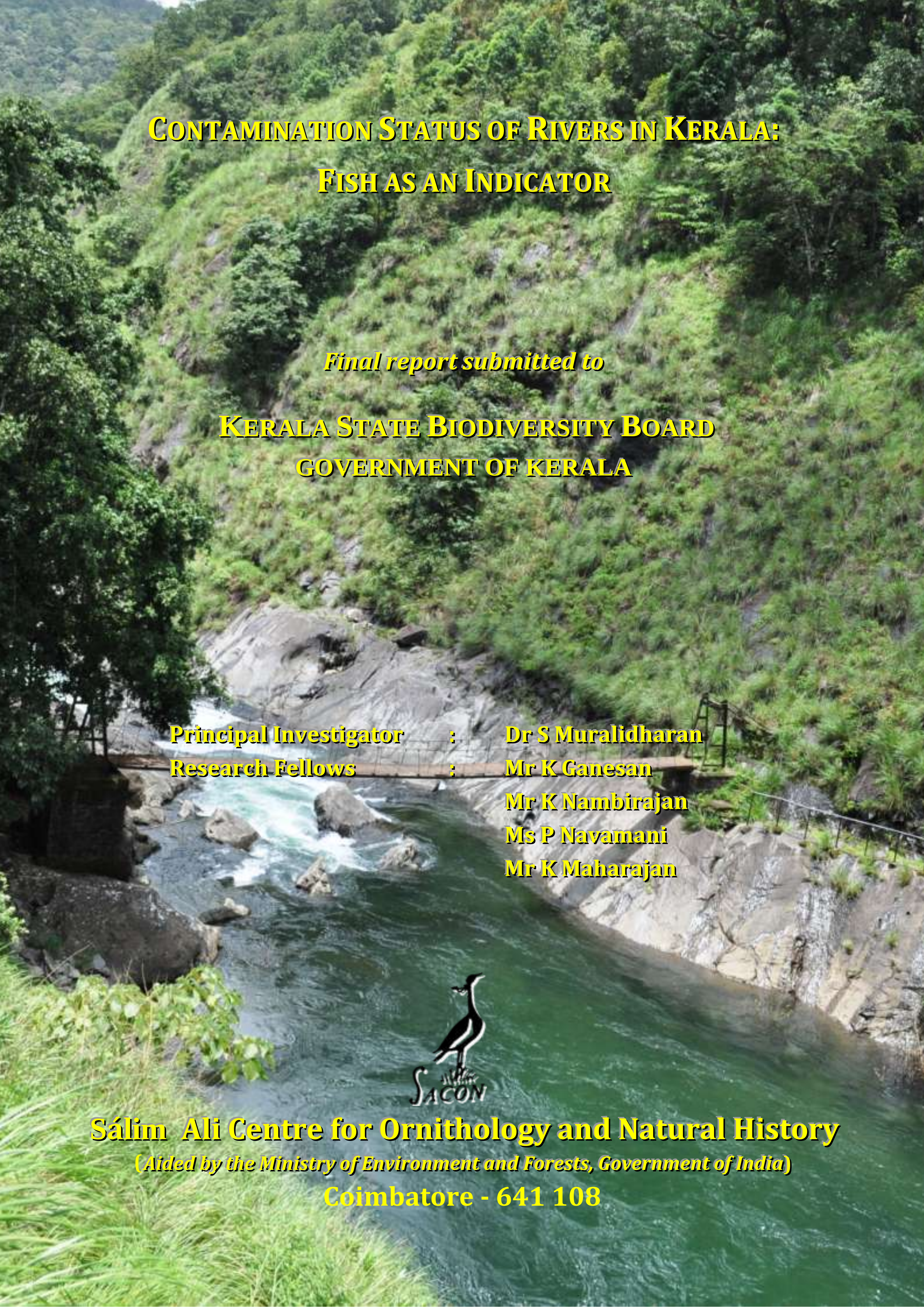

CONTAMINATION STATUS OF RIVERS IN KERALA:
FISH AS AN INDICATOR

Final report submitted to

KERALA STATE BIODIVERSITY BOARD
GOVERNMENT OF KERALA

Principal Investigator	:	**Dr S Muralidharan**
Research Fellows	:	**Mr K Ganesan**
		Mr K Nambirajan
		Ms P Navamani
		Mr K Maharajan

Sálim Ali Centre for Ornithology and Natural History
(*Aided by the Ministry of Environment and Forests, Government of India*)
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Cover front	:	A view of Kunthipuzha River, Silent Valley National Park
Cover back - above	:	Pamba River near Sabarimala
Cover back - below	:	Mortality of fishes - Chalakudy River
Photos	:	Mr K Ganesan, Mr SP Santha Kumar and Dr CP Shaji
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1. PREAMBLE

1.1 Pesticides

Intensive use of synthetic pesticides in agriculture and public health operations has resulted in serious environmental hazards (Singh *et al.,* 2006) over the years. Water bodies adjoining agricultural fields receive runoff loaded with pesticides (Bouregeois *et al.,* 1993, Nayak *et al.,* 1995 and Kalavathy *et al.,* 2001) and inhabiting organisms including fish (Saravanan *et al.,* 2003 and Park *et al.,* 2004) suffer. It may be noted that the annual consumption of pesticides mainly for agricultural in India has crossed 50,583 metric tons (MT) from a mere 5,000 MT during 1960s (www.indiastat.com retrieved on 9th May 2013). Several studies have shown that pesticides could cause health problems such as endocrine dysfunction, birth defects, nerve damage, lower sperm count, breast and testicular cancer in humans (Ahmed *et al.,* 1996, Soto *et al.,* 1998, John *et al.,* 2001, Brody and Rudel, 2003, Garry *et al.,* 2004, Bedi *et al.,* 2005 and Aulakh *et al.,* 2006).

Among the different groups of pesticides, organochlorines (OCs) were the first group to be invented. OC pesticides are non-polar, lipophilic, toxic and highly persistent compounds. These synthetic pesticides reach the aquatic environment via soil percolation, air drift or surface runoff, leaching and disposal of empty containers. Unfortunately these OCs, for example Dichlorodiphenyltrichloroethane (DDT), Hexachlorocyclohexane (HCH), endosulfan, heptachlor, dieldrin and their transformation products (TPs) remain for years in the environment. Most of them are now banned for agriculture but their residues are still present in many biological and non-biological components (Vijayan *et al.,* 2008, Dhananjayan and Muralidharan, 2010 and Mudiam *et al.,* 2011). They can also bioaccumulate and biomagnify through food chains and produce harmful effects at every level (Muralidharan *et al.,* 2009). On the other hand organophosphate (OP) and synthetic pyrethroids (SPs) are more toxic but less persistent lasting only for days, weeks or months in the environment (Wania and Mackay, 1996 and WHO, 2000). Hence, they have almost replaced OCs except a few. However, the OPs are potent cholinesterase (ChE) inhibitors. They can bind covalently with the serine residues in the active site of acetylcholinesterase (AChE), and prevent its natural function in the catabolism of neurotransmitters, eventually lead to death.

Pyrethroids are derived from natural pyrethrins with some modifications to enhance their environmental stability or alter their insecticidal activity. They are also synthesized. Since they guarantee as effective agents against a broad range of pests and stable under field conditions, they are used as insecticides in agriculture worldwide (Shan *et al.,* 1999 and Mak *et al.,* 2005). Many products such as Raid found commonly in retail stores for home use, contain pyrethroid such as Permethrin, to eliminate household pests such as ants and spiders (De Pasquale, 2010). Pyrethroids are reported to be present in environment due to their extensive usage also in agriculture and households. Several studies have proved that pyrethroids are highly toxic to a number of non-target organisms such as honeybees, freshwater fishes and aquatic arthropods even at very low concentrations mainly through their action on voltage-sensitive sodium channels (Oudou *et al.,* 2004, Kim *et al.,* 2005, Bradberry *et al.,* 2005, Tomlin, 2006, Palmquist *et al.,* 2008a&b and Palmquist *et al.,* 2011). Pyrethroids are also capable of inducing chromosomal aberrations, genotoxicity and micronucleus formation in rat bone morrow cells (Campana *et al.,* 1999, Fahmy and Abdallah, 2001 and Celik *et al.,* 2003 & 2005).

1.2 Heavy metals

Heavy metals in trace amounts are constituents of all life forms including fishes. But at higher concentrations, they are toxic and may lead to deleterious effects on organisms, particularly fishes and make them unsuitable as food (Muralidharan, 1995). Metals can enter into the food web through direct consumption of water or organisms, or through uptake processes, and get accumulated in fish and other wildlife (Paquin *et al.,* 2003). Fishes form major part of the human diet because of their high protein content, low saturated fat and also presence of omega fatty acids known to support good health (Dural *et al.,* 2007). There is a growing concern that metals accumulated in fish muscle tissues may represent health risk, especially for populations with high fish consumption rates (Liao and Ling, 2003, Burger and Gochfeld, 2009 and Diez *et al.,* 2009). Metals such as copper, iron, manganese and zinc play a role in physiological regulatory activities in organisms (Hogstrand and Haux, 2001), but metals, namely cadmium, chromium, lead, arsenic, nickel and mercury exhibit toxic effects on organisms (Mason, 1991 and Bhupander and Mukherjee, 2011a&b). Chromium (Cr) has been associated with various effects such as severe respiratory, cardiovascular, gastrointestinal, hepatic and renal damage and skin disorders in sensitive individuals (Health Protection Agency, UK 2007); Cadmium (Cd) has showed increased risk to kidney, bone and lungs and long-term exposure may also cause skeletal damage. First it was reported from Japan, where the *itai-itai* (ouch-ouch) disease (a combination of osteomalacia and osteoporosis) was related to cadmium exposure in the 1950s. The exposure was caused by cadmium-contaminated water used for irrigation in local rice fields (Jarup, 2003). Lead (Pb) has been implicated for various ailments such as headache, irritability, abdominal pain and severe neurological damage in man (Jarup, 2003). These health concerns become a greater issue when we consider the susceptible populations such as young children or women of child bearing age.

As fishes are constantly exposed to pollutants in contaminated water, they accumulate all contaminants including non essential metals in their tissues (Canli and Atli, 2003). Hence, consumption of contaminated fishes has been shown to cause potential risk to man (USEPA, 2000, Storelli, 2008, Imar and Carlos, 2011 and Michael, *et al.,* 2011). Therefore, studies have been conducted worldwide on the level of contamination in different species of fishes and their impact on human health (Cheung *et al.,* 2008, Roach *et al.,* 2008; Lin and Ming-Chao, 2009, Bhattacharyya *et al.,* 2010, Kumar *et al.,* 2010, Malik *et al.,* 2010, Mol *et al.,* 2010, Anim *et al.,* 2011 and Laar *et al.,* 2011). Further it may be noted that accumulated metals may eventually reach toxic levels even if the exposure may be extremely low. Even if no toxic effect occurs in an organism accumulating the metal, an effect may appear at a higher tropic level because of the ingestion of the accumulating organisms as foodstuff (Kneip and Lauer, 1973). Despite limitations, such as relatively high mobility, fish are generally considered to be the most feasible organisms for monitoring heavy metal and pesticide contamination in aquatic ecosystems (Beyer 1996).

Kerala State Biodiversity Board (KSBB), Govt of Kerala launched a major programme to document the fish diversity in all the 44 rivers in Kerala involving resource persons from colleges, institutions and fishermen community. One of the modules of the programme is on the contamination status of the rivers using fish as an indicator. We at the Division of Ecotoxicology, SACON were entrusted with the job by the Board. We have attempted to document the levels of select contaminants, namely pesticides and heavy metals. Efforts have also been made to assess the suitability of the fishes for human consumption.

2. BACKGROUND INFORMATION

2.1 Impact of Pesticides: National and International perspective

Production of pesticides in India started in 1952 with the establishment of a plant for Benzene Hexa Chloride (BHC) in Kolkata. Now, India ranks the 2nd largest manufacturer of pesticides in Asia after China, and 12th globally (Gupta 2004, Abhilash and Singh, 2009, Muralidharan *et al.,* 2009 and Vinay Malik, 2013). The worldwide consumption of pesticides is about two million tonnes per year, of which 24% is consumed in the USA alone, 45% in Europe and 25% in the rest of the world. India's share is just 3.75%. Average usage of pesticides in India is about 0.6 kg/ha, while in Korea and Japan, it is 6.6 and 12.0 kg/ha, respectively. Out of the total consumption of pesticides in India, 80% of them are insecticides, 15% are herbicides, 1.46% are fungicide and less than 3% are others. In comparison, the worldwide consumption of herbicides is 47.5%, insecticides is 29.5%, and fungicides, 17.5% and others account for 5.5% only (Gupta, 2004). The present annual (2011 - '12) consumption of chemical pesticides in the country has been estimated at 50,583 metric tons (MT). Kerala, one of the smaller states in the country consumed about 629 MT during 2011 - '12 (source: www.indiastat.com retrieved on 9th May 2013).

In India, the first report of pesticide poisoning was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with a pesticide, parathion (Karunakaran, 1958). This prompted the Special Committee on Harmful Effects of Pesticides constituted by the ICAR to focus attention on the problem (Wadhwani and Lall, 1972). Several years later, a multi-centric study was proposed to assess the pesticide residues in select food commodities collected from different states in the country (Toteja *et al.,* 1993). Organochlorine compounds such as PCBs, DDT, HCH and cyclodiene groups are among the most widely known class of contaminants because of their ubiquity, potential for magnification in the food chain and harmful biological effects. In India, various researchers have reported elevated levels of organochlorine pesticides in air, soil, surface and groundwater, benthic macro-invertebrates, fishes, crabs, snails, birds, fruits, vegetables, food-grains, milk, milk-products, eggs, honey and meat (Kaushik *et al.,* 1987, Kannan *et al.,* 1995, 1997a&b, Agnihotri *et al.,* 1996, Rao, 1996, Reddy *et al.,* 1997, Sharma *et al.,* 2003, Satyanarayan *et al.,* 2004, Kumar *et al.,* 2006, Raina *et al.,* 2008, Muralidharan *et al.,* 2009 and Kaushik *et al.,* 2011).

Persistent pesticides, namely DDT and dieldrin, although were used successfully in controlling a number of diseases and crop pests, were banned or restricted during 1970s in most of the technologically advanced countries. But in some developing countries, they are still being used due to their low cost and high effectiveness. In India, pesticides, namely DDT and HCH were used extensively until recently both for agricultural and vector control. Technical HCH-use was banned in developed nations in 1970s followed by many developing countries in 1980s (Li, 1999). A complete ban on the production and sale of HCH in India came into effect in April 1997 (Tomar and Parmar 1998). Except for termite control in agriculture and buildings, the import, manufacture and use of lindane (ϒ-HCH) was restricted since August 2007 (The Gazette of India 2007). According to Li (1999) historically, the most polluted continent by technical HCH is Asia, where the three most polluted countries, China, India and Japan are located. DDT was identified as a persistent organic pollutant (POP) during the Stockholm Convention on POPs in 2001. India, China and Russia opted for exemption from total ban on DDT (Dash *et al.,* 2007). India banned the agricultural use of DDT in 1989 (Anonymous 1991) with a ceiling to use a maximum of 10,000 tons of per annum for the control of malaria and kala-azar (Dash *et al.,* 2007). During the 9th Five Year Plan of India (1997-1998 to 2001 - 2002), the National Anti-Malaria Programme sprayed 42,200 metric tons of DDT (50% WP; including 11,600 metric tons for *kala-azar* disease control) (Sharma 2003). Due to their persistence, it is difficult to eliminate the OCPs by simply avoiding their use, as many years are required for them to disappear from the environment (Juhler *et al.,* 1999 and Kaushik *et al.,* 2011). Historical and ongoing trends in the use of OCPs in India have raised toxicological concerns for both wildlife and mankind (Dhananjayan and Muralidharan, 2010).

An extensive study on inland wetlands of India by Vijayan *et al.* (2004) documented threats and conservation issues in many wetlands across the country. The study pointed out two overwhelming issues of immediate concerns: huge loss of wetlands and contamination of the remaining ones. Compounding to the loss of wetlands is that almost all the wetlands studied from 14 states in the country were contaminated at varying levels. About 80% of fishes from the wetlands in the country, even from relatively remote regions had residues of HCH, while heptachlor epoxide was detected in about 78% and endosulfan in 66% of the wetlands studied. The most persistent DDT and its homologs (*p,p'*-constituents) were detected in 76% of fishes. In many cases, the fishes were not fit for human consumption, because of the high levels of heavy metals or pesticides. It is a cause for great concern that not even one of the several hundred fishes studied from 115 wetlands was free from pesticides or heavy metals (Vijayan *et al.,* 2004).

Measurement of chemicals in human diet provides the best estimates of the exposure and potential risk. The risk of consumers may then be evaluated by comparison with toxicologically acceptable intake levels (Kashyap *et al.,* 1994). Fatty food was the main source of these contaminants. Chlorpyrifos, a common contaminant of urban streams (U.S. Geological Survey, 1999), is highly toxic to fish, and has caused fish kills in waterways near treated fields or buildings (US EPA, 2000). Herbicides can also be toxic to fish. Weed-killers such as Ronstar and Roundup are also toxic to fish (Folmar *et al.,* 1979 and Shafiei and Costa, 1990). In addition to direct acute toxicity, some herbicides may produce sublethal effects on fish that lessen their chances for survival and threaten the population as a whole. Glyphosate or glyphosate-containing products can cause sublethal effects such as erratic swimming and laboured breathing, which increase the chance of fishes being eaten by predators (Liong *et al.,* 1988). 2, 4-D an herbicide is reported to have caused physiological stress responses in Sockeye Salmon (McBride *et al.,* 1981) and reduced food-gathering abilities in Rainbow Trout (Little, 1990).

There are reports on the concentrations of heavy metals (Kannan *et al*., 1993), organochlorine pesticides and polychlorinated biphenyls (PCBs) (Kannan *et al.,* 1994; Kannan *et al.,* 1997a), and butyltin compounds (Kannan *et al.,* 1997b) in Ganges river dolphins and their prey. The continuing use of organochlorine pesticides and PCBs in India is of concern (Kannan *et al.,* 1992, Kannan *et al.,* 1997a, Kannan *et al.,* 1997b and Tanabe *et al.,* 1998). The Ganges river basin is densely populated and heavily polluted by fertilizers, pesticides and industrial and domestic effluents (Mohan, 1986). Neither the rivers in rest of the country are safe.

Senthilkumar *et al.* (2000) reported levels of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in sediments, aquatic organisms, birds, eggs of birds and bat collected from coastal areas of South India. Misra and Bakre (1994) studied the levels of organochlorine pesticides in a few biological and non-biological components in Rajasthan. Reports on the impact of agricultural chemicals on agro-ecosystem components and avifauna are also available (Rajendran *et al.,* 2003 and Muralidharan *et al.,* 2004). Muralidharan *et al.* (2002 and 2008) and Dhananjayan *et al.* (2011a&b) reviewed the existing situation on the use of pesticides and their impact on birds. Singh (2001 and 2005) documented organochlorine residues in sediments of Gomati river, Lucknow. Ramesh *et al.,* (1991) reported HCH, DDT and other OC residues in sediments of river Kaveri, Tamil Nadu. Pillai, (1986) reported pesticide residues in soil, water and air in Delhi area, India. Guzella *et al.* (2005) reported total HCH, DDT, HCB, PCB and PAH in the sediments of Hubli estuary, West Bengal, Northeast India. Hans *et al.* (1999) reported OC pesticide residues in agricultural produce (paddy and wheat straw) from dry bed of the river Ganges, Kanpur. Sankar *et al.* (2006) reported the organochlorine residues and heavy metals in fishes and shellfish from Calicut region, Kerala, India.

The bioaccumulation of pesticides in fishes (Kannan *et al.,* 1995, Satyanarayan *et al.,* 2004, Vijayan *et al.,* 2004, Malik *et al.,* 2007, Singh and Singh, 2008a&b and Muralidharan *et al.,* 2009), food stuffs (Tanabe *et al.,* 1991) and endocrine disrupting chemicals (Barse *et al.,* 2007, Micheletti *et al.,* 2007 and Pojana *et al.,* 2007) have also been reported. Fishes exhibit low metabolism and uptake all the chemicals rapidly from water, sediment and food to different degrees (Muir *et al.,* 1990 and WHO, 1993). Kannan *et al.* (1992) and Kumari *et al.* (2001) reported high concentration of HCH in fish and food products and higher dietary intake through fishes to man.

