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Tissue concentration of organochlorines, organophosphate and plasma cortisol in captured fish of polluted river Gomti at Jaunpur during post-monsoon season

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Abstract: *The objectives of the present investigation was to monitor the HCHs (isomers of hexachlorocyclohexane), DDTs (metabolites of dichlorodiphenyltrichloroethane), chlorpyrifos in tissue bioaccumulation and plasma levels of cortisol between the sampling sites of the unpolluted ponds of Gujartal, Jaunpur (reference site) and the polluted rivers the Gomti (Jaunpur) and the Ganga (Varanasi) as stress hormone of some edible catfishes during non-breeding season owing to industrialization. The insecticides were measured by the gas liquid chromatography (GLC) and plasma cortisol by Enzyme-Linked Immunosorbents Assay (ELISA). The results indicated that the presence of HCHs, DDTs and chlorpyrifos was much higher in the catfishes captured from the river Gomti than those of the river Ganga when compared to reference site. The plasma level of cortisol was low in the fishes captured from both the rivers. In conclusion, the fishes from polluted rivers showed high degree of contamination which caused stress and ultimately decreased the growth of edible fishes of riverine sources.*

Key words: Insecticides, Bioaccumulation, Wild catfishes, Cortisol, Stress hormone, River pollution.

Introduction

There is a ban on some insecticides in most of the industrialized nations including India; the occurrence of high concentrations of these compounds in ecosystems owing to continuous use prompted the need for the measurement of bioaccumulation in fishes of reverine systems. Pesticide residues in wild captured fish have been reported by workers (Kannan *et al.,* 1995; Antunes and Gil, 2004; Ferreira *et al.,* 2004; Abdallah and El-Greisy, 2006; Coat *et al.,* 2006; Amado *et al.,* 2006; Antunes *et al.,* 2007a, b. Afful *et al.,* 2010; Dhananjayan and Murlidharan, 2010; Pandey *et al.,* 2010; Fianko *et al.,* 2011; Kelly *et al.,* 2011). Bioaccumulations of insecticide residues in the dolphins found in the Ganges (Kannan *et al.,* 1994; Senthilkumar *et al.,* 1999a; Saeed *et al.,* 2009; Suneetha *et al.,* 2010; Sarvanan *et al.,* 2010; Salah and Mahmoud, 2011; Margarita, 2011) and fish as well as in food stuffs (Tanabe *et al.,* 1991; Kannan *et al.,* 1995) have also been reported. It has been documented that 40% of pollution in the Ganga owing to sewage discharge and 13% to chemical waste released from factories. The data indicated that fish, birds, reptiles, mammals and other species inhabiting the polluted environment with a number of known and unknown synthetic compounds (Lenardon *et al.,* 1984; Senthilkumar *et al.,* 1999a, b, 2001) suffer from reproductive problems (Colborn and Clemment, 1992; Colborn *et al.,* 1993). Recently, thirteen organochlorines pesticides in eighteen fish species have been reported having high concentrations (Zohu *et al.,* 2007).

Recently, bioaccumulation of HCHs and DDTs in ovary and low levels of plasma estradiol-17α in the catfish and carp of the polluted Gomti and the Ganga from north India has been reported, disturbing the reproductive physiology of some freshwater food fishes during pre-monsoon (Agnieszka and Witczak, 2010; Wan *et al.,*

2010; Mohammed, 2011). Degenerated ovarian follicles and abnormal ovaries also have been reported in the fish living for prolong durations in Lake Van and Karasu river, eastern Turkey owing to chemical exposure (Unal *et al.,* 2007; Mohammed, 2011).

Cortisol has been reported two peaks in the annual reproductive cycle of *H. fossilis* (Lamba *et al.,* 1983) and its role well documented in reproduction in female teleost (Bry, 1985). The cortisol in fish has been identified as metabolic hormone (Vijayan *et al.,* 1994; Shankar *et al.,* 2007; Shanker and Kulkarni, 2007) having multifaceted action. It has been also considered as an important stress hormone produced in fish (Barry *et al.,* 1995; Wendelaar Bonga, 1997; Stouthart *et al.,* 1998; Jentoft *et al.,* 2002; William and Berlinsky, 2006). One of the responses of fish to most forms of environmental stress is reduction of the growth rate, a response which may have important economic bearing on the aquaculture industry (Pickering, 1990). Glucocorticoids can either inhibit or stimulate reproductive physiology depending upon the timing of the annual cycle of species (Brann and Mahesh, 1991). The hormone cortisol plays a pivotal role in teleost stress response but relatively few species have been studied in depth with respect to the level of cortisol in the blood under different stages of development and environmental conditions (Pottinger and Carrick, 2000). The seasonal study carried out in fish adrenal tissue in relation to reproductive activity indicate that the adrenal cells are hyperactive during breeding phase of the fish and it is also reported that cortisol is shown to interfere with reproductive function in mature and maturing rainbow trout (Chakraborti *et al.,* 1987). Stress induced facilitation of the cortisol response in 17α-hydroxylase deficient in C*yprinus carpio* has also been reported (Agnieszka and Witczak, 2010).

Most of the studies are restricted up to total pesticide residues or only reproductive steroid hormones during the breeding seasons; but reports are not available for non-breeding season under insecticide stress of the fishes inhabiting the polluted rivers the Gomti and the Ganga owing to industrialization. Since pesticide causes reproductive and metabolic dysfunctions, it is necessary to monitor the tissue bioaccumulation of HCHs, DDTs, chlorpyrifos and plasma levels of cortisol in some edible catfishes of the un-polluted ponds of Gujartal, Jaunpur (considered as reference site) and the polluted rivers the Gomti and the Ganga during non-breeding season. The objective of this study is to compare the insecticide bioaccumulations in brain, liver and abdominal muscles, and plasma levels of cortisol as stress hormone during non-breeding season. Taking the above facts into account the persistence of chemicals like organochlorine insecticides (OCs) and organophosphates (OPs) in these rivers, and their eventual incorporation in the food chain finally to humans are necessitated. It is imperative to monitor the presence of residues of toxic chemicals and stress-hormone owing to contaminants in order to keep wild fish species in the riverine resources edible for human beings.

Materials and Methods

Characteristics of fish sampling sites from unpolluted pond (reference site) and polluted rivers the Gomti and the Ganga : The ponds of Gujartal are situated about 30 km away from the Gomti river (city of Jaunpur) and have an area of nearly 21 hectare. These include several ponds like nursery, rearing and water areas having no chance of being externally mixed with any type of pollutants. Hence this site is considered as reference site. The captured fish of this site have been given in Fig. 1.

