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Research Article

Bioaccumulation and Biochemical Studies of Toxicants in Fish on AChE

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Abstract

The rivers Ganges have been worst victim of environmental degradation as many hazardous and toxic substances are carried through sewage and industrial effluents to open water bodies. The present paper deals with the study related to occurrence and bioaccumulation of some organochlorine pesticides and heavy metals in the riverine sediment and the muscles of two cat fish species, *Channa punctatus* and *Aorichthys* aor procured from Ganges at Allahabad. The levels of these toxicants were determined to find out the extent of contamination and accumulation in the aforesaid samples from Ganges. Their contents in decreasing order were found to be HCH>DDT>heptachlor>endosulphan>aldrin>endrin for pesticides and Zn>Pb>Cr>Cu>Cd for heavy metals. Though level of these detected were under the permissible level but the results were indicative of rising trend in these samples. The in vivo effect of subacute concentrations of toxicants were also evaluated by treating fish with sublethal levels for 96h and assaying the activity of acetylcholinestearse (AChE) isolated from the muscle of the fish. The data indicated significant alterations in the levels of AChE, a known biomarker of toxicity, which exhibited direct correlation with the doses applied. The results could be used as an indicator for better environmental management with special reference to the water quality and human health.

Keywords: Organochlorine pesticides (OCP); Heavy metals; Accumulation; Toxicity; Acetylcholinesterase (AChE)

Introduction

It is a well-known fact that organochlorine pesticides are dual weapons, but because of their persistent nature they are more detrimental than beneficial. These pesticides are hydrophobic, highly volatile in nature, able for long distance transport and can also bioaccumulate in aquatic organisms and then to higher trophic levels of food chain. Besides different human anthropogenic activities several other natural ways for release of these in different ecological compartments like soil, water, and air and in environment are pesticide drift, soil erosion and rainfall, affecting many other organisms away from the first target [1,2]. Only 0.1% reaches the specific target after their application into the field [3]. The adversities of these pollutants on aquatic ecosystems are either acute or chronic due to gradual accumulation or slow degradation in different ecological compartments or in body tissues [4]. Being slowly metabolized in partially closed surface area they can have much higher adverse effects on the inhabitants of that area [5]. Livings being are always in continuous contact with water through different activities like irrigation, washing, bathing, drinking [6] and can be directly affected by these toxicants. Indirectly humans using these fish resources as food may therefore be highly impacted because it is well known that fish accumulate these contaminants in tissue and organs when exposed to polluted water [7,8]. Organochlorine pesticides like DDT, HCH, endosulphan are put under the persistent organo chlorines pesticides by Stockholm Convention. OCP are also linked to thinning of egg shells of aquatic birds, crocodiles and on reproductive development [9]. Many agencies like Arctic Monitoring and Assessment Program, Integrated Atmospheric Deposition Network, European Monitoring and Evaluation Program [10-12]

for monitoring the levels of theses in air, sediment, water, as well as in aquatic organisms provides the current status of these at the local levels [13]. Models like BETR, mechanistics, kinetics, has been useful in assessing the transport and fate of these pesticides in different compartments [14]. Fish has shown to bioaccumulate these toxicants through two major routes ie dermal and ingestion/oral. Currently freshwater fish/ loaches and several other aquatic organisms have been used as model for assessing the bioaccumulation of these at the regional or local levels [15]. Not only pesticides but heavy metals are also the potent polluters of aquatic environment. Several heavy metals are used as cofactors like Zn, Ni, Mn, Cu, Fe but other heavy metals like Cd, Pb, Hg though not useful have found to be tremendously toxic to aquatic organisms [16,17]. Heavy metal toxicity is mainly due to generation of free radicals which can cause locomotry respiratory, osmoregulatory, neurodegenerative or endocrinology related problems in fish [18,19]. Discharge from industries like forging, leather, printing etc contains these metals in the ionic forms which easily pass through the cell membranes of the aquatic organisms and deposited on the mucous membranes sometimes rupturing them [20]. The significant use of different metals like copper in electronics has resulted in aquatic pollution. Chromium is mainly used in tanning industry and small amount required for carbohydrates, lipids and sugar metabolism [21,22]. Entry of toxicants in different ecological compartments is shown in (Figure 1).

