Assessment of pesticide residues in water, sediments and muscles of *Cyprinus Carpio* from Head Balloki in the River Ravi

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Abstract: The present study investigated the presence and potent source of pesticides and specific pesticide bearing effluent release points on the River Ravi at Balloki Headworks (BH). The levels of selected organochlorine and nitrogen containing pesticide residues were assessed in water, sediments and muscle tissues of *Cyprinus carpio* of five different weight groups collected from five different sampling sites on the River Ravi at BH by using HPLC (Reverse phase chromatography). Six pesticide residues viz., endosulfan, carbofuran, cypermethrin, prophenophos, triazophos and deltamethrin were detected in muscle tissues of *Cyprinus carpio* The endosulfan and profenofos were the most abundant pesticides recorded in the fish tissue.Profenofos and cypermethrin were dominant pesticides recorded in the water samples from the River Ravi at BH. Cypermethrin and triazophos were not detected in the sediment samples.

[Mahboob S, Niazi F, Sultana S. **Assessment of pesticide residues in water, sediments and muscles of *Cyprinus Carpio* from Head Balloki in the River Ravi**. *Life Sci J* 2013;10(11s):32-38]. (ISSN:1097-8135). http://www.lifesciencesite.com. 6

Keywords: Fish; HPLC; River Ravi, pesticides; sediments; water

Introduction:

Unsystematic utilization of chemical pesticides, especially has influenced on man and atmosphere and improved the load of chemicals in the atmosphere due to non- biodegradability (Tripathy, 1992; Kumar et al. 2005). In spite of their extremely essential utilization in agriculture, pesticides produces an amount of pesticide residues in agriculture and dairy products, food and water causing a huge public health concern. Further, these compounds give a serious hazard with regards to environmental pollution and accidental poisoning (Ntow, 2005). Effluents with pesticides have resulted marked increase in the level of expansion retardation, death and damage or harmful to tissue in fish (Jhingran, 1991; Dikshit et al. 1990; Panday et al. 2000). Utilization of pesticides has become inevitable to control pests and fungus to avoid heavy losses to agricultural products. Numerous pesticides are being used and demands for more variety of pesticides are increasing to kill and control the toxic effects of pests. This extensive use of pesticides has resulted in widespread distribution of them in the air, soil and aquatic environment (Mahboob et al. 2011). Organochlorine compounds are used in great quantities and there is no doubt that the use of OCPs is vital in agriculture, but the fear is growing about their toxic and harmful effects of these chemicals that pose a probable risk to the environment. All organochlorine pesticides are very noxious to fish as compared to pyrethroid, carbamate and organophosphate under some environmental conditions (Mahboob et al. 2009). It has been reported that high rate of toxicity of poisonous substances does not depend on environmental conditions e.g. oxygen, pH, temperature, and residue molecules (Singh and Mishra, 2009. Because of their extensive use, their residues are found in various environmental matrices such as soil, sediments, water, air, vegetation and biota (Kang et al. 2001). These OCPs containing groups of DDT (dichlorodiphenyltrichloroethane), isomers of BHC (hexachlorobenzene), aldrin, dieldrin, endrin, chlordane, toxaphene, endrin, heptachlor, heptachlor epoxide, methoxychlor and HCH (hexacyclochlorobenzene) (Tanabe et al. 1994). After the application of pesticides, they finally reach to the aquatic ecosystem through agricultural run-off from land, pollutes ground water, bottom sediments, urban runoff, industrialized plant waste, community water treatment, and atmospheric drop out through rain making the life of the target as well as non-target species misery, and between them fishes are most terrible victims (Akhtar et al. 2009).

Fish is very sensitive to endosulfan and other organochlorines and kill fish. Rao *et al*., (1998) studied the relative toxicity and metabolism of endosulfan in Indian major carps. Dhasararathan *et al.*, (2000) reported a drop in carbohydrate, lipid and protein contents and also enhance the concentration of enzymes as a result of treatment with endosulfan. The majority of organochlorines is banned in Pakistan but are still in use and are causing remaining

and other toxic effects to fauna and flora. The wide use of pesticides for agricultural performance represents thousands of molecules with an enormous variety of physicochemical properties that are hazardous to living organisms. It is due to contamination of a number of aquatic ecosystems, including sediments, water and biota. The present study was planned to estimate the level of pesticide residues in water, sediments and in *Cyprinus carpio* and their biomagnifications at different locations in the River Ravi at Balloki Headworks.