Peter (2008) studied the concentration of OC residues in the muscle tissues of 164 fishes comprising five species collected from Sewri, Mahul and Nhava, Mumbai. Total HCH residues were the highest (673 ppb) in *Otolithes rubber*. *Coilia dussimierie* had the maximum concentration of total DDT (61.7 ppb).

Jayanthi (2012) reported the OC pesticide residues in select species of commercially important marine fishes, received from Cochin and Rameshwaram, and sold at Coimbatore market. Among the species of fishes analysed, concentration of γ HCH and *p,p'-DDT* were higher in *Scoberomorus commersonn* (4.8 ± 1.0 ppb) and *Sardinella longiceps* (13.2 ppb), respectively. However, endosulfan residues were detected at lower concentrations (< 2.2 ppb). Based on the human consumption survey, the daily per capita consumption of marine fishes in Coimbatore city was 47 g/ day. Based on this, the average dietary intake concentration of organochlorines irrespective of the species preference was estimated to be 4.31µg/person/day. Higher dietary intake of OC residues mainly through *Scoberomorus commersonn* (5.65 µg/ person/ day) followed by *Sardinella longiceps* (4.73 µg/person/day) was reported. These are the species which are the most preferred and highly consumed by the local people. These fishes are rich in fat content and thereby accumulate higher quantum of residues. Calculated dietary intake values were below the ADI limits prescribed by existing statutory guidelines (Health Canada 1996, European Union 2000 and FDA 2000 and 2001).

2.2 Impact of metals: National and international perspective

Freshwater fishes alone account for approximately one quarter of all living vertebrate species and it is estimated that there are 44,000 scientifically named species of fresh water biota. They are potentially an indirect source of metals entering the human body, but they may also suffer from a wide range of metabolic, physiological and ecological factors. For example, of those species considered in the World Conservation Union's (IUCN) Red List for 2000, 20% of amphibians and 30% of fishes (mostly freshwater fishes) were considered threatened (FAO, 2007).

Accumulation of heavy metals in fishes is influenced by water quality parameters such as hardness and acidity. Moreover, the life cycle of an organism is also an important consideration in examining the effects of heavy metals, as the concentration of heavy metals in body tissues vary with age or size of the organism (Atchinson *et al.,* 1977; Chernoff and Dooley, 1979).

Fishes are sensitive to metal contamination in water and may significantly damage certain physiological and biochemical process when they enter the organs of these animals (Namcsok *et al.,* 1987). Chronic exposure of fish to sub lethal trace metal levels can cause disturbed ion regulation, reduced growth and swimming speed (Alsop *et al.,* 1999, Hollis *et al.,* 1999).

Several authors have described the haematological alterations, biochemical changes and hampering of locomotion in fishes like Rainbow Trout and Salmon at a concentration of 0.012 ppm of lead (Hodson *et al.,* 1978). Kunhert *et al.* (1976) reported significant inhibition in activity of enzyme Na/K ATPase, which is involved in osmoregulation in the kidney of *Salmo gairdneri* after exposure at 2.5 ppm Cr (VI) for 48 hrs. Singh and Singh (1979) found that 0.003 ppm of cadmium inhibiting the oxygen consumption of *Mystus vittatus* by 50% during an exposure of 12 hr.

Determining the levels of heavy metals in freshwater fishes has received considerable attention in and around the world. Some of the important contributions relevant to the present study are as follows: Rauf *et al.* (2009) studied the concentration of Cd and Cr in three major corps collected from Ravi river, Pakistan. They concluded that fish liver had significantly high levels of Cd (1.57 - 4.26 ppm) and Cr (1.14 -6.23 ppm) than the gills (Cd: 0.53 - 1.10 ppm, Cr: 0.52 - 1.46 ppm).

Burger and Gochfeld, (2006) from New Jersey, USA reported levels of As, Cd, Mn, Pb, Hg and Se in commercial fishes and suggested that the levels of most metals were below those known to cause adverse effects. However, the levels of As, Pb, Hg and Se in fish were in the range known to cause sub-lethal effects in sensitive predatory birds and mammals, and in some fishes the levels exceeded the standards set by WHO/FAO (1992), Environmental Protection Agency (EPA, 1993) and FDA (1999).

Black *et al.* (1981) reported high levels of Cu in Torch Lake Michigan and other substances found in the lake may be complexing with Cu to form relatively non-toxic compounds. Complexation may partly account for the relatively good hatching rates of Perch despite elevated Cu concentrations found in Torch lake water (Ellenberger *et al.,* 1994). But mixture of salts of metals, especially Cu and Zn were observed to produce an additive toxicity in the Rainbow Trout and Atlantic Salmon. Copper and its compounds are ubiquitous in the environment and are frequently found in surface water. Copper ion precipitate gill secretions, causing death by asphyxiation. Similarly it is the same in the case of iron. Zinc is an abundant element and it constitutes approximately 0.04 g/kg of earth’s crust. Its occurrence in sewage is expected because of its extensive use in making house hold appliances and by leaching from galvanized pipes (Pandey *et al.,* 1995).

Studies reported concentration of Pb in muscle, gill and liver of the fish *Catla catla* in the range of 0.7-2.39 ppm, 0.74-2.25 ppm and 0.89-2.68 ppm (dry weight) respectively (Korai *et al.,* 2008). While 9.87% the samples did not exceed the UK limit of 1.0 ppm (1979), 44.46% were well below 4.88 ppm (USEPA 1990) and remaining 45.67% were lower than the (USFDA, 1997) level of 1.3 ppm.

Saeed and Shaker (2008) reported the levels of Fe, Zn, Cu, Mn, Cd and Pb in *O.niloticus* (Tilapia) fish tissues, water and sediments collected from northern Delta Lakes, Egypt. They found that the edible part of *O.niloticus* from Lake Edku and Manzala contained the highest level of Cd while fish from Manzala Lake contained the highest level of Pb. They reported that Nile Tilapia caught from these two Lakes could pose health hazards to consumers.

In India, concentrations of Cr, Cd, Cu, Fe, Mg, Mn, Ni, Co, Zn and Al in four commercially important marine fishes from Parangipettai Southeast coast were within the permissible safety levels for human consumption according to FAO, (1983), European Commission, (2001); Food and Drug Administration (FDA, 2001) standards (Raja *et al.,* 2009). Lakshmanan *et al.* (2009) reported the concentrations of Zn, Pb, Cr, Co and Cd in five of the most commercially important fishes in the same study site. The results revealed that the muscle concentrations of Cr, Zn, Pb, Cd and Co ranged from 0.415 ± 0.27 to 1.168±1.49; 0.103 ± 0.14 to 0.807 ± 0.13; 0.062 ± 0.00 to 1.569 ± 1.41; 0.004 ± 0.00 to 0.114 ± 0.14 and 0.006 ± 0.00 to 0.014 ± 0.00 ppm respectively.

Another study evaluated the levels of Cd, Pb, Hg, Cr, As, Zn, Cu, Co, Mn, Ni, and Se in commercially important species of fish, shellfish and fish products from fish markets in and around Cochin, India. Results showed that different metals were present in the samples at different levels but within the maximum residual levels prescribed by the European Union (EU) and USFDA and the fish and shellfish, in general, were safe for human consumption (Sivaperumal *et al.,* 2007).

Ayyadurai *et al.* (1994) investigated heavy metals namely Cu, Fe, Mn, Zn and Hg present in water and fin fish, *Oreochromis mossambicus* during 1990-91 at three locations in River Cauvery, South India. The accumulation of these metals was the maximum in liver as compared to other organs of the fish. The mean concentrations of these metals in the muscle were 1.28, 6.3, 0.86, and 6.36 ppm for Cu, Fe, Mn and Zn respectively while that of Hg was 0.065 ppm which is below the stipulated toxic limit.

In India, varying levels of Cd concentration have been reported to be present in aquatic ecosystem which is more than 5 ng/ml in the Yamuna river water at Agra, Delhi, Etawah and Mathura (Ajmal *et al.,* 1985) and 0.50 - 114.8 ppm in the Yamuna river sediments at Agra and Delhi but the water around the industrial areas have been found to contain much higher levels of cadmium (Kaushik *et al.* 2003).

Muralidharan (1995) studied heavy metal contamination in 16 species of fishes collected from Keoladeo National Park, Bharatpur, Rajasthan, India and found the contamination levels to be significantly varying among various types of herbivorous, carnivorous and omnivorous fishes. The average load of cadmium in the fishes was 0.18 ppm. Levels of both copper and lead were at high level (0.74 ppm). Zinc with 7.19 ppm was the highest in concentration of all the metals. Further the variation in contamination level ($p<0.05$) among species was reported to be dependent on the contamination level of the water, sediment and invertebrates and also the duration of stay of the fishes in the contaminated water.

The heavy metal (Cu, Fe, Zn and Cr) accumulation in fish samples of sewage fed ponds in Rahara was studied. Among the metals studied, Fe had higher concentration, while Cr showed the lowest concentration. Copper concentration in fish was about 0.02mg/g and found to be the lowest. Concentration of Zn was less and the accumulation of Cr in all the samples was very low which might be due to its low solubility and availability in water. The average value of Cr was 0.01 mg/g in fish samples. The values in all the samples were below the standard limits of and did not seem to pose any serious threats to the consumers or the fishes themselves (Pandey *et al.,* 1995).

Krishnamurthi and Nair, (1999) documented the concentration of metals in fishes from Thane and Bassien creeks of Bombay, India. Irrespective of species, Cu and Zn contributed 42-58% of the total metals accumulated. In general, most of the metals showed relatively higher concentration in liver than the other tissues. In the muscle tissue maximum average concentration of Cu was observed in *A.tenuispinis* (63.1 ppm). The pattern of accumulation of different metals was Zn> Cu> Ni > Pb >Cd. Irrespective of the species and the area from where the fishes were caught, generally the liver or alimentary canal showed the highest concentration of any given metal. The bioaccumulation of different metals at higher levels in the intestine and liver of fish suggested that the metal accumulation was effective through the food chain rather than by direct contamination.

In a study conducted in Hoogley Estuary, India during 1977-1981, 127.8 ppm of Cu and 20 ppm of Cr (average) were recorded in various tissues of fish. High concentration of Zn (218.5 ppm) was observed in the muscle tissue of *Mastacembelus pancalus* and it indicated exposure to increased concentration of the metal. Although muscle is not a suitable body part to determine the extent of the heavy metal contamination reflected by the low concentration of the metal in majority of the samples, the levels in muscle tissue show the fitness for human consumption (Kaviraj, 1989).

Jayakumar (2001) reported the levels of Cu, Cd, Cr and Pb in 940 fishes comprising 12 species collected seasonally from Aliyar, Amaravathy and Thirumoorthy reservoirs and Ukkadam, Kurichi and Singanallur wetlands in Coimbatore, Tamil Nadu. *Rasbora daniconius* recorded the maximum load of metals (Cu 0.68 ppm; Pb 1.34 ppm and Cr 0.29 ppm). Of all the sites, Kurichi and Ukkadam were the most contaminated, with considerable concentrations of metals.

Assessment of heavy metal contamination in Grass Carp (*Ctenopharyngodon idella*) by Misra *et al.* (2002) revealed that Pb (0.6 to 1.45 ppm), Cu (0.37 to 1.02 ppm), Cr (0.46 to 1.18 ppm), Cd (0.35 to 0.324 ppm) and Zn (0.8 to 1.17 ppm) concentrations in muscle tissue of the fish were found to be low to cause any adverse effects to fishes. Further, the levels were reported to be within prescribed limits and safe for human consumption.

Selvam (2002) documented quantitatively the levels of a few heavy metals in select species of fishes namely *Channa punctatus*, *Channa striatus*, *Heteropneustes fossilis*, *Labeo rohita* and *Cirrhinus mrigala* from Keoladeo National Park, Bharatpur, Rajasthan. Of all the species, *Labeo rohita* (Cu 0.70 ppm; Pb 0.74 ppm; Zn 8.0 ppm; Cd 0.08 ppm) and *Channa striatus* (Cu 0.34 ppm; Pb 0.73 ppm; Zn 6.64 ppm; Cd 0.04 ppm) recorded the maximum metal load.

Sindhu (2006) documented the levels of Cu, Cd and Zn in 136 individuals comprising five species of marine fishes received from Cochin coast. Among the five species examined, invariably *Sardinella longiceps* recorded the highest levels of Cu (1.70 ± 0.09 ppm), Cd (0.16 ± 0.05 ppm) and Zn (38.90 ± 5.83 ppm) while *Cynoglossus macrolepidotus* had the lowest levels of Cu (0.89 ± 0.04 ppm) and Zn (3.85 ± 0.68 ppm).

Jayakumar (2007) comprehensively documented the levels of heavy metals in 889 fishes comprising 19 species collected from 90 inland wetlands across 10 Indian states. Among the wetlands, Pb contamination was found to be the highest in Misamari Beel (8.33 ± 0.39 ppm) of Jorhat dt., Assam and lowest in Draksha Rama (1.46 ± 0.47 ppm) of East Godhavari dt., Andhra Pradesh. Anarag dam in Jharkhand dt., Bihar had the maximum concentration of Zn (38.94 ± 5.79 ppm) and Cu (39.63 ± 5.89 ppm) and minimum in Mandhakhali Kere (Zn 4.86 ± 1.27; Cu 4.94 ± 1.29 ppm) of Mysore dt., Karnataka. Among the species, *Cyprinus carpio* collected from Madhya Pradesh recorded the highest concentration of Pb (9.01 ± 0.24 ppm) followed by *Channa orientalis* (7.70 ± 0.58 ppm) from Assam. *Puntius dorsalis* recorded the maximum concentrations of Cu (39.75 ± 3.10 ppm) from Bihar and minimum in *Ompak bimaculatus* (Cu 1.02 ± 0.22 ppm) from Karnataka. *Heteropneustes fossilis* of Tamil Nadu observed the highest levels of Cd (2.21 ± 1.60 ppm) and lowest (0.16 ± 0.01 ppm) in *Mystus bimaculatus* of Andhra Pradesh. Cr concentration was the highest in *Ompak bimaculatus* (2.54 ± 1.18 ppm) of Gujarat and lowest in *Notopterus notopterus* of Tamil Nadu. Significant variations in concentrations ($p<0.05$) noticed among these species could be related to a number of factors. *Cyprinus carpio,* a subsurface dweller and a voracious detrivorous feeder could have accumulated Pb through its feeding habit and habitat. *Heteropneustes fossilis* a bottom dweller and an omnivorous feeder could have accumulated higher levels plausibly through biotransformation of metals from food and sediments. Further its air breathing adaption enables it to exist in almost any kind of water. Other factors such as species specific differences, bioavailability of chemicals in food and water and the physico-chemical parameters of the aquatic environment also could influence. Carnivorous fishes had significantly higher concentration ($p<0.05$) of Cu, Zn and Cd than planktivorous. Further, Pb contamination was higher in omnivorous fishes as they prey on a variety of aquatic organisms and they are expected to accumulate more metals in a significant manner.

The levels of Ar, Cu, Hg, Ni and Zn were determined in muscle tissues of fishes from Kolkata wetland and estimated the health risk posed by fish ingestion. Results indicated that levels of metals were below the permissible limits set by the Joint FAO/ WHO Expert Committee on Food Additives. When compared with guideline values, although fish from Kolkata wetlands are safe for human consumption, there are high probability of contracting cancer if As and Ni exposure continues throughout the life time of an individual (Bhupander Kumar and Mukherjee, 2011a).

Chitra *et al.* (2013) documented the levels of heavy metals such as Cu, Cd, Cr, Zn, As, Ni, Pb and Se in nine species of fishes collected from five urban wetlands of Coimbatore, namely Valankulam, Kurichi, Coimbatore Big tank, Singanallur and Sulur and assessed their suitability for human consumption with various statutory body guidelines (BIS, ICMR, FSSAI and WHO). Levels of Cu were the highest in the liver tissues of *Channa striata* (0.97± 0.75 ppm) and lowest in the muscle tissues of *Stolephorus indicus* (0.01 ppm). Total Cr was the highest in gill tissues of *I.punctatus* (0.12±0.03 ppm) and lowest in muscle tissues of *Oreochromis mossambicus* (0.004±0.003 ppm). Pb contamination was found to be highest in gills of *C.striata* (0.08 ppm) and lowest in muscle of *Cyprinus* sp (0.003 ppm). Nickel accumulation was highest in gills of *C. striata* (0.13 ppm) and lowest in muscle tissues of *L. rohita, C. striata, C. catla* (0.03 ppm). Arsenic contamination was detected only in the gills of *Cyprinus* sp in a measurable amount (0.08 ppm). In other species, arsenic level was below detection limit. Among the wetlands studied, Coimbatore big tank and Kurichi were highly contaminated. Study revealed that the order of metal contamination in freshwater fishes was $Zn > Cr > Ni \approx Cd > Cu > Se > Pb > As$. The levels of certain metals reported in the present study are above the permissible limit and they have to be looked in to with concern as the public consume them. Consumption of these contaminated fishes showed high risk potential for human. However, the physiological mechanisms, species difference, physico-chemical properties of surviving water, availability and absorption of the metal are also to be admitted while considering the current levels.

Although, in India we have information on the levels of different type of contaminants in fishes, they are largely scattered. No comprehensive data on any specific timescale is available even at regional level.

2.3 Objective

- ✓ Assess the contamination status of the rivers of Kerala with respect to pesticides and heavy metals using fish as an indicator.

3. METHODOLOGY

3.1 Study area

Kerala, the land of rivers, has a cultural history, which indeed is closely intertwined with the river valley social life. It is the land of 44 rivers, 41 of them follow westward, join the Arabian Sea and three others flow eastward (table 1). Most of the rivers originate from the Western Ghats biodiversity hotspot. The rivers of Kerala are small, when considered in terms of length, breadth or annual stream flow. The size of the rivers coupled with very high population density make them susceptible to environmental onslaughts.

Kerala is divided into three physiographic zones, namely lowlands (0 - 7.5 m), midlands (7.5 - 75 m) and highlands (> 75m). In each river one survey site was fixed for each of these zones (three for one river). The sampling stations for collection of fishes were selected by the coordinators based on (i) being representative of the particular river reach, (ii) having reasonable access and fishability, (iii) having local fisher folk, and (iv) having velocities sufficient to carry fish downstream to the gill net. Each survey site was approximately 200 meter in length, although longer and shorter lengths were used to accommodate differences in stream size and unusual channel structure or habitat as adopted by Biju Kumar *et al.* (2010).

Despite social, economic, cultural and ecological significances, rivers represent the highly threatened ecosystem in Kerala State due to rampant sand mining, overexploitation of resources, encroachment, construction of dams, debilitation of watersheds, invasion of alien species and pollution. Despite the importance of the rivers in terms of direct, indirect and existence values, the river systems in Kerala are not frequently monitored either for studying their ecological status or for recording fish - the major component of biodiversity. The River Fish Monitoring Programme of Kerala State Biodiversity Board (KSBB) is an attempt to continuously monitor the health of the riverine ecosystems of Kerala.

