Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Human health risk assessment of screened pesticides residues in two edible fish species of river Ganga, India

Zeshan Shah (zeshanomer11@gmail.com)
Aligarh Muslim University
Zubair Nengroo
Aligarh Muslim University

Research Article

Keywords: Pesticide residue, Bioaccumulation, Health risk assessment, Edible fish, Ganga, India

Posted Date: December 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-131466/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Study was carried out to determine the concentration and bioaccumulation of pesticide residues in edible fish from river Ganga, India, to assess human health risk via consumption of fish. Two commonly edible fish species bagrid catfish and common carp were collected. The n-hexane extract of the muscle tissues were characterized by gas chromatography coupled to mass spectrometry and quantified by electron capture detector for pesticide residues. Bioaccumulation factors (BAFs) in common carp of pesticides were found to be higher than those in bagrid fish. Daily exposures of pesticides for consumers via fish consumption were determined by calculating and comparing estimated daily intake (EDI) with ADI values. The EDI results in our study were insignificantly high from ADI values. Non-carcinogenic and carcinogenic risks were evaluated by Target hazard quotient (THQ) and risk ratio (R), respectively. Hazard quotients (THQ) were found to be lower than the set 1.0, inferring non-carcinogenic risk by consumption of fish from the river. Regard to contaminants carcinogenic affects the total risk ratio (R) value of pesticides were found lower than threshold of tolerable risk except of heptachlor indicating carcinogenic risk via consumption of fish. The results demonstrate that due to increased pollution in the ecosystem required more attention.

1. Introduction

Agrochemical pollution being a global concern in past few decades due to their persistence in the environment, bioaccumulation in organisms and having a toxic impact on humans and other organisms. Bioaccumulation of residues has a wide range of acute and chronic adverse impacts on reproduction, physiology and biochemical parameters (Shah and Parveen, 2020).

Among all aquatic ecosystem organisms fish is considered as suitable bio-indicator of monitoring environmental contamination pollution. Fish uptakes pollutants directly through the water via gills, integuments and indirectly from the food web. The pollutants present in the fish not only indicate environmental persistence but are also transferred to other organisms through the trophic web. Fish as nutrition is an important source of not only proteins but also omega-3-polyunsaturated fatty acids which are a cure for cardiovascular diseases (Jabeen and Chaudhry, 2011) and used in the pharmaceutical and cosmetic preparations (Nengroo and Rauf 2019). Consumption of fish from the contaminated environment causes accumulation of pollutants in the human body.

River Ganga is important not only for irrigation purposes but also provides basic nutrition for common people (Sharma et al., 2014). With the increase in population India has been undergoing rapid industrialization and economic development. Use of pesticides for agricultural purposes had increased to hundred times to sustain more population. Enormous quantities of pesticides are being applied along with Ganga river basin agricultural fields.

These residues finally find their way into the river by flash floods, drainage and surface runoff. Large numbers of reports are available that ground waters in India are highly polluted with pesticides. Kaushik

et al., 2008 reported organochlorine pesticides in Ghaggar river of Haryana. Sankararamakrishnan et al., 2005 reported organochlorine and organophosphate pesticides in river Ganga at Kanpur station. In the present study we choose river Ganga at Narora as sampling site. These pesticides residues are accumulated in aquatic organisms and pose a potential carcinogenic risk for humans.

Therefore the objective of the present study is to clarify the concentration and accumulation of pesticides in water and edible fish of river Ganga. Further the assessment of daily exposure and human health risk via consumption of fish from the river. The study provides a broader overview of pesticide status in edible fish of river so that effective measures to be taken to reduce the potential human health risk.

2. Materials And Methods

2.1. Study site and the Sampling:

Figure 1 displays the details of sampling site, thirty fish and twenty-five water samples were collected at Narora site of the river Ganga in August 2019. The fish samples included 10 bagrid catfish and 10 common carp, belonging to bagridae and cyprinidae families, commonly consumed fishes of India (Gupta, 2015, Nongbri and Syiem, 2012). The fish and water samples were analyzed for pesticide residues. All of the collected samples were stored in an ice-box and are immediately transported to the laboratory.

