Contamination of fresh water fish "Schizothorax niger" with chlorpyrifos from "Dal Lake" basins, India

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Abstract: Dal Lake a Sub-Himalyan urban Lake is one of the most beautiful lakes of India and second largest in Jammu & Kashmir. Intensive farming practiced in the surrounding area of Dal Lake and its floating gardens leads to an enhanced vulnerability of crops to pests and indiscriminate use of pesticides. Possible transfer of these hazardous molecules from vegetable fields to the aquatic environment of the Lake, poses a potential threat to the aquatic species and human health as well. In the present investigation conducted from 2008 to 2010, 135 samples of fish including 81 samples of *schizothorax niger* (Algaad / Kasheer Gaad) and 54 samples of *Cyprinus carpii* (Punjab Gaad) were collected from three basins of Dal Lake namely Hazratbal, Nigeen and Cheshmashahi basin. The samples were analyzed for seven commonly used pesticides viz. Butachlor, γHCH, Chlorpyrifos, Hexaconazole, Endosulfan 1, Endosulfan 2 and Dichlorvas. Detection and quantification of pesticide residues was performed by GC-MS/MS (Thermofinnigan Polaris Q type) equipped with Ni ECD. It was found that 73 samples (54.07%) out of 135 were contaminated with chlorpyrifos an organophosphate pesticide with mean concentration of (0.0009 ± 0.0010ng/kg) with concentration ranging from undetected to 0.003ng/kg. The highest concentration was found in Hazratbal basin in 2009 (0.002 ± 0.001ng/kg). The results also reveal that level of pesticide was higher in pesticide use season than non use season except in 2009 when levels were same. With respect to basins the results show that mean concentration of chlorpyrifos level was higher in pesticide application season than non application season except in Nigeen basin in 2008 and 2009 where levels were same (0.001±0.001ng/kg) and in Hazratbal basin in 2010 where levels were same (0.001±0.001ng/kg). The results indicate a sub acute exposure of chlorpyrifos in a locally consumed *Schizothorax niger* and not in *Cyprinus carpii*. These findings suggest that low dose exposure to pesticides through food chain like fish can be a major contributor for presence of pesticide residual levels in human blood.

Key Words: Pesticide, Chlorpyrifos, Schizothorax niger, Gas chromatogram Mass Spectrometer.

Introduction: Pesticide use has brought tremendous benefit to mankind by increasing food production and controlling the vectors of man and animal diseases. At the same time use of these pollutants has posed potential health hazards to the life of non-target organisms like fishes and river bodies are one of the main recipients of pesticide residues generated on farming fields. (Afful et al.,2010)

Due to an increasing awareness of healthy diet, fish has a prominent place for its energy and bionutritive value because of omega-3- fatty acids predominantly present in fish. Moreover, fish is also a significant source of oligoelements. Since major part of the world´s food is being supplied from fish source, it is therefore, essential to secure the health of fishes.(Tripathi et al., 2002). Fish forms an integral part of diet in the people of Jammu & Kashmir. There is every likelihood of bioaccumulation of these noxious chemicals in the tissues of the fish which may lead to biomagnifications in humans on the top of the food chain. Since human health has been a matter of primary concern so many studies which have so far been done arrived at the conclusion that pesticide exposure is associated with chronic health ailments. These primarily include dermatologic, respiratory, memory disorder (Arcury, 2003, O malley,1997), neurodeficits (Kamel et al.,2003, Firestone et al., 2005), birth defects and miscarriages (Engel et al.,2000, Cordes, 1988, Das et al., 2001, Eskenazi et al., 1999, Garcia, 2003, Moses, 1989, Schwartz et al., 1986, Stallstones, 2002, Strong et al.,2004, Von MaeleFabry, 2003). Moreover, bioaccumulation of pesticide residues show an association to cause cancer, depression, seizure disorder, liver and kidney dysfunction (Daniels et al., 1997, Sandhu, 1992, Ekbom et al., 1996, Straub et al., 1999, Beseler et al., 2008).

Dal Lake one of the famous lakes of India with its pristine glory is a source of potable and recreational water as well as a source of cheap, affordable protein in the form of fish. At the moment, there is no baseline data regarding the pesticide residue content in the fish samples in the Dal Lake. It is therefore, important to

determine the levels of pesticide residues in fish samples from Dal Lake to help establish a baseline data for pesticide residue content in the Dal Lake basins. The two fish species analyzed in the work are widely consumed by the local population hence the study may help to evaluate the health risk residue level of pesticides detected in the fish poses on the population.