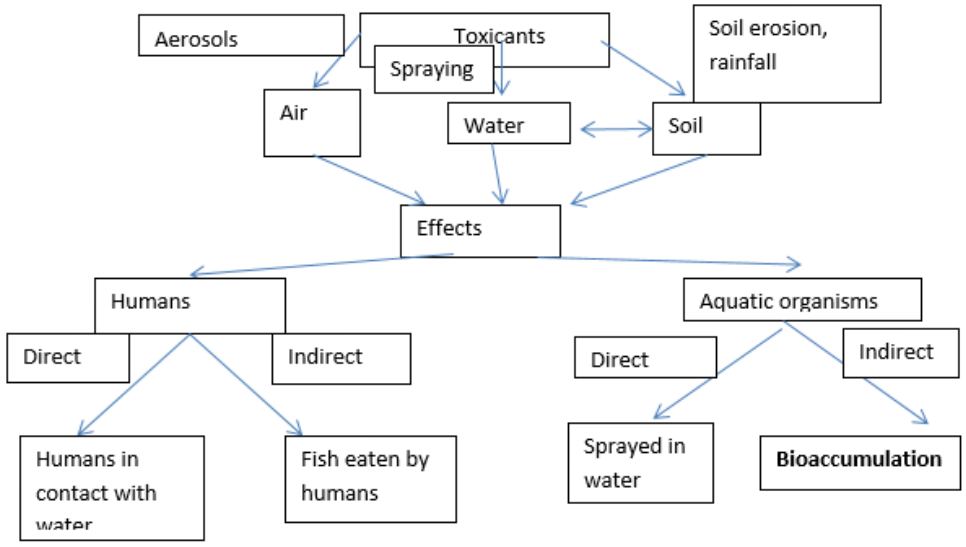

Figure 1: Entry of Pollutants and effects on Humans and aquatic organisms.

Toxicity caused by these pollutants may be at cellular, molecular or morphological level. The biological changes due to these toxicants can be used as a barometer for assessing the adverse effects of these pollutants [23]. Acetylcholinesterase (AChE) inhibition in fish is used as a biomarker of the species' exposure to toxicants present in the aquatic biota [24]. It is an enzyme which hydrolyzes the neurotransmitter acetylcholine into acetic acid and choline. The impaired function of this enzyme results in nervous or musculoskeletal system deformities finally to paralysis and death [25,26]. There are numerous studies on bioaccumulation, but very few have been extrapolated in laboratory based on the accumulation data. In this study we have integrated our bioaccumulation results with biomarkers like AChE study in vivo by exposing the fish with two different sublethal concentrations of maximum accumulated pesticides HCH, DDT and heavy metals Zn, Cu, Cr, Cd and Pb detected in the study.

Materials and Methods

Samples of sediment, water and fish *Channa punctatus (C.punctatus)* and *Aoricthys.aor (A.aor)* were collected bimonthly throught the year from river Ganges at Allahabad, India.

Organochlorine pesticide analysis: The reagents used for pesticide analysis were anhydrous sodium sulphate, petroleum ether (60-800C), acetonitrile, hexane, diethyl ether, florisil (60-100 mesh), and whatmann filter paper number 1 and 42. All reagents used were of Merck.

Pesticide extraction from sediment: The extraction of sediment was performed by procedure given by FAO. Sediment samples were first dried and grinded before extraction. In a beaker 50g grinded soil sample was mixed with 100ml of solvent mixture of hexane:acetone (1:1). After shaking them in beaker, mixture was filtered through whatmann filter paper number 1. First step was repeated for re-extraction of pesticides and then the filtrate was transferred through a seperatory funnel. After vigorous shaking 5ml of 2% NaCl and 300ml of distilled water was added and kept for some time to separate into solvent and aqueous phase. On clear separation lower aqueous portion was discarded and the solvent phase was washed twice with 100ml portion of distilled water. Finally column plugged tightly with 2.5gm anhydrous sodium sul-

phate and 2gm of 15% activated alumina. The column was eluted with 10ml hexane (HPLC). The concentration of samples was done using flat rotatory evaporator. The dried extract was then dissolved in 2ml of hexane. The cleaned up extract was stored at 40C for analysis on gas chromatograph.

Pesticide extraction from fish muscle: The extraction of pesticides from fish muscle was done in four steps described by USFDA 1994.