In the laboratory, fishes were dissected and dorsal muscle tissues of the fish were taken and analyzed for pesticide residues. Collected muscle samples were freeze-dried, grounded to fine powder, and stored at −20 °C before the process of extraction begins. The impurity particulates in the collected water samples were separated by filtration through 0.45-μm hydrophilic filters.

2.2. Sample extraction and clean up:

Pesticides in water samples were described by the method of Muir and Sverko (2006). 2,4,5,6-tetrachloro-*m*-xylene (TCmX) as recovery surrogate was added in 1 litre of filtered water sample. Liquid-liquid extraction with dichloromethane (35 ml) was performed. Na_2So_4 column was used to remove water in the organic phase further n-hexane was used as an organic solvent. The column was packed from bottom to top with neutral silica, neutral alumina, and anhydrous sodium sulfate, to remove impurities in the extract. The extract was finally blown to dryness by purity nitrogen and the residues were redissolved with 20 μl of *n*-hexane.

Matrix-solid phase dispersion method (Villaverde-de-Saa *et al*., 2013) was done to extract pesticides in fish muscle. Samples (0.5 g) was grounded to fine powder with known amount of surrogate (TcmX) and 0.5 g octadecylsilane bonded silica (C18) as dispersion sorbent in a mortar and pestle for 5 min to get a homogeneous mixture. The homogeneous mixture was transferred into (0.22 μm) membrane filter polyethylene (10 ml) syringe. The column was packed with neutral silica, acidic silica, florisil acidic alumina and sample mixture from bottom to top with other membrane filter placed on top. 15 ml of

dichloromethane was used to elute the packed column at a flow rate of 2 ml min^{-1}. High purity nitrogen at the gentle stream was used to dry eluent, followed by dry residues redissolution with 200 µl of n-hexane and addition of internal standard (pentachloronitrobenzene). Qualitative as well as quantitative analysis of analytes were done with GC-MS and GC-ECD.

2.3. Lipid:

Determination of lipid content was done gravimetrically (Smedes, 1999). Two grams of fine grounded muscle powder was dissolved in twenty millilitres of water, cyclohexane and 16 ml isopropyl mixture. Ultrasonic extraction was done, mixture reached statically separated equilibrium, and the organic phase was collected. Extraction was repeated with18 ml of cyclohexane and 3 ml isopropyl alcohol and combined with earlier then dried under a gentle nitrogen stream. The residue was weighed.

2.4. Gas Chromatography and Mass Spectrometry:

3 µl of Sample were injected into a gas chromatography (Agilent 7890A) instrument equipped with an electron capture detector (GC-ECD) (Agilent Technologies, USA) the analytical capillary column was DB-5 (30 m × 0.25 mm i.d. × 0.25-µm film thickness, Agilent). Nitrogen was used as carrier gas with the flow rate of (1 ml min^{-1}). Injector and detector temperatures were adjusted at 250 and 300 °C. Started at 80 °C with 1 min hold, and the oven temperature was raised to 150 °C at 20 °C min^{-1} rate and finally to 300 °C (5 min hold) at the rate of 5 °C min^{-1}.

The instruments were calibrated with calibration standards during analysis. Each sample was analyzed in duplicate. The recoveries of TCmX (surrogate standard) were 75 ± 6% in water samples and 68 ± 6% in fish samples. The recoveries of pesticides ranged from 73 to 100% in water samples and from 66 to 84% in fish samples. The method detection limits (MDLs) concentration of analytes were confirmed whose signal-to-noise (S/N) ratio was three and ranged from 0.05 to 100 µg L^{-1} in water samples and from 0.01–100 µg g^{-1} in fish samples. Concentration detected less than MDLs in samples were treated as not detected (nd).