Table 1 List of rivers in Kerala included for the study and their major tributaries

S.No	Name of the river	Districts in which river basin is located	Length of the river (km)	Main tributaries
West Flowing Rivers				
1.	Manjesehwaram	Kasargod	15	Pavuru
2.	Uppala	Kasargod	50	Uppala
3.	Shiriya	Kasargod	67	Kallanje Thodu, Kanyana ThoduEramathihole & Kumbla
4.	Maugral	Kasargod	34	Nettipadi and Muliyar
5.	Chandragiri	Kasargod	105	Payashani and Chandragiri
6	Chithar	Kasargod	25	Kalnad, Bekal and Chittari
7.	Nilleswaram	Kasargod and Kannur	46	Aryangal and Baigotehole
8.	Kariangode	Kasargod and Kannur	64	Mundore, Padimalahole and Ariakkadavuhole
9.	Kavvayi	Kasargod and Kannur	31	Nil
10.	Peruvamba	Kasargod and Kannur	51	Macharu Thodu, Mathamangalam & Challachal
11.	Ramapuram	Kasargod and Kannur	19	Nil
12.	Kuppam	Kannur	82	Cheriya Thodu & Kuttikilpuzha
13.	Valapattanam	Kannur	110	Valiyapuzha and Venipuzha
14.	Anjarakandy	Kannur	40	kappu Thodu and IdumbaThodu
15.	Thalasseri	Kannur	28	Dharmadom puzha
16.	Mahii	Kannur and Kozhikode	54	Nil
17.	Kuttiyadi	Kozhikode	74	Onipuzha, Thottilapalam and Kannathil
18.	Korapuzha	Kozhikode	40	Agalapuzha and Pannurpuzha
19.	Kallayi	Kozhikode	40	Nil
20.	Chaliyar	Kozhikode, Malappuram and Wayanad	169	Karimpuzha, Kanchirapuzha And Cherupuzha
21.	Kadalundi	Malappuram and Palakkad	130	Olipuzha Veliar
22.	**Tirur***	**Malappuram**	**48**	**Vallilapuzha**
23.	Bharathappuzha	Palakkad, Malappuram and Thrissur	209	Gayathri Puzha, Chittor Puzha, Kalpathi Puzha and Thootha Puzha
24.	Keecheri	Thrissur	51	Choondal Thodu
25.	Phuzhakkal	Thrissur	29	Para Thodu Nadu and Thodu(etc.)
26.	Karuvannoor	Thrissur	40	Manali Kurumali and Chimmani
27.	Chalakudy	Thrissur, Palakkad and Ernakulam	130	Parambikulam, Sholayar and Karappara
28.	Periyar	Idukki and Ernakulam	244	Muthirapuzha, Idamalayar Mangalapuzha and Perinjankutty

S.No	Name of the river	Districts in which river basin is located	Length of the river (km)	Main tributaries
29.	Muvattupuzha	Ernakulam and Kottayam	121	Kallar Thodupuzha and Kothamangalam
30.	Meenachil	Kottayam	78	Kadapuzha, Kalathukadavu and Kurisumalai
31.	Manimala	Kottayam and Pathananthitta	90	Kokayar Elakkal Thodu
32.	Pamba	Pathanamthitta, Idukki and Alappuzha	176	Kakkiyar, Kallar, Arudai and Pamba
33.	Achenkovil	Pathanamthitta, Idukki and Alappuzha	128	Kallar
34.	Pallikal	Kollam, Pathanamthitta and Trivandrum	42	-
35.	Kallada	Kollam, Pathanamthitta and Trivandrum	121	Kulathupuzha and Chendruni
36.	Itthikkara	Kollam and Trivandrum	56	Vattaparambu and Vattam Thodu
37.	Ayroor	Kollam and Trivandrum	17	Nil
38.	Vamanapuram	Kollam and Trivandrum	88	Nil
39.	Mamam	Kollam and Trivandrum	27	Nil
40.	Karamana	Trivandrum	68	Kaviar Thodiyar
41.	Neiyyar	Trivandrum	56	Kallar and Karavaliyar
East Flowing Rivers				
42.	Kabani	Wayanad	-	Panamaram, Mananthavady, Babali and Noolpuzha
43.	Bhavani	Palakkad	-	Siruvani and Varagar
44.	Pambar	Kottayam	-	Thrithamala, Eravikulam, Myladi and Chenkaloor

*Fish sample was not received from Tirur River

Source: Biju Kumar *et al.* (2010)

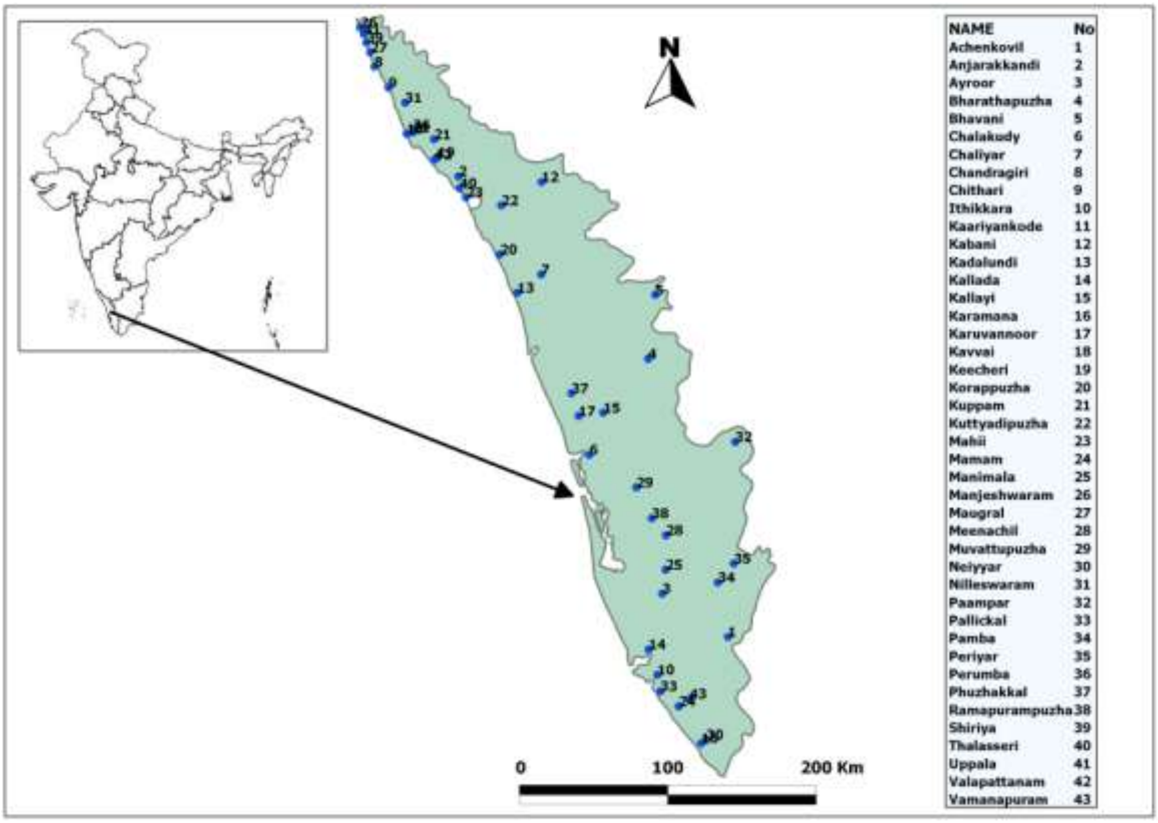


Fig 3.1 Map showing the rivers of Kerala state

3.2 Sample receipt

Eight hundred and ninety five individuals belonging to 28 species of fishes (fig 3.2a-q) were received from 43 rivers of Kerala (fig 3.1) through the project coordinators. Most of the fishes were collected from three different locations, namely high, low and mid land during the course of the river. Details are furnished in table no. 2 and 4. It is to be noted that number of species and individuals collected varied among the rivers.

The samples, on receipt at the laboratory were cleaned off dirt in tap water and muscle tissue was dissected, minced into smaller pieces and a sub sample was taken from the homogenate. About 10 and 2 g of the homogenate were weighed separately using a top loading electronic balance (Mettler AE420) and transferred to clean specimen vials for pesticide and metal analysis respectively, and stored in freezer at -20°C till further processing.

Fig 3.2a-q : Select species of fishes included in the study

a. Xenentoden cancila

Photo credit : Dr C P Shaji

b. *Rasbora dandia*

Photo credit : Mr Shibu Bhaskar

c. *Puntius filamentosus*

Photo credit : Dr C P Shaji

d. *Puntius denisonii*

Photo credit : Mr Shibu Bhaskar

e. *Pristolepis marginata*

Photo credit : Mr Shibu Bhaskar

f. *Puntius conchonius*

Photo credit : Dr C P Shaji

g. *Puntius mahecola*

Photo credit : Dr C P Shaji

h. *Oreochromis mosambica*

Photo credit : Ms Mocy Vincent

i. *Osteochilichthys nashil*

Photo credit : Dr C P Shaji

j. *Nandus nandus*

Photo credit : Dr C P Shaji

k. *Garra mullya*

Photo credit : Mr Shibu Bhaskar

l. *Etroplus suratensis*

Photo credit : Dr Dinesh

m. *Etroplus maculatus*

Photo credit : Dr C P Shaji

n. *Devario malabaricus*

Photo credit : Dr C P Shaji

o. *Channa striatus*

Photo credit : Dr C P Shaji

p. *Channa gachua*

Photo credit : Dr C P Shaji

q. *Barilius bendelisis*

3.3 Chemical processing for pesticides

Multi-residue extraction method was followed for extracting pesticide residues. Ten grams of tissue was taken and ground with pestle and mortar to get a homogenous mixture. Then the homogenate was transferred into a 50 ml centrifuge tube (Oakridge, Tarsons) and 20 ml of Acetonitrile (Merck) was added to it and shaken vigorously for a minute. To the centrifuge tube, 4 g of anhydrous magnesium sulphate ($MgSO_4$) (Himedia), 1 g of sodium chloride (Merck) were added and centrifuged at 10,000 rpm for 10 min. Then the organic layer of the sample (4 ml) was transferred into a 15 ml centrifuge tube which contained 100 mg of primary secondary amine and 600 mg of $MgSO_4$ and again centrifuged at 5,000 rpm for five min. From the above extract, 2 ml was transferred into test tube, evaporated to near dryness and reconstituted with 1 ml of HPLC grade n-Hexane and stored in deep freezer at -20^{0}C until final qualitative and quantitative analyses were carried out.

3.4 Chemical processing for metals

Microwave Digestion System (Milestone, MLS 1200) equipped with Microwave Digestion Rotar (MDR- 300/10) and Exhaust Module (EM 45) was used for digesting the samples. About 2 g of tissue samples were digested with 10 ml of concentrated nitric acid (69%) for 10 min and 1 ml of perchloric acid (70%) for 5 min and 5 ml of 30% hydrogen peroxide (bleaching agent) for 10 min at 250 W power supply. After ensuring complete digestion, vessels were cooled to room temperature and the digested solution was transferred into standard measuring flask and made up to 25 ml with metal free double distilled water. Samples were stored in well cleaned polythene bottles in refrigerator till final analysis with spectrophotometer.

3.5 Chemical analysis for pesticides

Qualitative and quantitative analysis of organochlorines, organophosphates and synthetic pyrethroids were made with Gas Chromatograph (Agilent Model 7890A Series) equipped with GC-MS 5975 Quadruple and HP-5ms fused silica capillary column (15m x 0.25mm I.D x 0.25µm film thickness) coated with 5% phenyl and 95% dimethyl polysiloxane. While Helium (IOLAR) was the carrier gas (1.2 ml/min), chromatographic operating conditions were as follow: detector 325^{0}C; injector: 325^{0}C; oven temperature was programmed as 70^{0}C -1min; 8^{0}C/min - 280^{0}C - 9.2 min. All the samples were analyzed for residues of organochlorine pesticides, namely alpha-hexachlorocyclohexane (α-HCH), beta-hexachlorocyclohexane (β-HCH), delta hexachlorocyclohexane (δ-HCH), gamma-hexachlorocyclohexane or lindane (γ-HCH), o,p’-dichlorodiphenyltrichloroethane (o,p’-DDT), p,p’-dichlorodiphenyltrichloroethane (p-p’-DDT), o,p’-dichlorodiphenyldichloroethylene (o,p’-DDE), p,p’-dichloro diphenyldichloroethylene (p,p’-DDE), o,p’-dichlorodiphenyldichloroethane (o,p’-DDD), p,p’-dichlorodiphenyldichloroethane (p,p’-DDD), α-endosulfan, β-endosulfan, endosulfan sulphate, heptachlor, heptachlor epoxide (HE), dieldrin, chlordane and mirex, organophosphate pesticides, namely malathion, methyl parathion, chlorpyrifos, quinalphos, phenthoate, primiphos ethyl and ethion and synthetic pyrethroids, namely permethrin I, permethrin II, fenvalerate I and fenvalerate II. An equivalent mixture manufactured by Sigma-Aldrich Chemicals (Accustandard - United States) was used as standard. Concentrations of individual compounds were quantified from the peak area of the sample to that of the corresponding external standard. Recoveries of the compounds from fortified samples (50 ppb) ranged from 91 to 102% and the results were not corrected for per cent recovery and expressed in wet weight basis.

3.6 Chemical analysis for metals

Determination of cadmium, chromium and lead was carried out using Inductively Coupled Plasma equipped with Mass Spectrophotometer (ICP-MS PerkinElmer Nexion 300D). Performance of the instrument was checked by analyzing standard reference solutions (PerkinElmer multi elements standards) concurrently to check the precision of the instrument. After appropriate dilutions of stock standard solutions, a seven level calibration curve (1, 5, 10, 25, 50, 75, 100 ppb) was prepared. The detection limit for Cd, Cr and Pb was 0.1 ppb, 0.2 ppb and 1.0 ppb, respectively. Duplicate method blanks were processed and analyzed alongside the samples to check any loss or cross contamination.

3.7 Suitability for human consumption

The annual per capita consumption of fish in India is 9.8 kg, whereas the recommended intake is 13 kg (MoSPI, 2011; www.mospi.gov.in). Bearing this factor in mind, assuming that a person consumes 250 g of fish per week the Average Daily Intake (ADI) of pesticides and metals through consumption of fishes was calculated as shown below:

$$\text{ADI} = \text{Average concentration of the pesticide/ metal in the tissue} \times 250\ \text{g} / 7$$

4. RESUTLS AND DISCUSSION

4.1 Pesticide residues in fishes in the rivers of Kerala

Samples of fishes were pooled species wise depending on the number and weight of fishes received from each river. Pooled samples of two hundred and sixty five fishes comprising twenty eight species (table 2) from forty three rivers of Kerala were analysed. Data have been compiled to check the overall load of pesticides, species and river wise, with reference to groups, namely organochlorines, organophosphates and synthetic pyrethroids. Further, within a group, levels of individual pesticides and their isomers and metabolites have been compiled to understand the implications with reference to usage pattern or policy of the government in existence.

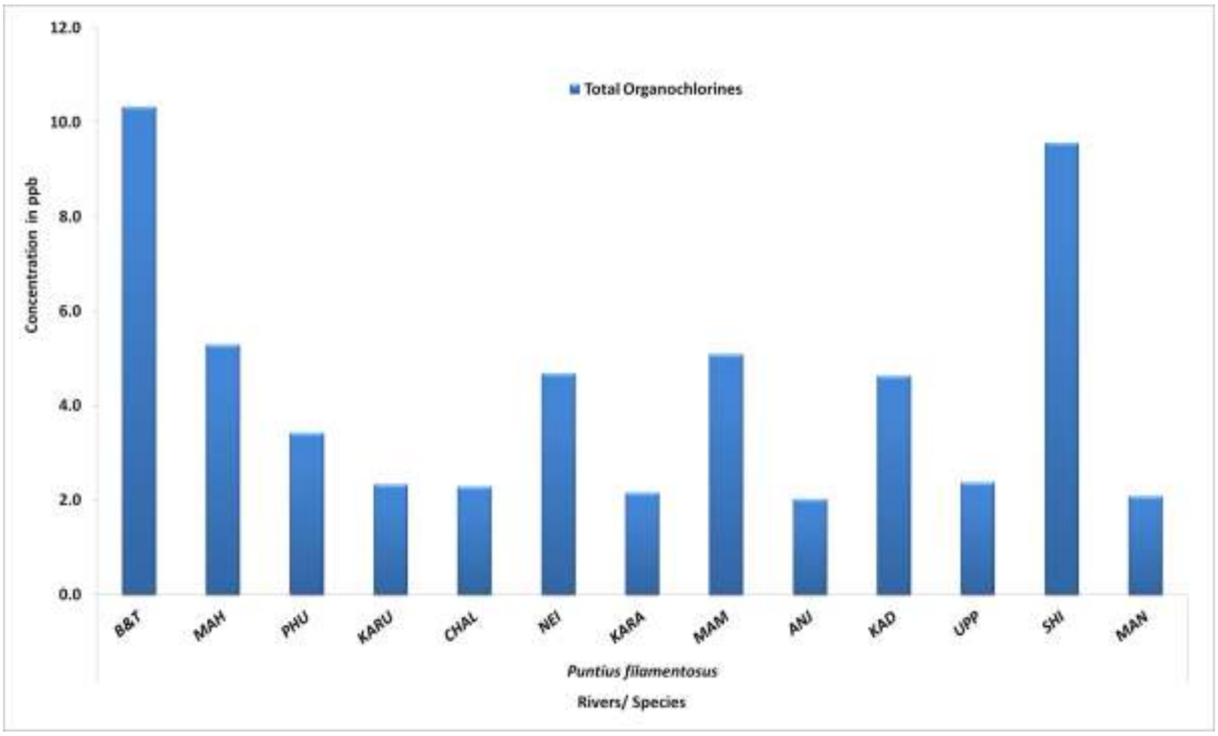
Table 2 List of fish species analysed for pesticide residues

S.No.	**Name of the fish species**	**Vernacular Name**	**No. of samples analysed**
1	*Barbodes subnasqutus*	Kuruva, Mundothipparal	1
2	*Barilius bakeri*	Pullippavukan	7
3	*Barilius bendelisis*	Pavukan	2
4	*Barilius canarensis*	Pullippavukan	5
5	*Barilius* sp		2
6	*Channa gachua*	Vatton, vattudi, Thodan	2
7	*Channa striatus*	Varal, Bral, Kannan	2
8	*Devario malabaricus*	Thuppalamkothi, Ozhukkilatti	8
9	*Devario* sp		15
10	*Etroplus maculatus*	Pallathi, Pootta	7
11	*Etroplus suratensis*	Karimeen	4
12	*Garra mullya*	Kallotti, Njezhu, Kallunthi, Kallemkari	16
13	*Garra* sp		5
14	*Hypselobarbus curmuca*	Kuzhikuthi, Kooral	1
15	*Mugil cephalus*	Thirutha	4
16	*Nandus nandus*	Muthukkila, Muthukki, Urakkamthoongi	1
17	*Ompak bimaculatus*	Manglanchi, Thonnan vala	1
18	*Oreochromis mosambica*	Silopi	1
19	*Osteochilichthys nashil*	Mammalu	2
20	*Pristolepis marginata*	Aattuchembelli, Andikalli	2
21	*Puntius conchonius*	Paisepparal	2
22	*Puntius denisonii*	Chenkaniyan	2
23	*Puntius filamentosus*	Poovalipparal, valekodiyan	133
24	*Puntius mahecola*	Urulan paral	1
25	*Puntius* sp		2
26	*Rasbora dandia*	Kananjon	10
27	*Rasbora* sp		23
28	*Xenentoden cancila*	Koyla, Kolan	4
Total			**265**

Total organochlorine pesticide residue load was found to be the maximum in *Puntius filamentosus* (10.32 ppb) collected from highland areas of Bharathappuzha-Thutha, followed by the same species in Manjesehwaram river (9.56 ppb), while it was mimimum (2.01 ppb) in the same species collected from Anjarakkandi river (Fig 4.1 a, b & c). Total organophosphate residues were detected in four out of 265 samples (1.51 %) analysed with the highest in *Barilius bakeri* (5.85 ppb) received from highlands of Anjarakandi and lowest in *Puntius filamentosus* (2.05 ppb) from midland areas of Karuvannoor.

