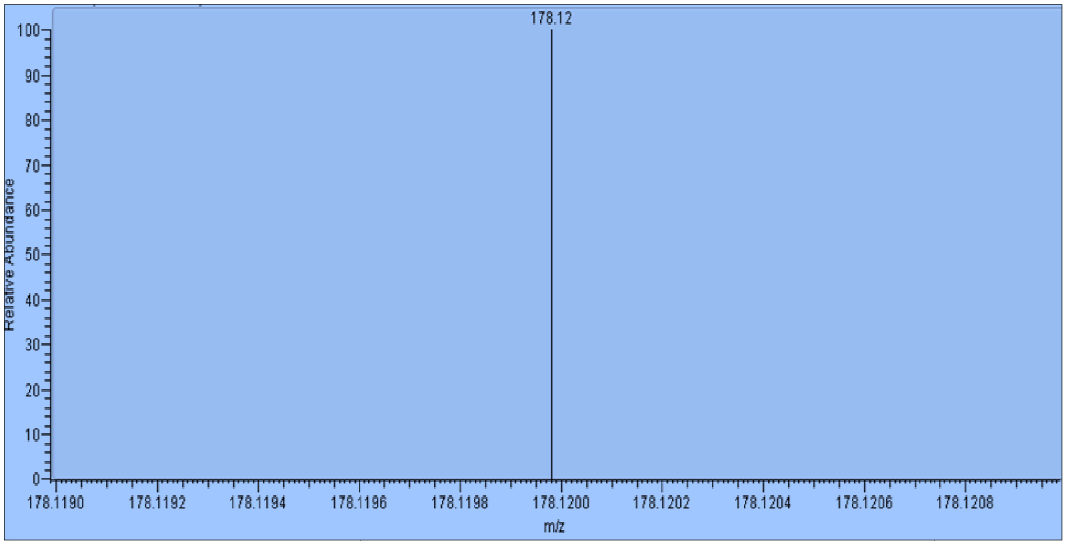

Fig. 3. Mass spectra of naphthalene

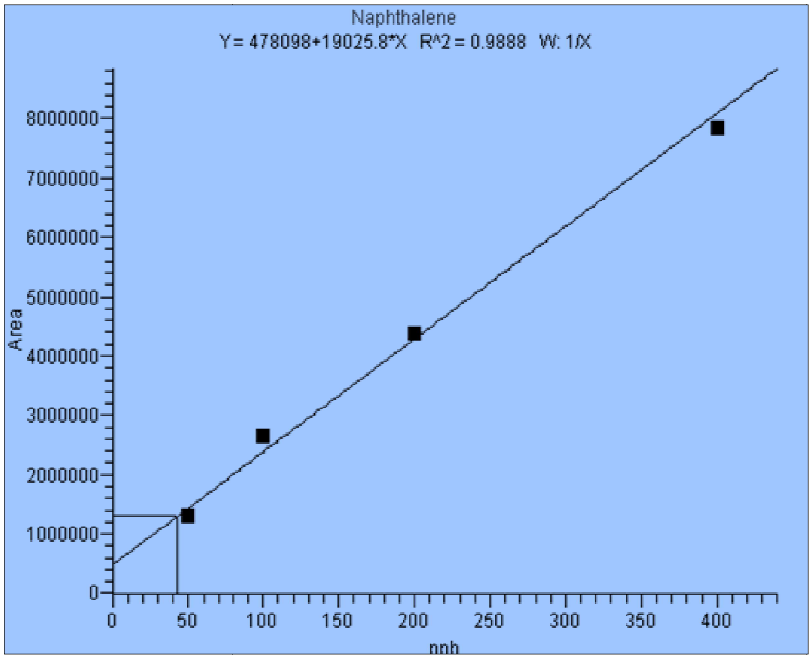

Fig. 4. Calibration curve of naphthalene

Tissue extracts were concentrated by evaporation, collected in stoppered KD tubes and analyzed for their Naphthalene content using GC-MS (Gas Chromatography- Mass Spectrophotometry). The use of GC and GC-MS to estimate the degree of bioconcentration has been widely accepted [22]. It can identify a wide variety of compounds in many different matrices and in the presence of interfering compounds [23]. For this purpose, a GC-MS/MS (Gas Chromatograph – Mass Spectrometer) of make Thermo Scientific, USA and model TSQ Quantum MS Trace GC Ultra with a Capillary column TR5-MS (30m x 0.25mm i.d., 0.25 µm) was used. Standard chromatogram, mass spectra and calibration curves were obtained for pure Naphthalene, as shown in Figs. 2 to 4.

This was followed by actual analysis of samples using GC-MS.

3. RESULTS AND DISCUSSION

Tissue extracts showed increasing concentrations of Naphthalene over intervals of 5 days. Figs. 5 to 8 show a few chromatograms for Naphthalene in kidney, intestine, liver and gill tissues of *Rasbora*.