2.5. Data analysis:

Biota-water accumulation factor (BAF) is calculated by the following equation:

$$\mathrm{BAF} = Cl/c$$

Where C_l is the pollutant concentration in the fish (µg g^{-1}) normalized by lipid content of fish and c is concentration of pollutant in water (µg l^{-1}).

In order to determine potential human health risk of tested fishes, the estimate daily intake (EDI), THQ and R were calculated. The calculation formulas of EDI, THQ and R are listed as follows:

$$\mathrm{EDI} = \frac{C \times W_F}{W_{AB}}$$

Where,

C = Concentration of pollutant in food (μg g^{-1}), W_F = Average daily fish consumption in India is 55 g day^{-1} $person^{-1}$, and W_{AB} = Average adult body weight (70 kg).

The target hazard quotients (THQs), and carcinogenic risk ratio (R) were used in risk assessment. The THQ>1 denotes that the daily exposure seems to cause human health hazard effects.

$$\mathrm{THQ} = \frac{\mathrm{EF}\times\mathrm{ED}\times\mathrm{FIR}\times\mathrm{C}}{\mathrm{RFD}\times\mathrm{WAB}\times\mathrm{TA}} \times 10^{-3}$$

$$\mathrm{R} = \frac{\mathrm{EF}\times\mathrm{ED}\times\mathrm{FIR}\times\mathrm{SF}\times\mathrm{C}}{\mathrm{WAB}\times\mathrm{TA}} \times 10^{-3}$$

Where,

E_F = frequency of exposure (350 days $year^{-1}$), E_D = duration of exposure (70 years), F_{IR} = Average daily fish consumption in India is 55 g day^{-1} $person^{-1}$, R_{FD} = oral reference dose (mg kg^{-1} day^{-1}), T_A = average life exposure time (365 days $year^{-1}$ × lifetime, assuming 70 years), and SF = oral cancer slope factor (mg kg^{-1} day^{-1}) $^{-1}$.

Concentrations used in the present study of risk calculations are on a wet weight basis. Oral reference dose (R_{FD}) and oral cancer slope factor (SF) values were used from (US EPA, 2009) for risk assessment.

3. Results And Discussion

3.1. Concentration of pesticides in water samples:

Table 1 depicts the mean value (μg/l) concentration of pesticide residues detected in analyzed water samples. In India, 60,000 MT of pesticides are being annually used of which maximum consumption occurs along river Ganga basin (Kumar et al., 2013). Pesticides used in the agricultural fields could easily concentrate in the water through surface runoff streams and tributaries. Besides the continuous agricultural activities done along the Ganga basin, the dry beds of the river are used to grow vegetables and fruits, also add pesticides to the river during monsoon season. In the present study eight pesticides were detected, chlordane were found in higher quantity with 0.104 μg/l and heptachlor were found in a lower quantity of 0.006 μg/l. Chlordane, dimethoate and malathion were detected in all of the analyzed samples while atrazine, heptachlor were detected in 8 of the 25 water samples. No obvious change in the trend of detection and concentration were found for malathion, heptachlor and chlordane which shows

their historical use and persistence from the past. Dimethoate, atrazine, dichlorvos, azinphosmethyl and cypermethrin were found in the present study, they are newly introduced pesticides and are being used in increasing trend along the basin (Indiastat, 2018). Rehana et al., 1996 reported 0.20 µg/l of dimethoate from the same site. While Agnihotri et al., 1994 reported 79 ng/l heptachlor near Farrukhabad site. Similar results were reported by Sankararamakrishnan et al., 2005, Amrita *et al*., 2009 along with Kanpur and Lucknow sites of river Ganga. Reports showed that river Ganga waters suffered more containment pollution compared to other rivers of the country (NGRBA, 2011). Pollution was mainly due to human activities like agricultural and drainage along the river basin.

Table 1
Biometric data of the two fishes collected from River Ganga.

