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A Comparative Study of the Bioaccumulation of Naphthalene and PCB 126 in a Few Tissues of *Rasbora daniconius*

Advait Bhagade*

Department of Zoology, St. Francis De Sales College, Nagpur - 440 006, Maharashtra, India

***Corresponding author:** Assistant Professor in Zoology, St. Francis De Sales College, Nagpur - 440 006, Maharashtra

e-mail: bhagade.sfscollege@gmail.com **Contact:** +91-9423681555 / 9561591744

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ABSTRACT

The effects of bioaccumulation of the PAH (Polcyclic Aromatic Hydrocarbon) Naphthalene and the PCB (Polychlorinated Biphenyl) 126, both of which are constituents of pollutant substances, in four different tissues of freshwater fish *Rasbora daniconius*, were compared by bioassay techniques of analysis using GC-MS. PCB 126 was found to have a much higher degree of bioaccumulation in all tissues studied, as compared to Naphthalene.

Key words: PCB 126, GC-MS, Polcyclic Aromatic Hydrocarbon, *Rasbora daniconius*

A large quantity of work has been carried out to analyze the effects of various pollutants on fish. Major part of the work has been carried out on marine fish and lesser so on fresh water fish. It is known that the pollutant substances or their constituents present in water are easily able to enter the bodies of fish through various points of entry. Further, the pollutants are seen to bioaccumulate in the bodies of fish, and biomagnify over different trophic levels of the food chain, ultimately reaching the diet of man. Study of degrees of bioaccumulation of various pollutants, and their effects in organisms, has thus become an important part of ecological studies. The pollutants include a variety of substances, such as pesticides, heavy metals, etc. In the present study, the bioaccumulation of PAH Naphthalene and of the PCB Congener PCB 126 (3, 3', 4, 4', 5-Pentachlorobiphenyl) have been studied in gill, intestine, kidney and liver tissue of freshwater fish *Rasbora daniconius*. Further, a comparison of the extent of bioaccumulation of these two substances in various tissues of the fish has been carried out.

MATERIALS AND METHODS

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

Neither of the pollutant substances under study is not soluble in water. A stock of the test solution was prepared using 1 mg /ml of the pollutant with Dichloromethane (MERCK) as an organic solvent and then diluted for use as per requirement. The test organisms were then exposed to varying concentrations of each solution, by performing acute bioassays, at 24, 48, 72 and 96 hours intervals. Naphthalene of MERCK make, and PCB 126 of Dr. Ehrenstorfer (Germany) make, were used for the present work. A sub lethal concentration, based on the LC50 values obtained during acute bioassay, was arrived at. 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, using continuous flow through method, with tissue samples taken at intervals of 5, 10, 15, 20, 25 and 30 days. Various

methods have been used with the aim to analyze the effects of various pollutants. Though there are many references available in literature regarding the use of bioassays for evaluation of soil and sediment toxicity (Couillard *et al*. 2009, Britta-Eklund *et al*. 2010), and integrating bioassays with other techniques using fish and invertebrates for assessing environmental risks and damage (Martínez-Gómez *et al*. 2010), only some references are available for bioassays using the pollutant substance for direct exposure of test organisms. Bioassays to determine acute toxicity after 96 hour exposures to determine LC50 values (Vieira 2008), and use of continuous flow through bioassays (Brenniman *et al*. 1976), are some of these methods.

The use of GC and GC-MS to estimate the degree of bioconcentration has been widely accepted (Maack and Sonzogni 1988). Literature available confirms that PAHs and PCBs are toxic and bioaccumulate in organisms (Nakata *et al*. 2003, Greco *et al*. 2010).

Naphthalene is a white crystalline solid having the chemical formula $C_{10}H_8$, obtained from distillation of coal tar and other hydrocarbon mixtures, has wide applications, and is known from its primary use in mothballs. PCBs, which are found as a group of 209 congeners, are odorless, tasteless, clear to pale-yellow, viscous liquids. The chemical formula of biphenyl is C_{12} H_{10}, where the 10 Hydrogen positions are variously substituted by Chlorine to obtain the different congeners. The chemical formula of PCB 126 is C_{12} H_7C_{l5}.

Bioaccumulation study was carried out initially by means of acute bioassays of 96 hour duration to determine LC_{50} values as per standard methods available in literature (Davis and Mason 1973, Lee *et al*. 2005), and based on such values, a subsequent chronic bioassay of 30 days using a dosing apparatus with a continuous flow through method (Murty 1986), to introduce sublethal doses of the pollutant substances, 0.5 µg l^{-1} Naphthalene and 12.5 µg l^{-1}PCB 126 respectively, separately, for 30 days each, into the water. Appropriate controls were also run in parallel.