Extraction: 50g fish muscle (boneless) was mixed with 250g sodium sulphate and grinded in metallic blender at high speed with three consecutive volumes (200, 150 and 100ml) of petroleum ether. The filtrate from each portion was combined in a 500ml conical flask and was evaporated to near dryness on a rotatory evaporator.

Partitioning: The extract was then partitioned in three steps. In first portion extract was mixed with 30ml saturated solution of acetonitrile in petroleum ether, shaken 100 times and lower portion was transferred to 2nd separating funnel containing 100ml petroleum ether, 40ml of saturated solution of NaCl, 600ml distilled water, shaken 100 times and lower portion of separating funnel 2nd was transferred to 3rd separating funnel containing 100ml petroleum ether, shaken and lower layer discarded. Its upper layer was mixed to separating funnel 2, 100ml distilled water added, shaken, lower layer discarded and upper layer collected and was evaporated to dryness on a rotatory evaporator.

Clean Up: 20g activated florisil was topped with 4g anhydrous Na_2SO_4. The column was eluted with 200ml of 6% and 15% diethylether. All the glassware's were cleaned with liquid soap followed by tap water, distilled water and acetone. The glassware's were then kept in oven at a temperature of 220^{0}C for 24h.

Analysis: Gas liquid Chromatography Analysis: Pesticides residue in all extracts were estimated quantitively by NUCON Gas Chromatograph equipped with Ni^{63} Electron Capture Detector (ECD).

Operating parameters: Purified nitrogen gas grade1 99.9% pure gas was used as carrier gas, and its flow rate is optimized at usually 60ml/min Temperature of injection port (210^{0}C), column (190^{0}C) and detector (220^{0}C) is adjusted accordingly and stabilized properly. When the instrument is thermally stabilized (3-4h) we injected appropriate volume (2-10µl) of the working standard mixture of pesticides with the help of 10 µl micro syringe (Hamilton) carefully and waited till the component with maximum retention time is detected on chromatogram usually 1-1/2 h. Now as the standard run is over, we injected unknown sample volume concentrated extract (2µl-fish extract, 1ml- sediment extract) on the same column efficiently with many times washed and rinsed microsyringe with hexane. The different peaks of the samples were identified with those of standards. The pesticides for which the GC was standardizes were p-p'- dichlorodiphenyl trichloroethane (p-p'-DDD), p,p'-dichlorodiphenylethane (p-p'-DDE), α, ß, γ isomers of hexachlorocyclohexane, dieldrin, aldrin and endosulphan.

Preparation of standards for GLC: 1mg of pure pesticide standard A.R. grade 100% is dissolved in 10ml hexane. From this different concentration of pesticide is prepared. These standard solutions are kept in vaccum dessicator at -15^{0}C for a period of six months in case of organochlorine pesticides.

The fish were exposed to two different sublethal concentrations of effectors for 96h. After the stipulated periods of treatment, the fish were dissected and muscle was excised out. The muscle was thoroughly washed in normal cold saline (0.15M, 4-6oC), blotted dry and quickly weighed. For isolating AChE, a membrane bound enzyme, sodium phosphate buffer (50mM, pH 8.0) containing tritonX-100 (0.5%, v/v) a non-ionic detergent was used in extraction buffer. The homogenates of each tissue were kept for 30min in cold with intermittent stirring and centrifuged at 10,000 Xg for 30min in a refrigerated high-speed centrifuge. The clear supernatant of each tissue homogenate was thus collected and used as the source of enzymes and other cellular constituents. The estimation of biochemical indices (protein content, AChE activity) were done in these cell free fractions of tissue homogenates.

Acetylcholinesterase: (Acetylcholinesterase, EC 3.1.1.7, AChE) was assayed by the method of Ellman et al., (1961). The reaction mixture contained sodium phosphate buffer (50mM, pH 8.0), acetylthiocholine iodide (ATI) (0.5mM), 5,5' dithiobis-(2-nitrobenzoic acid) (DTNB, 0.5mM, pH 8.0) and suitable amount of enzyme preparation (100-200µg cytosolic protein). The increase in absorbance was monitored at 412nm for 3min against blank at room temperature (26±2^{0}C). The measurements were made in triplicates in each tissue homogenate.

Heavy metal analysis: The reagents used for analysis of were nitric acid, perchloric acid, sulphuric acid and metals/salts of copper (Cu), chromium (Cr), cadmium (Cd), Zinc (Zn) and lead (Pb).