	Bagrid fish	**Common carp**
Scientific name	*Rita rita*	*Cyprinus carpio*
n[a]	10	10
Fish length (cm)	22.13 ± 4.13	27.33 ± 6.42
Fish weight (g)	350.71 ± 20	380.66 ± 17
Dietary habit[b]	Carnivorous	Omnivorous
Trophic level[b]	3.7	3.1
Lipid % (Muscle)	14.36 ± 1.19	8.49 ± 3.68
a Number of Samples		
b Fish base		

3.2. Concentration of pesticides in tissue samples:

Pesticide concentration detected in fish samples is given in Table 2. Mean concentrations (wet weight, ww) ranged from 0.167 µg g^{-1} for heptachlor to 0.045 µg g^{-1} for azinphosmethyl in bagrid cat fish. Mean concentration in common carp ranged from 0.182 µg g^{-1} for dichlorvos to 0.017 µg g^{-1} for malathion. Aktar et al., 2009 reported 13 pesticides from muscle tissues of fish from river Ganga with 0.1 µg g^{-1} of dimethoate and 5.4 µg g^{-1} of malathion. Dimethoate and malathion in the present study were reported lower than the early findings. All the examined samples contain residues except for azinphosmethyl, only 4 samples reported concentration in bagrid catfish. In common carp, only 6 out of 15 examined samples show concentrations of malathion and dimethoate residues. Heptachlor and chlordane were reported at below permissible limit by (Samanta, 2006) in fish from the river at West Bengal site. Due to continuous exposure, contaminants accumulate and get concentrated in the muscle tissues compared to water. Accumulation of pesticides depends upon different physiological,

environmental and feeding habits. The diverse feeding habit of fishes prefers more accumulation of pesticides.

Table 2
The concentration of pesticide residues in surface water (ug/l) and fish tisuues (µg/g ww) from river Ganga, India.

Pesticide	$LogK_{ow}$	Surface water	Bagrid	Common carp
			Muscle tissues	
Chlordane	6.16	0.104 ± 0.33 (0.54 – 0.301)	0.101 ± 0.25 (0.67 – 0.278)	0.091 ± 0.52 (nd-0.187)
Dimethoate	0.78	0.082 ± 0.15 (0.051–0.191)	0.069 ± 0.64 (0.063–0.079)	0.079 ± 0.64 (0.071–0.095)
Malathion	2.36	0.055 ± 0.80 (nd-0.109)	0.081 ± 0.32 (0.075–0.097)	0.017 ± 0.71 (nd-0.111)
Atrazine	2.61	0.051 ± 0.19 (nd-0.104)	0.131 ± 0.19 (0.076–0.189)	0.087 ± 0.23 (0.055–0.104)
Heptachlor	6.10	0.006 ± 0.20 (nd-0.0024)	0.167 ± 0.20 (0.143–0.201)	0.142 ± 0.20 (0.123–0.209)
Dichlorvos	1.43	0.059 ± 0.17 (nd-0.132)	0.142 ± 0.11 (0.111–0.267)	0.163 ± 0.13 (0.109–0.267)
Azinphosmethyl	2.75	0.065 ± 0.80 (0.055–0.102)	0.045 ± 0.10 (nd-0.201)	0.138 ± 0.19 (0.117–0.197)
Cypermethrin	6.60	0.076 ± 0.93 (0.061–0.097)	0.052 ± 0.47 (nd-0.177)	0.182 ± 0.52 (0.154–0.235)

Data shown as mean ± standard deviation; maximum and minimum concentrations are in parenthesis.

3.3. Bioaccumulation factors:

The bioaccumulation factor is the ratio of chemical contaminant found inside the tissue of the fish to that found in the surrounding water. Pesticides being lipophllic, the concentration inside the tissue is normalized with lipid content. In the present study, the BAF of heptachlor was found higher in both fishes than the other pesticides. Higher BAF of heptachlor might be accredited to poor water solubility and relative high log K_{ow} (Table 2). BAF of common carp were found to be higher than that found in bagrid catfish. From the studies conducted it is known that bioaccumulation of chemical contaminant in the fish is due to the cumulative effect of many physiological and environmental conditions such as trophic level, fish age, total lipid percentage and environmental concentration of the contaminant. In this study the lipid content of bagrid catfish is much higher than common carp and BAFs were also higher than found in later. Some earlier studies reported that no relationship between lipid percentages found in the fish and concentration of lipophllic chemicals in the fish Gobas, 1999.