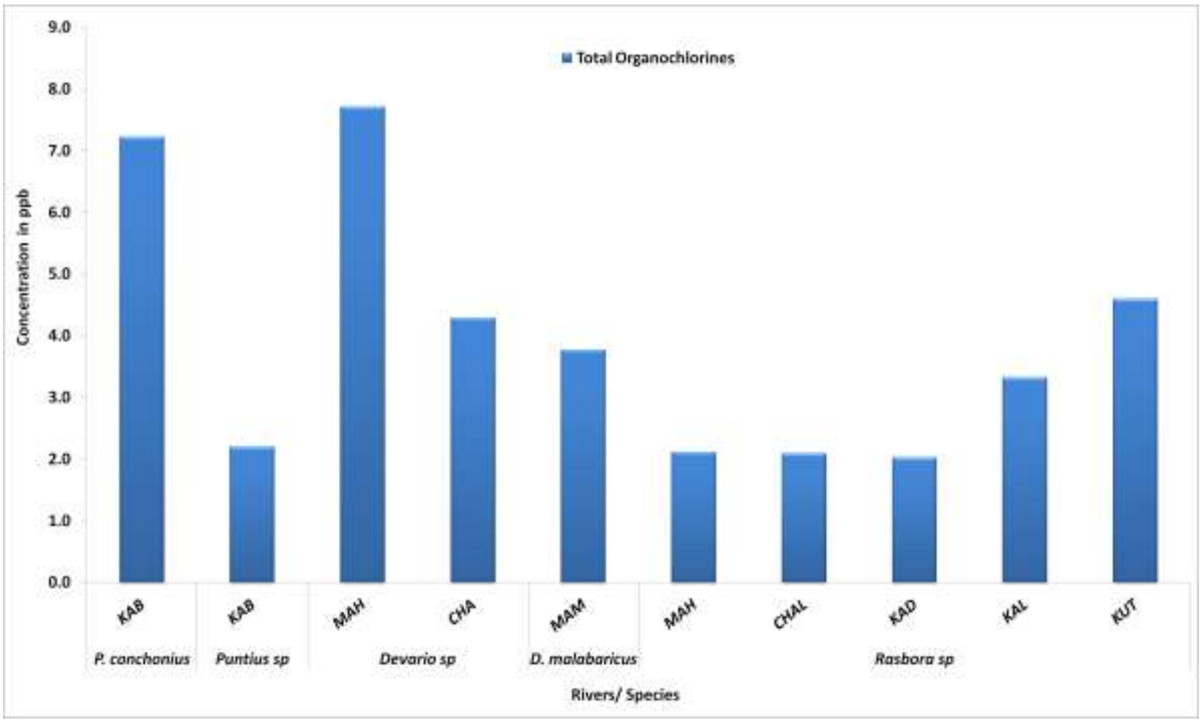
Among the total synthetic pyrethroids, Permethrin-II was the only compound detected in three out of 265 samples (1.13%) with the highest in *Rasbora* sp (6.24 ppb) collected from lowlands in Chaliyar, and the lowest in *Puntius filamentosus* (2.51 ppb) collected from midland areas of Kallada. The OC residue levels did not vary significantly ($p>0.05$) among the species and rivers, and frequency of occurrence of OC pesticide residues was the maximum (5.26%) in *Puntius filamentosus*.

Fig 4.1a Levels of total organochlorine residues in fishes in the rivers of Kerala



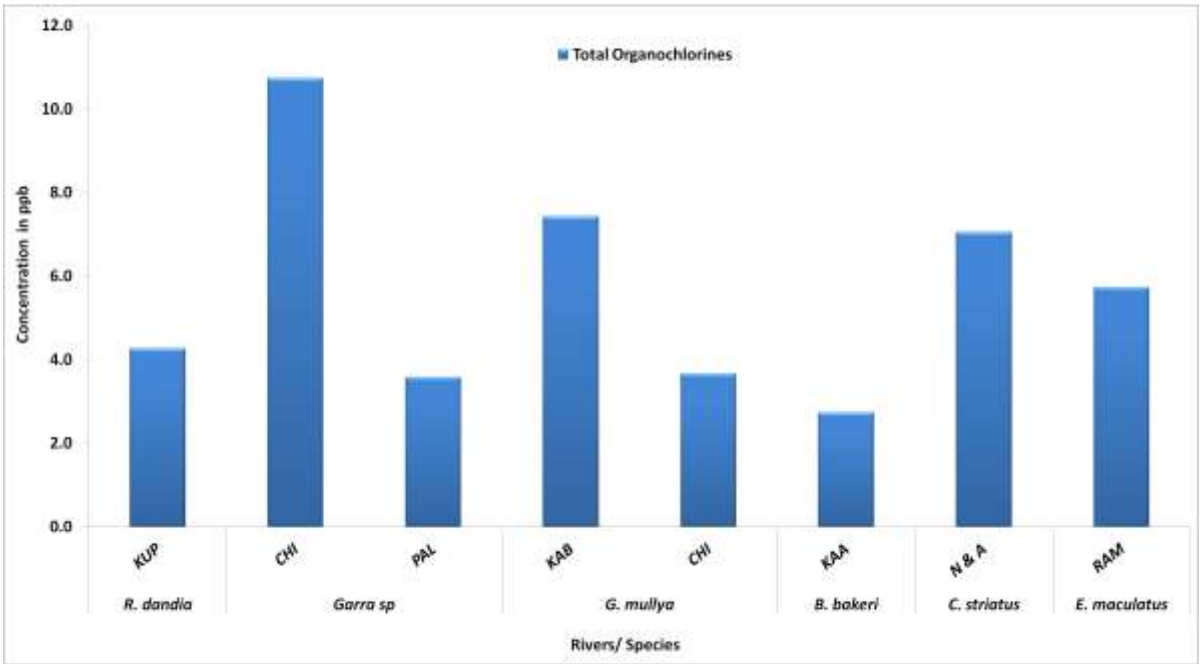
B & T- Bharathappuzha-Thutha; MAH - Mahii; PHU - Phuzhakkal; KARU - Karuvannoor; CHAL - Chalakudy; NEI - Neiyyar; KARA - Karamana; MAM - Mamam; ANJ - Anjarakkandi; KAD - Kadalundi; UPP - Uppala; SHI - Shiriya and MAN - Manjesehwaram

Fig 4.1b Levels of total organochlorine residues in fishes in the rivers of Kerala



KAB - Kabani; MAH - Mahii; CHA - Chaliyar; MAM - Mamam; CHAL - Chalakudy; KAD - Kadalundi; KAL - Kallayi and KUT - Kuttiyadipuzha

Fig 4.1c Levels of total organochlorine residues in fishes in the rivers of Kerala



KUP-Kuppam; CHI-Chithar; PAL-Pallickal; KAB-Kabani; KAA-Kaariyankode; N&A-Nilleswaram-Arayi and RAM- Ramapuram puzha

4.1.1 Residues of major metabolites/ isomers of organochlorine pesticides in fishes in the rivers of Kerala

a) HCH and its isomers

Among the four isomers of HCH, namely alpha (α), beta (β), gamma (γ) and delta (δ), only residues of γ and δ HCH were detected in twelve and one species of fish respectively collected from 12 out of 43 rivers in Kerala. While levels of α and β HCH were at below detection limit (fig 4.2), γ HCH was recorded the maximum in *Puntius filamentosus* (7.74 ppb) collected from midland areas of Bharathappuzha-Thutha, followed by midland samples of Mahii (6.94 ppb) and Neiyyar river (6.75 ppb) in the same species. The minimum was also in the same species from lowland areas of Chalakudy (2.41 ppb). Absence of α HCH in environmental samples might indicate that there has been no recent use of technical mixtures of HCH (Doong *et al.,* 2002). Concentration of δ HCH was detected only in *Garra* sp (3.58 ppb) collected from Pallickal and BDL in other samples. Residues of total HCH were detected in 5.28% of samples analysed and it ranged between BDL and 7.74 ppb. The other species those recorded detectable amount of γ HCH residues include *Devario* sp. (4.85 ppb- Mahii), *Puntius filamentosus* (4.62 ppb - Kadalundi; 4.61 ppb - Phuzhakkal and 4.27 ppb - Kuppam), *Channa striatus* (4.16 ppb - Nilleswaram-Arayi) and *Garra mullya* (3.66 ppb - Chithar).

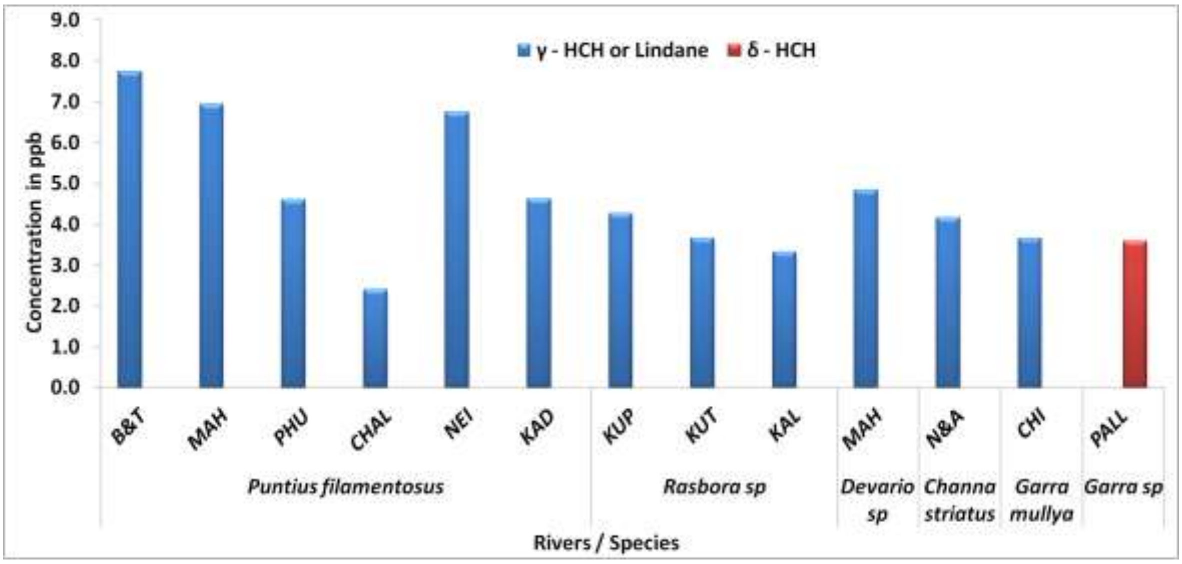
Technical HCH is a broad spectrum organochlorine insecticide that was available for decades and used throughout the world for agricultural and non-agricultural purposes because of its effectiveness and low cost (Li *et al*. 1998 and Raina *et al.,* 2008). The isomeric forms of HCH are toxic and recalcitrants. They exhibit both acute and chronic toxicity, particularly β isomer acts as an environmental estrogen (Walker *et al*. 1999). Use of HCH continued unabated until it was banned in 1997 in India, but restricted use was allowed for lindane (99% γ HCH) till 2011. Illegal use for control of mosquitoes and other insect pests was reported by Raina et *al.* (2008) and Lal *et al.* (2010). However, HCH is totally banned vide Gazette Notification No S.O. 637(E) dated 25/03/2011, for manufacture, import or formulate and use w.e.f. 25th March, 2013 (http://cibrc.nic.in/ retrieved on 17th April 2013). The residues of total HCH recorded in the present study (BDL- 7.4 ppb) are lower than the levels recorded in nine species of freshwater fishes (51.7 ppb) from different inland wetlands in Karnataka (Dhananjayan and Muralidharan, 2010) and six species of fishes, namely *Cyprinus carpio communis*, *Ciprinus carpio nudus*, *Ciprinus carpio specularis*, *Oreochromis mossambicus* and *Barbus carmuca* from various reservoirs (BDL to 77.68 ppb), namely Avalanche, Upper

Bhavani, Emerald, Kamaraj Sagar, Pykara and Maravakandy, Ooty lake and rivers such as Coonoor and Moyar in Nilgiris district (Vijayan and Muralidharan, 1999).

Even the highest level of HCH reported in the present study (7.74 ppb: *Puntius filamentosus* - Bharathappuzha-Thutha rivers) is lower than the concentrations reported in freshwater fishes of Andhra Pradesh (21 ppb: Amaraneni and Pillala, 2001) and comparable with the levels reported in fresh water fishes of Punjab (7 ppb: Kaur *et al.* 2008). Kumari *et al.,* (2001) reported high concentration of HCH in fishes (147.6 ppb) of river Ganga at four sites in Bihar: Buxar, Patna, Mokama and Rajmahal and possibilities of higher dietary intake of pesticides to human beings through consumption of fishes. Total HCH residues recorded in the present study are lower than the levels reported in *Platicephalus* sp. (5.4 ppb), comparable to *Epinephelus* sp. (7.37 ppb) and higher than *Lutjanus rivulatus* (70.56 ppb) collected from rivers in Calicut (Ferok, Korappuzha and Purakkattiri) (Sankar *et al.* 2006).

Further, the levels of total HCH reported in the present study are lower than the levels reported in fish samples collected from Madhya Pradesh, Rajasthan, Ludhiana, Kolkata and Bombay (0.01 - 8.5 ppm) (Bhinge and Benerji 1981; Battu *et al.* 1984; Raizada *et al.* 1989). Species-specific differences in lipid content of the fishes and level of contamination in the water and food will also influence the accumulation pattern of organochlorine residues among the species.

Fig 4.2 Concentration of HCH isomers in fishes in the rivers of Kerala



B&T- Bharathappuzha - Thutha; MAH- Mahii; PHU- Phuzhakkal; CHAL- Chalakudy; NEI- Neiyyar; KAD- Kadalundi; KUP- Kuppam; KUT- Kuttiyadipuzha; KAL- Kallayi; N&A- Nilleswaram-Arayi; CHI- Chithar and PAL- Pallickal

b) DDT and its metabolites

Two isomers (*o,p′-DDT* and *p,p′-DDT*) and two metabolites (*p,p′-DDE* and *p,p′-DDD*) of DDT were detected in fishes collected from six out of 43 rivers in Kerala (fig 4.3). Σ DDT residues were detected in 2.26% of samples in the range of BDL to 9.56 ppb. The species those recorded DDT residues were *Puntius filamentosus* from Bharathappuzha-Thutha, Kabani, Anjarakkandi, Uppala and Shiriya rivers, *Puntius conchonius* from Kabani and *Etroplus maculatus* from Ramapuram puzha .

Residues of *o,p′-DDT* were detected only in <0.5% of the samples analysed. Levels of *p,p′-DDT* were 2.60 and 2.58 ppb in *Puntius filamentosus* from Angadi mogar areas of Shiriya and, midland areas of Bharathappuzha-Thutha rivers respectively. *Puntius conchonius* received from Orappu areas of Kabani river detected 2.16 ppb of *p,p′-DDT*. Levels of these chemicals were BDL in fishes from other rivers. Residues of *p,p′-DDE* was the maximum in *Puntius filamentosus* (6.96 ppb) collected from Angadi mogar areas of Shiriya river, followed by o,p′-DDT and *p,p′-DDD* (each 2.87 ppb) in *Etroplus maculatus* collected from lowland areas of Ramapuram puzha.

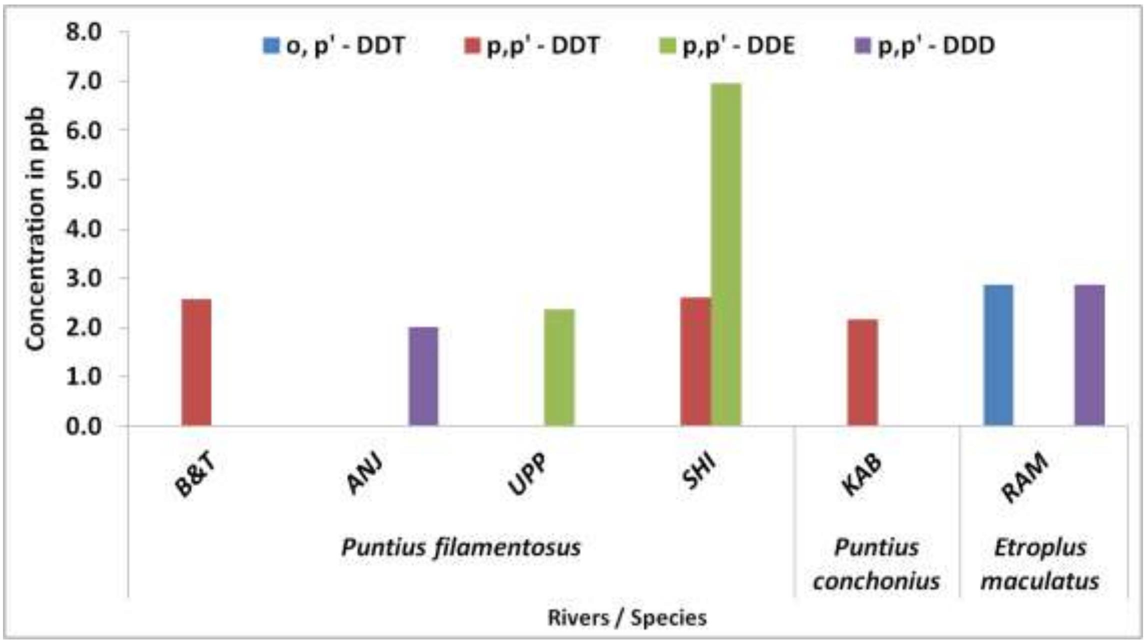
Concentration of DDE has ecological implications because this metabolite is the most persistent and toxic than its parent compound, the DDT. Furthermore, DDE is known to alter metabolic functions in organisms by acting as an antiandrogen, binding to androgen receptors and inhibiting transcriptional activation, which causes reproductive abnormalities (WHO 2004). In India, the use of DDT in agriculture was banned in 1989. But a maximum of 10,000 tons of DDT per annum for the control of malaria is being permitted (Dash *et al.,* 2007). DDT can accumulate and biomagnify in organisms (Walker 2001) due to its lipophilicity (K_{ow} = 5.7 - 6.36) and persistence ($T_{1/2}$ = 10 - 15 years). Their long persistence is the reason that residues of DDT and its metabolites can still be detected in the environment. The degree of persistence of DDE varies considerably, as persistence depends on the medium and many environmental parameters.

Levels of Σ DDT (BDL - 9.56 ppb) recorded in the present study are lower than the levels reported in 1050 fishes (BDL to 23.72 ppb) comprising six species of fishes studied from reservoirs, namely Avalanche, Uppar Bhavani, Emerald, Kamaraj Sagar, Pykara and Maravakandy, Ooty lake and rivers such as Coonoor and Moyar in Nilgiris district (Vijayan and Muralidharan, 1999) and freshwater fishes (3.4 - 12.3 ppb) of different inland wetlands in Karnataka (Dhananjayan and Muralidharan, 2010). Further, *p,p′-DDT* gets converted in to *p,p′-DDE* due to the activity of mixed function oxygenase enzyme through metabolic functions.

Concentrations of *p,p'-DDE* (6.96 and 2.37 ppb in *Puntius filamentosus* from Shiriya and Uppala rivers) recorded in our study are higher than the levels reported in fishes of Ganges Estuary, Bangladesh (1 - 2 ppb) (Jabber *et al.* 2001). However, the total DDT is lower than the levels reported in fishes (72.6 - 1666 ppb) from Ganga River (Kumari *et al.* 2001) and comparable with the levels reported in freshwater fishes of Punjab (Kaur *et al.* 2008).

DDT residues reported in the present study are marginally higher than the levels reported in fresh water fish, *Scatophagus argus* (6.91 ppb) collected from Calicut areas (Ferok, Korappuzha and Purakkattiri) (Sankar *et al.* 2006). Further, the levels of total DDT are lower than the levels reported in fishes collected from Madhya Pradesh, Rajasthan, Ludhiana, Kolkata and Bombay (0.02 - 34 ppm) (Bhinge and Benerji 1981; Battu *et al.* 1984; Raizada *et al.* 1989). The present pattern of presence of DDT compounds in the fishes from forty three rivers of Kerala indicates that the availability or use of DDT in the state might be very limited. However, considering the facts that DDT has a high half-life of 10 - 20 years, it degrades to DDE and DDD (Sericano *et al.,* 1990) and the DDE accounts for 50- 70% of the total DDT burden in the environment (Newsome and Andrews, 1993), DDT and its metabolites bioaccumulate and are reported to be probable human carcinogens (Fairey *et al.,* 1997), fresh inputs of DDT into the environment through the usage for public health needs, has to be monitored. In this context it is to be noted that we still have instances of DDT poisoning in birds in India.