Water collection: Water samples were collected in polyethylene bottles (500ml), acidified with concentrated nitric acid (5ml) and then evaporated on sand bath till volume concentrated to 50ml.

Sediment collection and analysis: Sediment samples were dried, powdered and sieved. 5gm samples were digested with a mixture of concentrated nitric acid (35ml), perchloric acid (5ml) and sulphuric acid (2.5ml) in a ratio of 7:1:0.5 at 75-80^{o}C for 4-5h on heating mantle till a clear solution is obtained.

Fish muscle: Fish samples were rinsed in deionised water to remove surface adherents that could have adsorbed metals. Then 30gm of muscle (boneless) was chopped and kept in 25ml nitric acid overnight. The samples were then digested by adding 10ml sulphuric acid on heating mantle till a clear light yellow solution was obtained.

The volume of all digested samples was then made up to 100ml. heavy metals analyzed in samples by atomic absorption spectrophotometer (GBC Avanta∑) using different cathode lamps.

Preparation of standards:

Preparation of 1000µg/ml standard Cadmium (Cd): The 1000µg/ml standard Cd was prepared by dissolving 1.0g of cadmium metal in 20ml of 5N hydrochloric acid containing 0.5ml of concentrated nitric acid and diluted to 1 litre.

Preparation of 1000µg/ml standard Chromium (Cr): The 1000µg/ml standard chromium was prepared by dissolving 1.0g of chromium metal in 50ml of concentrated hydrochloric acid and diluted to 1 litre.

Preparation of 1000µg/ml standard Copper (Cu): The 1000µg/ml standard copper was prepared by dissolving 1.0g of copper metal in 50ml of 6N nitric acid and diluted to 1 litre.

Preparation of 1000µg/ml standard Lead (Pb): The 1000µg/ml standard lead was prepared by dissolving 1.0g of lead metal in 20ml of 6N nitric acid and diluted to 1 litre.

Preparation of 1000µg/ml standard Zinc (Zn): The 1000µg/ml standard zinc was prepared by dissolving 1.0g of zinc metal in 40ml of 5N hydrochloric acid and diluted to 1 litre.

Exposure to subacute concentrations of pesticides (HCH, DDT) and heavy metals (Cu, Cr, Cd, Pb, Zn): The healthy fish were equally distributed in four aquaria of 1x1 ft, The subacute concentrations of toxicants were used for the exposure of *C. punctatus* and *A.aor* for 96h. The equal volume of acetone was maintained in control aquaria as pesticide added to the experimental aquaria was dissolved in acetone. All aquaria were constantly aerated during the period of exposure by aerator and the fish were fed properly. The water was changed after 24h each and replenished with fresh toxicants.

Result and Discussion

The aquatic system have been worst victim of environmental degradation as many hazardous and toxic substances such as pesticides and heavy metals and other chemicals are carried through sewage and industrial effluents including urban and agricultural total run off to open water bodies. These substances being highly persistent get deposited either in the sediment or water and ultimately contaminate the whole aquatic cycle. The extensive use of organochlorine pesticides, heavy metals and their ability to accumulate in aquatic food chain pose serious threat to the environmental equilibrium. The present study is related to occurrence and bioaccumulation of these toxicants in the water, sediment and muscles of two cat fish species, *Channa punctatus* and *Aorichthys aor* and the sublethal effect of these on the biomarker enzyme acetylcholinesterase AChE in laboratory.

Evaluation of organochlorines in sediments and muscles of fish

The order of annual accumulation of different pesticides in two ecological compartments of aquatic ecosystem ie sediment and fish are HCH>DDT>Endosulphan>Heptachlor>aldrin>endrin. The highest accumulation was of HCH, DDT, endosulphan and lowest of aldrin, endrin in all the compartments. Heptachlor accumulation is on average among all the pesticides. Pesticides accumulation was higher in sediment than fish (Table 1).

Table1: Annual accumulation of pesticides (µg/Kg wet weight) in the sediment and muscle of the freshwater fish species procured from river Ganges at Allahabad.