3.4. Risk assessment of pesticides in the fish:

Fish consumption is known as the most common way for contaminants into human body Hoekstra et al., 2013. EDIs were calculated to assess potential pesticide exposure to humans with the concentration (mean values) of determined pollutants in the fish. Table 3 compares our calculated results with the ADI value issued by USEPA 2009. From the results the daily exposure to pesticides via consumption of fish from the river, potential risks are present as the calculated results are insignificantly high. Table 3 shows the health risk assessment figures for the two fishes. The THQs values for both the fishes were less than 1.0. The HI values of pesticides to humans via consumption of fish indicated lower health risk from the study area. The calculated *R* values of pesticides related to consumption of fish were lower than 1×10^{-4} indicating their cancer risks were negligible (USEPA 2009). Nevertheless, the calculated R value of heptachlor were found of high value than 1×10^{-4} in both two fishes, therefore which suggested the cancer risk of heptachlor associated with consumption of fish cannot be ignored.

Table 3
Health hazard index for pesticide in fish Bagrid and Common carp.

Name of pesticide	ADI (ug/kg/d)	EDI (ug/kg/d)	
		Bagrid	Common carp
Chlordane	0.016	0.079	0.071
Dimethoate	0.01	0.054	0.062
Malathion	0.007	0.063	0.013
Atrazine	0.005	0.102	0.068
Heptachlor	0.002	0.131	0.111
Dichlorvos	0.002	0.111	0.128
Azinphosmethyl	0.001	0.035	0.108
Cypermethrin	0.02	0.040	0.143

Table 4
Non-carcinogenic (THQ) and carcinogenic risks (R) of pesticide residues.

Chemical	Rfd $(mg/kg/day)^{-1}$	SF $(mg/kg/day)^{-1}$	THQ		R	
			Bagrid	Common carp	Bagrid	Common carp
Chlordane	0.00006	0.35	1.36E06	1.31E04	2.87E05	4.39E05
Dimethoate	0.0002		2.59E01	2.97E01		
Malathion	0.02	0.0038	3.05E03	6.40E04	2.31E07	4.86E08
Atrazine	0.035	0.22	2.81E03	1.87E03	2.17E05	1.44E05
Heptachlor	0.0005	4.5	2.51E01	4.49E06	5.66E04	4.81E04
Dichlorvos	0.0005	0.29	2.13E01	2.45E01	3.10E05	3.56E05
Azinphosmethyl	0.2		3.39E04	1.03E04		
Cypermethrin	0.01		3.91E03	1.37E02		

4. Conclusion

Bagrid and common carp two commonly consumed fish from river Ganga were collected from Narora site and were analyzed for pesticide residues. Residues found in the muscle tissues of fish show a slight change in concentration than found in water samples. More concentration of detected pesticides was found in Common carp than bagrid. The EDIs in our study were all insignificant and slightly higher than the ADI value. The HI of the detected pesticides was lower than 1.0, which implying lower human non-carcinogenic risk via consumption of fish from the river. Carcinogenic risks of detected pesticides to human exposure were negligible, but for heptachlor exposure to carcinogenic risk were high. Finally consumption of fish from the study river cannot be ignored with carcinogenic risk because of enormous use of pesticides in the agricultural basin.

Declarations

5. Acknowledgement:

Authors acknowledge Chairperson Department of Zoology for providing requisite facilities; simultaneously authors would like to acknowledge SICART, Sardar Patel Centre for Science and Technology Gujarat, India for providing GC-MS facilities.

References

Gupta, S., 2015. Rita rita (Hamilton, 1822), a threatened fish of Indian subcontinent. *Int. Res. J. Biol. Sci*, *4*(11), 70-73.