Materials and Methods The study was conducted at the Quality Control and Quality Assurance laboratory of Indian Institute of Integrative Medicine Jammu (CSIR Lab) from 2008 to 2010.

Sampling: Three Dal lake basins (Hazratbal basin, Nigeen basin and Cheshmashahi basin) were taken for within the lake study **fig 1.**

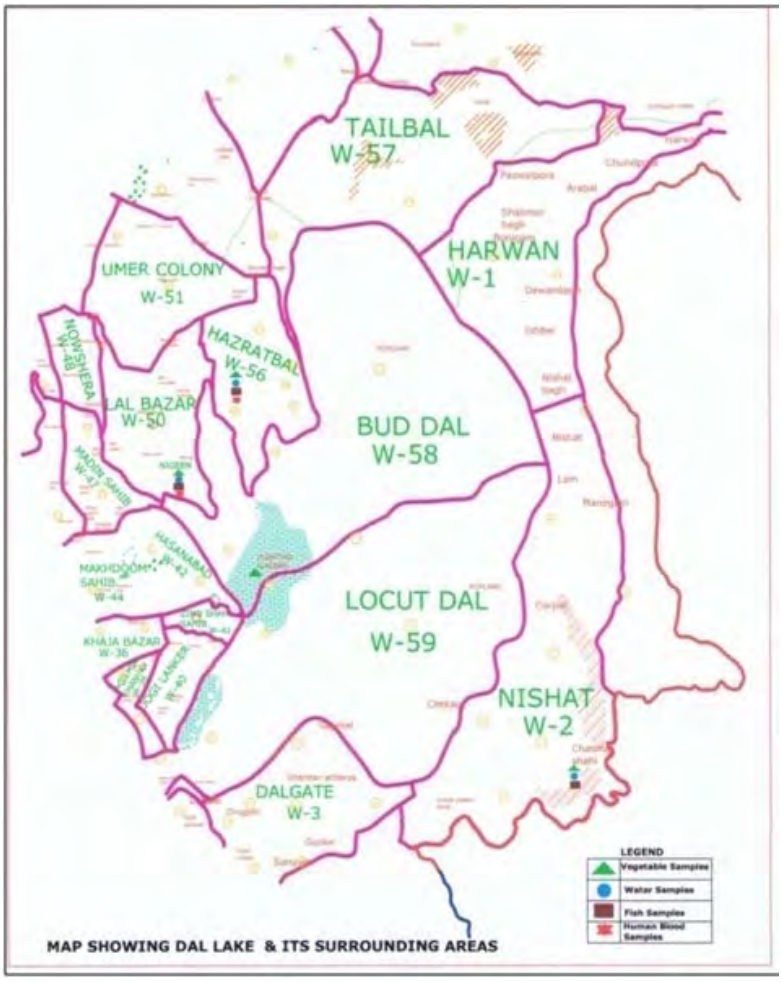

Figure 1: Map of Dal Lake showing different sampling sites of fish

Three samples of freshwater fish for each basin at different places were collected once every month for four months from March to June for pesticide application season and in December for pesticide non application season per year for three years from 2008 to 2010 for direct estimation of pesticides **Table 1**. From every respective basin 15 fish samples were collected with a total of 45 samples each year from 2008 to 2010 amounting to a total of 135 samples for three consecutive years. Two commonly consumed species of fish *Schizothorax niger* and *Cyprinus carpii* were taken from all the three basins as discussed in **Table 1.**

Table 1: Pattern of fish samples collected from the basins of Dal Lake from 2008 to 2010 i.e., 45 samples per year from three basins.

	March	April	May	June	December
Hazratbal basin	3	3	3	3	3
Nigeen basin	3	3	3	3	3
Cheshmashahi basin	3	3	3	3	3

Preparation of samples: The fish samples were scaled (if scaly) and the head removed using a stainless knife. The flesh and other edible portions were removed from the bone and entrails. These edible portions were cut into small cubes or pieces and frozen until analysis.

Extraction:

i. 25-50g of thoroughly ground and mixed sample of fish was taken in a homogenizing beaker.

ii. The sample was homogenized in high speed blender or homogenizer until thoroughly mixed.

iii. 100g anhydrous sodium sulphate (Na_2SO_4) was added to combine with water and to disintegrate the sample.

iv. The sample was thoroughly mixed with a spatula and blender until well mixed. Sides of the blender jar were scrapped down to break up the caked material with a spatula.

v. 150ml of petroleum ether was added and homogenized at high speed for 2 minutes.

vi. Petroleum ether supernatant was decanted in a glass fitted funnel with filter paper, into a 500ml flask fitted with a suction apparatus.

vii. The residue in the blender cup was re-extracted with two 100ml portions of petroleum ether blended for 2 minutes each time, filtered and combined with the first extract.

viii. The combined extract was passed through column (25mm x 50mm long) of anhydrous Na_2SO_4 and collected as petroleum ether extract.

ix. The above extract was concentrated on a rotary vacuum evaporation at steam bath temperature to obtain fat, taken up for clean-up procedure.