Fig.4.3 Concentration of DDT isomers in fishes in the rivers of Kerala



B&T- Bharathappuzha - Thutha; ANJ- Anjarakkandi; UPP- Uppala; SHI- Shiriya; KAB- Kabani and RAM- Ramapurampuzha

c) Cyclodiene insecticides

Residues of eight cyclodiene pesticides (α and β endosulfan, endosulfan sulphate, heptachlor, heptachlor epoxide, dieldrin, chlordane and mirex) were tested in the fish tissues (fig 4.4a and 4.4b). In all the samples analysed, while levels of α endosulfan was BDL, β endosulfan was detected in *Channa striatus* (1.77 ppb) collected from Nilleswaram-Arayi rivers (Kasargod and Kannur dt). Endosulfan sulphate was the maximum in *Garra* sp (7.08 ppb) collected from midland areas of Chithar (Kasargod Dt), followed by *Rasbora* sp (5.54 ppb) collected from lowlands of Kuttiyadipuzha river (Kozhikode Dt), while it was minimum in highland areas of Kadalundi, Malappuram Dt (2.03 ppb) in the same species (*Rasbora* sp). The other species those recorded detectable amount of endosulfan sulphate residues include *Devario* sp from highland (3.61 ppb) and midland (4.98 ppb) areas of Chaliyar river (originates in the Western Ghats range of Elambalari Hills located near Cherambadi town in the Nilgiris Dt of Tamil Nadu and flows through Malappuram Dt and enters Arabian sea in Kozhikode Dt), *Garra mullya* (4.28 ppb) from Meencolli areas of Kabani river (originates in Wayanad Dt of Kerala and flows eastward to join Kaveri river in Karnataka), *Puntius filamentosus* (3.64 ppb) from low land areas of Mahii, *Devario* sp (2.86 ppb) from Mahii river (near Kannur), *Puntius filamentosus* from Neiyyar (2.59 ppb) (Agasthiya mala, Trivandrum dt) and Mamam (2.40 ppb) (originates in Panthalacode hills in Trivandrum). Heptachlor residue was the maximum in the *Puntius conchonius* (5.07 ppb) collected from Orappu areas of Kabani river and minimum in *Puntius filamentosus* (2.04 ppb) received from midlands of Chalakudy river. Other notable species those had detectable levels of heptachlor include *Devario malabaricus* (3.77 ppb) collected from midland areas of Mamam, *Garra mullya* (3.14 ppb) from Kabani, *Rasbora* sp from Mahii (2.11 ppb) and Chalakudy (2.09 ppb), *Puntius filamentosus* from Mamam (2.68 ppb), Karuvannoor (2.33 ppb), Phuzhakkal (2.22 ppb) and Karamana (2.15 ppb). The *Barilius bakeri* collected from Bheemanadi area of Kaariyankode river alone recorded 2.73 ppb of heptachlor epoxide. Residues of other pesticides, namely α-Endosulfan, dieldrin, chlordane and mirex were found to be BDL in all species of fishes. Of all the fishes studied, while total endosulfan was detected in 4.15% of fishes, Σ-heptachlor was in 4.91% of fishes.

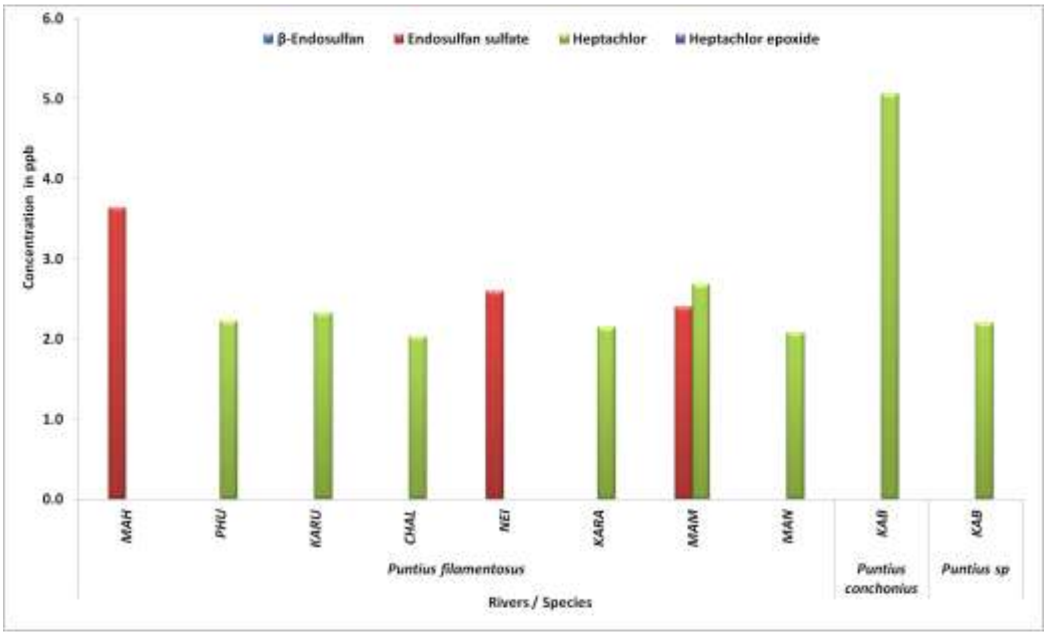
Endosulfan is an off-patent organochlorine insecticide and acaricide that is being phased out globally. Endosulfan became a highly controversial agrochemical due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor (Vinay Malik, 2013). Because of its threats to human health and the environment, a global ban on the manufacture and use of endosulfan was negotiated under the Stockholm Convention in April 2011. It may be noted that Kerala government banned endosulfan way back in 2002 particularly in Kasargod district, Kerala. More than 80 countries, including the European Union, Australia and New Zealand, several West African nations, the United States, Brazil and Canada had already banned it or announced phase outs by the time the Stockholm Convention's ban was agreed upon. It is still used in India, China and few other countries. The Supreme Court of India has banned the toxic endosulfan in the country with effect from May, 2011, with certain exemptions for five additional years (Vinay Malik, 2013).

The maximum concentration of total endosulfan recorded in *Garra* sp (7.08 ppb) from river Chithar in the present study is lower than the levels reported in Kolkata (Kole *et al.,* 2001). This level is also lesser than the levels that could produce histopathological changes in fishes particularly gill tissues (Khare *et al.,* 2002). Similarly, concentrations of total heptachlor recorded in the present study are lower than the concentrations reported (110 ppb) in the fishes of the Ganges Estuary, Bangladesh (Jabber *et al.,* 2001). However, residues of total endosulfan (BDL - 7.08 ppb) recorded are higher (BDL - 4.3 ppb) and heptachlor residues (BDL - 5.07 ppb) are lower than the levels (10.8 ppb) recorded in 156 individuals comprising nine species of fishes collected in ten inland wetlands in Karnataka (Dhananjayan and Muralidharan, 2010).

Concentrations of total endosulfan and heptachlor are lower than the levels reported by Vijayan and Muralidharan, (1999) in 1050 fishes comprising six species of fishes, namely *Cyprinus carpio communis*, *Ciprinus carpio nudus*, *Ciprinus carpio specularis*, *Oreochromis mossambicus* and Barbus *carmuca* studied from various reservoirs (BDL - 16.24 ppb), namely Avalanche, Uppar Bhavani, Emerald, Kamaraj Sagar, Pykara and Maravakandy, Ooty lake and rivers such as Coonoor and Moyar in Nilgiris district.

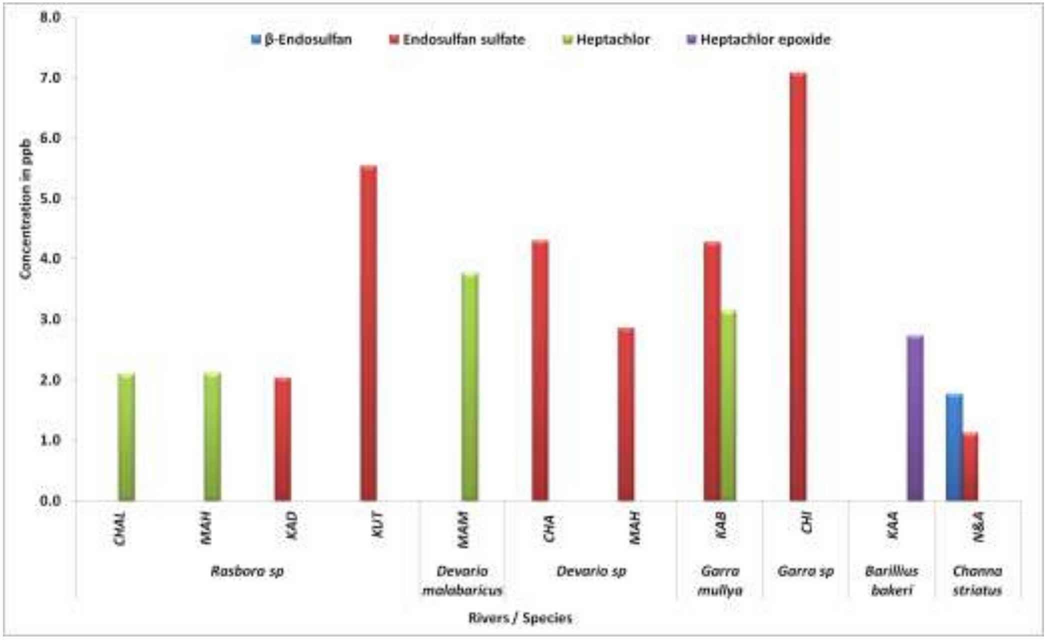
Further the maximum concentrations of total endosulfan reported in this study are lower than the concentrations reported in freshwater fishes of Andhra Pradesh (76.5 ppb; Amaraneni and Pillala, 2001). While Kaur *et al.* (2008) reported levels of endosulfan to be below detection limit, in a few species of fishes in Punjab, Kumari *et al.* (2001) reported high concentration of endosulfan in fishes (401.7 ppb) of River Ganga at four sites in Bihar: Buxar, Patna, Mokama and Rajmahal and possibilities of higher dietary intake of pesticides to human beings through consumption. When total cyclodiene residues reported in the present study are compared with the levels reported in the fishes of Rivers in Calicut (Ferok, Korappuzha and Purakkattiri), they are lower than the levels in *Platicephalus* sp. (5.4 ppb), comparable with *Epinephelus* sp. (7.37 ppb) and higher than the levels in *Lutjanus rivulatus* (70.56 ppb) (Sankar *et al.,* 2006). Although we have phased out many OCs due to their ill effects in India, whatever we used till recently are unfortunately expected to be in the environment for many more years. As the OC pesticides have adverse effects not only on fishes and other aquatic life forms, but also on man, their regular monitoring is strongly advised.

Fig 4.4a Concentration of cyclodiene insecticides in fishes in the rivers of Kerala



MAH-Mahii; PHU- Phuzhakkal; KARU-Karuvannoor; CHAL- Chalakudy; NEI- Neiyyar; KARA-Karamana; MAM-Mamam; MAN- Manjesehwaram and KAB-Kabani

Fig 4.4b Concentration of cyclodiene insecticides in fishes in the rivers of Kerala



CHAL-Chalakudy; MAH-Mahii; KAD-Kadalundi; KUT-Kuttiyadipuzha; MAM-Mamam; CHA-Chaliyar; KAB-Kabani; CHI-Chithar; KAA-Kaariyankode and N&A-Nilleswaram-Arayi

4.1.2 Residues of organophosphate pesticides in fishes in the rivers of Kerala

Among the fishes collected from 43 rivers in Kerala, malathion was detected in three species, namely *Barilius bakeri* (5.85 ppb) from highlands of Anjarakkandi, *Barilius canarensis* (2.62 ppb) from highlands of Valapattanam and *Rasbora* sp (2.76 ppb) from lowland areas of Chalakudy. Only one sample (*Puntius filamentosus* - 2.05 ppb) recorded residues of phenthoate. Levels of other OP pesticides namely, methyl parathion, chlorpyrifos, primiphos ethyl, quinalphos and ethion were below detection limit in all the fishes studied.

Concentrations of malathion recorded (5.85 ppb) in this study are lower than the levels reported from freshwater fishes of Andhra Pradesh (45.3 ppb; Amaraneni and Pillala, 2001), different tributaries of the Nile River in Egypt (8.31 ppb; Malhat and Nasr, 2011) but higher (BDL - 3.05 ppb) than the levels reported in fishes from various points in Ganga river around Kolkata, namely Berhampore, Palta, Dakshineswar, Uluberia and Diamond harbour in three successive seasons, between 2001 and 2005. Although, considerable quantities of OP pesticides are used, as they degrade comparatively faster in the environment, we are unable to measure their residues and assess the impact. Nevertheless, it is to be noted that within a short span of time they are capable of creating deleterious effects such as reproductive failure, neurotoxicity, kidney/ liver damage and birth defects in addition to being a possible carcinogen (US EPA, 2000).

4.1.3 Residues of synthetic pyrethroids in fishes in the rivers of Kerala

All the samples were analysed for residues of permethrin and fenvalerate. Permethrin residues were detected only in three samples (<1%) which include *Puntius filamentosus* from midland areas of Kallada (2.51 ppb), *Rasbora dandia* from highland areas of Valapattanam (3.53 ppb) and *Rasbora* sp from low land areas of Chaliyar (6.24 ppb). Levels of fenvalerate residues were BDL in all the other samples.

The ill effects of pyrethroid insecticide, fenvalerate on the histology and histochemistry in the liver of catfish (*Clarias gariepinus*) after exposure to 1/10 of lethal concentration for 5 and 10 days were investigated by Sakr *et al.* (2005). The results showed that the histopathological changes induced in the liver were mainly represented by cytoplasmic vacuolization of the hepatocytes, blood vessel congestion, inflammatory leucocytic infiltration, necrosis and fatty infiltrations. Studies on the levels of pyrethroids in fishes in India are very few.

Although many of the OC group of pesticides have been banned, still sometimes we find unsafe levels of residues in the environment. Further, many short-lived pesticides belonging to OP and SPs are being used quite extensively in the name of replacements to POPs. But the impact created by these substances on the aquatic ecosystems, and particularly fishes is not assessed fully. Neither there is any programme in place in India to monitor the fate of these chemicals in the environment on long-term basis.

4.1.4 Dietary intake of pesticide residues to man

Based on the OC, OP and SP pesticide residues recorded in fishes collected from all the rivers, an attempt was made to assess their suitability for human consumption. It is to be noted that the annual per capita consumption and recommended intake of fish is 9.8 kg and 13 kg, respectively in India (MoSPI, 2011; www.mospi.gov.in). However, considering the local situation, assuming that a person consumes 250 g of fish per week, the average daily intake of pesticides through fishes was calculated as explained by FAO/WHO (1991). The calculated dietary intake levels represent the quantum of pesticides entering the human body through consumption of these twenty eight species of fishes, selected for the present study. The levels were compared with the Allowable Dietary Intake (ADI) concentration levels proposed by various statutory agencies across the world. Apart from this, the maximum permissible residue levels of the pesticides proposed by International Regulatories and Advisories have also been compared with the residue levels documented in the present study.

In India, although information on the residue levels of pesticides in water, soil, sediment, fishes and birds are available, information on the daily dietary intake of pesticides through consumption of fishes is scarce, except a few (Kumari *et al.,* 2001; Muralidharan *et al.,* 2009; Dhananjayan and Muralidharan, 2010 and Jayanthi, 2012). Vijayan *et al.* (2004) documented many persistent contaminants in about 66 species of fresh water fishes from 173 wetlands spread across 14 states in India. The study also looked at the dietary input to human beings in contrast to the WHO guidelines.

The calculated dietary intake (CDI) concentration of γ HCH residues ranged from 0.09 to 0.28 µg/kg. The maximum dietary intake through consumption of *Puntius filamentosus* was 0.28 µg/kg from Bharathappuzha-Thutha, followed by 0.25 and 0.24 µg/kg through the same species from Mahii and Neiyyar rivers respectively and minimum through the same species from the river Chalakudy (0.09 µg/kg). The dietary intake of δ HCH through *Garra* sp (Pallickal River) was 0.13 µg/kg (Table 3a). Similarly dietary intake concentrations of total DDT residues were maximum (0.34 µg/kg) and minimum (0.07 µg/kg) through *Puntius filamentosus* from Shiriya and Anjarakkandi rivers respectively. Dietary intake levels through *Etroplus maculatus* from Ramapuram puzha are 0.20 µg/kg (Table 3b). The maximum intake concentrations of endosulfan sulphate and heptachlor residues were 0.25 µg/kg through *Garra* sp from Chithar and 0.18 µg/kg through *Puntius conchonius* from Kabani respectively (Table 3c).

The dietary intake concentrations of OP pesticides, namely malathion and phenthoate residues were 0.21 µg/kg and 0.07 µg/kg through *Barilius bakeri* from Anjarakkandi and *Puntius filamentosus* from Karuvannoor respectively (Table 3d). Intake of synthetic pyrethroid, permethrin residues was 0.22 µg/kg through *Rasbora* sp from Chaliyar (Table 3e). Levels of pesticide residues in the present study are well below the MRL limits formulated by Food Safety and Standards Authority of India (FSSAI) and the dietary intake concentration levels of these pesticide residues are far below the limits established by international statutory agencies, namely United States Environmental Protection Agency (USEPA), European Union, Health Canada, Germany, Food and Agricultural Organisation and World Health Organisation (FAO/ WHO Codex Alimentarius commission). However, effects that result from long-term exposure through diet to low doses are often difficult to distinguish although acute toxic effects are easily recognised.

In conclusion, the estimated dietary intake of select pesticides covered in the study did not entail a serious risk. But precautions must be taken when generalizing this negative result to people with high dietary exposure level or an intake of other pesticides.