NGRBA, 2011. National Ganga River Basin Authority (Ministry of Environment and Forests, Government of India). Environmental and Social Management Framework (ESMF). Volume I - Environmental and Social Analysis January 2011. http://moef.nic.in/downloads/public-information/Draft%20ESA%20Volume%20I.pdf., 7-10.

Hoekstra, J., Hart, A., Owen, H., Zeilmaker, M., Bokkers, B., Thorgilsson, B. and Gunnlaugsdottir, H., 2013. Fish, contaminants and human health: quantifying and weighing benefits and risks. *Food and chemical toxicology*, *54*, 18-29.

Samanta, S., 2006. Organochlorine Pesticide Residues in Fish of River Ganga in West Bengal. *Pesticide Research Journal*, *18*(1), 104-108.

Malik, A., Ojha, P. and Singh, K.P., 2009. Levels and distribution of persistent organochlorine pesticide residues in water and sediments of Gomti River (India)—a tributary of the Ganges River. *Environmental Monitoring and Assessment*, *148*(1-4), 421-435.

Agnihotri, N.P., Gajbhiye, V.T., Kumar, M. and Mohapatra, S.P., 1994. Organochlorine insecticide residues in Ganga river water near Farrukhabad, India. *Environmental monitoring and assessment*, *30*(2), 105-112.

Villaverde-de-Sáa, E., Valls-Cantenys, C., Quintana, J.B., Rodil, R. and Cela, R., 2013. Matrix solid-phase dispersion combined with gas chromatography–mass spectrometry for the determination of fifteen halogenated flame retardants in mollusks. *Journal of Chromatography A*, *1300*, 85-94.

Kumar, B., Verma, V.K., Naskar, A.K., Chakraborty, P., Kumar, S. and Mukherjee, D., 2013. Human health risk from hexachlorocyclohexane and dichlorodiphenyltrichloroethane pesticides, through consumption of vegetables: estimation of daily intake and hazard quotients. *Journal of Xenobiotics*, *3*(1), e6-e6.

Rehana, Z., Malik, A. and Ahmad, M., 1996. Genotoxicity of the Ganges water at Narora (UP), India. *Mutation Research/Genetic Toxicology*, *367*(4), 187-193.

Aktar, M.W., Paramasivam, M., Sengupta, D., Purkait, S., Ganguly, M. and Banerjee, S., 2009. Impact assessment of pesticide residues in fish of Ganga river around Kolkata in West Bengal. *Environmental monitoring and assessment*, *157*(1-4), 97-104.

Sankararamakrishnan, N., Sharma, A.K. and Sanghi, R., 2005. Organochlorine and organophosphorous pesticide residues in ground water and surface waters of Kanpur, Uttar Pradesh, India. *Environment International*, *31*(1), 113-120.

Jabeen, F. and Chaudhry, A.S., 2011. Chemical compositions and fatty acid profiles of three freshwater fish species. *Food chemistry*, *125*(3), 991-996.

US EPA (2009). Risk-based concentration table. Philadelphia PA: United States Environmental Protection Agency, Washington.

Shah, Z.U. and Parveen, S., 2020. A review on pesticides pollution in aquatic ecosystem and probable adverse effects on fish. *Pollution research*, 39 (2), 309-321.

Gobas, F.A., Wilcockson, J.B., Russell, R.W. and Haffner, G.D., 1999. Mechanism of biomagnification in fish under laboratory and field conditions. *Environmental science & technology*, *33*(1), 133-141.

Muir, D. and Sverko, E., 2006. Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: a critical appraisal. *Analytical and bioanalytical chemistry*, *386*(4), 769-789.

Smedes, F., 1999. Determination of total lipid using non-chlorinated solvents. *Analyst*, *124*(11), 1711-1718.

INDIASTAT., 2018. www.Indiastat.com.

Nongbri, B.B. and Syiem, M.B., 2012. Analysis of heavy metal accumulation in water and fish (*Cyprinus carpio*) meat from Umiam Lake in Meghalaya, India. *International Multidisciplinary Research Journal*, *2*(2).