Clean-up

i. The extracted sample was transferred to a 125ml separatory funnels with the aid of 15ml petroleum ether and 30ml acetonitrile (saturated with petroleum ether).

ii. The mixture was shaken vigorously for 1 minute, the layers were allowed to separate and the acetonitrile layer was drained into a beaker. This procedure was repeated thrice.

iii. The solution thus obtained was transferred to 1 litre separatory funnel containing 650ml water; 40ml saturated NaCl solution and 100ml petroleum ether.

iv. The extract in the separating funnel was mixed thoroughly for 30-45 seconds.

v. The layers were allowed to separate and the aqueous layer was drained into another 1litre separating funnel.

vi. 100ml petroleum ether was added to the second 1litre separating funnel and the mixture was shaken vigorously for 15 seconds and layers allowed to separate. Aqueous layer was discarded, and the petroleum ether layer was combined with the previous one in the first separatory funnel and washed with two 100ml portions of water. The washing was discarded.

vii. The petroleum ether layer was passed through column (25mm x 50mm long) of anhydrous sodium sulphate (Na_2SO_4).

viii. The dried petroleum extract (dried by passage through Na_2SO_4 column which absorbs water) was evaporated to 10ml in a rotary vacuum evaporator.

Florisil column clean-up

i. A washed cotton swab was placed at the bottom of a chromatographic column and it was rinsed with petroleum benzene. The column (10mm x 120mm long) was filled with 4g activated Florisil topped with anhydrous sodium sulphate to about 2cm. The column was pre-wetted with 40-50ml petroleum ether.

ii. Concentrated petroleum ether solution of sample extract (about 10ml in step 8 of clean-up) was transferred to the column. The container was rinsed with two 5ml, portions of petroleum ether and rinsings transferred to the column.

iii. The column was eluted with 200ml eluting solvent (6% ethyl ether in petroleum ether i.e. ethyl ether = 12ml and petroleum ether =188ml) for recovery of organochlorine pesticides.

iv. For recovery of organophosphorus residues, elution was done with 200ml hexane and dichloromethane (4:1, v/v, Hexane = 160ml, DCM = 40ml).

v. The elute in the round bottomed flask was collected and concentrated under rotary vapour to complete dryness.

vi. 2ml of ethyl-acetate was added to round bottom flat and shifted in labelled glass stoppered vials for analysis in GC-MS/MS.

Gas Chromatography Analysis (GC- Analysis)

The analysis includes a qualitative and quantitative estimation of pesticides with the help of Gas Chromatograph Mass Spectrometer (Thermo Finnigan Polaris Q type) equipped with Ni Electron Capture Detector (ECD). The GC operating parameters were: Injector temperature 80 - 2700C, first ramp temperature @ 250C/min to 2000C, second ramp temperature 200^{o} C/min to 230^{o} C for 1 min and final temperature @ 20 ^{o} C/min to 280^{o}C used for 10 min. Purified helium gas was used as carrier gas and a known volume (25µl) of the sample was injected in. A column of 30 m length, 0.25mm i.d and 0.25µm film thickness (liquid stationary phase of Dimethyl-polysiloxane) was used. Different peaks of the sample were identified by comparing their retention times with those of standards obtained from pesticide from Pesticides India Limited. Seven commonly used pesticides were chosen and extraction procedures were performed for residual pesticide analysis by GC-MS/MS. The pesticides included were Butachlor (herbicide), Hexachlorocyclohexane, γHCH, chlorpyrifos, Hexaconazole, Endosufan-I, Endosulfan-2 and Dichlorvos (DDVP).

Results:

The results revealed that chlorpyrifos an organophosphate pesticide was present in fresh water Schizothorax niger a local fish which is very widely consumed by the kashmiri population. The mean concentration of chlorpyrifos residual level detected in samples was (0.0009±0.0010ng/kg) with concentration ranging from undetected to 0.003ng/kg. The status of detected pesticide residues in fish samples taken from selected basins of Dal Lake during 2008 to 2010 as given in **Table 2** reveals that the highest concentration was found in Hazratbal basin in 2009 (0.002±0.001ng/kg). For the other two basins the mean level was (0.001±0.001ng/kg) for all the years.