Table 3a Comparison of calculated dietary intake concentration with the acceptable dietary intake (ADI) stipulated by various statutory agencies

Isomers of Hexachlorocyclohexane (HCH)

Name of the species	Name of the River	γ - HCH or Lindane (µg/kg)	δ – HCH (µg/kg)	ADI limit (µg/kg or ppb)
Puntius filamentosus	Bharathappuzha-Thutha	0.28	-	FDA limit for γ HCH : 300 ppb (Barasa *et al.* 2007) FSSAI limit for ∑ HCH: 250 ppb (http://www.fssai.gov.in/ 2011) ∑ HCH : 18 µg/person (Health Canada limit, 2007)
	Mahii	0.25	-	
	Phuzhakkal	0.16	-	
	Chalakudy	0.09	-	
	Neiyyar	0.24	-	
	Kadalundi	0.17	-	
Rasbora sp	Kuppam	0.15	-	
	Kuttiyadipuzha	0.13	-	
	Kallayi	0.12	-	
Devario sp	Mahii	0.17	-	
Channa striatus	Nilleswaram-Arayi	0.15	-	
Garra mullya	Chithar	0.13	-	
Garra sp	Pallickal	-	0.13	

Table 3b Comparison of calculated dietary intake concentration with the acceptable dietary intake (ADI) stipulated by various statutory agencies

Isomers and metabolites of DDT

Name of the species	Name of the River	*o, p* - DDT (µg/kg)	*p, p* - DDT (µg/kg)	*p, p* - DDE (µg/kg)	*p, p* - DDD (µg/kg)	∑ DDT (µg/kg)	ADI limit (µg/kg or ppb)
Puntius filamentosus	Bharathappuzha - Thutha	-	0.09	-	-	0.09	∑ DDT : 300 µg/person (IARC, Health Canada, 2007) ∑ DDT: 7000 ppb (on a whole product basis) (FSSAI (2011): http://www.fssai.gov.in/
	Anjarakkandi	-	-	-	0.07	0.07	
	Uppala	-	-	0.08	-	0.08	
	Shiriya	-	0.09	0.25	-	0.34	
Puntius conchonius	Kabani	-	0.08	-	-	0.08	
Etroplus maculatus	Ramapuram puzha	0.10	-	-	0.10	0.20	

Table 3c Comparison of calculated dietary intake concentration with the acceptable dietary intake (ADI) stipulated by various statutory agencies

Cyclodiene Insecticides

Name of the species	Name of the River	β-Endosulfan (μg/kg)	Endosulfan Sulphate (μg/kg)	Heptachlor (μg/kg)	Heptachlor Epoxide (μg/kg)	ADI limit (μg/kg or ppb)
Puntius filamentosus	Mahii	-	0.13	0.00	-	∑ Endosulfan: 100 ppb European Union (Di Muccio *et al.,* 2002). ∑ Endosulfan: 200 ppb http://www.fssai.gov.in/ (FSSAI, 2011). Heptachlor: 450 μg/person IARC (Falandysz *et al.,* 2001). Heptachlor: 50 ppb Tolerance limits of Germany (Falandysz *et al.,* 2001). Heptachlor epoxide: 100 ppb Tolerance limits of Sweden (Santerre *et al.,* 2000) ∑ Heptachlor: 300 ppb FDA Action limit (Santerre *et al.,* 2000). ∑ Heptachlor: 200 ppb European Union (Stefanelli *et al.,*2004) ∑ Heptachlor: 150 ppb FSSAI http://www.fssai.gov.in/ (2011)
	Phuzhakkal	-	0.00	0.08	-	
	Karuvannoor	-	0.00	0.08	-	
	Chalakudy	-	0.00	0.07	-	
	Neiyyar	-	0.09	0.00	-	
	Karamana	-	0.00	0.08	-	
	Mamam	-	0.09	0.10	-	
	Manjesehwaram	-	0.00	0.07	-	
Puntius conchonius	Kabani	-	0.00	0.18	-	
Puntius sp	Kabani	-	0.00	0.08	-	
Rasbora sp	Chalakudy	-	0.00	0.07	-	
	Mahii	-	0.00	0.08	-	
	Kadalundi	-	0.07	0.00	-	
	Kuttiyadipuzha	-	0.20	0.00	-	
Devario malabaricus	Mamam	-	0.00	0.13	-	
Devario sp	Chaliyar	-	0.15	0.00	-	
	Mahii	-	0.10	0.00	-	
Garra mullya	Kabani	-	0.15	0.11	-	
Garra sp	Chithar	-	0.25	0.00	-	
Barillius bakeri	Kaariyankode	-	0.00	0.00	0.10	
Channa striatus	Nilleswaram-Arayi	0.06	0.04	0.00	-	

Table 3d Comparison of calculated dietary intake concentration with the acceptable dietary intake (ADI) stipulated by various statutory agencies

Organophosphates

Name of the species	Name of the River	Malathion	Phenthoate	ADI (µg/kg or ppb)
Rasbora sp	Chalakudy	0.10	-	20 ppb (Lu 1995 and FAO/WHO, 2002) & 30 ppb (FAO, 1998)
Barillius bakeri	Anjarakkandi	0.21	-	
Barillius canarensis	Valapattanam	0.09	-	
Puntius filamentosus	Karuvannoor		0.07	-

Table 3e Comparison of calculated dietary intake concentration with the acceptable dietary intake (ADI) stipulated by various statutory agencies

Synthetic Pyrethroids

Name of the species	Name of the River	∑ Permethrin	ADI (µg/kg or ppb)
Puntius filamentosus	Kallada	0.09	50 ppb (WHO, 2002)
Rasbora sp	Chaliyar	0.22	
Rasbora daniconius	Valapattanam	0.13	

4.2 Metal contamination in fishes in the rivers of Kerala

As explained in the methodology two hundred and sixty two muscle tissue samples (table 4) were analysed for metals, namely cadmium (Cd), chromium (Cr) and lead (Pb). It may be noted that *Puntius filamentosus* (138 individuals) was the most dominant species followed by *Rasbora* sp (20 individuals) and *Garra mullya* (15 individuals). The data have been compiled by species and river wise to check the overall load of these metals. Further species with less than three individuals were not taken into the account for statistical analysis. Suitability of fishes for human consumption in terms of Acceptable Daily Intake (ADI) have also assessed.

Table 4 List of fish species analysed for metals

S.No.	Name of the fish species	Vernacular Name	No. of samples analysed
1	*Barbodes subnasqutus*	Kuruva, Mundothipparal	1
2	*Barilius bakeri*	Pullippavukan	6
3	*Barilius bendelisis*	Pavukan	2
4	*Barilius canarensis*	Pullippavukan	5
5	*Barilius* sp		2
6	*Channa gachua*	Vatton, vattudi, Thodan	2
7	*Channa striatus*	Varal, Bral, Kannan	4
8	*Devario malabaricus*	Thuppalamkothi, Ozhukkilatti	7
9	*Devario* sp		14
10	*Etroplus maculatus*	Pallathi, Pootta	7
11	*Etroplus suratensis*	Karimeen	5
12	*Garra mullya*	Kallotti, Njezhu, Kallunthi, Kallemkari	15
13	*Garra* sp		5
14	*Hypselobarbus curmuca*	Kuzhikuthi, Kooral	1
15	*Mugil cephalus*	Thirutha	4
16	*Nandus nandus*	Muthukkila, Muthukki, Urakkamthoongi	1
17	*Ompak bimaculatus*	Manglanchi, Thonnan vala	1
18	*Oreochromis mosambica*	Silopi	1
19	*Osteochilichthys nashil*	Mammalu	2
20	*Pristolepis marginata*	Aattuchembelli, Andikalli	2
21	*Puntius conchonius*	Paisepparal	1
22	*Puntius denisonii*	Chenkaniyan	2
23	*Puntius filamentosus*	Poovalipparal, valekodiyan	138
24	*Puntius mahecola*	Urulan paral	1
25	*Puntius* sp		0
26	*Rasbora dandia*	Kananjon	9
27	*Rasbora* sp		20
28	*Xenentoden cancila*	Koyla, Kolan	4
Total			**262**

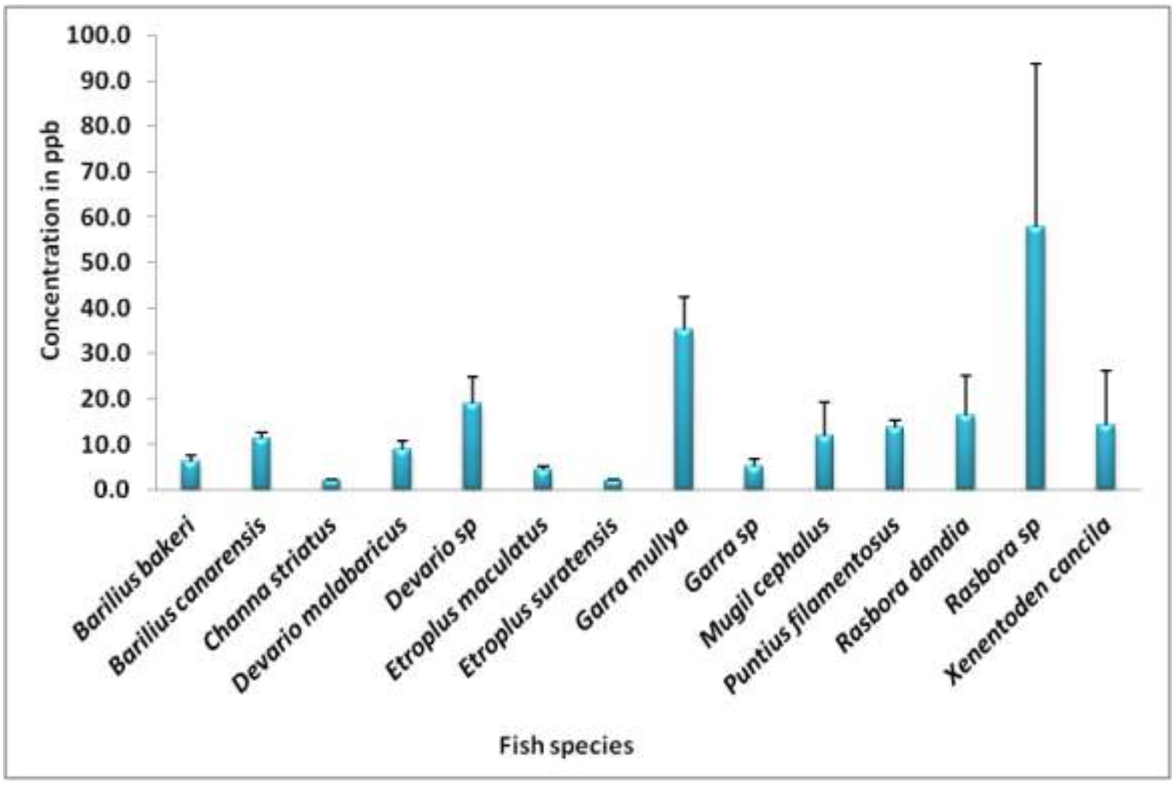
4.2.1 Metal contamination in fishes in the rivers of Kerala

4.2.1a Variation in the levels of metal contamination among the species of fishes collected from various rivers in Kerala

i) Cadmium

Mean cadmium concentration was the maximum in *Rasbora* sp. (57.69 ± 36.13 ppb) and minimum in *Channa striatus* (2.02 ± 0.0 ppb) closely followed by *Etroplus suratensis* (2.03 ± 0.13 ppb). Other species of fishes which had notable levels were *Garra mullya* (35.23 ± 7.07 ppb), *Devario* sp (19.09 ± 5.69) and *Rasbora dandia* (16.44 ± 8.69) (fig 4.5). Cadmium concentration varied significantly among the species (p<0.05) included in the study.

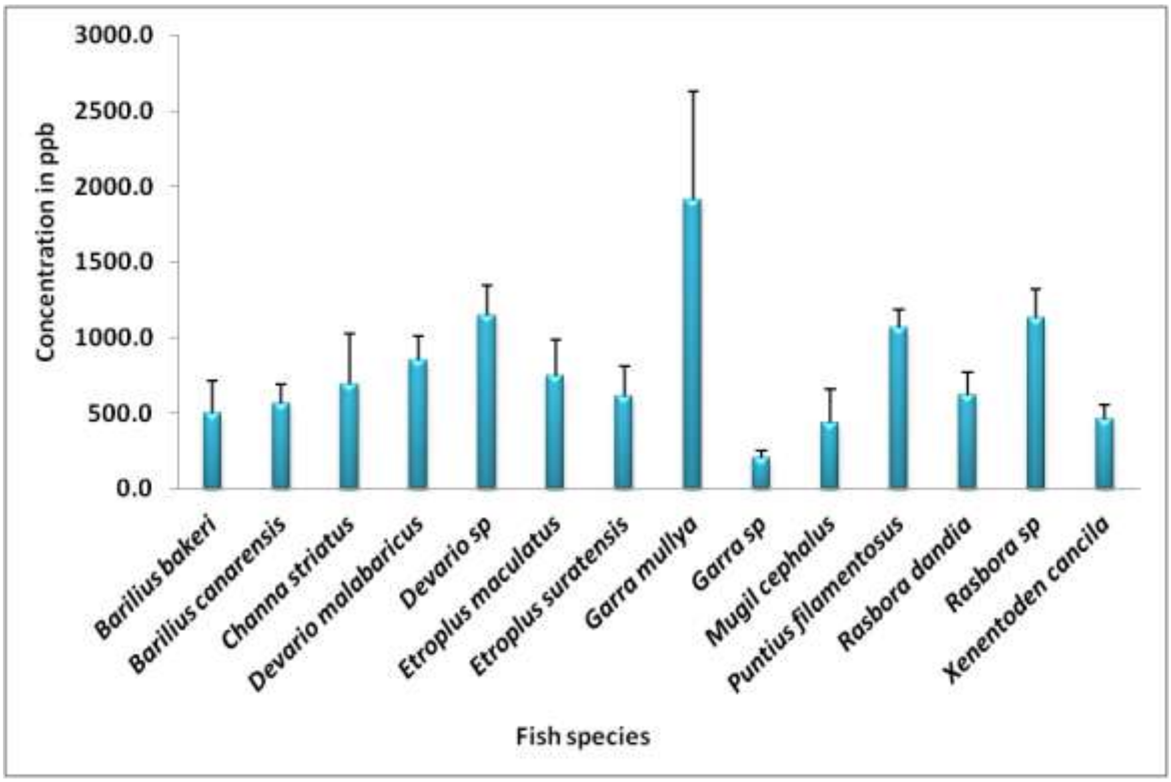
Fig 4.5 Levels of cadmium among the species of fishes collected from various rivers in Kerala



ii) Chromium

The highest mean concentration of chromium was observed in *Garra mullya* (1909.66 ± 718.22 ppb) and lowest in *Garra* sp (204.67 ± 50.07 ppb). Other species of fishes which had notable concentrations were *Devario* sp (1144.65 ± 198.56 ppb), *Rasbora* sp (1127.29 ± 190.40 ppb), *Puntius filamentosus* (1068.73 ± 116.85 ppb), *Devario malabaricus* (846.67 ± 166.96 ppb), *Etroplus maculatus* (748.25 ± 236.48 ppb), *Channa* *striatus* (694.40 ± 332.01 ppb), *Rasbora dandia* (619.11 ± 153.86 ppb), *Etroplus suratensis* (611.33 ± 199.32 ppb) and *Barilius canarensis* (560.75 ± 128.31 ppb) (fig 4.6).

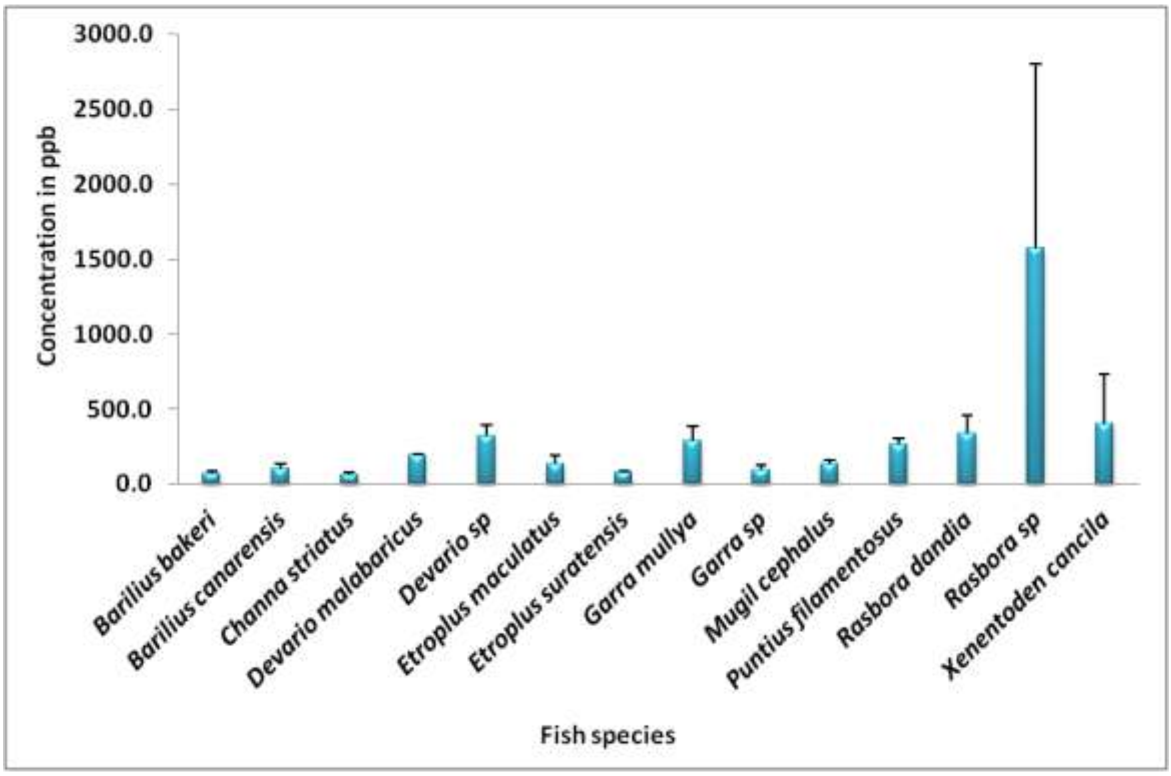
Fig 4.6 Levels of chromium among the species of fishes collected from various rivers in Kerala



iii) Lead

Mean lead (Pb) concentration was observed to be the maximum in *Rasbora* sp. (1574.95 ± 1226.28 ppb), while the mimimum was in *Barilius bakeri* (68.46 ± 15.97 ppb) followed by *Channa striatus* (66.12 ± 13.89 ppb). Variation in lead concentration was significant among the species ($p<0.05$) studied (fig 4.7).

Fig 4.7 Levels of Lead among the species of fishes collected from various rivers in Kerala



4.2.1b Variation in the levels of metal contamination in fishes among the 43 rivers in Kerala

i) Cadmium

On an average, cadmium contamination was the maximum in the fishes collected from Kuttiyadipuzha (78.21 ± 72.59 ppb), while minimum was observed in fishes of Pamba (2.02 ± 0.37 ppb) followed by Manimala (2.37 ± 0.0 ppb) and Pallickal (2.12 ± 0.45 ppb) rivers (fig 4.8). Cadmium concentration in fishes varied significantly among the rivers ($p<0.05$).

The levels of Cd recorded in the present study are lower than the levels reported from Thane and Basin creeks of Mumbai (30-400 ppb) by Krishnamurti and Nair (1999); sixteen species of fishes (BDL - 180 ppb) from Keoladeo National Park, Rajasthan by Muralidharan (1995); four species of fishes (BDL - 570 ppb) from Keoladeo National Park, by Selvam (2002); *Labia rohita* and *Catla catla* collected from Aliyar (240 ppb), Amaravathy (360 ppb) and Thirumoorthy (180 ppb) reservoirs and *Mystus vittatus,* and *Tilapia mosambica* of Ukkadam (300 ppb), Kurichi (880 ppb) and Singanallur (660 ppb) urban wetlands in Coimbatore, Tamil Nadu (Jayakumar 2001); 11 species of fishes (130 -

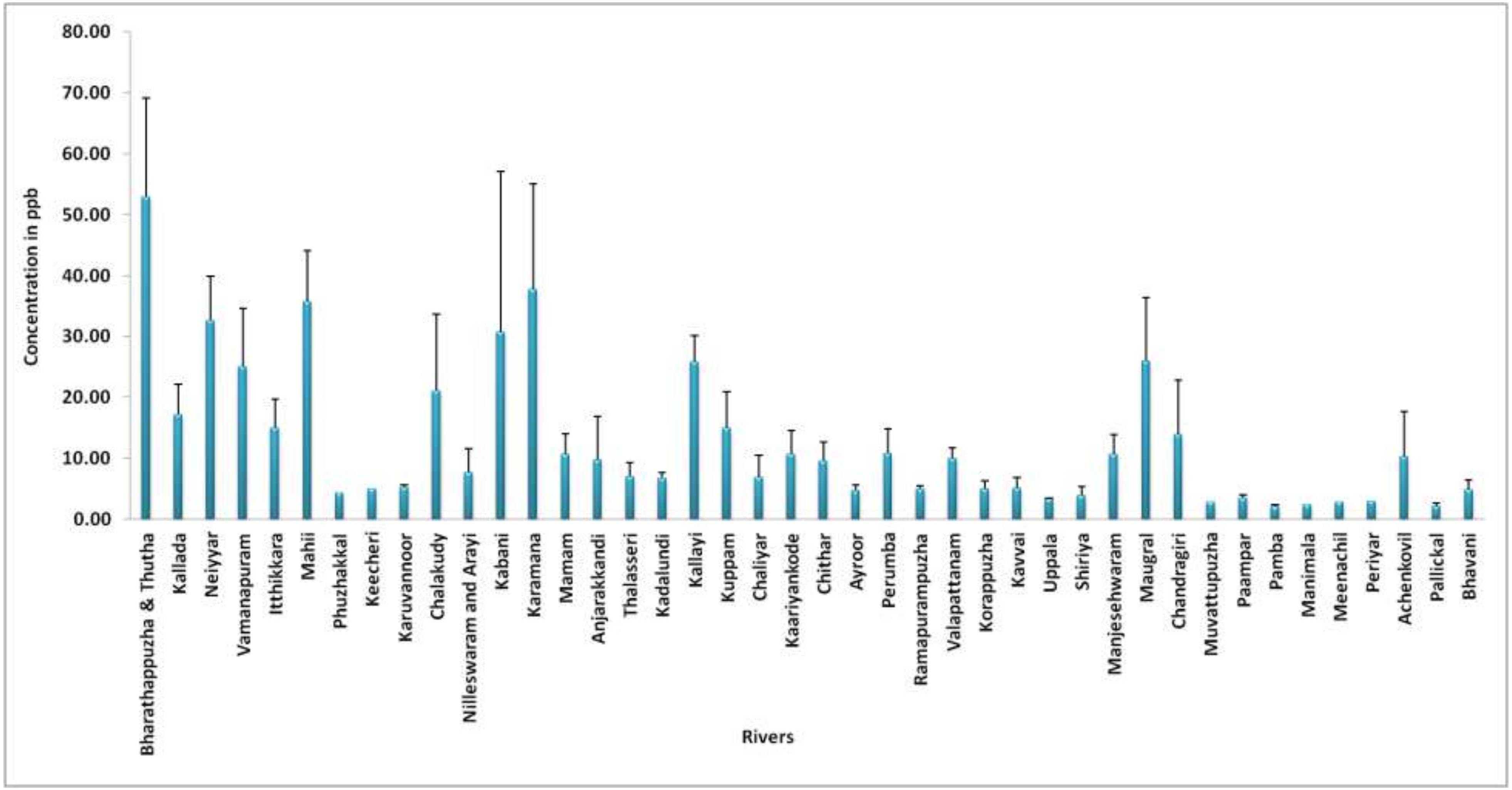
1160 ppb) from nine wetlands in Andhra Pradesh; five species of fishes (650 - 870 ppb) from six wetlands in Assam; 10 species of fishes (590 - 920 ppb) from nine wetlands in Bihar; 11 species of fishes from (380 - 920 ppb) 11 wetlands in Gujarat; 13 species of fishes (100 - 800 ppb) from 12 wetlands in Karnataka; five species of fishes (890 - 6050 ppb) from five wetlands in Maharashtra; eight species of fishes (450 - 810 ppb) from six wetlands in Madhya Pradesh; five species of fishes (BDL - 840 ppb) from four wetlands in Uttar Pradesh; eight species of fishes (BDL - 920 ppb) from seven wetlands in West Bengal and 11 species of fishes (540 - 1530 ppb) from 17 wetlands in Tamil Nadu (Jayakumar 2007). The levels of Cd observed in the present study were higher than the levels reported in fishes of Assi, Nigeria (BDL - 726 ppb) and Assiut City, Egypt (621 ppb) and lower than the levels reported from Nala Deg (4250 ppb), Pakistan (Ahmed and Bibi, 2010) and four fish farms of Qassim region (150 ppb), Saudi Arabia (Abdullah El-Ghasham *et al.,* 2008).