Based on these observations, concentrations of Naphthalene were calculated in terms of µg g^{-1} wet weight of the tissues. These calculations have been shown in Table 2, and the same have been represented graphically in Fig. 9.

The above observations indicate that Naphthalene was initially highest in the gill, i.e., 0.01µg g^{-1}, at the end of 5 days, and accumulated to 0.02 µg g^{-1} for the first 10 days. The accumulation increased to 0.05 µg g^{-1} till the 15th day and then gradually increased over the next 15 days, and then stabilised so that Naphthalene was highest at 0.08 µg g^{-1} in the gill tissue of *Rasbora* at the end of 30 days. Naphthalene accumulated very slowly in the liver, so that the build up was very insignificant as compared to other tissues, at 0.04 µg g^{-1} at the end of 30 days. The kidney tissue showed only a gradual build up to 0.10µg g^{-1} of accumulated Naphthalene at the end of 30 days.

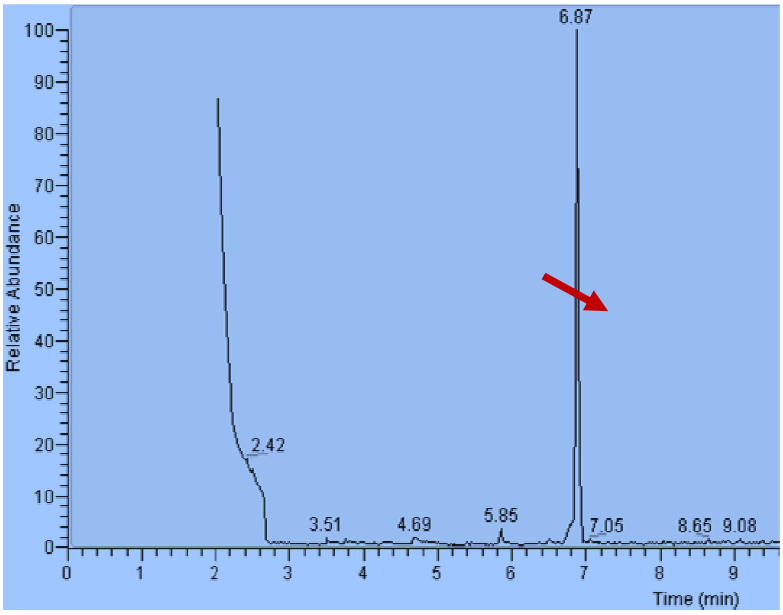

Fig. 5. Chromatogram showing naphthalene in rasbora liver (30 Days)