Nengroo, Z.R. and Rauf, A., 2019. Fatty acid composition and antioxidant activities of five medicinal plants from Kashmir. *Industrial Crops and Products*, *140*, 111596.

Figures

Figure 1

Sketch map of the sampling site.

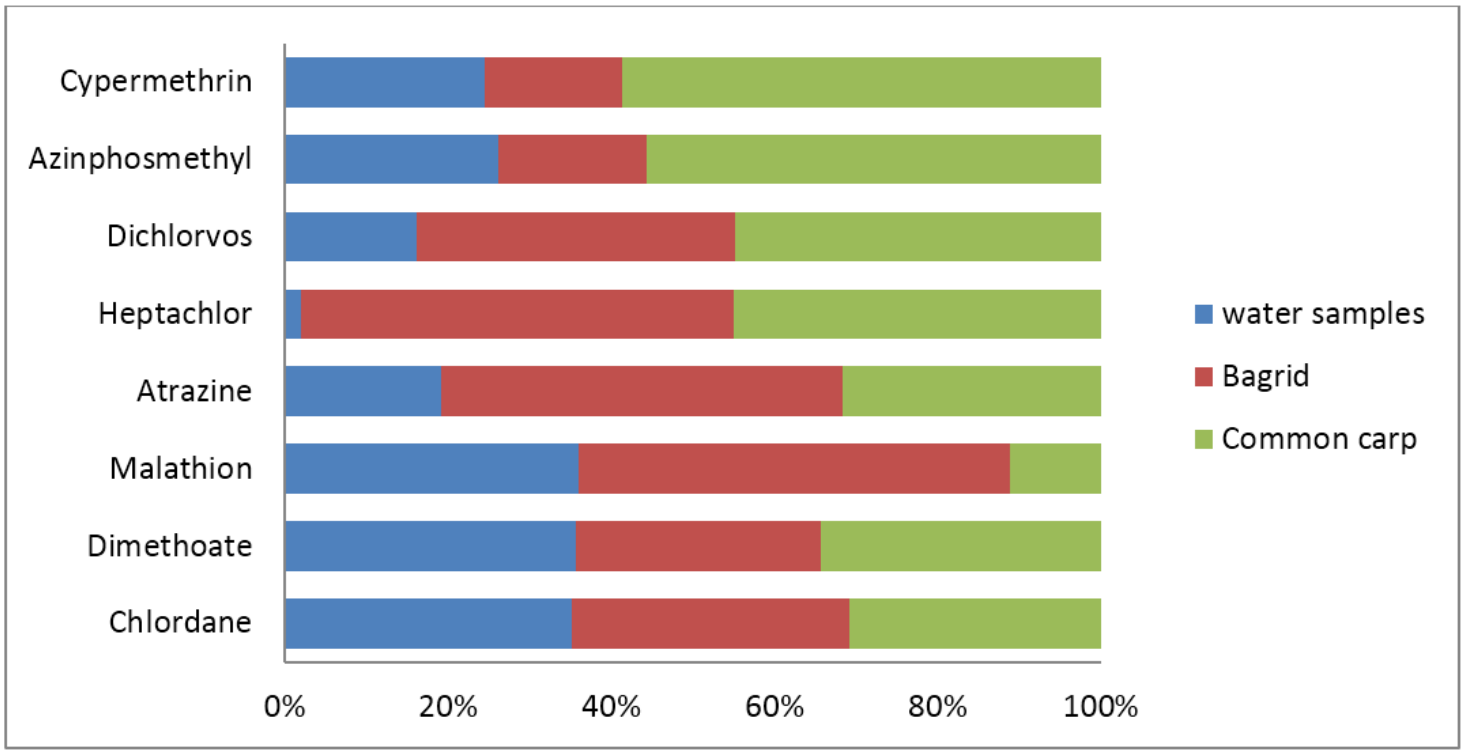

Figure 2

Relative abundance of pesticides in water samples, muscle tissue Bagrid and muscle tissue Common carp.