Cadmium is toxic to all life forms, including plants, fishes, birds, mammals and microorganisms (Eisler 1985, Eisler 2000, Nordberg *et al.,* 2007 and ATSDR 2008). It is widely used in mining, metallurgical operations, electroplating industries, manufacturing of vinyl plastics, metallic and plastic pipes (Passow *et al.,* 1961). Fish can accumulate cadmium from the water and/ or foods contaminated with cadmium. It is important to note that bioaccumulation and magnification occur when a substance cannot be easily metabolized or excreted. Cadmium exhibits this persistence (ATSDR Medical Fact Sheet 2008). Cadmium biomagnifies in the food chain and can accumulate in humans (Eisler 1985, Edmonds and Peplow 2000 and Nordberg *et al.,* 2007).

Sublethal effects such as decreased growth, inhibited reproduction and population alterations in fishes may occur due to chronic exposures and can be pronounced or probable when cadmium concentration exceeds 3 mg/L in fresh water (Eisler 1985). However, some studies have concluded that sublethal cadmium exposure did not reduce growth but did cause alterations to appetite and metabolism. Compared to pre-exposure conditions, fish exposed to cadmium consumed less food to achieve the same growth rate (McGeer *et al.,* 2000). This is consistent with other studies those observed hypoactivity from exposure to certain metals, suggesting that reduced activity permitted a greater proportion of consumed energy to be directed towards growth (Wilson *et al.,* 1994 and McGeer *et al.,* 2000). Therefore, a sensitive measure of chronic sublethal effects may be measurement of behaviour, particularly spontaneous and basal activity levels. Cadmium’s toxicity to freshwater fish can be altered by water chemistry variables, such as alkalinity, hardness and natural organic matter (Stuart and Levit, 2010).

Cadmium has been shown to increase in agricultural soils and cause contamination and impair kidney function in people eating foods from those soils (Olsson *et al.,* 2005). Similarly, consumption of a cadmium-contaminated rice and fish diet in North central Sri Lanka has been shown to cause chronic renal failure (Bandara *et al.,* 2008). Cadmium has been demonstrated to persist in the human body for up to 38 years, underscoring the importance of decreasing human exposure to cadmium (ATSDR 2008). It causes cancer, birth defects and genetic mutations in man (Eisler 2000 and Nordberg *et al.,* 2007). Humans accumulate cadmium primarily in the kidneys and liver (ATSDR 2008). Due to slow excretion, cadmium accumulates in the body over a lifetime and its biologic half-life may be up to 38 years (ATSDR: http://www.atsdr.cadmiumc.gov/csem/cadmium/cadmiumbiologic_fate.html; accessed on 25th May 2013). This study further indicates that the levels of cadmium observed in the present study (2.02 - 57.70 ppb) in fishes from rivers in Kerala may alter the physiology of fishes and inhibit reproductive steroid hormones either alone or synergistic with other injurious agents that are present in the environment.

Fig 4.8 Levels of cadmium in fishes in the rivers of Kerala



ii) Chromium

While fishes collected from Bharathappuzha-Thutha had the highest levels of chromium (5364.30 ± 1654.01 ppb), fishes from Pallickal (229.81 ± 36.87 ppb) had the lowest. Other rivers those recorded notable concentrations of Cr include Maugral (3256.76 ± 1620.62 ppb), Karamana (1923.26 ± 475.65 ppb) and Kallayi (1863.27 ± 415.51 ppb). Fishes from twenty seven out of 43 rivers (63%) studied had an average concentration ranging between 230 and 935 ppb (fig 4.9).

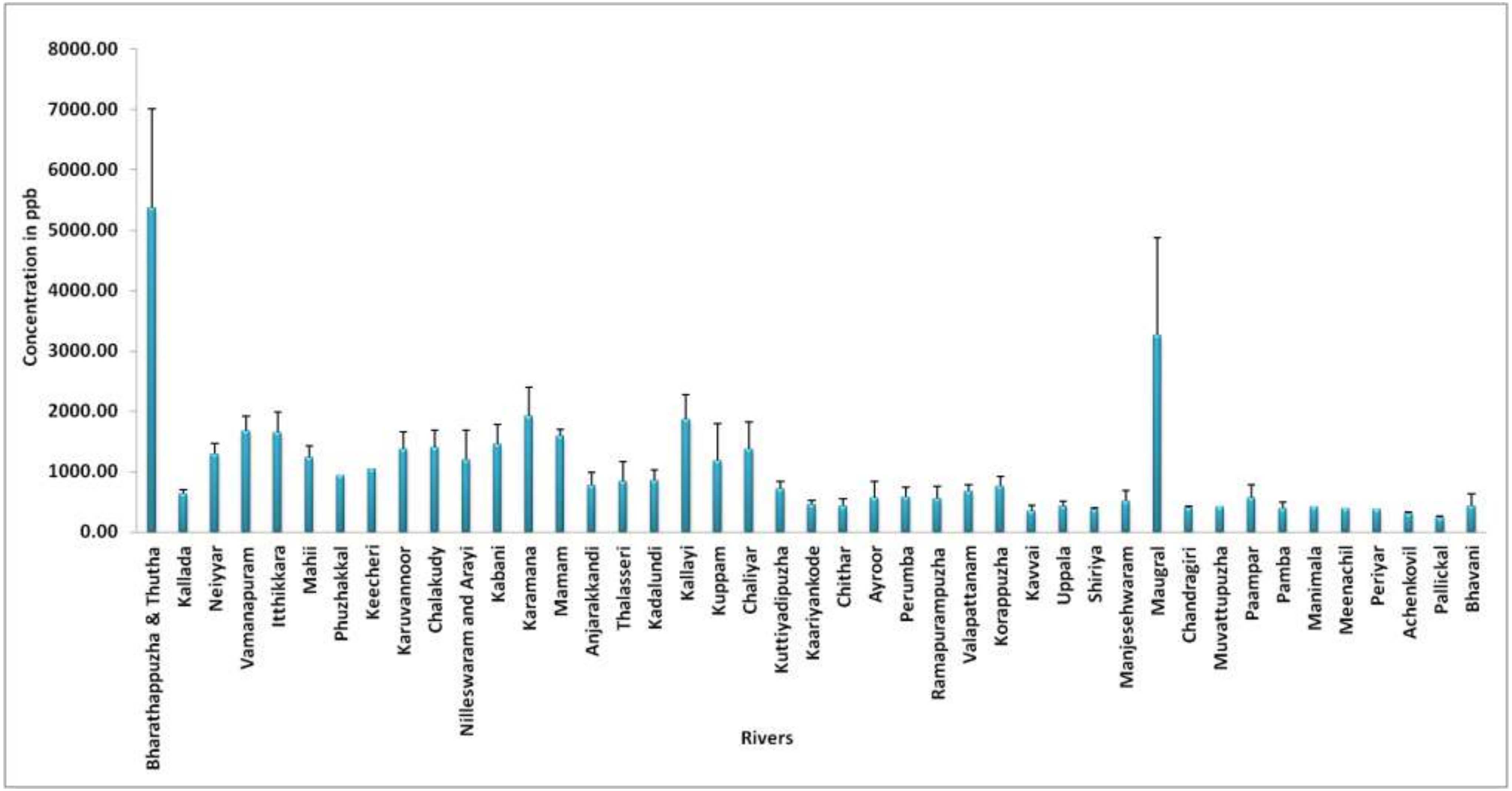
Levels of Cr recorded in the present study are lower than the levels reported from Keoladeo National Park, Rajasthan in sixteen species (3.26 ppm) of fishes (Muralidharan 1995); 110 fishes (20.0 ppm) from Hoogley estuary (Kaviraj 1989); higher than the levels reported in five species of fishes (380 ppb) from Mahul, Nhava and Sewri areas in Mumbai (Vijayan *et al.,* 2008); 12 species of fishes collected from Aliyar (40 ppb), Amaravathy (110 ppb) and Thirumoorthy (970 ppb) reservoirs and Ukkadam (870 ppb), Kurichi (880 ppb) and Singanallur (670 ppb) - urban wetlands in Coimbatore, Tamil Nadu (Jayakumar 2001); 11 species of fishes (70-1650 ppb) from nine wetlands in Andhra Pradesh; five species of fishes (40-900 ppb) from six wetlands in Assam; 10 species of fishes (20 - 680 ppb) from nine wetlands in Bihar; 11 species of fishes from 11 wetlands in Gujarat (250 - 1800 ppb); 13 species of fishes (10 - 1540 ppb) from 12 wetlands in Karnataka; five species of fishes (BDL - 950 ppb) from five wetlands in Maharashtra; eight species of fishes (550-2830 ppb) from six wetlands in Madhya Pradesh; five species of fishes (BDL-1.07 ppm) from four wetlands in Uttar Pradesh; 8 species of fishes (410 - 1690 ppb) from eleven wetlands in West Bengal and 11 species of fishes (BDL - 1610 ppb) from 17 wetlands in Tamil Nadu (Jayakumar 2007). A study carried out in Assi, Nigeria (Nwani *et al.,* 2010) also reported lower levels of chromium (280 - 1120 ppb) than the levels reported in the present study.

Chromium is an element that exists in several different forms in soils, rocks, rivers and seawater. Metallic chromium is mined for use in steel and other metal products. “Trivalent (Cr III)" chromium is naturally occurring and is considered essential for good health. The normal intake through foods up to 70-80 micrograms per day is considered safe. "Hexavalent (Cr VI)" chromium does not occur naturally, but is produced by certain industrial processes. It is the most toxic form of chromium, and is shown to cause cancer. Many chromium-containing compounds are used for electroplating, wood preservation, leather tanning and textile processing (ATSDR, 2012 http://www.atsdr.cdc.gov/ Accessed on 26th May 2013).

Krishnaja and Rege (1982) reported that Cr^{6+} induces chromosomal aberrations in the gills of Mud Skipper (*Boleophthalmus dussumieri*) at 1 mg/kg of body weight. In juvenile Coho Salmon (*Oncorhynchus kisutch*), disease resistance and serum agglutinin production both decreased after 2 weeks in water containing 0.5 ppm of chromium (Sugatt 1980). The Cr uptake and effects in teleosts were modified significantly by many biological and abiotic variables, including water temperature and pH, the presence of other contaminants or compounds, and sex and tissue specificity. In rainbow trout, only males showed significant changes in liver enzyme activity during exposure to 0.2 ppm Cr^{6+} for 6 months; the effects were intensified by the presence of Ni and Cd salts in solution (Arillo *et al.* 1982). In Rainbow Trout, acute Cr poisoning caused morphological changes in kidney and stomach tissues at pH 7.8, but only in the gills at pH 6.5 (Van der Putte *et al.* 1981). Chromium uptake in trout increased when 10 ppb of ionic cadmium was present in solution (Calamari *et al.* 1982)-again demonstrating that uptake patterns are not necessarily predictable for single components in complex mixtures.

Chromium plays an important role in glucose metabolism in fishes by acting as an essential cofactor for insulin. Excess chromium can damage the liver, kidney, nerves and it may cause irregular heart rhythm in fishes (Pechova and Pavlata, 2007). The present study reports the levels of total chromium in 28 species of fishes studied from Kerala rivers. *Garra mullya* had the highest level of Cr accumulation and *Garra* sp (0.2 mg/L) the lowest. Although these levels may not cause any adverse effects in fishes, they could show impact on reproduction, either indirectly via accumulation in the reproductive organs, or directly on the free gametes (sperm or ovum) which are released into the water. Control of reproduction in fish is complex and regulated by a wide range of factors and low-level pollution could affect any part of this pathway. Steroid hormones are very important and play essential roles in maintaining reproductive functions (Kime *et al.,* 1996; Rurangwa *et al.,* 1998). While long-term exposure of fish to low-level pollutants might not show any obvious or visible effect on the fish itself, it could exert deleterious effects on the reproductive organs leading to a decline in numbers of offspring and hence to eventual extinction of fish stocks (Kime, 1995). Pituitary damage, testicular degeneration and decrease in fry numbers due to exposure to heavy metals have already been reported (Fernicola *et al.,* 1985 and Popek *et al.,* 2006). Cr has been associated with various effects such as severe respiratory, cardiovascular, gastrointestinal, renal damage and skin disorders in man (HPA UK, 2007).

Fig 4.9 Levels of chromium in fishes in the rivers of Kerala



iii) Lead

While the fishes of Kuttiyadipuzha measured the highest mean concentration of lead (2577.24 ± 2468.51 ppb), fishes of Pamba (34.50 ± 4.83 ppb) recorded the lowest. Fishes of rivers which measured comparable concentrations were Chalakudy (481.79 ± 123.55 ppb), Karamana (471.37 ± 245.96 ppb), Mahii (457.83 ± 89.60 ppb), Vamanapuram (420.94 ± 133.49 ppb), Maugral (320.57 ± 145.40 ppb) and Chandragiri (309.83 ± 147.25 ppb) (fig 4.10). The levels of Pb recorded in the present study are lower than the levels reported in sixteen species of fishes (BDL - 4100 ppb) from Keoladeo National Park, Rajasthan (Muralidharan, 1995) and higher than the levels reported in four species of fishes (BDL - 730 ppb) in the same park by Selvam (2002). Levels are comparable with *Labeo rohita* and *Catla catla* collected from Aliyar (2180 ppb), higher than Amaravathy (760 ppb) and Thirumoorthy (1170 ppb) reservoirs and lower than *Mystus vittatus* and *Tilapia mosambica* of Ukkadam (2190 ppb), Kurichi (2770 ppb) and Singanallur (2590 ppb) - the urban wetlands in Coimbatore, Tamil Nadu (Jayakumar 2001).

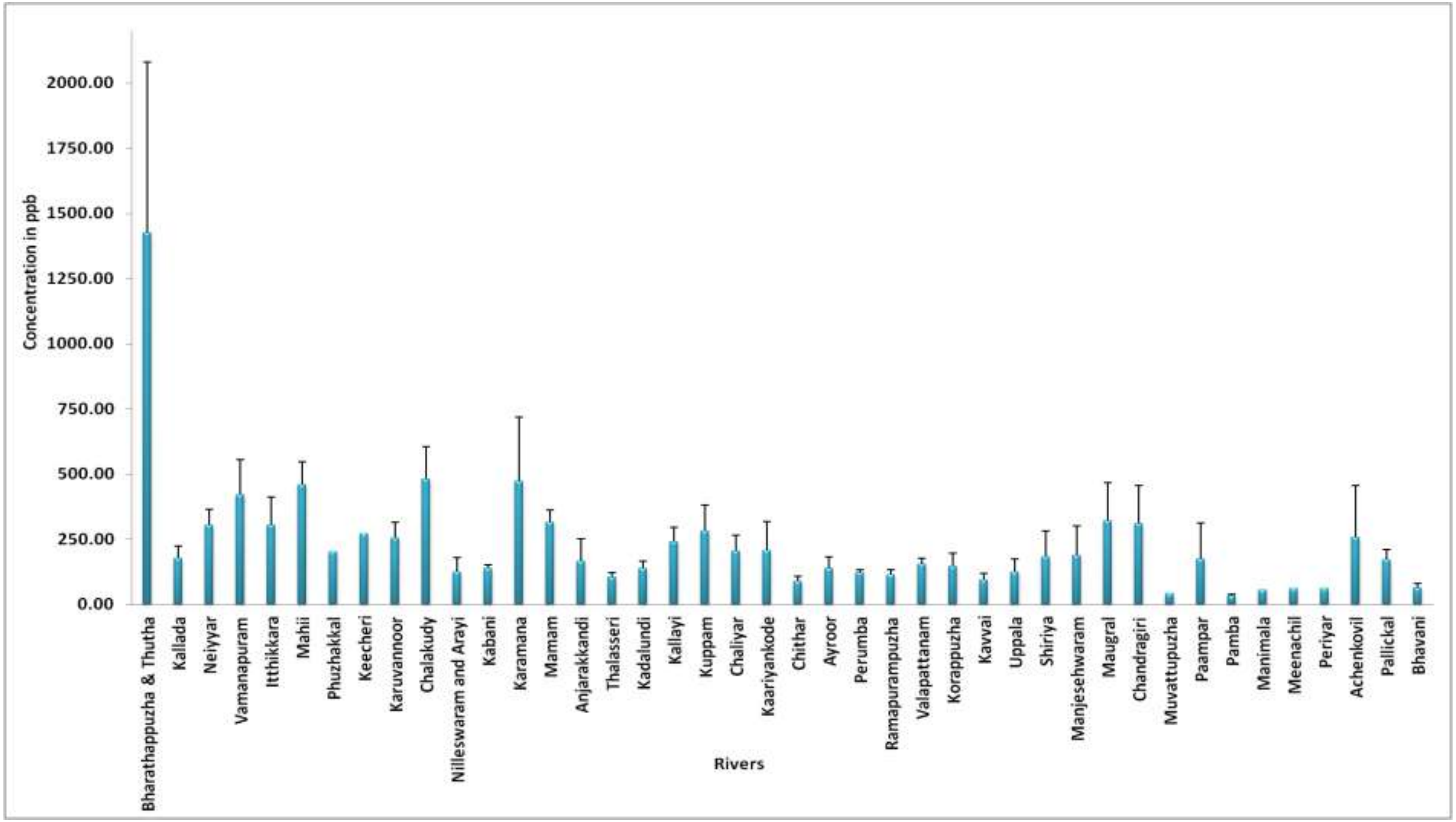
Further, present study results are lower than the levels recorded in 11 species of fishes (0.81 - 6.36 ppm) from nine wetlands in Andhra Pradesh; five species of fishes (4.05 - 8.33 ppm) from six wetlands in Assam; 10 species of fishes (4.33 - 7.77 ppm) from nine wetlands in Bihar; 11 species of fishes (4.90 - 7.91 ppm) from 11 wetlands in Gujarat; 13 species of fishes (3.96 - 5.50 ppm) from 12 wetlands in Karnataka; five species of fishes (4.45 - 6.62 ppm) from five wetlands in Maharashtra; eight species of fishes (3.79 - 8.02 ppm) from six wetlands in Madhya Pradesh; five species of fishes (BDL - 7.51 ppm) from four wetlands in Uttar Pradesh; eight species of fishes (4.01 - 6.47 ppm) from eleven wetlands in West Bengal and 11 species of fishes (2.87 - 8.60 ppm) from 17 wetlands in Tamil Nadu (Jayakumar 2007). In the international context, the levels of Pb recorded in the present study are higher than the levels reported in Spotted Wolffish (BDL - 120 ppb); four species of fishes from Assi, Nigeria (100 - 500 ppb) (Nwani *et al.* 2010); Aba river, Nigeria (BDL - 64 ppb) (Ubalua, 2007) and lower than *T.mossambica* (6.7 ppm) reported from Honolulu, Hawaii (Lowe *et al.,* 1985). Lead (Pb) has been implicated for various ailments such as headache, irritability, abdominal pain and severe neurological damage in man. These health concerns become a greater issue when we consider the susceptible populations such as young children or women of child bearing age (Jarup, 2003). In addition to inflicting ill effects on consumers, lead can directly or indirectly affect fishes. Although there are published information on the ill effects of metals on fishes (Jarup

2003, Sakr 2005, Sivaperumal *et al.,* 2007, Storelli 2008 and Bhupander *et al.,* 2011a&b), the present study did not have the same in its scope.