The river Gomti, one of the major tributaries of the river Ganga originates from Pilibhit district of Uttar Pradesh of north India flowing through the districts of Pilibhit, Shajahanpur, Sitapur,

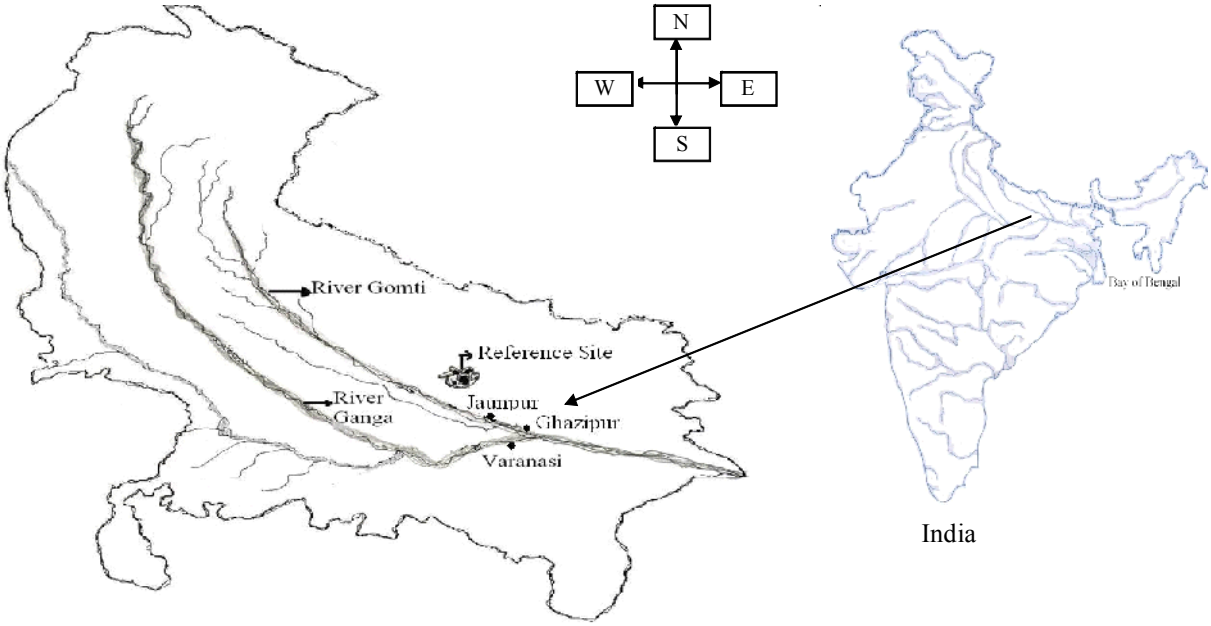

Fig. 1.

Lucknow, Barabanki, Sultanpur, Jaunpur and Ghazipur in Uttar Pradesh and drains a catchments area of about 25,000 km^2 and traverses a total distance of about 730 km before finally merging with the river Ganga in Ghazipur district which is about 30 km northeast of Varanasi. Lucknow, Sultanpur and Jaunpur are the three major urban settlements on the banks of the river. The river, receives the untreated waste water and effluents from Lucknow, Jagdishpur, Sultanpur and Jaunpur as it winds its way through more than 40 wastewater drains. The different range of insecticides due to effluent discharge (aldrin 0.7 - 16.0; α-BHC 0.3 - 39.0; β-BHC 0.2 - 3,674.0; γ-BHC 0.03 - 240.0; δ-BHC 9.0 - 497.0; ΣBHC 0.4 - 2,183.0; *o,p*'-DDT 148.0 - 1,324.0; *o,p*'-DDT BDL - 11.0; *p,p*'-DDT BDL-11.0; *p,p*'-DDD BDL - 2.0; *p,p*'-DDE 1.0 - 3.0; ΣDDT 1.0 - 1,337.0; endosulfan 0.3 - 667.0) in Gomti river water (ng/L) have been reported by Singh *et al*. (2005).

The sacred river Ganga originates from the Gangotri glacier in Garhwal in the Himalayas and runs through industrially developed and agricultural based vast states of Uttar Pradesh, Bihar, Jharkhand and West Bengal before emptying into the sea in the Bay of Bengal traversing a total distance of about 2,570 kms. Among the various pesticides selected for their residue level monitoring in water of the river Ganga, the organochlorines (DDT, BHC and endosulfan) have been most frequently detected all through the study period as compared to the organophosphorous pesticides (Haldar *et al*. 1989; Nayak and Das, 1995; Ahmad *et al*., 1996). The main reason for this seems to be the stability and persistence nature of the organochlorines in the natural aquatic systems.

Collection of samples : The original research reported herein was conducted under ethical guidelines for the treatment of animals in behavioral research and teaching (Animal Behavior, 1998). For the comparative study of HCHs, DDTs, chlorpyrifos in brain, liver and abdominal muscles and plasma cortisol levels were collected from the captured catfishes of reference site (*Rita rita* and *Bagarius bagarius*) and polluted rivers the Gomti (*Rita rita* and *Bagarius bagarius*) and the Ganga (*Rita rita* and *Clupisoma garua*) during non-breeding season

in order to assess the status of pollutants causing stress. Details of different catfish species which were captured from different sampling sites during non-breeding season have been given in Fig. 1. Fish were captured by drag net with the help of local fishermen from each sampling site. In all thirty fish were used in this study from all sites. Blood was taken by caudal vein in separate heparinized culture tubes for pesticide residue and hormone assay and fish were kept in the ice-box and brought to laboratory for the collection of brain, liver and abdominal muscles. Tissues were kept at -20^{0}C till subsequent analysis. Within two days all the samples were extracted and cleanup for the analysis and sent to Indian Institute of Toxicology Research (IITR), Lucknow (UP) for GLC. Heparinized blood was centrifuged at 5,000 rpm for 15 min at 4^{0}C and plasma was separated for hormone assay.

Extraction and cleanup procedure for organochlorines : HCHs (α, β, γ and δ isomers) and DDTs (*o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE and *p,p'*-DDT metabolites) were analyzed using the methods as described by Kannan *et al*. (1995) with certain modifications.

Extraction and cleanup procedure for organochlorines insecticides : HCHs (α, β, γ and δ) and DDTs (*o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE and *p,p*'-DDT) were analysed following the method described by Dale *et al.* (1965) later with modifications as given below. The fatty tissues from five fish of one species of each site was taken separately in duplicate (500 mg fatty tissues- brain and ovary or 1000 mg non-fatty tissues- liver), were homogenized separately in a homogenizer cup by using 7 ml of formic acid and 5 ml *n*-hexane (GLC grade only). Each homogenate was transferred separately in the conical flask by rinsing the cup and teflan pestle twice with 10 ml of *n*-hexane each time. The homogenate was shaken on shaker for 1 h at 37^{0}C. The upper layer of *n*-hexane was separated in another conical flask and the aqueous layer was again extracted with 10ml of *n*-hexane two times (20 ml) by similar fashion. The organic layer was collected together. The residue of formic acid from the extract was removed by shaking the organic phase with distilled water (50ml) in separating funnel. This extract was demoisturised by passing through anhydrous sodium sulfate bed. The demoisturised extract was concentrated on rota evaporator up to 1 ml and finally volume was made up to 2 ml in volumetric flask with help of *n*-hexane by rinsing the rota evaporator flask. The cleanup procedure for required pesticide was done by adding 2ml concentrate sulphuric acid in the above extracted 2 ml extract. This mixture was vortexed and centrifuzed to separate the aqueous and non-aqueous layer. The upper *n*-hexane layer was separated in 2 ml clean volumetric flask with the help of Pasteur pipette for individual samples. This concentrated sample was applied on GLC for identification and quantification of HCHs and DDTs.