Table 2: Mean concentration of chlorpyrifos residual levels in fish samples taken from selected basins of Dal Lake.

	2008				**2009**				**2010**			
	Pesticide level ng/kg				**Pesticide level ng/ kg**				**Pestcide level ng/ kg**			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min.	Max
Hazratbal Basin	.001	.001	.000	.002	.002	.001	.000	.003	.001	.001	.000	.002
Nigeen Basin	.001	.001	.000	.003	.001	.001	.000	.002	.001	.001	.000	.003
Cheshmashahi Basin	.001	.001	.000	.003	.001	.001	.000	.003	.001	.001	.000	.002

The mean concentration of chlorpyrifos in pesticide application (March to June) and non application season (December) is present in **Table 3.**

Table 3: Mean concentration of chlorpyrifos in fish samples taken during pesticide application season and non application season for years (2008-2010).

Year			Pesticide Use Season	Pesticide Non Use Season
2008	Pesticide Level ng/Kg	Mean	.001	.000
		SD	.001	.001
		Min	.000	.000
		Max	.003	.002
2009	Pesticide Level ng/Kg	Mean	.001	.001
		SD	.001	.001
		Min	.000	.000
		Max	.003	.002
2010	Pesticide Level ng/Kg	Mean	.001	.000
		SD	.001	.001
		Min	.000	.000
		Max	.003	.002

The results reveal that level was higher in pesticide application season (0.001ng/kg) than non-application season (0.000ng/kg) in 2008 and 2010. In 2009 the pesticide level was same (0.001ng/kg) in both the seasons. **Table 4** represents the mean level of chloropyrifos detected in fish samples in all the selected basins during seasons of pesticide use and non-use for years 2008 to 2010.

Table 4: Mean levels of chlorpyrifos detected in fish samples in all the selected basins during pesticide application and non-application seasons for years 2008 -2010.

Year			Hazratbal Basin		Nigeen Basin		Cheshmashahi Basin	
			Pesticide Use Season	Pesticide Non Use Season	Pesticide Use Season	Pesticide Non Use Season	Pesticide Use Season	Pesticide Non Use Season
2008	Pesticide Level ng/kg	Mean	.001	.000	.001	.001	.002	.000
		S. D	.001	.000	.001	.001	.001	.001
		Min.	.000	.000	.000	.000	.000	.000
		Max.	.002	.001	.003	.002	.003	.001
2009	Pesticide Level ng/kg	Mean	.002	.001	.001	.001	.001	.000
		SD.	.001	.001	.001	.001	.001	.001
		Min.	.000	.000	.000	.000	.000	.000
		Max.	.003	.002	.001	.002	.003	.001
2010	Pesticide Level ng/kg	Mean	.001	.001	.001	.000	.001	.000
		S.D	.001	.001	.001	.001	.001	.000
		Min.	.000	.000	.000	.000	.000	.000
		Max.	.002	.002	.003	.002	.002	.001

The results show that mean concentration of chlorpyrifos level is higher in pesticide application than non application season except in Nigeen basin in 2008 and 2009 where levels are same(0.001± 0.001ng/kg) and in Hazratbal basin in 2010 where levels are same (0.001± 0.001ng/kg). **Fig 2** presents the mean concentration of chlorpyrifos in two species of fish viz schizothorax and Cyprinus species taken from selected basins of Dal Lake from years 2008 to 2010. The results show that chlorpyrifos was present only in Schizothorax niger species and not in Cyprinus carpii. These two species of fish are most commonly consumed by the people because of its good biological value and a satisfactory amino acid balance. Hazratbal is a major basin in Dal Lake for its fish productions. **Fig 2** reveals that concentration of chlorpyrifos does not go beyond 0.002ng/kg in Schizothorax in any basin during years 2008 to 2010. Cyprinus carpii (Punjab Gaad) did not show any trace of chlorpyrifos residual level.