Pesticides	Annual accumulation of pesticides (µg/Kg wet weight)		
	Sediment	Muscle of fish species	
		C. punctatus	*A. aor*
HCH	35.05±2.1	9.92±0.81	10.83±1.10
Endosulphan	1.6±0.07	0.94±0.08	0.77±0.05
DDT	12.0±1.3	5.81±0.95	6.58±0.81
Aldrin	1.45±0.01	0.92±0.07	0.75±0.04
heptachlor	0.75±0.04	0.96±0.08	0.85±0.05
Endrin	0.23±0.02	0.08±0.01	0.04±0.01
Total OCP	51.33±3.7	18.63±1.71	19.93±1.82

The extraction of organochlorines from sediment and muscles of two fish species and their determination were done as has been described in Materials and methods. The values are the average of three independent experiments. HCH=Hexachlorocyclohexane, DDT=Dichlorodiphenyltrichloroethane

The percentage distribution of pesticides between sediment and fish shows almost 20% increase rate of accumulation for HCH, 10% for DDT in sediment. In fish the accumulation of HCH was higher in *C.punctatus* and DDT in *A.aor*. However the other OCP detected in sample was less than 10% in sediment and 20% in fish (Table 2).

Table 2: Percent distribution of organochlorine pesticides in the sediment and the muscles of two freshwater fish species procured from river Ganges at Allahabad.

Pesticides	Distribution of pesticides (%)		
	Sediment	Muscles of freshwater fish species	
		C. punctatus	*A. aor*
HCH	70.2	55.33	50.83
DDT	21.7	22.25	33.74
Other OCP	8.3	18.33	14.33

The data indicated that the major contributor of total OCP was DDT and HCH in sediment and fish muscle.

The accumulation of pesticides into the sediment of river and muscles of two fish species were determined as mentioned in the Materials and Methods. Values are the average of three independent experiments.

The results show the highest concentration of pesticide present in sediment as compared to that present in the flesh of two fish species. In fish higher concentration of pesticide accumulation was recorded in the flesh of *A. aor* than *C. punctatus*, which is directly related to their feeding behaviour. As organochlorine pesticides are insoluble in water they get settled at the bottom, adsorb to sediments or remain suspended in water and when bottom feeder fish feed on these contaminated zooplankton and plankton, it gets in them.

The bioaccumulation of these pesticides in aquatic organisms depends on species, age, sex, feeding habitat, position in trophic level and the rate of mobilization, uptake and elimination [27,28]. The interaction of these pesticides with sediments depends on the charge, ions, detritus organic matter associated with sediment which sometimes increases the total dissolved solids (TDS) and conductivity of water.

The high levels of HCH in the sediment of river Ganges may have served as a sink to persistent organochlorine pesticides. Drought, leaching of the surface soil during monsoon can also result in increase of OCP in sediment [29-32]. The presence of pesticides in soil is controlled by many factors like adsorption on the solid phase, decomposition and volatilization. The results show that pesticides concentration in sediment is more than that present in fish. There are mainly two ways how these pesticides reach the soil, (1) by fall out on account of crop spraying for insect / pest control or (2) by their direct application for the treatment of soil or for the control of soil dwelling pests, nematodes and pathogens of bacterial and fungal diseases. The organic content in the sediment may be considered as the probable reason for attracting the pesticides from water. The organochlorine pesticides are non-polar and therefore are less soluble in water and tend to remain adsorbed on the suspended solids. Therefore, the pesticide residues in sediment tell the data of not only the pesticides present in sediment but also in water. The movement of the pesticides from water to sediment and to lipid compounds is possible. The presence of pesticides in sediments of Ganges at Allahabad in the present study is in agreement with the studies of Miles and Harris [33], Rajendra and Subramanian [34] and Darko et al. [35]; Sarafioska et al. [36]. A study by Bossi et al. [37] showed high concentrations of pesticides in May-June followed by decreasing concentartions in July-August and then slight increase in September-October. The endosulphan detected in present study may be due to soil leaching. Aldrin can undergo photolysis or metaboloized to dieldrin (more toxic than aldrin) in plants and animals by epoxidation of double bonds carried out by microsomal enzymes making it more polar and less lipid soluble [38,39]. Among pesticides high concentrations of DDT has been reported by [40,41]. Camenzuli et al. [14] reported higher concentrations of pesticides HCH and DDT in sediment may be due to soil organic carbon, pesticide drift, emissions, agriculture use.