Quantitative analysis: The quantitative analyses of OC (HCHs and DDTs) were performed by Gas Liquid Chromatography (Nucon 5765) equipped with ^{63}Ni electran capture detector (ECD). The GLC column (6 inch x 1/8 inch i.d.) filled with 80-100 mesh, Gas Chrome coated with a mixture of 1.5 / SP-2250 and 1.95% SP-2401. Oven temperature was 190^{0}C. The injector and detector temperature were kept at 250^{0}C. Nitrogen IOL-AR grade I was kept as carrier gas (flow rate 60 ml/min). The volume of injection for each unknown samples and standard were 2-5 ml depending upon concentration of pesticides in samples. Pesticides were estimated from individually resolved peak of samples with corresponding peaks of standards.

Extraction and cleanup procedure for organophosphate : The method of extraction was used with little modification as have been described earlier (Lino and Noronha da Silveira, 1994). Known amount of each tissue from five individual separate groups in duplicate (0.5 g

fatty tissue- brain, 1.0 g non-fatty tissue - liver and abdominal muscles) were collected separately and then homogenized with 5 ml of *n*-hexane. The content was then transferred into conical flask by adding the wash (10 ml) of Teflon pestle twice with *n*-hexane (20 ml). The homogenate was shaken 1 hr for the extraction of pesticides at 37^{0}C. The *n*-hexane layer was decanted and residue was re-extracted with 10 ml of *n*-hexane twice after 30 min shaking. The extracted contents were filtered with Whatman filter paper No. 1. Now contents were passed through the anhydrous sodium sulfate bed and concentrated on rota evaporator up to 5 ml. This concentrated extract was passed through the bed of anhydrous sodium sulfate (1 g), activated Florisil (4 g) and anhydrous sodium sulfate (1 g) prepared in column 400 mm x 20 mm i.d. for pesticides cleanup. The contents was eluted with 200 ml of diethyl ether : *n*-hexane mixture (6 : 94) for the extraction of chlorpyrifos. Now the contents were concentrated into 1 ml on evaporator and finally made up the volume to 2 ml by rinsing the evaporatory flask with *n*-hexane for the analysis and quantification of chlorpyrifos by GLC.

Quantitative analysis : The quantitative analyses of organochlorines (HCHs and DDTs) and organophosphate (chlorpyrifos) were performed by Gas Liquid Chromatography (Nucon 5765) equipped with ^{63}Ni electron capture detector (ECD). The GLC column (6 inch x 1/8 inch i.d.) filled with 80-100 mesh, Gas Chrome coated with a mixture of 1.5 / SP-2250 and 1.95% SP-2401. Oven temperature was 190^{0}C. The injector and detector temperature was kept at 250^{0}C. Nitrogen Indian Oil Limited-Analytical Reagent (IOL-AR grade I) was kept as carrier gas (flow rate 60 ml/min). The volume of injection for each unknown samples and standard were 2-5 ml depending upon concentration of pesticides in samples. Pesticides were estimated from individually resolved peak of samples with corresponding peaks of standards. The confirmation of chlorpyrifos was done on NPD detector in same column and conditions.

Recovery studies: Representative samples of each matrix were spiked with known concentrations of the HCHs, DDTs and chlorpyrifos, and kept at least for 3 h. The samples were extracted and cleanup for analysis using GLC equipped with ECD system. The pesticide residues in fish tissues were calculated and the recoveries were between 93.02 - 95.5% for individual HCH, DDT and chlorpyrifos. The recoveries factors were 1.0342, 1.0492 and 1.0523 for HCH, DDT and chlorpyrifos respectively. Detection limit was 0.1 ng /g (ppb) for all studied insecticides.

Estimation of Cortisol hormone from plasma by Enzyme-Linked Immunosorbents Assay (ELISA): The methodology used for the extraction of free steroid hormone was same as described by Singh and Kime (1995), and the method was followed as given in ELISA kit (DiaMetra, Italy).

Steroids were extracted twice from plasma (400 ml or less as appropriate) with 5 ml of distilled dichloromethane. The details of ELISA have been described elsewhere (Shankar and Kulkarni, 2007).

Statistical analysis: Insecticide residues were expressed ng/g (ppb) of tissues and ng/ml plasma cortisol [Mean ± standard error of the mean (SEM), n = 5]. Results were analyzed by Students *t*-test (Bruning and Kintz, 1977) and two / one - way analysis of variance (ANOVA TW / OW) by Microsoft Excel tool pack data analysis (ANOVA) two factor with replication.

Results and Discussion

Analyses of variance have revealed that the tissue concentrations of insecticides and plasma cortisol varied in the catfishes captured from the reference site and the polluted rivers during non-breeding season. The summary of ANOVA have been given in the Figs. 2-7.

Comparison of bioaccumulation of HCHs, DDTs and chlorpyrifos in catfishes between reference site and polluted rivers the Gomti and the Ganga during non-breeding season

Bioaccumulation of HCHs : The catfish captured from the Gomti has high bioaccumulations for α, β, γ and δ isomers of HCH in liver, brain and abdominal muscles as compared to the same species of reference site. The percentage of δ-HCH of the ΣHCH was high in liver, brain and abdominal muscles with low percentage of b and ä isomers in both the catfish captured from polluted rivers Gomti and Ganga during non-breeding season. The preferential order of tissue bioconcentrations of ΣHCH was recorded in catfish captured from polluted rivers Gomti (liver > brain > muscles) and Ganga (brain > muscles > liver). The ΣHCH was higher in the brain of catfish captured from the Ganga as compared to those of the Gomti (Fig. 2). Another species of catfish, captured from the Gomti and the Ganga showed significantly high tissue bioaccumulation of isomers of HCH than the catfish captured from reference site. The preferential order of tissue bioaccumulation for the polluted fish was brain > muscles > liver. The γ-HCH was in high percentage of bioaccumulation of ΣHCH (Fig. 3). The *B. bagarius* has higher bioaccumulation of HCH than *R. rita* captured from same polluted the Gomti and the Ganga respectively.

Bioaccumulation of DDTs: The catfish captured from the river Gomti showed high bioaccumulations of *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD and *p,p'*-DDT metabolites of DDT in liver, brain and abdominal muscles as compared to catfish from reference site and polluted river Ganga. The percentages of bioaccumulations were different in different tissues. It was much higher in polluted fish than those of the reference site. Among the metabolites of ΣDDT, the percentage of *p,p'*-DDT was maximal as compared to the other metabolites. The preferential order of tissue bioaccumulation of ΣDDT varied in the catfish captured from polluted river Gomti (muscles > brain > liver) and Ganga (muscles > liver > brain), but the magnitude of bioaccumulation was very high in catfish captured from the river Gomti. The pesticide residue in the catfish captured from the Gomti revealed that ΣDDT > ΣHCH in catfish captured from the Ganga (Fig. 4). The preferential order of ΣDDT bioaccumulation (muscles > brain > liver) was noticed in both the catfishes captured from the polluted rivers as compared to the fishes captured from reference site. In general, *p,p'*-DDT was high among all metabolites of DDT in all tissues of both the catfishes captured from polluted rivers (Fig. 5).