Studies show that the path of hydrocarbons through the fish includes entrance through the gills, metabolism by the liver, transfer of hydrocarbons and their metabolites to the liver, and, finally, excretion through kidneys. The gall bladder is recognized as a major storage site of hydrocarbons and their metabolites (Lee *et al*. 1972). The tissues gill, intestine, kidney and liver of the test organisms, i.e. fresh water fish *Rasbora daniconius* were obtained every 5 days over a period of 30 days. Tissues were weighed, and extracts were obtained after clean up using Dichloromethane through Celite columns. Analysis of these extracts, using GCMS for analysis, has shown bioaccumulation of the above compounds in different tissues over a period of time.

RESULTS AND DISCUSSION

I] Observations

Exposure to naphthalene

Mild bioaccumulation of naphthalene was observed in the tissues of *Rasbora daniconius*, as can be seen from the Chromatograms obtained after analysis of their extracts on GC-MS (Fig 1, 5). The bioaccumulation of Naphthalene in Gill, intestine, kidney and liver tissues of *Rasbora daniconius* has been shown in (Table 1). A graphical representation of accumulation of Naphthalene in the four tissues studied has been given in (Fig 6). Thus, over the period of 30 days of the chronic bioassay, naphthalene accumulated to a minimum, i.e. 0.04 µg g^{-1} in the liver tissue, whereas it was seen to accumulate the most in the intestine tissue at 0.33 µg g^{-1} wet weight of the tissue.

Table 1 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

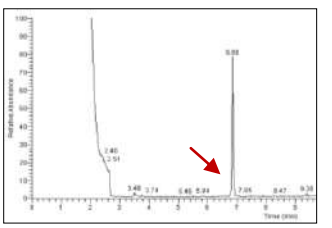

Fig 1 Standard Chromatogram of Naphthalene

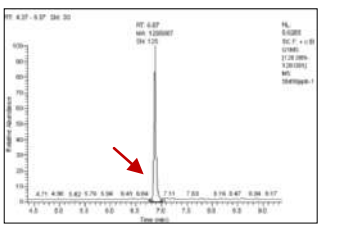

Fig 2 Chromatogram showing Naphthalene in Rasbora Kidney (30 days)

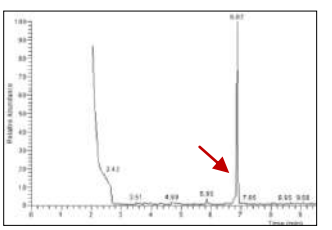

Fig 3 Chromatogram showing Naphthalene in Rasbora Liver (30 Days)

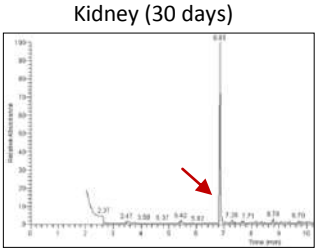

Fig 4 Chromatogram showing Naphthalene in Rasbora Intestine (20 Days)

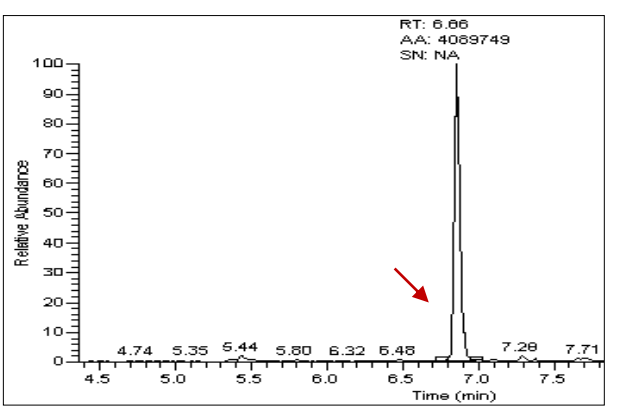

Fig 5 Chromatogram showing Naphthalene in sample Rasbora Gill (20 Days)

Table 2 Bioaccumulation of PCB 126 in *Rasbora daniconius*

Exposure time, days	Values in µg g^{-1} wet weight of tissue			
	Gill	Intestine	Kidney	Liver
5	52.68	0	0	0.00
10	59.52	5.08	0.47	1.64
15	60.66	11.00	0.54	8.45
20	61.95	15.79	0.62	15.63
25	63.84	19.42	0.68	16.24
30	64.68	23.95	0.78	17.14