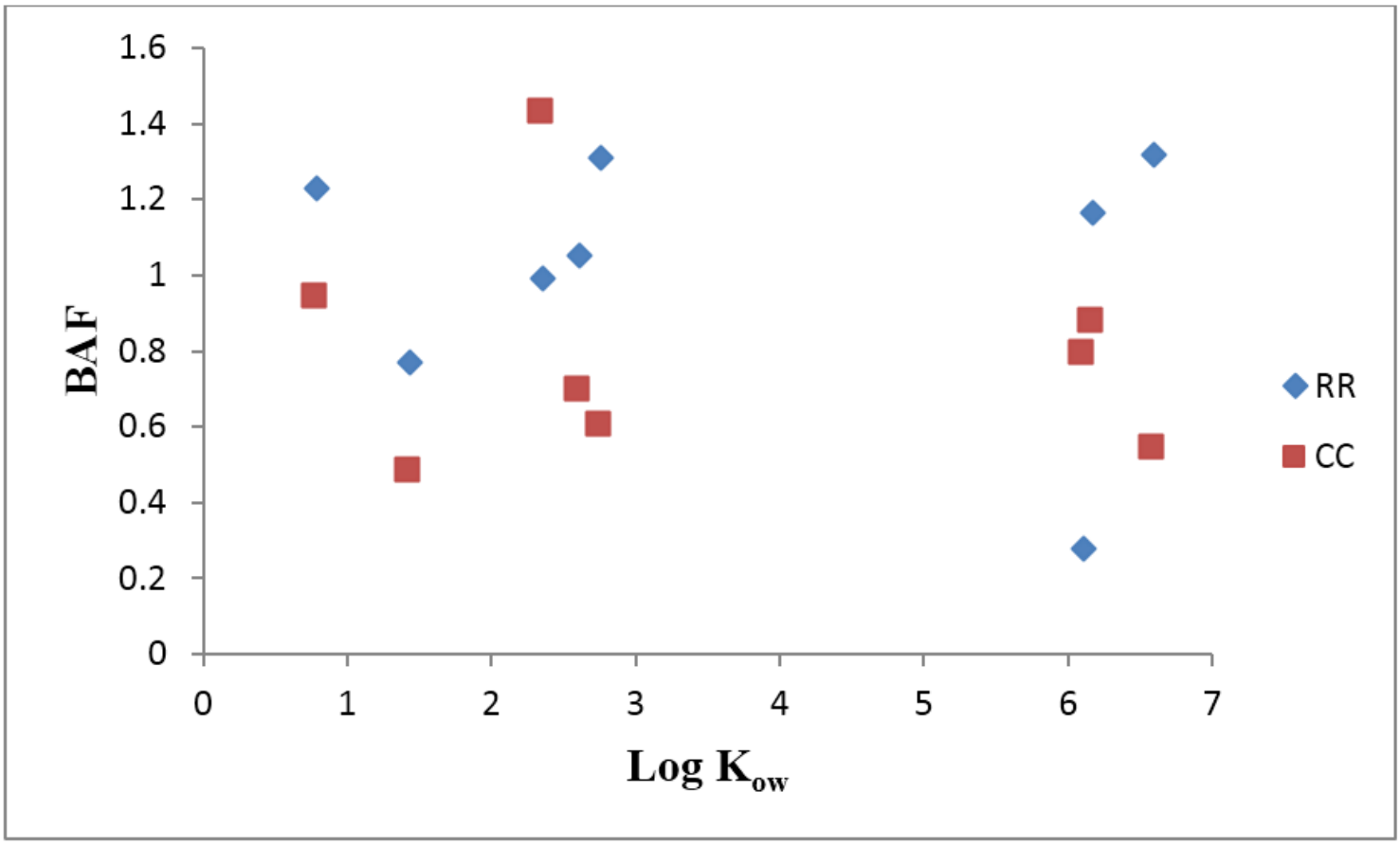

Figure 3

Bioaccumulation factors of the residues in the two fishes