Bioaccumulation of chlorpyrifos: The tissue bioaccumulation of chlorpyrifos was low compared to organochlorines in the captured catfishes from polluted rivers. The preferential order of tissue bioaccumulation was noticed for catfish (muscles > brain > liver) captured from polluted rivers Gomti and Ganga (liver > brain > muscles). The bioaccumulation of chlorpyrifos in the tissues of catfish captured from reference site was below detection limit (BDL) while it was detected in the catfish captured from polluted river Gomti and catfish captured from the river Ganga (Fig. 6).

Comparison of plasma cortisol in the catfishes of reference site and polluted rivers the Gomti and the Ganga during non-breeding season: The levels of plasma cortisol declined in the catfish captured from the Gomti and the Ganga when compared to those of reference site. Similarly, the catfishes captured from the Gomti and the Ganga respectively showed decrease in the plasma cortisol level as compared to catfish captured from reference site. The magnitude of plasma cortisol was low in the captured fish from the Ganga as compared to the Gomti (Fig. 7).

Recently (Singh and Singh, 2008) it has been reported the presence of exceeded level of MRL

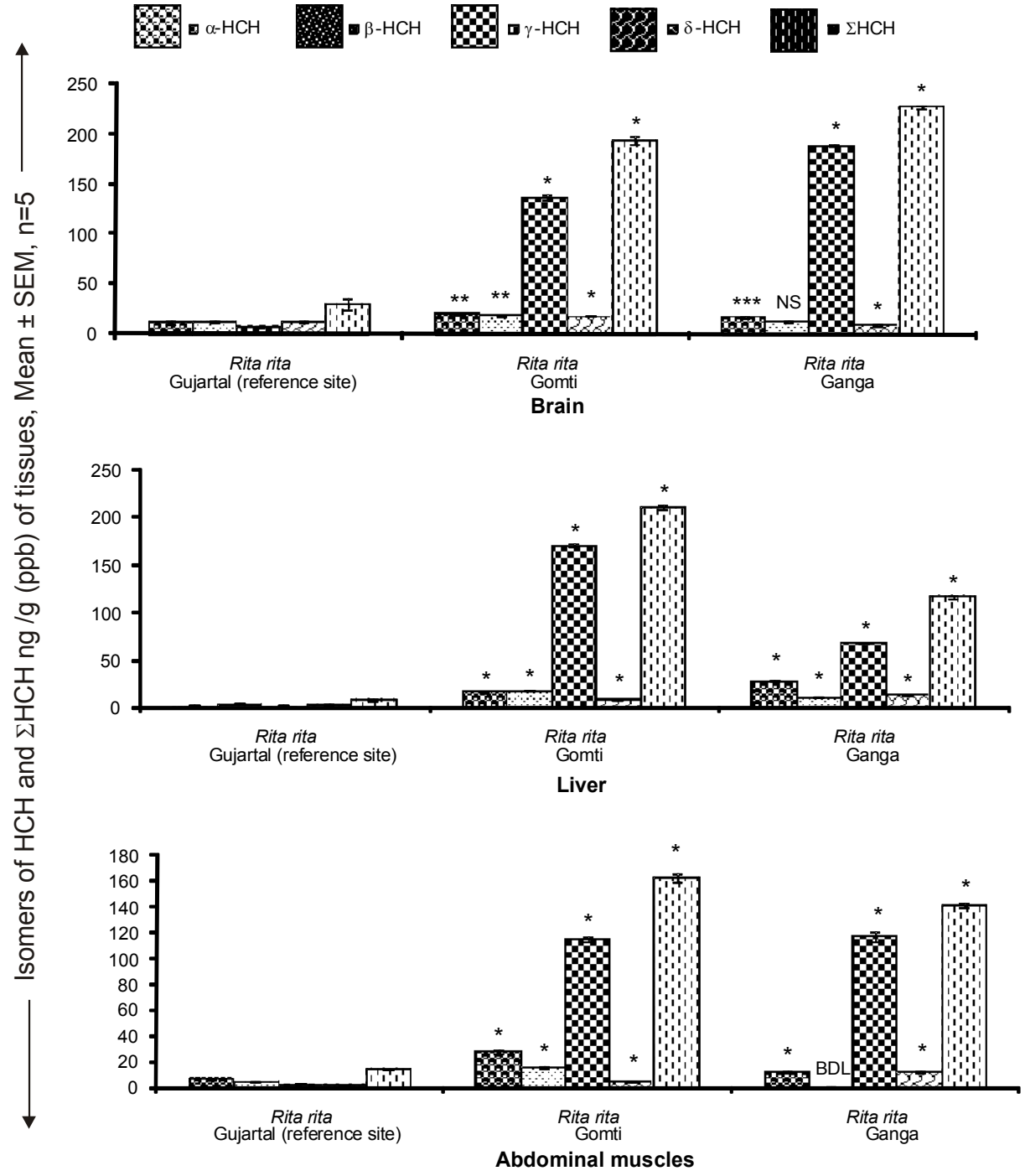

Fig. 2. Comparison of isomers of HCH in brain, liver and abdominal muscles of captured catfish between reference site and polluted rivers Gomti and Ganga during non-breeding season. ΣHCH = α+β+γ+δ isomers of HCH. BDL-below detection limit. Results of isomers of HCH of fish tissues from reference site versus fish captured from rivers Gomti and Ganga were compared by Students t-test. The level of significance (P)- *P< 0.001; **P< 0.005; ***P< 0.02; NS. NS- not significant. ANOVA (TW): tissue F-4424.67 P< 0.001; isomers of HCH F-456.52 P< 0.001; tissues x isomers of HCH F-175.61 P< 0.001.