Exposure to PCB 126

A significant bioaccumulation of PCB 126 was observed on analysis of the tissues of *Rasbora daniconius*, as is evident from the Chromatograms obtained after analysis of tissue extracts on GC-MS (Fig 7, Fig 11). The bioaccumulation of PCB 126 in Gill, intestine, kidney and liver tissues of *Rasbora daniconius* has been shown in (Table 2). A graphical representation of accumulation of PCB 126 in the four tissues studied has been given in (Fig 12). Thus, over the period of 30 days of the chronic bioassay, PCB 126 accumulated to a minimum, i.e. 0.78 µg g^{-1} in the kidney tissue, whereas it was seen to accumulate the most in the gill tissue at 64.68 µg g^{-1} wet weight of tissue.

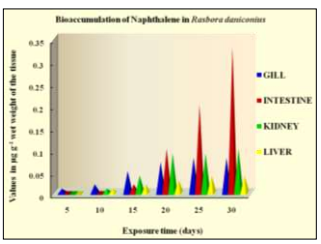

Fig 6 Graph showing Bioaccumulation of Naphthalene in tissues of *Rasbora daniconius* over a 30 day exposure period

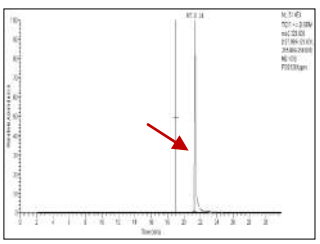

Fig 7 Standard chromatogram of PCB 126 (3,3',4,4',5-pentachlorobiphenyl)

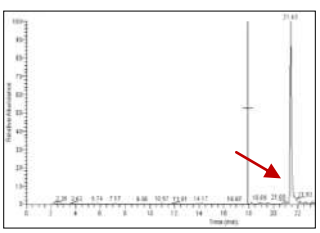

Fig 8 Chromatogram showing PCB 126 in *Rasbora* Liver (10 Days)

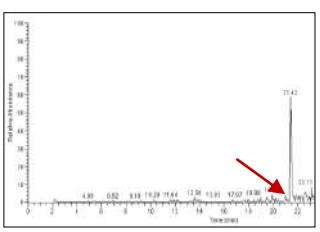

Fig 9 Chromatogram showing PCB 126 in *Rasbora* Gills (30 Days)

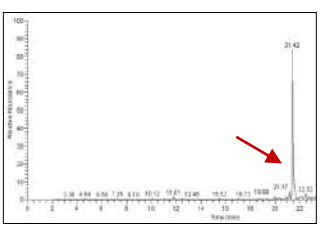

Fig 10 Chromatogram showing PCB 126 in *Rasbora* Intestine (30 Days)

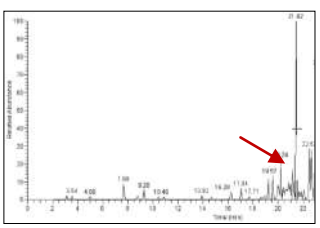

Fig 11 Chromatogram showing PCB 126 in *Rasbora* Kidney (30 Days)

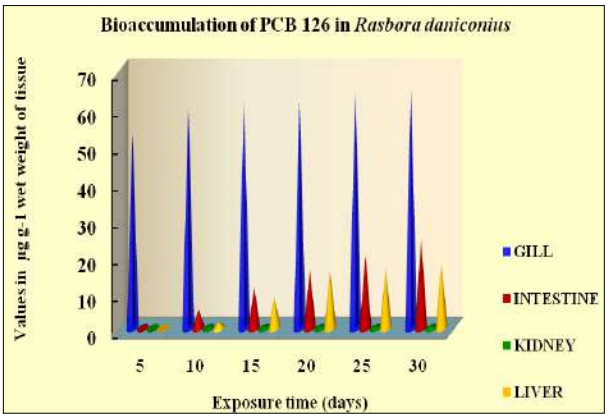

Fig 12 Graph showing PCB 126 in tissues of *Rasbora daniconius*

Gill tissue

The build up of concentration of naphthalene was gradual in the gill tissue of *Rasbora* over a 30 day period from 0.01 µg g^{-1} to 0.08 µg g^{-1} wet weight of tissue. On the other hand PCB 126 accumulated to a large extent in the gill tissue of *Rasbora*, at an initial high of 52.68 µg g^{-1} at the end of 5 days, and then showed gradual increase to 64.68 µg g^{-1} wet weight of tissue at the end of 30 days. The accumulation of naphthalene was thus very insignificant as compared to that of PCB 126. A graphical representation of comparative accumulation of naphthalene and PCB 126 in gill tissue of *Rasbora* has been shown in (Fig 13).