Materials and Methods

2.1 Study area and sample collection

The River Ravi coursing along the India–Pakistan border meanders substantially in the alluvial plains of the Amritsar and Gurdaspur districts of Punjab and then enter into Lahore, Pakistan. After passing through Lahore the River takes a turn at Kamalia and then debouches into the Chenab River, south of the town of Ahmadpur Sial. In the trans-boundary Ravi River flowing from India to Pakistan, in urban areas of Lahore the pollution levels in the river discharge are reportedly very high, which is attributed to careless disposal of large amounts of industrial and agricultural wastewater and faulty drainage system in both countries. A 72 km stretch of the Ravi River from Lahore Siphon to Bulloki Headworks indicates heavy contamination of the water and sediment with different pesticides. The river sediments are highly contaminated and have become a secondary source of pollution of the river water, even though some control over unauthorized discharges into the river have been checked. The study was conducted on the fish specimen collected from the River Ravi from Head Bulloki. The effect of pollution was evaluated on fish samples of *Cyprinus carpio* of five different weight groups, sediments and water from five different locations nearby Head Bulloki in triplicates. Sampling was done at random and single time and available fish sizes were broadly categorized into five categories, W1 (950 to 1000 g), W2 (1150 to 1200 g), W3 (1350 to 1400g), W_4 (1550-1600g) and W_5 (1750-1800g).

Water and sediment samples were taken for six months on a monthly basis from seven sampling sites along the right and left banks of River Ravi at Head Balloki: “L1”= before HB downstream, Right bank (R B), “L2”= HB downstream, Left bank (L B), “L3”= After HB Bridge (R B), “L4”= After HB Bridge (L B) and “L5= Balloki Headworks, respectively. Sediment and water samples were collected in triplicate from above mentioned five different points at Head Bulloki (HB) River Ravi. Three water samples from each sampling site on each sample collection day were collected in prewashed glass bottles (1.5 L) with Teflon screw caps. Prior to sampling, 100 ml of water was taken by immersing the glass bottles in the water body, and these bottles were rinsed at least two times. After rinsing, the bottles were re-immersed in the water body and filled to maximum. Each water sample was fortified at the site of collection with dichloromethane (25 ml in 1L of distilled water) to prevent biological activity in the water. Bottles were labeled and brought to the Fisheries Research Laboratory, Department of Wildlife and Fisheries, Government College University, Faisalabad. The sediment core sample was obtained with a self gravity diving and immediately sectioned with at different depths using stainless plates after sampling.All sediment samples were immediately transferred to the laboratory and frozen at -25 ^{O}C until used.All equipments used for sample collection, transport, and preparation, were free from all types of pesticides.

2.2 Preparation of Water Samples

The extraction of pesticide from the collected water samples was done by liquid extraction (Ahad et al., 2006, Akhtar, 2013) with some modifications. One liter of water sample was poured in a separatory funnel (2 L) and 25 ml of dichloromethane (Merck) was added by dispenser flask. Separatory funnel was sealed with a lid and kept upright in a stand until two distinct layers were formed. The lower oily layer was collected in a round- bottomed flask through a small funnel with a cotton wool plug and anhydrous sodium sulphate in its opening<AQ does this mean the bottle was sealed with a cotton wool plug and anhydrous sodium sulphate. Ten drops of propylene glycol (Merck) in ethyle acetate (1:1) solution were added to the round- bottomed flask. The extract was evaporated using a rotary evaporator at 55 ºC under a vacuum hood at optimum rotation speed to dryness. Nitrogen or air steamed over activated charcoal was used to dry the extract completely.

2.3 Reconstitution or Clean-up

1400 µl of n-Hexane was added to the round bottom flask to reconstitute the contents, and mixed thoroughly. The contents were transferred to glass vials for analysis by a gas chromatograph equipped with an electron capture detector.

2.4 Detection and Quantification of Pesticides

A multi residue method using a gas chromatograph was used to determine the presence of six pesticides in the water samples (Anwar, 2008; Akhtar, 2013). The Gas Chromatograph was equipped with electron capture detector, nitrogen (N2) flow rate at 30-32 ml/min., and variable temperature arrangements (Injector temperature (220ºC); Oven temperature was maintained at 150ºC for 4 mins, then raised to 290ºC at a rate of 8ºC/ minute,<AQ Unclear what is meant by 8ºC/ minute>

and then held for 10 minutes. The temperature of the detector was 300ºC).