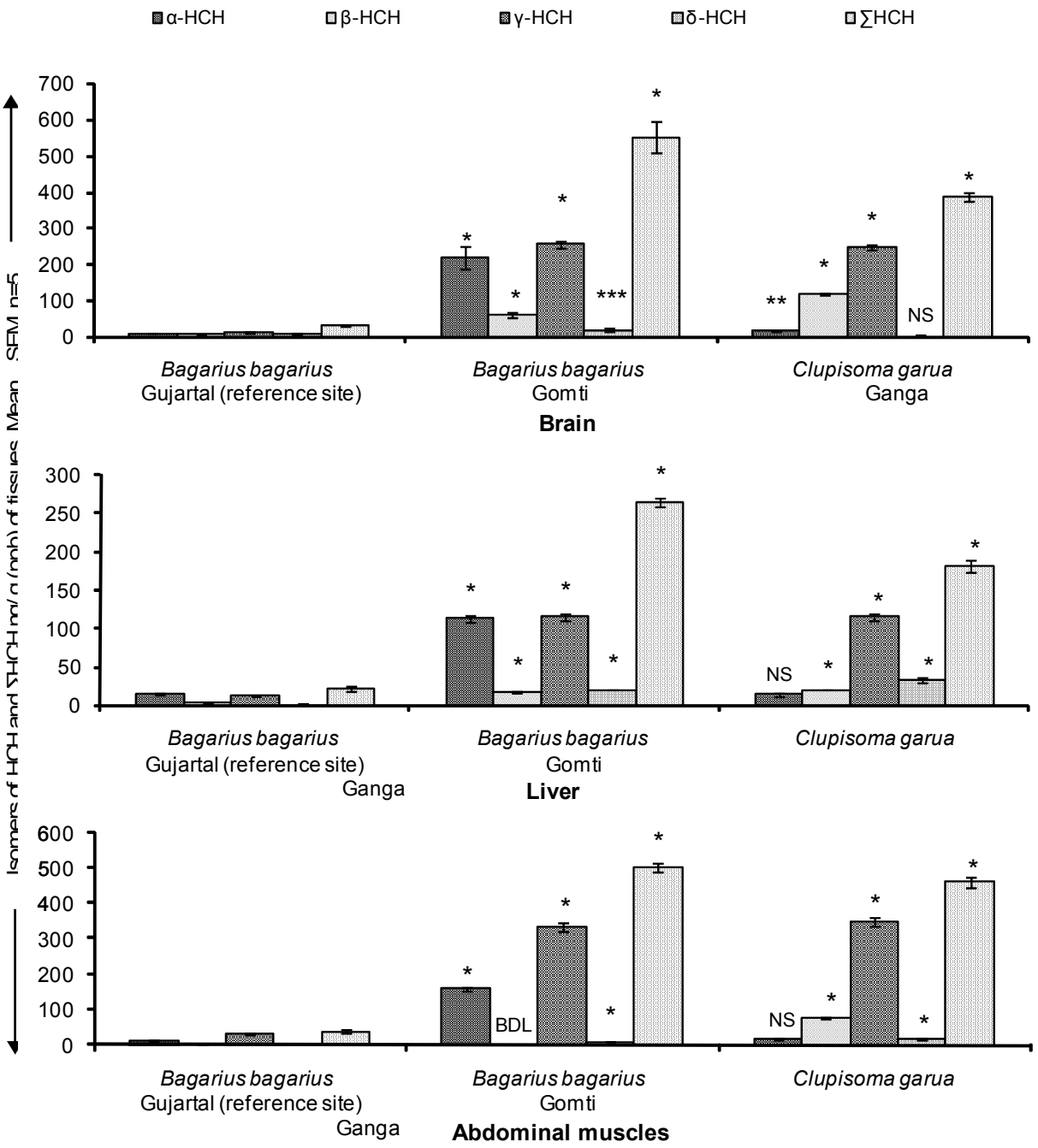

Fig. 3. Comparison of isomers of HCH in brain, liver and abdominal muscles of captured catfish between reference site and polluted rivers Gomti and Ganga during non-breeding season. ΣHCH = α+β+γ+δ isomers of HCH. BDL-below detection limit. Results of isomers of HCH of fish tissues from reference site versus fish captured from rivers Gomti and Ganga were compared by Students t-test. The level of significance (P)- *P< 0.001; **P< 0.005; ***P< 0.05; NS. NS- not significant. ANOVA (TW): tissue F-630.74 P< 0.001; isomers of HCH F-248.81 P< 0.001; tissues x isomers of HCH F-39.86 P< 0.001.

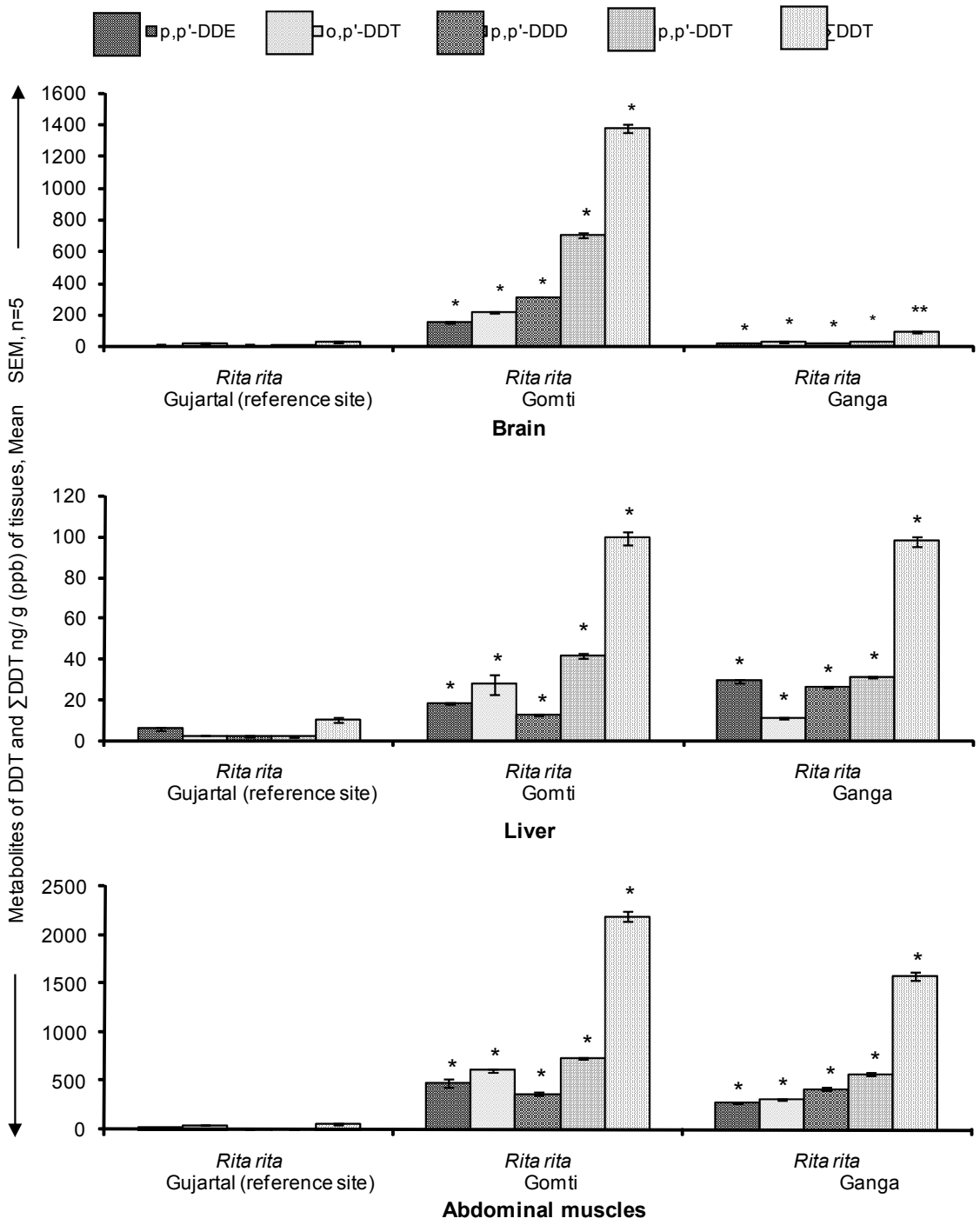

Fig. 4. Comparison of metabolites of DDT in brain, liver and abdominal muscles of captured catfish between reference site and polluted rivers Gomti and Ganga during non-breeding season. ΣDDT = p,p' -DDE + o,p'-DDT + p,p'-DDD + p,p'-DDT. Results of metabolites of DDT of fish tissues from reference site versus fish captured from rivers Gomti and Ganga were compared by Students t-test. The level of significance (P)- *P< 0.001; **P< 0.005. ANOVA (TW): ANOVA (TW): tissue F-2074.20 P< 0.001; metabolites of DDT F-5525.53 P< 0.001; tissues x metabolites of DDT F-683.04 P< 0.001.