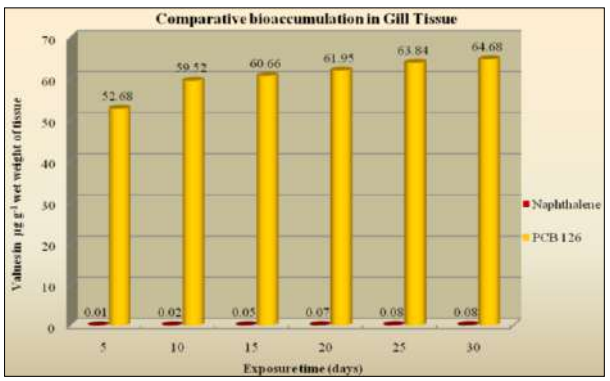

Fig 13 Graph showing comparative bioaccumulation of Naphthalene and PCB 126 in Gill tissue of *Rasbora daniconius*

Intestine tissue

Accumulation of naphthalene was not detectable in this tissue in the first ten days of exposure. There was a gradual build up through the remaining days, till accumulation was detected at a maximum of 0.33 µg g^{-1} wet weight of tissue at the end of 30 days. It was also observed that accumulation of naphthalene over the 30 day exposure period was the maximum in the intestine amongst all tissues. However, in comparison, the accumulation of PCB 126 showed a gradual build up, and was significantly high at 23.95 µg g^{-1} wet weight of tissue over the 30 day period. A graphical representation of comparative accumulation of naphthalene and PCB 126 in intestine tissue of *Rasbora* has been shown in (Fig 14).

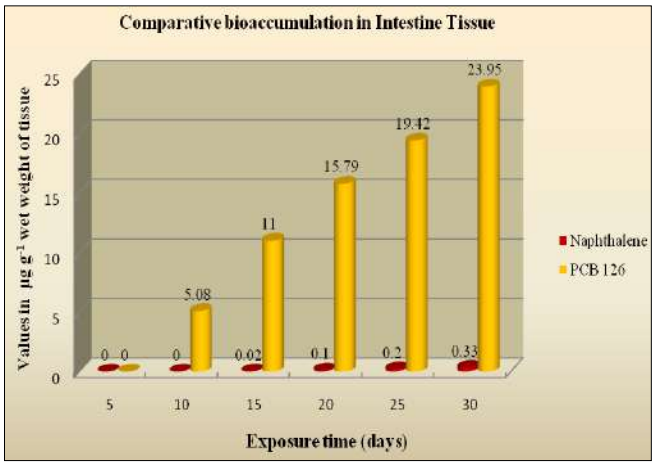

Fig. 14: Graph showing comparative bioaccumulation of Naphthalene and PCB 126 in Intestine tissue of *Rasbora daniconius*

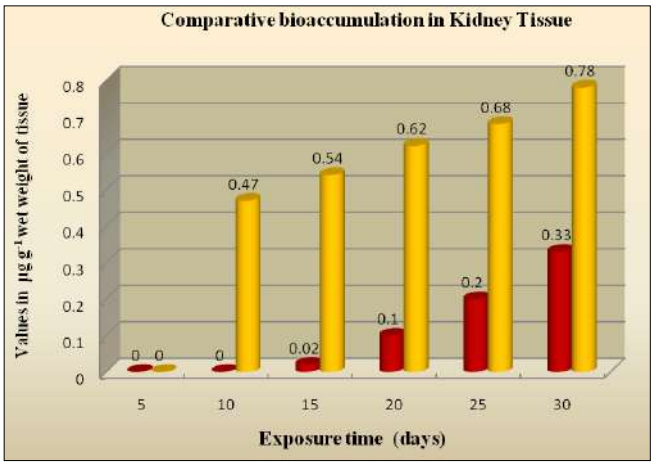

Fig. 15: Graph showing comparative bioaccumulation of Naphthalene and PCB 126 in Kidney tissue of *Rasbora daniconius*

Kidney tissue

Accumulation of both naphthalene and PCB 126 appeared to be gradual though not very significant. Naphthalene could not be detected in the first 10 days of the 30 days' exposure, and was seen to be 0.02 µg g^{-1} on day 15, which rose to 0.33 µg g^{-1} wet weight of tissue on day 30. The accumulation of PCB 126 was more than twice that of naphthalene over the 30 day period, though a significant 0.47 µg g^{-1}on day 10 itself, with only a gradual increase up to 0.78 µg g^{-1} wet weight of tissue on day 30 of exposure. A graphical representation of comparative accumulation of Naphthalene and PCB 126 in kidney tissue of *Rasbora* has been shown in (Fig 15).