These pesticides in water may be present in dissolved, precipitated (when present in excess) and suspended forms (when adsorbed on suspended particles). The solubility of pesticides in water besides their chemical structure (polarity) depends on pH, temperature, salt concentration and organic matter content on medium and on partition between water and sediment phases and biotic activity. Although in aquatic ecosystems especially in rivers the pesticides joining the course move and distribute into various inter compartments viz. sediments, aquatic flora and fauna etc.

The predominant concentration of HCH in all the fish samples in the present study suggests that different HCH containing formulations of pesticides are used or as vector control. These pesticides reach the water by direct or indirect application. The indirect sources include runoff from agricultural fields, spray drift, rain water, sewage and effluents from various industries manufacturing pesticides or using them in their process, whereas direct application include the control of unwanted weeds, insect's pest infecting water plants, undesirable fish in fish culture ponds to restock with more desirable fishes. Germen et al. [2] reported the trend of accumulation of organochlorine pesticides to be PCB>DDT>HCH and Afful et al. [7] to be of γ-HCH, δ-HCH, heptachlor, aldrin, γ-chlordane, α-endosulfan, dieldrin and p,p'-DDT respectively, in different fishes. In contrast findings by Buah-Kowfie et al. [23] showed higher accumulation in fish than sediment which was directly related to the feeding habitat of fish Clarias gariepinus [42].

Evaluation of heavy metals in water, sediments and muscles of fish

The range of heavy metal accumulation in all the three ecological compartments were water 0.001-0.043, sediment 0.08-14.62 and muscles of fish *C. Punctaus* 0.001-9.50, *A.aor* 0.001-14.13. The decreasing trend in all the cases were Zn>Pb>Cr>Cu>Cd. Highest accumulation of Zn was seen in sediment and fish *A.aor*. In water the concentration detected was almost negligible (Table 3).

Table 3: Annual accumulation of heavy metals (µg/Kg wet weight) in the water, sediment and fish muscle.

Heavy metals	Annual accumulation of heavy metals (µg/Kg wet weight)			
	Water	Sediment	Muscles of freshwater fish species	
			C. punctatus	*A. aor*
Cu	0.017±0.002	2.48±0.11	0.64±0.06	0.92±0.07
Cr	0.012±0.001	3.84±0.13	0.062±0.002	0.072±0.002
Cd	0.008±0.001	0.86±0.08	0.038±0.001	0.042±0.001
Pb	0.043±0.002	6.12±0.35	2.42±0.12	3.12±0.57
Zn	0.072±0.003	14.62±1.51	9.50±0.75	14.13±1.35

The extraction of heavy metals from water, sediment and muscles of two fish species and their determination were done as described in Materials and methods. The values are the average of three independent experiments. Cu=Copper, Cr=Chromium, Cd=-Cadmium, Pd= Lead, Zn=Zinc

The trend of percent distribution was same as that of accumulation of heavy metals. The difference in percent accumulation of Zn with Cr and Cd was around more than 70% in fish. Almost similar level of percentage difference seen for Zn with Cu but variable for Pb. Around 30% difference (decrease) was seen in sediment than fish for Zn and less than 5% between sediment and water (Table 4 & Table 5).

Table 4: Percent distribution of heavy metals in water, sediment and the muscles of two freshwater fish species procured from river Ganges at Allahabad.

Heavy metals	Distribution of heavy metals (%)			
	Water	Sediment	Muscles of freshwater fish species	
			C. punctatus	*A. aor*
Cu	11.18	8.88	5.08	4.92
Cr	7.89	13.75	0.49	0.39
Cd	5.26	3.08	0.31	0.22
Pb	28.29	21.92	19.19	16.7
Zn	47.37	52.36	75.34	75.64

The heavy metals from water, sediment and the fish tissues were extracted from and detected into the muscles of fish species as described in Materials and Methods. Cu=Copper, Cr=Chromium, Cd=Cadmium, Pd= Lead, Zn=Zinc.

Table 5: Reference levels of heavy metals and Organochlorine Pesticides.