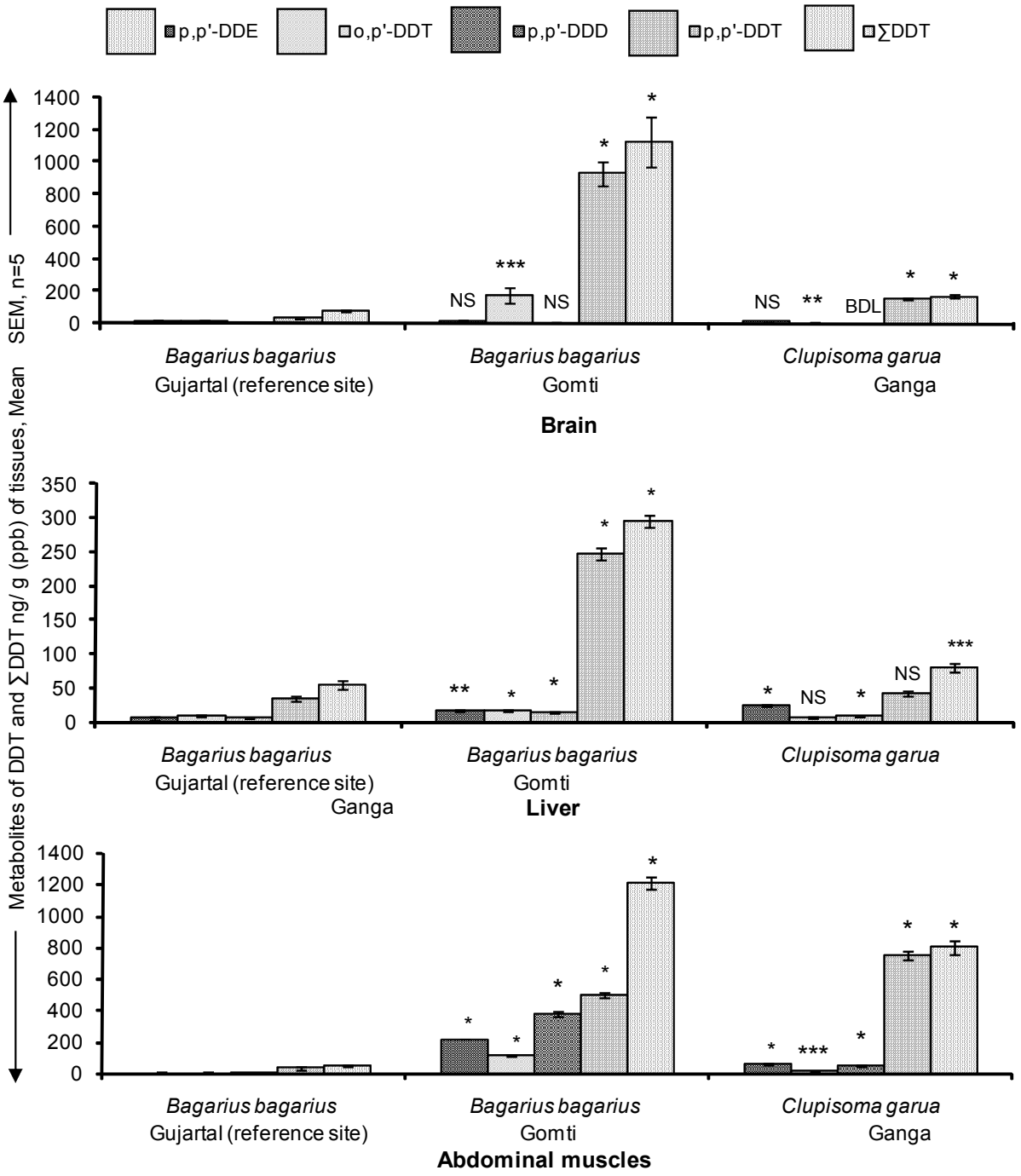

Fig. 5. Comparison of metabolites of DDT in brain, liver and abdominal muscles of captured catfish between reference site and polluted rivers Gomti and Ganga during non-breeding season. ΣDDT = p,p' -DDE + o,p'-DDT + p,p'-DDD + p,p'-DDT. BDL-below detection limit. Results of metabolites of DDT of fish tissues from reference site versus fish captured from rivers Gomti and Ganga were compared by Students t-test. The level of significance (P)- *P< 0.001; **P< 0.005; ***P< 0.02; NS. NS- not significant. ANOVA (TW): tissue F-345.17 P< 0.001; metabolites of DDT F-339.55 P< 0.001; tissues x metabolites of DDT F-73.37 P< 0.001.