Maximum value for Pb in fish muscles measured in this study (BDL - 1.58 ppm) was lower than 2 ppm the maximum permissible level set by the European Union (Biggeri *et al.,* 2006). The mean concentrations of this metal in fish samples from 43 rivers of Kerala were lower than the lethal concentrations of Pb in water for cyprinids (100 mg/L), although low levels of Pb could cause some adverse effects on fish health and reproduction (Delistraty and Stone, 2007).

Lead continues to pose serious threats to the health of many children as well as adults world over. Concern about Pb exposure as a significant public health problem has increased as evidences have mounted regarding adverse health effects at successively lower levels. This issue is complicated by the fact that there is no demonstrated biological function of lead in human. Lead potentially induces oxidative stress and evidence is accumulating to support the role of oxidative stress in the physiology of lead toxicity.

Fig 4.10 Levels of lead in fishes in the rivers of Kerala



4.3 Suitability for human consumption in terms of metal levels in fishes in the rivers of Kerala

Dietary intake of metals by man through consumption of fishes was calculated for all the species and rivers as described in the methodology (MoSPI, 2011).

Table 5 Average dietary intake of Cd, Cr and Pb (µg) through consumption of fishes - Species wise

S.No.	Name of the Fish	Cd	Cr	Pb
1	*Barilius bakeri*	0.22	17.79	2.44
2	*Barilius canarensis*	0.40	20.03	3.80
3	*Channa striatus*	0.07	24.80	2.36
4	*Devario malabaricus*	0.32	30.24	6.79
5	*Devario* sp	0.68	40.88	11.48
6	*Etroplus maculatus*	0.15	26.72	4.82
7	*Etroplus suratensis*	0.07	21.83	2.83
8	*Garra mullya*	1.26	68.20	10.21
9	*Garra* sp	0.18	7.31	3.45
10	*Mugil cephalus*	0.43	15.61	4.75
11	*Puntius filamentosus*	0.49	38.17	9.58
12	*Rasbora dandia*	0.59	22.11	12.06
13	*Rasbora* sp	2.06	40.26	56.25
14	*Xenentoden cancila*	0.51	16.26	14.25
WHO/FAO (1972) Tolerable daily dietary intake limit		**50**	**400**	**500**
WHO/FAO (1989)		**1000**	**NA**	**210**
FAO/WHO (1992)		**50**	**150-1000**	**500**
EC (2005)		**50**	**NA**	**200**
Food Safety & Standards Authority of India (FSSAI, 2011)		**1500**	**NA**	**5000**

WHO/FAO - Joint committee on World Health Organisation and Food and Agricultural Organisation
NA - Not Available

Table 6 Average dietary intake of Cd, Cr and Pb (µg) through consumption of fishes - River wise

S.No.	Name of the River	Cadmium	Chromium	Lead
1	Bharathappuzha-Thutha	1.89	191.58	50.90
2	Kallada	0.61	22.40	6.32
3	Neiyyar	1.16	46.09	10.89
4	Vamanapuram	0.89	59.88	15.03
5	Itthikkara	0.53	58.58	10.83
6	Mahii	1.28	44.17	16.35
7	Phuzhakkal	0.15	33.39	7.22
8	Keecheri	0.18	37.27	9.65
9	Karuvannoor	0.18	49.14	9.11
10	Chalakudy	0.75	50.09	17.21
11	Nilleswaram-Arayi	0.27	42.46	4.49
12	Kabani	1.10	51.89	4.93
13	Karamana	1.35	68.69	16.83
14	Mamam	0.38	56.88	11.22
15	Anjarakkandi	0.35	27.68	5.95
16	Thalasseri	0.25	29.75	3.75
17	Kadalundi	0.24	30.42	4.89
18	Kallayi	0.92	66.55	8.57
19	Kuppam	0.53	41.99	10.05
20	Chaliyar	0.24	48.82	7.30
21	Kuttiyadipuzha	2.79	25.30	92.04
22	Kaariyankode	0.38	16.13	7.40
23	Chithar	0.34	15.09	3.20
24	Ayroor	0.17	20.26	4.89
25	Perumba	0.39	20.81	4.30
26	Ramapuram puzha	0.18	19.72	4.04
27	Valapattanam	0.36	24.25	5.48
28	Korappuzha	0.17	27.06	5.21
29	Kavvai	0.18	12.41	3.38
30	Uppala	0.12	15.42	4.41
31	Shiriya	0.14	13.49	6.54
32	Manjesehwaram	0.38	18.26	6.74
33	Maugral	0.93	116.31	11.45
34	Chandragiri	0.49	14.04	11.07
35	Muvattupuzha	0.10	14.86	1.53
36	Paampar	0.12	20.00	6.20
37	Pamba	0.07	13.93	1.23
38	Manimala	0.08	14.87	2.01
39	Meenachil	0.10	13.96	2.16
40	Periyar	0.10	13.49	2.15
41	Achenkovil	0.37	10.76	9.13
42	Pallickal	0.08	8.21	6.15
43	Bhavani	0.17	15.16	2.27
WHO/FAO (1972) Tolerable daily dietary intake limit		**50**	**400**	**500**
WHO/FAO (1989)		**1000**	**NA**	**210**
FAO/WHO (1992)		**50**	**150-1000**	**500**
EC (2005)		**50**	**NA**	**200**
Food Safety & Standards Authority of India (FSSAI, 2011)		**1500**	**NA**	**5000**

WHO/FAO - Joint committee on World Health Organisation and Food and Agricultural Organisation
NA - Not available

The dietary intake level of heavy metals by man through the consumption of various species of fishes shows that the cadmium input is the highest through consumption of *Rasbora* sp (2.06 µg) and lowest through *Channa striatus* and *Etroplus suratensis* (0.07 µg). Other notable daily calculated inputs were through consumption of *Garra mullya* (1.26 µg), *Devario* sp (0.68 µg), *Rasbora dandia* (0.59 µg) and *Xenentoden cancila* (0.51 µg). The maximum dietary input of chromium was through consumption of *Garra mullya* (68 µg) and minimum through *Garra* sp (7.31 µg). The calculated dietary input range of lead through fishes varied between 2.36 µg (*Channa striatus*) and 56.25 µg (*Rasbora* sp) per day.

Among the various rivers, consumption of fishes from Kuttiyadipuzha contributed the highest level of cadmium input to man (2.79 µg) than the fishes from other rivers. Input of chromium appeared to be the maximum through consumption of fishes from Bharathappuzha-Thutha (191.58 µg) and minimum through the fishes from Pallickal (0.08 µg). The input of lead ranged from 1.23 µg to 92.04 µg/ day/ person through consumption of fishes from Pamba, Bharathappuzha-Thutha rivers respectively.

In consumer health point of view, the dietary intake levels of Cd, Cr and Pb observed in the current study (table 5 and 6) are far below the guideline values proposed by agencies such as the European Commission (2001), the Joint FAO/ WHO (2005) and Food Safety and Standards Authority of India (FSSAI, 2011). Hence we may consider that the fishes are safe for human consumption. Even though the current levels of metals do not appear to be harmful to man, the physiological mechanisms, increased consumption, species difference, physico-chemical properties of surrounding water, availability and absorption of the metals are also to be taken in to account while considering the impact. Further, chronic exposure will also move towards deleterious effects. The present study recommends periodical monitoring of contaminants in fishes.

5. SUMMARY AND CONCLUSION

- ✓ Samples of fishes from 43 rivers of Kerala were analysed for select pesticides under different groups, namely organochlorines, organophosphates and synthetic pyrethroids and metals, namely cadmium (Cd), chromium (Cr) and lead (Pb).
- ✓ Data have been compiled to check the overall load of pesticides and metals among the species and rivers.
- ✓ Total organochlorine pesticide residue load was found to be the maximum in *Puntius filamentosus* (10.32 ppb) from highland areas of Bharathappuzha-Thutha, while it was mimimum in the same species (2.01 ppb) from Anjarakkandi river.
- ✓ Among the four isomers of HCH, namely α, β, γ and δ analysed, only γ and δ HCH residues were detected in twelve and one species of fish respectively collected from 12 out of 43 rivers in Kerala, while levels of other two isomers (α & β) were BDL.
- ✓ Gamma (γ) HCH was the maximum in *Puntius filamentosus* (7.74 ppb) collected from midland areas of Bharathappuzha-Thutha, while it was minimum in low land areas of Chalakudy (2.09 ppb) in the same species.
- ✓ Residues of two isomers (*o,p′*-DDT and *p,p′*-DDT) and two metabolites of DDT (*p,p′*-DDE and *p,p′*-DDD) were detected in fishes from six out of 43 rivers in the state.
- ✓ Σ DDT residues were detected in 2.26% of total number of fishes analysed. Residues of *o,p′*-DDT were detected in <0.5% of the fishes studied. Levels of *p,p′*-DDT were 2.60 and 2.58 ppb in *Puntius filamentosus* from Angadi mogar areas of Shiriya and mid land areas of Bharathappuzha-Thutha rivers respectively.
- ✓ Residues of *p,p′*-DDE was the maximum in *Puntius filamentosus* (6.96 ppb) collected from Angadi mogar areas of Shiriya river, followed by *o,p′*-DDT and *p,p′*-DDD (each 2.87 ppb) in *Etroplus maculatus* collected from low land areas of Ramapuram puzha.
- ✓ While, levels of alpha (α) endosulfan were BDL, trace amounts of beta (β) endosulfan were detected in *Channa striatus* (1.77 ppb) collected from Nilleswaram-Arayi rivers (Kasargod and Kannur dt). Endosulfan sulphate was the maximum in *Garra* sp (7.08 ppb) collected from midland areas of Chithar (Kasargod Dt), and minimum in highland areas of Kadalundi, Malappuram Dt (2.03 ppb) in the same species (*Rasbora* sp).

- ✓ Other species those recorded detectable amounts of endosulfan sulphate residues include *Devario* sp from highland (3.61 ppb) and midland (4.98 ppb) areas of Chaliyar river (originates in the Western Ghats range in Elambalari Hills located near Cherambadi town in the Nilgiris Dt of Tamil Nadu and flows through Malappuram Dt and enters Arabian sea in Kozhikode Dt), *Garra mullya* (4.28 ppb) from Meencolli areas of Kabani river (originates in Wayanad Dt of Kerala and flows eastward to join Kaveri river in Karnataka), *Puntius filamentosus* (3.64 ppb) from low land areas of Mahii, *Devario* sp (2.86 ppb) from Mahii river (near Kannur), *Puntius filamentosus* from Neiyyar (2.59 ppb) (Agasthiyar malai, Trivandrum Dt) and Mamam (2.40 ppb) (originates in Panthalacode hills in Trivandrum Dt).

- ✓ Heptachlor residue was the maximum in *Puntius conchonius* (5.07 ppb) collected from Orappu areas of Kabani river and minimum in *Puntius filamentosus* (2.04 ppb) received from midlands of Chalakudy river.

- ✓ Of all the fishes studied, while total endosulfan was detected in 4.15% of fishes, Σ heptachlor was in 4.91% of fishes.

- ✓ Among the cyclodiene pesticides tested, only residues of β-endosulfan, endosulfan sulphate and total heptachlor were at detectable levels in 8.3% of fishes studied. Residues of other pesticides, namely α-Endosulfan, dieldrin, chlordane and mirex were found to be BDL in all species of fishes.

- ✓ OC residue levels did not vary significantly ($p>0.05$) among the species and rivers, and frequency of occurrence of OC pesticide residues was the maximum (5.26%) in *Puntius filamentosus*.

- ✓ Total organophosphate residues were the highest in *Barilius bakeri* (5.85 ppb) received from highlands of Anjarakandi and lowest in *Puntius filamentosus* (2.05 ppb) collected from midland areas of Karuvannoor.

- ✓ Malathion was detected in three species, namely *Barilius bakeri* (5.85 ppb) from highlands of Anjarakkandi, *Barilius canarensis* (2.62 ppb) from highlands of Valapattanam and *Rasbora* sp (2.76 ppb) from lowland areas of Chalakudy. Only one sample (*Puntius filamentosus*) had residues of phenthoate (2.05 ppb).

- ✓ Residues of other OP pesticides, namely methyl parathion, chlorpyrifos, primiphos ethyl, quinalphos and ethion were below detection limit in all the fishes studied.

- ✓ Among the synthetic pyrethroids tested, Permethrin-II was the only compound detected in three out of 265 samples (1.13%). While the highest was in *Rasbora* sp (6.24 ppb) collected from lowland areas of Chaliyar, the lowest was in *Puntius filamentosus* (2.51 ppb) collected from midland areas of Kallada.

- ✓ On an average, cadmium contamination was the maximum in the fishes collected from Kuttiyadipuzha (78.21 ± 72.59 ppb), while minimum was observed in fishes of Pamba (2.02 ± 0.37 ppb) followed by Manimala (2.37 ± 0.0 ppb) and Pallickal (2.12 ± 0.45 ppb) rivers. Among the species, mean cadmium concentration was the maximum in *Rasbora* sp. (57.69 ± 36.13 ppb) and minimum in *Channa striatus* (2.02 ± 0.0 ppb) followed by *Etroplus suratensis* (2.03 ± 0.13 ppb). Cadmium concentration varied significantly among the rivers and species ($p<0.05$).

- ✓ While fishes collected from Bharathappuzha-Thutha had the highest levels of chromium (5364.30 ± 1654.01 ppb), fishes from Pallickal (229.81 ± 36.87 ppb) had the lowest. Fishes from twenty seven out of 43 rivers (63%) studied had an average concentration ranging between 230 to 935 ppb. The highest mean concentration of chromium was observed in *Garra mullya* (1909.66 ± 718.22 ppb) and lowest in *Garra* sp (204.67 ± 50.07 ppb). Chromium concentration varied significantly among the rivers and species ($p<0.05$).

- ✓ Fishes of Kuttiyadipuzha measured the highest mean concentration of lead (2577.24 ± 2468.51 ppb), while fishes of Pamba (34.50 ± 4.83 ppb) recorded the lowest. Mean lead (Pb) concentration was observed to be the maximum in *Rasbora* sp. (1574.95 ± 1226.28 ppb), while the mimimum was in *Barilius bakeri* (68.46 ± 15.97 ppb) followed by *Channa striatus* (66.12 ± 13.89 ppb). Pb concentration varied significantly among the rivers and species ($p<0.05$).

- ✓ The study also looked at the dietary input of chemicals to human beings through consumption of fishes and compared with guidelines suggested by various statutory agencies. The calculated dietary intake (CDI) of γ HCH residues ranged from 0.09 to 0.28 µg/kg. The maximum dietary intake through consumption of *Puntius filamentosus* was 0.28 µg/kg from Bharathappuzha-Thutha and minimum through the same species from the river Chalakudy (0.09 µg/kg). The dietary intake of δ HCH through *Garra* sp (Pallickal River) was 0.13 µg/kg.

- ✓ Similarly dietary intake of total DDT residues was the maximum (0.34 µg/kg) and minimum (0.07 µg/kg) through *Puntius filamentosus* from Shiriya and Anjarakkandi rivers respectively.

- ✓ The intake of endosulfan sulphate and heptachlor residues was the maximum through consumption of *Garra* sp (0.25 µg/kg) from Chithar and *Puntius conchonius* (0.18 µg/kg) from Kabani respectively.
- ✓ Dietary intake of OP pesticides, namely malathion and phenthoate residues was 0.21 µg/kg and 0.07 µg/kg through consumption of *Barilius bakeri* from Anjarakkandi and *Puntius filamentosus* from Karuvannoor respectively. Intake of residues of synthetic pyrethroid, permethrin was 0.22 µg/kg through *Rasbora* sp from Chaliyar.
- ✓ Estimated dietary intake of select pesticides covered in the study through consumption of fish might not entail any serious risk to man. But precautions must be taken when generalizing this negative result to people with high dietary exposure level or intake of other pesticides.
- ✓ Dietary intake level of heavy metals through the consumption of various species of fishes shows that the cadmium input was the highest through consumption of *Rasbora* sp (2.06 µg), while the lowest was through *Channa striatus* and *Etroplus suratensis* (each 0.07 µg).
- ✓ Maximum dietary input of chromium was through consumption of *Garra mullya* (68 µg) and lowest through *Garra* sp (7.31 µg). Calculated dietary intake range of lead through consumption of fishes varied between 2.36µg (*Channa striatus*) and 56.25µg (*Rasbora* sp).
- ✓ Among the various rivers, consumption of fishes from Kuttiyadipuzha contributed the highest level of cadmium (2.79 µg) than the fishes from other rivers. Chromium input appeared to be the highest through consumption of fishes from Bharathappuzha-Thutha (191.58 µg) and minimum through the fishes from Pallickal (0.08 µg). Input of lead ranged from 1.23 µg to 92.04 µg/ day/ person through consumption of fishes from Pamba, and Bharathappuzha-Thutha rivers respectively.
- ✓ Dietary intake of pesticides and heavy metals in the fishes are within the limits prescribed by Food Safety and Standards Authority of India (FSSAI) and international statutory agencies, namely United States Environmental Protection Agency (USEPA), European Union, Health Canada, Food and Agricultural Organisation and World Health Organisation (FAO/ WHO Codex Alimentarius commission) for human consumption. However, these levels may pose health issues if the exposure is continuous and if per capita consumption goes up substantially. Moreover, chronic exposures of pesticides and metals have also to be admitted.

- ✓ It is necessary to bear in mind that the quantum of pesticide residues in fishes detected may not reflect the impending threat because the ill effects differ among pesticides.

- ✓ Apart from accumulation in animal tissues through food chains, high rainfall and laterite soil found in many areas in the state could facilitate speedy leaching of toxic chemicals leading to contamination of water bodies not only in the hills but also in the plains.

- ✓ Levels of Cd, Cr and Pb recorded in the present study in fishes of Kerala may not cause any severe adverse effects to fishes. However, the low-level contamination could have an impact on reproduction, either indirectly via accumulation in the reproductive organs, or directly on the free gametes (sperm or ovum) which are released into the water.

- ✓ While long-term exposure of fish to low-level pollutants might not show any obvious or visible effects on the fish itself, it could exert deleterious effects on the reproductive organs leading to a decline in number of off springs and eventually to extinction of fish stocks. Pituitary damage, testicular degeneration and decrease in fry numbers after exposure to heavy metals have already been established.

- ✓ The data clearly show that fishes studied from all the rivers have one or the other type of pesticides or metals, although the range of levels varies. Although in most cases the levels are low, it might affect the reproductive system of the species concerned. More importantly, regular consumption of these fishes would lead to health problems. It is therefore suggested that steps may be taken to prevent further pollution of the rivers with pesticides and heavy metals.

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