Heavy Metals			
	Water (mg/l)	Sediment (mg/g)	Fish(mg/g)
Cu	1	8	
Cr	0.05		0.15
Cd	0.05[a], 0.008	2	0.2
Pb	0.05[a], 0.01	22	1.5
Zn	5.0[a], 3.0	40	150
Organochlorine pesticides			
γHCH	0.002mg/l (2µg/l)		
DDT	0.001mg/l (1µg/l)		
Aldrin/Dieldrin	0.00003mg/l (0.03µg/l)		
Endrin	0.00006mg/ l(0.06µg/l)		

Sediment mg/g Canadian EPA (1976). Fresh water WHO mg/l (1993), Fresh water fish WHO mg/g (1993), a WHO (1984) guideline values, Pesticide (mg/l) FAO/WHO (2001)

The levels of metals detected in the study can be due to many reasons: (i) precipitation in the alkaline pH, (ii) increase in temperature proportionately decreases the oxygen concentration of water and hence toxicity increases resulting in physiological and morphological imbalances (iii) decreased particle size and increased organic matter results in adsorption and deposition (iv) metal contaminated feeds due to discharge of contaminants into the riverine system without any pretreatment.

Bioaccumulation studies of heavy metals in aquatic organisms were done by several researchers like Bonsignore et al. [43], Bawuro et al. [44], Rajkumar et al. [45] etc. The trend of heavy metals accumulation in the presented study was Zn>Pb>Cr>Cu>Cd which is different from the findings given by Rajeshkumar etal. [45] were Pb>Cu>Cr>Cd and by Bawuro et al. [44] Zn>Cu>Pb>Cd in carnivores and herbivore fish. These metals have toxic effect on animal reproduction, development and immunological function and are capable of producing acute and sub chronic toxic effects in mammals [46-49]. The copper concentration of 100mg/l has been detected in mining areas [50]. Bottom dwelling fishes are found to exhibit higher concentration of heavy metals than pelagic fishes. These fish are found in large quantity and so more susceptible to biomagnification [51]. The prolonged thermal and chemical protective actions of these substances against the pests create a risk of contaminating the environment and agricultural products. All the toxicants detected in the study were below the permissible limits (Table 6).

Table 6: Effect of pesticides and heavy metals on the activity of acetylcholinesterase (AChE) in muscles of freshwater fish.

Effectors (pesticides and Heavy metals)	LC50 values (mgL^{-1})	Concentration used (mgL^{-1})	Activity of AChE remaining (%) in Muscles of Fish	
			C.punctatus	*A.aor*
Pesticides				
DDT	0.15 & 0.18	0.03 & 0.036	65	59
HCH	0.13 & 0.15	0.025 & 0.03	62	53
Heavy metals				
Cu	1.37 & 1.51	0.27 & 0.30	72	68
Cr	1.05 & 1.23	0.21 & 0.24	68	59
Cd	6.5 & 7.4	1.3 & 1.5	57	48
Pb	98.5 & 102.3	19.7 & 20.4	58	42
Zn	42.5 & 46	8.5 % 9.2	69	58

Effect of Effectors on the AChE activity in fish muscle

The conclusions drawn from Table 7 on the basis of acute toxicity test were that HCH was more toxic than DDT. The inhibition was found to be between 38-47% in fish exposed to pesticides. For heavy metals the range of inhibition was between 28-52%. Maximum inhibition was in *A.aor* (48%) for Pb and lowest for Cu (28%) in *C. punctatus*. The AChE activity at two different subacute levels of effectors (pesticides and heavy metals) was found to show a stochastic decrease in activity at higher concentrations. The trend of % decrease in AChE activity for heavy metals in *C.punctatus* was Pb>Cd>Cr>Zn>Cu and *A.aor* was Pb>Cd>Zn>Cr>Cu. The % decrease for pesticides in both the fish was HCH>DDT.

The extraction of AChE from the pesticides and heavy metals treated fish tissues and the determination of enzyme activity in the tissue homogenates were done as described in Materials and Methods. The values are the average of three independent experiments. Cu=Copper, Cr=Chromium, Cd=Cadmium, Pd= Lead, Zn=Zinc