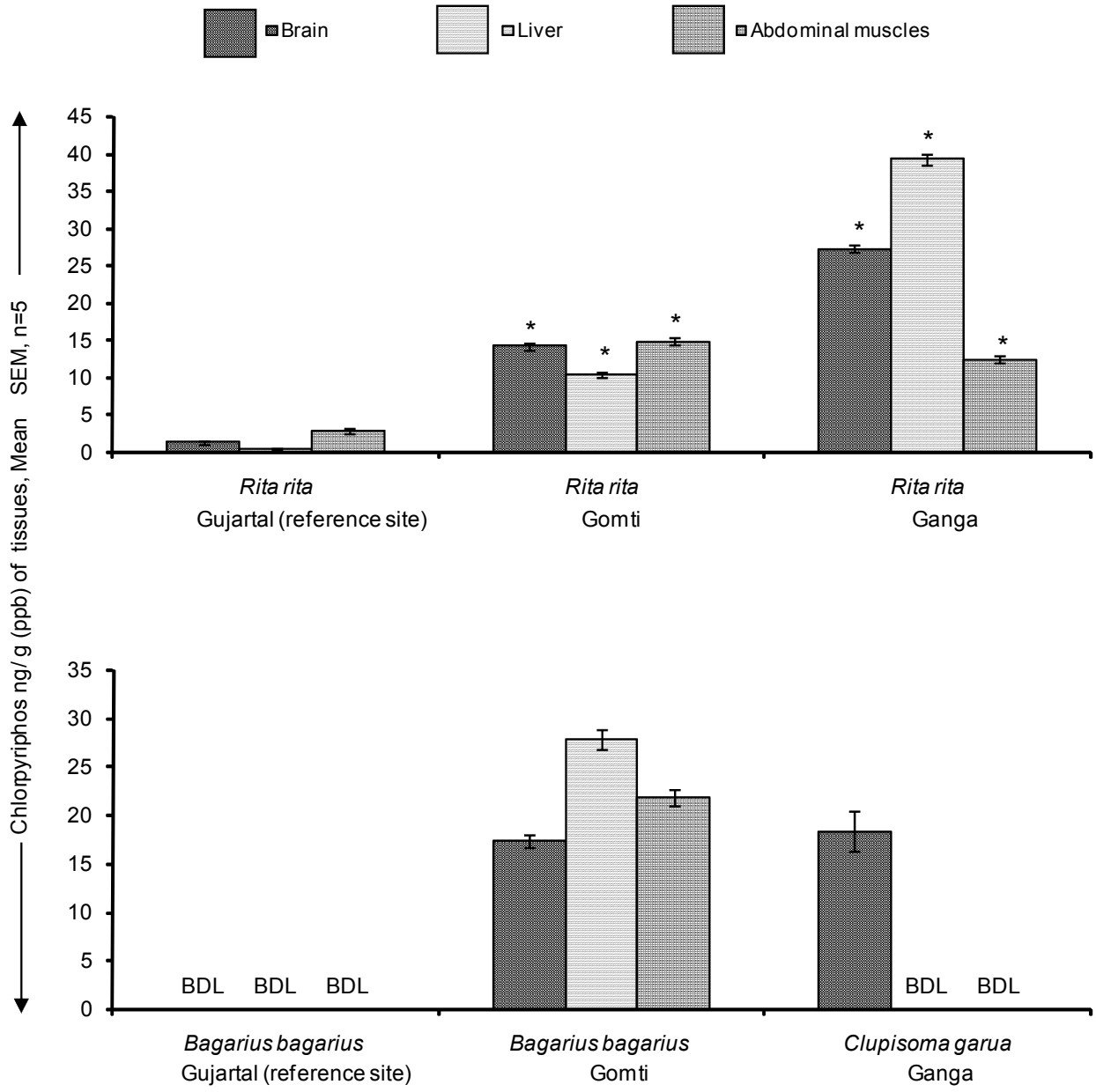

Fig. 6. Tissue bioaccumulations of chlorpyrifos in brain, liver and abdominal muscles in catfishes, captured from the reference site (R. rita and B. bagarius) and polluted rivers Gomti (R. rita and B. bagarius) and Ganga (R.rita and C. garua) during non-breeding season. Results of bioaccumulation of OP in fish tissues of reference site versus fish captured from polluted rivers Gomti and Ganga were compared by Students t-test. BDL- below detection limit.The level of significance (P)- *P< 0.001. ANOVA (TW): tissue F-667.04 P< 0.001; pesticide F-317.29 P< 0.001; tissue x pesticide F-191.55 P< 0.001

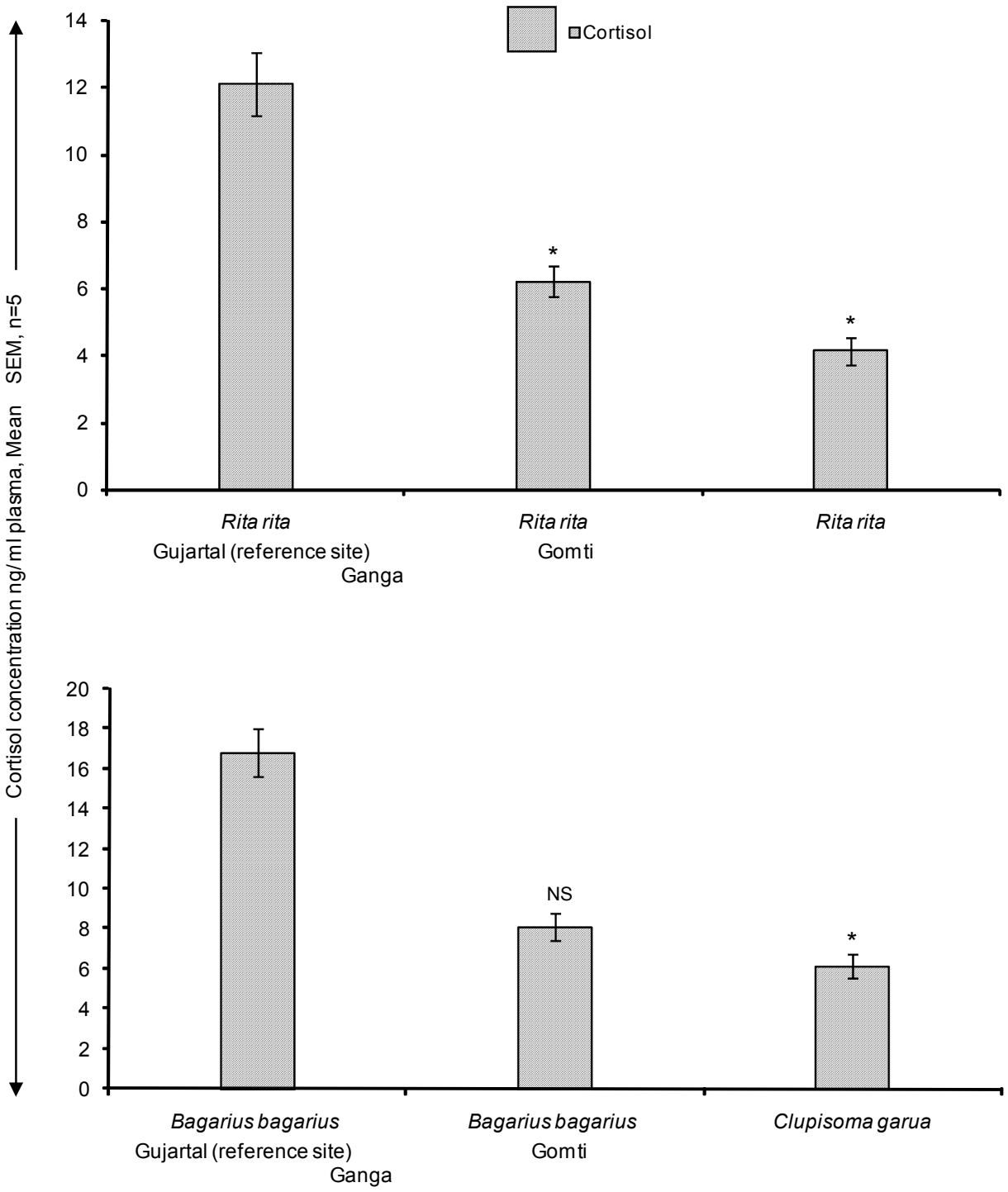

Fig. 7. Comparison of plasma levels of cortisol during non-breeding season in the catfish captured from the reference site (R. rita and B. bagarius) and polluted rivers Gomti, Jaunpur (R. rita and B. bagarius) and Ganga, Varanasi (R. rita and C. garua).Results were compared from non polluted fish to polluted fish by Students t-test. The level of significance (P)- *P< 0.001. ANOVA (OW): F-655.35 P< 0.001