***Procedure*:** Gas chromatography was turned on for the detection and quantification of pesticides. A 1µl standard solution of each pesticide under investigation in this study (DDT, DDE, Carbofuran, Cartap, Endosulfan and Endosulfan sulfate) was injected into the chromatograph and their retention times were determined.. Calibration curves of all standard pesticides were prepared with the help of computer software Turbochrome® (Perkin Elmer, Inc. USA). The limit of detection was calculated using Super cal-5 software. After running standard solutions of each pesticides, through the chromatograph 1 µl aliquot of concentrated elute was injected and residue peak(s) of elutes injected were identified on the basis of retention time. The retention time of all test solutions was within ±2% of standard pesticide solutions. The Height/area <AQ which was measured of residue peak(s) was measured, and the residue amount of test solution was determined, by comparison to the Height/area obtained from a known amount of appropriate reference/standard solution in the chromatograms.

2.4 Statistical Analysis of Data

Data on pesticide residue concentrations in water was analyzed using two-way classification (factorial experiment). Analysis of Variance and Duncan's Multiple Range tests for were performed to analyze differences between the parameters under study (Steel et al. 1996).

3. Results and Discussion

In the past ten years mostly in tropical countries or regions the agriculturist has increased significantly. It has been estimated that about one-third of the total area engaged by large-scale agricultural plantations which is positioned in tropical and sub-tropical regions are situated in developing countries (Henriques et al. 1997). To meet the growing demands of food supplies of burgeoning populations and to export cash crops the agriculture crops in such countries receives large inputs of the agrochemicals including pesticide (Lacher and Goldstein, 1997). Furthermore, the rigorous applications of pesticides which leads to the contamination of target fields, as a result of spray drift from aerial spraying and edge-of-field runoff due to irrigation and recurrent rainfall events may lead to non-point-source pesticide contamination of surface waters (Schulz, 2004). Furthermore, because of their comparatively high carcinogenicity to fish and aquatic invertebrates pyrethroid insecticides are considered as main concern pollutants among the different types of pesticides entering aquatic systems through non-point sources (Akhtar, 2013). The data acquired is discussed here about the authenticity of the results.

3.1 Pesticide residues in fish

After extraction and analysis, the data on pesticide residues in *Cyprinus carpio* are presented in Table 1. The different nature of pesticide residues was determined in the fish including organochlorine, organophosphate, pyrethroids etc. Other authors also reported the presence of endosulfan residues in fish samples collected from Ghana. The residue was in the range of 0.84-2.32 ng g^{-1} and 26% samples were found to be contaminated with endosulfan. Other organochlorine residues (DDT, dieldrin, lindane and DDE) were also detected in fish samples. Our results are in accordance with the reported results of Akhtar *et al*., (2012). The concentration of organochlorine residue in fish samples is much higher than the African water reservoir because still the organochlorine pesticide is an active ingredient and in use in the health sector to eradicate malaria but no sound proofs are available for their use in Pakistan.

Organophosphate pesticides are highly poisonous to water biota and the presence in a food chain is debatable locally as well as internationally. The residue of Organophosphorus was also assayed in fish samples under study. Triazophos and Profenophos were detected in fish samples by HPLC method. The concentration of triazophos was ranged from 0.35-2.64 mg kg^{-1}. More than 60% samples were contaminated with triazophos residues, whereas, 80% fish samples were contaminated with Profenophos. These compounds are highly soluble in water and may be a reason of high residues in fish samples collected from HB. Sun and Chen (2008) investigated the presence of chlorpyrifos residues in fish samples collected from Taiwan and detected the residues by GC-FPD. Only 23% samples were contaminated by chlorpyrifos. The farm fish contained higher residue than the wild fish samples. Our results are contradictory with the findings of above mentioned workers. It may be because of the fact that both these pesticides are extensively used to protect vegetable and fruits from different insects. Profenophos is also extensively used in cotton crop. Karen et al. (1998) mentioned that some aquatic macrophytes can absorb chlorpyrifos and help remove them from the aqueous environment.