AChE is an enzyme which helps in cholinergic transmission of neurotransmitter acetyl cholein at the post synaptic junction, and hydrolysed to acetyl Co-A and choline, where choline can again be used for synthesis of acetyl choline [52-54]. Potential sources of variation which can affect AChE activity are as follows: (i) differences in age, sex, reproductive status, and stressors such as water temperature, dissolved oxygen concentration, exposure to multiple contaminants [55] (ii) due to catecholamines or acetylcholine levels which through the adenylcyclase system, can increase cAMP affecting enzymes of glycogen breakdown and glycogen synthesis [56] (iii) due to higher accumulation of acetylcholine resulting in hyperpolarization of post synaptic membranes which causes disruption in the transmission of nerve impulses. The other reasons for AChE inhibitions are movement and seasons. Baslow, [25] reported increased inhibitions in active fish than sluggish fish. Seasonal variations were studied by Sumith et al. [57] in fish exposed to organophosphates and showed, increased inhibition during drier months ie july-september (Yala), when compared with Maha seasons. Similar findings reported by Menedez et al., [58] in fresh water fish *Cenesterodon decemmaculatus* where activity decreased upto 80% in summers and upto 60% in winters. Decrease in AChE activity in fish exposed to pesticides were reported by Marigoudar et al. [59] in *Labeo rohita* for cypermethrin, Milegla et al. [60] in *Clarius gariepinus* for organo phosphates and carbamates, Pereira et al. [61] in zebra fish muscle exposed to endosulphan whereas Ezemonye and Ikpesu [62] reported no change in activity in serum of *Clarias gariepinuss* exposed to endosulphan. In contrast increased activity were reported by Moraes et al. [63] in brain of fish upon exposure to imazapic and imazethapyr herbicides, Toni

et al. [64] for commercial herbicide bispyribac sodium after seven days of exposure. However, these authors have shown reduction in AChE activity in fish brain and muscle after prolonged (72 days) of exposure.

Heavy metals have shown to cause changes in circadian behavior and inverse relationship between exposure time and activity in addition to decreased AChE activity. Decreased activity by heavy metals exposure were reported by Cunha et al. [65] in marine gastropods for cadmium and copper, [66] for mercury and lead in brain of zebra fish, Haverroth et al. [67] in Zebra fish for Copper, Leitemperge et al. [68] in silver catfish *Rhamdia quelen* for copper, Kim and Kang et al. [22] for cadmium in brain and muscles of juvenile rock fish *Sebastes schgeleii*, Pan et al. [69], Zhang et al. [70] in fish Danio rerio for cadmium chloride and deltamethrin, Lee and Freeman [71] documented lead toxicity in Zebra fish, Green et al. [72].

The toxicity to aquatic biota by chromium is due to ionic states of these heavy metals which can easily pass through the cell membranes. Cr (Vl) can easily pass plasma membrane with the help of anion transporter phosphate whereas Cr (lll) cannot. Hexavalent state of chromium has been much toxic than trivalent stage.

The lethality of Cu in fish is due to several reasons; it mimics the sodium ion, blocks ions by ATP dependent enzymes, decreases the levels of sodium and chloride ions as a result increased diffusion loss takes place leading to osmoregulatory disturbances and death [73]. Sublethal toxic effects of copper in fish include degeneration of gill cells, decreased RBC and increased hematocrit at different sublethal levels of exposure [74,75]. Copper (ll) and (l) results in generation of reactive oxygen specie through lipid peroxidation and genotoxic [76]. Zinc toxicity in aquatic organisms is linked to embryo damage, fecundity, low hatching rate and high mortality. Zebra fish has the genes for AChE responsible for acetylcholine degradation, in brain [77]. Zinc esposure of more than 1ppm caused different circadian rhythms, decreased activity during day which increases in dark [78]. Lead and other stressors at sublethal levels showed muscular and neurodegenerative change, reproductive inhibitions in aquatic organisms, hypo demethylation of DNA [79]. Studies by Kumar et al. [80] showed lead toxicity in cat fish *Pangasius hypophthalmus*, can be mitigated to some extent when fish were fed on zinc diet. Cadmium toxicity decreases as water hardness increases [81]. Sublethal levels exposure may cause alterations to appetite and metabolism. It is an endocrine disruptor and cause DNA damage and stress in common carp *Cyprinus carpio* [82-90]. However it cannot be predicted that whether the OCPs and heavy metals acts as reversible or irreversible inhibitors, but yes the results prove that subacute concentrations of these effectors are also highly toxic to the fish. The bioaccumulation of pesticides and heavy metals were in the permissible limits and is directly related to the feeding habitat.

The data obtained from the present study gives an indication of the extent of aquatic contamination that may help to understand the behavior and fate of these persistent chemicals in the aquatic environment. The results may be important form public health and ecological standpoint and is useful for better water quality management and environmental health risk assessment.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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