of HCH and DDT in tissue bioaccumulation and decreased levels of estradiol-17β disturbing the reproductive physiology in the fishes captured from river Gomti and Ganga during breeding season. But, during non-breeding season the ng/g of tissue bioaccumulation of insecticides was lower than the fishes captured during breeding season which may be owing to the contaminants being swept away in the forceful flow of the river during rainy season. However, the MRL have been recorded for ΣHCH during non-breeding season in the captured fishes while ΣDDT was within MRL. The percentage of tissue bioaccumulations of γ-HCH isomer (lindane) was detected more than the other isomer which may be due to isomerization of α- and β-HCH. This transformation could have been caused by bacterial activity and ultraviolet radiation in the water column which ultimately sink to the sediments and finally get bioaccumulated in fish tissues. These findings get support from the report of Lenardon *et al*. (1984). Further it has been also demonstrated by El Beit *et al*. (1981) that γ-HCH is more resistance to biological and chemical degradation under aerobic conditions. Some researchers have shown that isomer α- and β-HCH has very high mammalian toxicity (Tomizawa, 1977), β-HCH toxicity in *P. reticulata* (Wester *et al.,* 1985) and γ-HCH toxicity in *H. fossilis* (Singh *et al.,* 1993). The bioaccumulation of ΣHCH was higher in the catfish captured from the river Gomti than the other catfishes captured from the river Ganga indicating that the Gomti is more highly polluted than the Ganga in relation to HCHs pollution. It is suggested that among catfishes there are degree of pesticide bioaccumulation of HCHs depending upon habit and habitat of the fish. The catfish which were captured from the bottom of the rivers showed high degree of bioaccumulation than the catfish which are not found at bottom of the river. The reason may be due to higher levels of presence of pesticide and water flow is less than the surface water. The results of Kaphalia *et al*. (1986) have shown that carnivorous fish have higher levels of insecticides than herbivores. The presence of HCHs and DDTs has been reported in river Gomti (Singh *et al.,* 2005) and Ganga (Nayak and Das*,* 1995; Haldar *et al.,* 1989; Ahmad *et al.,* 1996). It may be one of the major factors for bioaccumulation of different HCHs, DDTs and chlorpyrifos in fish tissues. The findings of the present investigations showing bioaccumulation of HCH, DDT and chlorpyrifos in catfishes of the Gomti and the Ganga support the above observations.

The DDTs studied in catfishes of the river Gomti and Ganga during non-breeding season have indicated its high content in abdominal muscles and brain. Results indicated that ΣDDT bioaccumulation was *R. rita* > *B. bagarius* > *C. garua* for insecticide bioaccumulation. These observations get support from the results of Kumari *et al*. (2001) who has demonstrated the presence of DDT residues in fish tissues captured from the river Ganga and concluded that catfish has the maximum DDT residues than the carp. Some researchers (Singh *et al.,* 1988; Singh *et al.,* 1997) have reported that nearly 85% of the total DDT produced in India is used for mosquito-control. Therefore, the levels of total DDT observed could be attributed to municipal-waste water discharge from the residential area into the river. It may be one of the causes of bioaccumulations in various tissues of fish ultimately reaching to the human being through the food chain. The DDT compound is also used for the control of sand flies (*Phleobotamus argintepes* and *P. papatasi*), the vector of kala-azar disease, in areas near the Gomti and the Ganga. Disposal of wastes from several insecticide manufacturing factories located along the banks of the Gomti and the Ganga have also contributed to pesticidal contamination (Haldar *et al.,* 1989; Singh *et al.,* 2005). Presence of HCHs and DDTs in bioaccumulation suggests its recent increased use in India.

Tanabe *et al*., 1991; Kannan *et al*. (1995) have reported the bioaccumulation of organochlorines in several species of fish collected from fish market in Bangkok, eastern and southern Asia and Oceania without considering either sex steroid or stress hormones during non-breeding phase. These authors have indicated the range of ΣHCH and ΣDDT including other insecticides which did not exceed MRL. The present studies have indicated that the catfish which were captured from the Gomti and the Ganga have exceeded the MRL levels for HCHs as well as decreased cortisol during non-breeding season causing the decreased growth of the fishes.

We have noticed the plasma cortisol was decreased more in the catfishes captured from the Ganga than from the Gomti, as compared with captured fishes of the reference site. Decrease in plasma levels of cortisol of the captured fishes is supported by other workers (Hontela *et al.,* 1992; Hontela, 1997, 98). The catfish (*Clarias batrachus*) exposed to mercuric chloride, emisan-6 or methylmercuric chloride for 90 days had very active corticotrophs, but at longer exposure times, especially with organic mercury, there was evidence of necrosis (Kirubagaran and Joy, 1991) which was attributed to the effect of long-term over stimulation resulted the lack of negative feed back caused primary action contaminants on interrenal biosynthesis. There is a possibility that fish captured from the polluted rivers containing high levels of HCHs, DDTs and chlorpyrifos caused the atrophied pituitary corticotrophs, leading to low plasma cortisol levels and inhibitory stress response. Above observation is supported by Hontela *et al*. (1992). A similar suppression of the cortisol response to stress together with disruption of pituitary morphology was found at sites where there are species had been exposed to bleached kraft mill effluents (Hontela *et al.,* 1997). Bleached kraft pulp mill effluent has been reported changes in reproductive functions and suppressed plasma cortisol levels in exposed white suckers (McMaster *et al.* 1994). Post-treatment of tissues with *o,p´*-DDD (dichlorodiphenyldichloroethane) or addition of the pesticide to the superfusion medium also suppressed the adrenocorticotrophs hormone response (Ilan and Yaron, 1983). Here it might be attributed to the presence of tissue bioaccumulation of insecticides disturbed the pituitary-interrenal axis at receptor level in the captured fishes of the river Gomti and Ganga during post-monsoon season. This is also suggested that these pollutants which caused reproductive dysfunctions through disruption of gonadal steroidogenesis (Singh and Singh, 2007, 2008), might also have similar effect on the stress response through disruption of interrenal steroidogenesis.

In conclusion, the catfishes of the Gomti have higher bioaccumulation of ΣHCH (above MRL) than ΣDDT captured during non-breeding season. The catfishes of the Ganga have higher ΣDDT than ΣHCH (below MRL, Table 1), indicating both the rivers are highly polluted as compared to reference site. The plasma level of cortisol was suppressed more in the catfishes captured from the river Ganga than the fishes of the river Gomti affecting the growth of the fishes. It is suggested that fish containing pesticide residues beyond the permissible limit must be avoided as food by human beings because it causes health as well as reproductive problems. It is also imperative to monitor insecticide pollution level in both Gomti and Gangetic ecosystem. Such measures would minimize their use in the catchments area and protect the riverine fishes from the adverse impact of insecticidal pollution on their growth and reproduction.

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