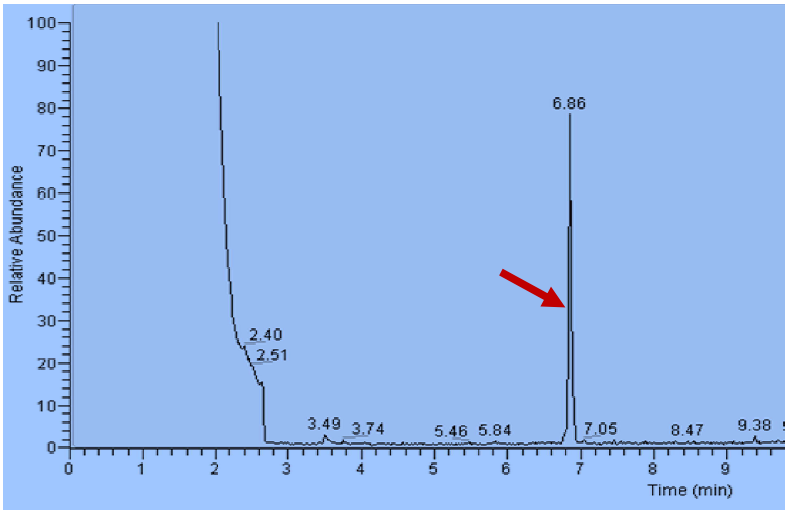

Fig. 6. Chromatogram showing naphthalene in rasbora kidney (30 days)

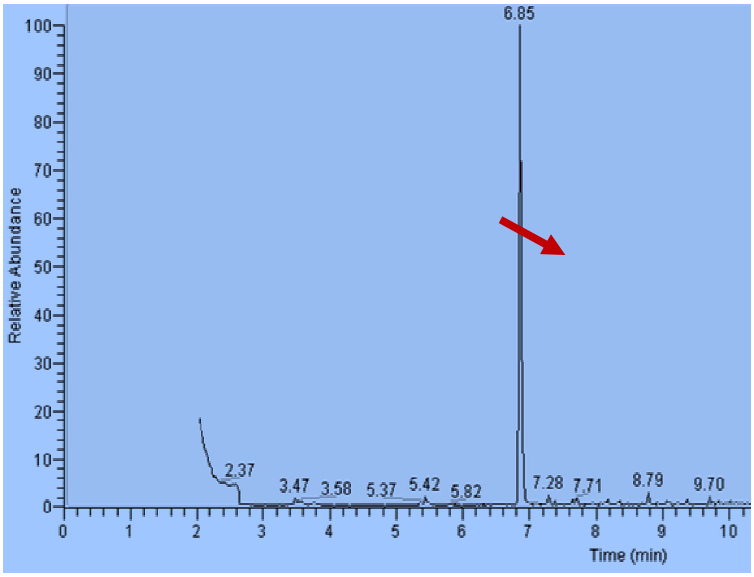

Fig. 7. Chromatogram showing naphthalene in rasbora intestine (20 Days)

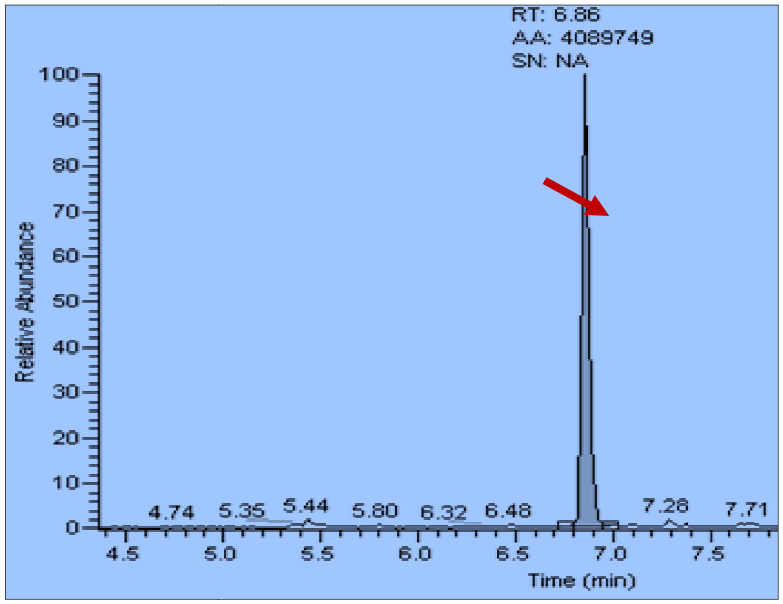

Fig. 8. Chromatogram showing naphthalene in sample rasbora gill (20 days)