Table 1 describes the highest concentration of profenofos is present in the samples collected from locations L2 closely followed by L5 in *Cyprinus Carpio.* No concentration of endosulfan and deltamethrin was found in fish collected from L1 and L2 and L3 locations, respectively. The concentration of deltamethrin in fish of L1, L4 and L5 was recorded as 1.36, 2.45 and 3.06 mg kg^{-1}. The pyrethroids are widely distributed in the HB sites hence fish lived in

the area may be polluted. It may be due to dilution factor or heavy rains or other hidden artifacts that's why the residue was not detected in a few samples of fish. The widespread use of these pesticides consequently leads to the exposure of manufacturing workers, field applicators, the ecosystem and finally the public to the possible toxic effects of these pesticides (Solomon *et al*. 2001). Velisek *et al*., (2007) studied the effect of deltamethrin on rainbow trout and found significantly lower values ($p < 0.05$) of plasma glucose, alanine aminotransferase, cholinesterase and significantly higher ($p < 0.05$) values of erythrocyte count, hemoglobin content, haematocrit and plasma total protein, albumins, ammonia, aspartate aminotransferase, creatinekinase and calcium compared to the control group. Similarly other authors also determined organochlorine residues in fish using gas chromatography with different solvents like acetone, ether, n-hexane and cleaned the extracts using Florisil column. In the present study the organochlorine residues were extracted in n-hexane and only endosulfan was found in fish samples collected from different locations of HB. The highest and minimum concentration of endosulfan was recorded as 13.62±1.50 and 6.94±0.72 mg kg^{-1} from location L2 and L4, respectively. The comparison of the means showed non-significant differences in sampling sites L2 and L5. The accumulation of this pesticide was increased with increased in body weight of the fish. Our results were found in agreement with the findings of Mahboob *et al.*, (2009).

The maximum and minimum concentration of cypermethrin in muscles of *C. carpio* was observed as 0.52 and 0.21 mg kg-1, respectively at L1 and L3. The comparison of means depicts significant differences among locations L1, L3 and L5 (Table 1). Among the classes of pesticide carbofuran is one of the most toxic agrochemical. The trading companies like FMC Corporation and Curater marketed carbofuran under the trade names Furadan. In agriculture the extensive use of this pesticide is in crops like soybeans, potatoes and corn insects. Carbofuran is considered as a systemic insecticide, that from the roots the plant absorbs it and from here the plant distributes it where insecticidal concentrations are attained throughout its organs. It is recognized that carbofuran also has contact activity against pests. The data given in Tables 1 showed the presence of carbofuran residues in all fish samples of *C. carpio*. The highest concentration of carbofuran was founded in L5 (8.53 mg kg^{-1}) of *C. carpio*. The sampling sites L2, L4 and L5 were statistically similar reference to the bioaccumulation of carbofuran in *C. carpio*. The statistical analysis depicted highly significant ($p<0.01$) differences in five different weight groups. Pearson correlation showed a similar findings. Among carbamates, carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is a broad spectrum systemic insecticide, nematicide, and acaricide commonly used throughout the world. As a result of its widespread use, carbofuran has been detected in ground, surface, and rain waters. The highest concentration of triazophos was found in L5 (0.64 mg kg-1). The minimum residue of triazophos was found in L1 i.e. 0.165 mg kg-1. Triazophos was in minimum concentration as compare to other residues in the fish. The statistical analysis showed the highly significant differences among the weight groups and locations (Table 1). World-wide research on fish collected from different water sources showed the presence of organochlorine and nitrogen containing pesticide residues. Mostly organochlorine residues were detected in fish samples as compared to nitrogen containing samples but no residue of carbofuran was found in fish samples (Sun *et al*., 2000). The correlation was also worked out among the various tested pesticides in five different weight categories of *C. carpio*. There was a nonsignificant relationship among the six pesticides in all weight groups in the muscles of this fish (Table 2). The overall comparison among the five different weight groups expressed that the maximum concentration of pesticide residues was recorded in W5. We are of the view that biomagnification of the pesticides has a definite relationship with the size and weight of the fish.