Discussion:

Chlorpyrifos (o,o- Diethyl-o-3,5,6-trichloro-2-pyridyl-phosphorothioate) is commonly known as Dursban and is a broad spectrum pesticide. It is the second largest selling OP agrochemical in India. Dursban is the most toxic organophosphorus compound for fish and is more toxic than organochlorine compounds (Veeraiah, 2001). In Kashmir Schizothorax niger is one of the prime cultured fresh water fish in polyculture and holds a great economic importance. The direct exposure of this species to toxic pesticide like chlorpyrifos is a matter of concern. The results show a trace of chlorpyrifos in Schizothorax niger and its absence in Cyprinus carpii which may probably be attributed to least dermal absorption of chlorpyrifos owing to thick, scaly layer over the body of Cyprinus carpii. On the contrary, Schizothorax niger has delicate thin layer of scales over its body eventually increasing susceptibility for chronic dermal absorption. Chlorpyrifos residual levels have also been detected in Taiwan fishery products (Sun et al., 2008). In a study conducted on fresh water fish Labeo rohita it was proved that the chlorpyrifos is highly toxic and has a detrimental impact on its behavioral response even at sub lethal concentration and any alterations caused by the pesticides may lead to variations of total proteins in fish (Rao et al., 2010). Similar results were obtained in a study conducted by Halappa et al 2009 on Cyprinus carpio. This is an indicator that despite the health risks associated with chronic exposure of chlorpyrifos, the amount of such pesticides in aquatic system should also be monitored to prevent the decreasing nutritive value of fish and fish mortality. A number of reports on the toxicity of chlorpyrifos on different aquatic animal models indicated that it is a potent neurotoxic agent (Rao et al., 2005). Chlorpyrifos is widely used for pest control and in agriculture sanitation industries worldwide (Lemus and Abdelghani, 2000). Many studies have assessed the

effect of chlorpyrifos on health and safety of mammals (Dishburger et al., 1997; Johnson et al., 1998; Clegg and Gemert, 1999 ab). A good number of studies have been done in which organochlorine (OC) pesticide residues have been detected in fish samples (Adeyemi et al., 2008). Organochlorine pesticides are non polar toxic compounds which show more persistence and cumulation than organophosphate pesticide. Chlorpyrifos is highly toxic to fresh water fish, aquatic invertebrates and estuarine and marine organisms (US, EPA, 1989). The pesticide gains entry into fish by ingestion, dermal absorption and respiration (Gold Bouchat, et al., 1995) The chemical bioaccumulates in fish until they are caught and eventually eaten by humans thereby subjecting them to great amount of health risk.

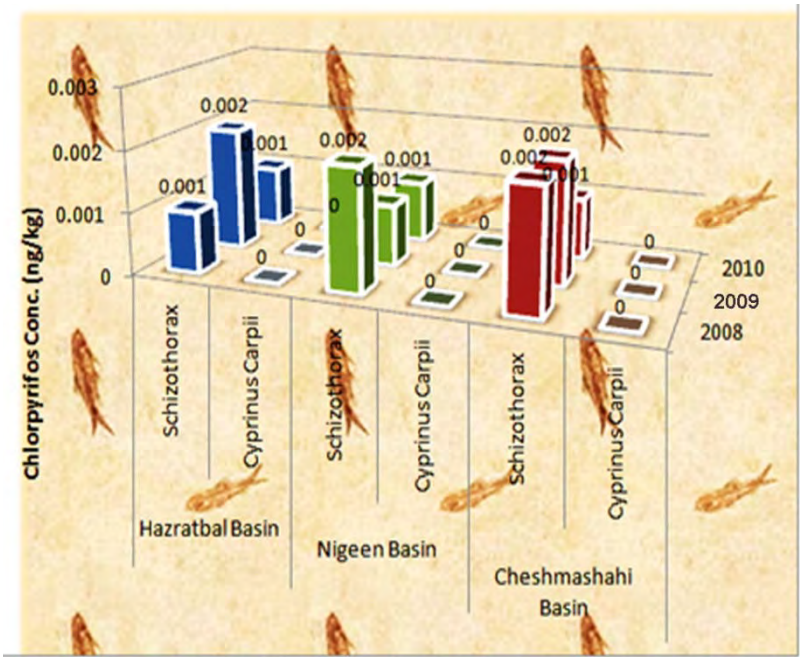

Figure 2: Mean concentration of Chlorpyrifos (ng/Kg) in two Species of Fish Samples *Schizothorax niger* and *Cyprinus Carpii* taken from Selected Basins during years 2008-2010

Conclusion:

The results of this work indicate that chlorpyrifos an organophosphate pesticide residues are present in a local fish "*Schizothorax niger*" collected from all the three basins of the Dal Lake. Although the concentration of chlorpyrifos is well below the tolerated limits established by authorities, based on these set standards, the fish from Dal Lake were found fit for human consumption. However, due to the bioaccumulation of these hazardous substances it becomes a potential risk to human health and is a matter of public health concern and stringent measures should be taken to prevent morbidity associated with chronic low dose exposure of pesticides.

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