Table 2. Bioaccumulation of Naphthalene in *Rasbora daniconius*

Exposure time, days	Values in µg g^{-1} wet weight of the tissue			
	Gill	**Intestine**	**Kidney**	**Liver**
5	0.01	ND	ND	ND
10	0.02	0.00	0.01	0.01
15	0.05	0.02	0.04	0.02
20	0.07	0.10	0.09	0.03
25	0.08	0.20	0.09	0.04
30	0.08	0.33	0.10	0.04

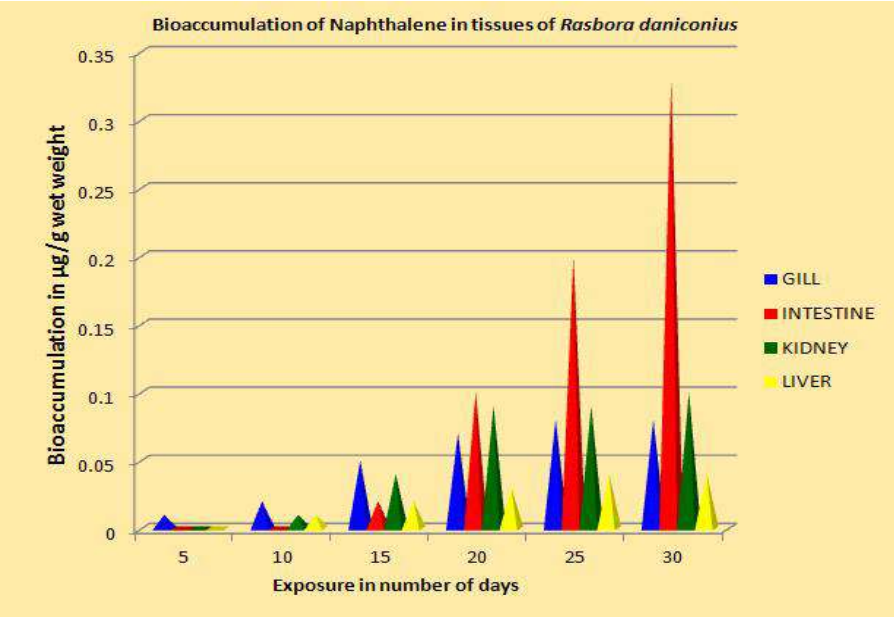

Fig. 9. Graphical representation of bioaccumulation of naphthalene in some tissues of *Rasbora daniconius*

Though Naphthalene could not be detected in the intestine tissue in the first 5 days of the experiment, there was a steady and strong build up of over the remaining 25 days, so that the level of Naphthalene at the end of 30 days in intestine tissue was highest among all tissues studied, at 0.33 µg g^{-1}.

4. CONCLUSION

The study indicates that Naphthalene from the surrounding medium enters the body through the respiratory process to bioaccumulate in the gills to a moderate extent, after which it is transported to other tissues from gills through the circulation, as is evident from the less yet stable bioaccumulation of Naphthalene in gill tissue over the complete period of study.

The bioaccumulation of the pollutant to a greatest extent found in the intestine tissue hints at a more direct absorption through the oral route, and the inability of this tissue to dispose of the pollutant accumulating in it. Normally, the pollutant would be transported to the liver for the process of biotransformation, and later to kidneys for excretion. However, the low bioaccumulation values of Naphthalene in liver as well as the kidneys indicate that Naphthalene accumulates in the intestine tissue instead of being transported to liver. Similarly, it appears that the pollutant accumulates to some extent in the gills but is not transported into the circulation to liver or kidney to any appreciable extent.

These characteristics of bioaccumulation of Naphthalene may give further insight into its effects on human health and life when man is exposed to Naphthalene thus bioaccumulated in food organisms.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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