3.2 Pesticide residues in water samples

Endosulfan was detected in some water samples in the range of 0.27-0.42 µg L^{-1}. The concentration of the organochlorine (endosulfan) was higher than the recommended maximum residue limits (MRL) i.e. 0.1 µg L^{-1} for single residues and 0.5 µg L^{-1} for cumulative residuals (Table 3). Darko and Acquaah (2007) determined organochlorine residues (DDT) in 78% samples of water with mean residue 0.012 ± 0.62 ng mL^{-1} collected from Ghana and also found endosulfan (0.064 ng mL^{-1}) but our results are contradictory with the findings of the above mentioned workers. The river is contaminated by organochlorine pesticide residues from nonpoint sources via runoff, atmospheric deposition, vector control practices and leaching due to agricultural applications. The sediments of river acts as a sink and whose re-suspension during the river mixing may increase pesticide bioavailability and accumulation in the fish. Pesticide pollution to the river and especially at Head Baloki is, therefore, likely to pose a danger to both aquatic organisms and humans.

Deltamethrin, and cypermethrin residues were also detected in water samples taken during the

present study. The residue concentration of deltamethrin in water samples was ranged from 0.45-0.006 ng mL^{-1},whereas, the concentration of cypermethrin was 1.41-0.0042 ng mL^{-1} (Table 3). Pyrethroids concentration was higher that recommended MRLs due to extensively used on vegetables, fruits and other crops. Pyrethroids are persistent compounds with high hydrophobicity (log K_{ow} in the range 5.7–7.6) and very low water solubility (of a few µg L^{-1}). In another study conducted by Feoa *et al*., (2010) found pyrethroids residues in water samples collected from Ebro River, Spain. The concentration of the detected pesticide (Deltamethrin) in three samples was determined as 0.73 ng L^{-1} to 57.2 ng L^{-1} and 2 ng L^{-1} to 58.8 ng L^{-1} for cypermethrin and deltamethrin, respectively. Our results are fairly in agreement with the findings of Feoa *et al.,* (2010) but we comparatively recorded these pesticide residues in less concentration.

3.3 Pesticide residues in sediment samples

Sediments act as secondary contamination source after water in the ecosystem. Thus, it is important to monitor and analyze pesticide residues in sediments that serve as the primary sink for a majority of pesticides used in agriculture. Sediment is one of the principal reservoirs of environmental pesticides, representing a source from which residues can be released to the atmosphere, groundwater and living organisms (Xue *et al*. 2006). Sediment samples were also collected from HB and analyzed using chromatographic techniques. Endosulfan, deltamethrin, profenofos and carbofuran were detected while triazophos and cypermethrin were absent in sediment samples in the current study. Endosulfan was ranged between 2.58-12.68 ppm (Table 4). Vagi *et al,* (2007) determined endosulfan (I & II) and other organochlorine in marine sediment using different organic solvent by ultrasonic extraction technique. The authors found that ethyl acetate is the best solvent to extract organochlorine residues. Our results are in favour of the above authors because in the present study ethyl acetate was used as a solvent. More than 95% recovery was found in the samples. Similar results were also reported in another study conducted by Darko and Acquaah (2007). In the present study we determined profenofos in the range of 3.74-24.06 ng g^{-1} (Table 4).

OP's are extensively used for the protection and production of crops in Pakistan and other Asian countries like China. World-wide many studies were conducted to find out the level of Organophosphorus in different matrices (Tse *et al*. 2004; Akhtar et al. 2012). Both group of authors evaluated OP's by using different solvent mixture for the extraction of pesticide residues from sediments. The analyses depicted the presence of organophosphate residues in sediment samples being analyzed using LC-MS and GC-MS. The range of OP was 65-6200 ng g^{-1} which is higher than our findings. The statistical analysis showed highly significant variation among the various samples (Table 4).

Triazophos was not detected in sediment samples collected from HB. The samples were also analyzed for pyrethroids and only deltamehtrin was detected in the range of 0.61-5.58 ppm (Table 4). Carbofuran was also found in the rang of 3.76-12.40 ppm. Triazophos was sprayed but rarely on rice, maize and other vegetables grown in the area that's why it was not detected in sediment samples or due to other artifacts.

Deltamethrin and carbofuran are extensively sprayed on many crops and all agriculture appliances like tractor are washed away on nearby rivers or canal. It may be the reason that these pesticides had been detected in sediment samples being the final sink of pesticides. Many scientists of the world determined the carbofuran residues in marine sand and water. Campbell *et al*. (1997) isolated and detected many pesticide residues including carbofuran in sand particles and water samples taken from Laysan Island in the Hawaiian Islands.