Liver tissue

Accumulation of naphthalene was very gradual and very insignificant in the liver tissue. Naphthalene was found to be 0.02 µg g^{-1} on day 10 of exposure and increased to 0.04 µg g^{-1} wet weight of tissue on day 25, following which it remained constant up to day 30. On the other hand, accumulation of PCB 126 was seen to build up rapidly from 1.64 µg g^{-1} on day 5 to 15.63 µg g^{-1} on day 20, after which it very slowly increased to 17.14 µg g^{-1} wet weight of tissue by day 30. A graphical representation of comparative accumulation of naphthalene and PCB 126 in liver tissue of *Rasbora* has been shown in (Fig 16).

Fish, being aquatic organisms, are directly exposed to pollutants present in the water primarily through gills, during respiration, which is a continuous process. Secondarily, direct exposure of the body to pollutants is also at the site of the alimentary canal, where they would enter through or with food. The metabolic pathway through which these substances may travel is first through the circulatory system, where they may be deposited by blood from gills and intestinal tissue, into the liver for biotransformation into less harmful and excretable substances, and then to the kidney for excretion to the outside. Accumulation of naphthalene in gill tissue was insignificant, indicating the ability of gills to metabolise or transfer Naphthalene to other tissues as can be seen from higher values of its accumulation in other tissues. On the other hand, accumulation of PCB 126 in gill tissue was seen to reach a very high initial value in the first 5 days of exposure itself indicating that gill tissue is unable to metabolise PCB 126 or send it to other tissues for further biotransformation.

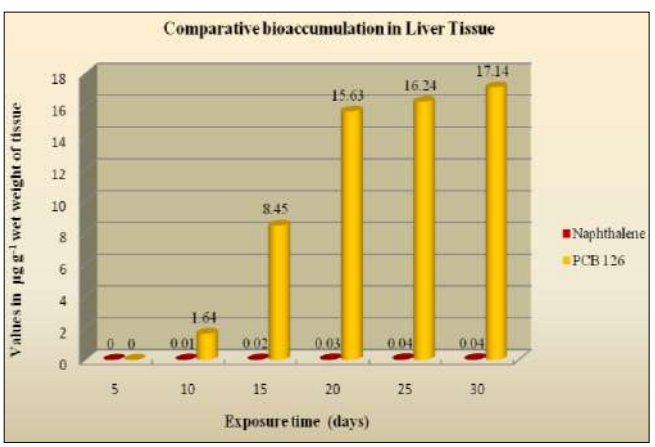

Fig 16 Graph showing comparative bioaccumulation of Naphthalene and PCB 126 in Liver tissue of *Rasbora daniconius*

The rate as well as magnitude of build up of Naphthalene in intestine tissue was seen to be insignificant as compared to PCB 126, although naphthalene showed highest concentration in intestine tissue. This indicates the inability of the intestine tissue of *Rasbora* to absorb and then transport naphthalene for transformation or excretion by liver and kidney. On the other hand, rate as well as magnitude of build up of PCB 126 was higher and significant, indicating high accumulation of the substance in the intestine tissue, in addition to its highest accumulation in gill tissue. Thus, intestine tissue also appears to be a major site of accumulation of PCB 126.

In case of the kidney tissue, no significant accumulation of naphthalene, and only a low accumulation of PCB 126

was seen. The kidney being the primary organ of excretion, it can be inferred that less amount of naphthalene was received by the kidney in the first place since most of it accumulated in the intestine, and that of the naphthalene received from other tissues, some partially accumulated in the kidney while some was excreted out from the fish bodies. A more than two fold accumulation of PCB 126 was observed in the kidney tissue, indicating the tendency of the kidney to accumulate rather than to excrete PCB 126.

The liver tissue was found not to accumulate Naphthalene to any significant extent, indicating that naphthalene was not absorbed from the intestine tissue, as is evident from its high accumulation in the latter. The low values of accumulation in liver tissue also indicate a possibly efficient biotransformation of whatever naphthalene must have been transported to the liver through the circulation from other tissues. PCB 126, at the outset, was seen to accumulate to a very high degree in gill tissue, indicating failure of the tissue to introduce it into circulation. PCB 126, from the significant values of its accumulation observed in Liver tissue, thus accumulated, and was not transformed after transport from other tissues.

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