Table 1: Comparison of concentration of various pesticides in the muscles of various weight groups of *Cyprinus carpio*

Location	Weight group	Endosulfan	Profenofos	Carbofuran	Deltamethrion	Cypermethrin	Triazophos
L1	W1	ND	0.02±0.000d	1.23±0.37c	1.36±0.07c	0.62±0.011a	0.17±0.017c
L2	W2	13.62±1.50a	13.72±0.86a	7.93±0.96a	ND	0.24±0.012d	0.18±0.012c
L3	W3	9.23±1.20b	9.03±0.69b	6.10±0.33b	ND	0.52±0.017b	0.40±0.08b
L4	W4	6.94±0.72c	6.94±0.42c	8.33±0.93a	3.45±0.10a	0.47±0.017c	0.43±0.017b
L5	W5	12.46±1.06a	12.60±0.61a	8.53±0.98a	2.06±0.47b	0.21±0.011d	0.64±0.008a

L1-L5= Locations W1=950-1000g; W2= 1150-1200g; W3=1350-1400; W4= 1550-1600g; W5= 1750-1800g. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 2: Pearson's correlation coefficient between weight category with different pesticides in *Cyprinus carpio*

Pesticides	Correlation coefficient	Pesticides	Correlation coefficient
Endosulfan	-0.047 NS 0.940	Deltamethrin	-0.364 NS 0.546
Profenophos	0.766 NS 0.131	Cypermethrin	-0.484NS 0.409
Carbofuran	0.643 NS 0.242	Triazophos	0.579 NS 0.306

Upper values indicated Pearson's correlation coefficient; Lower values indicated level of significance at 5% probability.; * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 3: Concentration of different pesticides (Pb) in water samples collected from different locations at the head Bulloki River Ravi.

Location	pH	Conductivity	DO_2	Endosulfan	Profenofos	Carbofuran	Deltamethrin	Cypermethrin	Triazophos
L_1	8.2b	3.07d	7.43c	0.42±0.03a	0.36 ± 0.03c	0.70±0.04c	0.033±0.00c	0.0042±0.00c	0.15±0.02c
L_2	8.4a	3.12c	7.54c	ND	1.40 ± 0.15a	0.45±0.04d	0.006±0.00d	1.36±0.10b	0.080±0.00d
L_3	8.1c	3.20a	7.67b	ND	1.13 ± 0.03b	0.85±0.03a	0.16±0.26b	1.41±0.05b	0.005±0.00d
L_4	8.3b	3.05d	7.72a	0.27±0.22b	0.36 ± 0.06c	ND	0.45±0.03a	0.066±0.06c	0.41±0.07a
L_5	8.4a	3.17b	7.52c	ND	1.40 ± 0.16a	0.82±0.03a	0.44±0.06a	2.68±0.66a	0.35±0.03b

Data is mean of triplicate analysis ± standard deviation; ND= Not detected. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 4: Concentration of different pesticides (Pb) in sediment samples collected from different locations in the Head Bulloki River Ravi.

Location	pH	Conductivity	Endosulfan	Profenofos	Carbofuran	Deltamethrin	Cypermethrin	Triazophos
L_1	8.4b	4.13d	2.60±0.34d	23.06±1.52a	4.76±0.20c	5.58±0.40a	ND	ND
L_2	8.6a	4.24b	3.33±0.18c	17.37±1.47b	3.76±0.29d	0.61±0.13c	ND	ND
L_3	8.3b	4.15d	2.58±0.03d	13.26±1.09c	5.65±0.31b	0.64±0.08c	ND	ND
L_4	8.5a	4.20c	12.68±0.10a	3.74±0.23d	12.40±1.04a	ND	ND	ND
L_5	8.6a	4.28a	3.44±0.22b	18.25±1.19b	5.23±0.31b	0.99±0.04b	ND	ND

Data is mean of triplicate analysis ± standard deviation; ND= Not detected. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Conclusion

It has been concluded the pesticide concentration levels of River Rav at Balloki Headworks are due to the continuous addition of pesticides from its tributaries. These tributaries carry a huge burden of concentrated industrial, domestic and agricultural waste substances that are carried into the Ravi River. Environmental Protection Agency and Non-profit organization should make an effort to create awareness through print and electronic media so that people may realize the harmful effects of pesticides and either reduce or stop their use. A constant monitoring program should be introduced by the Government of the Punjab to provide a hazard free environment to the aquatic biota and to ensure safe and healthy supply of fish for human consumption.

Acknowledgements

This project was supported by Deanship of Scientific Research, College of Science, Research Center, King Saud University, Saudi Arabia.

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