

## ANTENNAL DEFORMITIES OF CHIRONOMID LARVAE (DIPTERA: CHIRONOMIDAE) OCCURRING IN RICE FIELDS OF HOOGHLY DISTRICT, WEST BENGAL

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### Abstract

*Aquatic environments are under pressure by complex blends of contaminants whose effects are not always easy to assess. Due to this, organisms are sought in which early warning signs may be noticed upon the presence of potentially toxic xenobiotic substances. Thereby, the study evaluated the incidence of deformities and other morphometric variations in the antenna Chironomid larvae exposed to water from rice fields of Hooghly district. Morphological deformities of Chironomid (Diptera: Chironomidae) larvae have been proposed as a bioindicator of sediment quality and environmental stress. Chironomid larvae were collected from rice fields and physico-chemical parameters of water and sediment were recorded. Field data exhibit high incidence of deformity in Rishra compared with Serampore and Khanakul. Analysis of sediment and water indicate the presence of heavy metal pollutants like lead, zinc, copper and cadmium. These metals are responsible for deformation of chironomid larvae. Percentage of deformity positively correlated with heavy metals in industrial belt i.e. industrial effluents in the adjoining rice fields*

**Keywords:** Antenna, Chironomid larvae, Deformity, Pollution, Pesticides, sediment.

### INTRODUCTION

The chironomid larvae are considered as model organisms for bioassays because they spend most of their developmental occasion in sediments surface where they remain exposed to different toxicants; also, they are somewhat easy to culture and have a short life cycle. These criteria create them appropriate organisms for

ecotoxicological monitoring (Warwick 1985; Vermeulen 1995; Al-Shami et al. 2010). Rice fields are a unique man-made environment supporting a rather wide diversity of aquatic organisms which is closely related to environmental changes of rice agro-ecosystems (Ali 1996; Al-Shami et al. 2008). Species of chironomidae have been recorded in rice fields throughout the world including

India, Australia, and the USA (Stevens et al. 2006). Routine agricultural practices, such as ploughing, draining, fertilizing, and pesticide applications and wet and dry climate cycles influence diversity of inhabiting aquatic communities (Chesalmah et al. 1998). Morphological deformities in chironomid larvae represent more traditional and useful criteria for biological assessment of water quality. Hamilton and Sæther (1971) reported the relationship between morphological deformities in chironomid larvae and occurrence of heavy metals and pesticides within their habitat sediments. Bhattacharya et al. (1999) recorded high incidence of deformity in mouthparts of chironomid larvae occurring in the River Damodar flowing through the industrial zone of West Bengal, India. Such deformities could provide a useful tool for assessing aquatic pollution, specifically relating to industrial wastes and agricultural runoff (Wiederholm 1984; Warwick 1985, 1990; Warwick and Tisdale 1988; Janssens de Bisthoven et al. 1992; Vermeulen 1995; Hamalainen 1999; Bhattacharya et al. 2005; MacDonald and Taylor 2006; Al-Shami et al. 2010). Bhattacharya et al. (1999) demonstrated that high percentage of deformity in mouthparts due to heavy metal pollutants in the river water. Al-Shami et al. (2010) observed that metal induce morphological deformities in *Chironomus* spp. and also observed that concentration of metals, particularly Ni and Mn, were highly correlated with larval deformities. The objective of the present study was to investigate the use of chironomid mouthparts deformities to assess environment pollution in ricefields of Hooghly District.

### Materials and Methods

The study was conducted at three locations in Hooghly District, West Bengal, India (Fig. 1): (A) Khanakul is mainly an agricultural area. (B) Serampore is a pre-colonial town on the right bank of the River Hooghly while (C)

Rishra is an industrial town having polluted area with various types of periodically discharged industrial effluents into surrounding rice fields in addition to usage of large amount of pesticides in the rice fields.

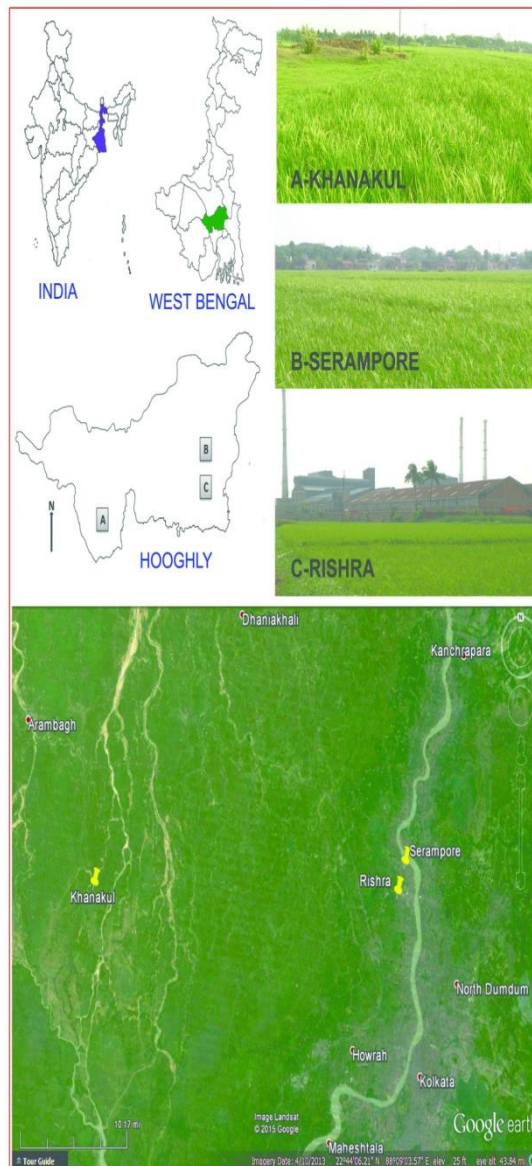


Fig.1. Map showing location of the three field sampling sites.

The samples were randomly collected from the three predetermined sites from July 2009 to September 2012. Adult chironomids were collected by sweep net around the sampling sites. Larvae were collected from mud bottom (10 cm) in the rice fields with mud scrapers and a scoop sampler (Chaudhuri and Chattopadhyay 1990). Each sample was transferred to a plastic bucket and

then washed with water and passed through a sieve (300- $\mu$ m pore). The physico-chemical parameters were measured at each sampling site in the rice fields on monthly basis during 2009 and 2012 as described in Bhattacharya et al. (2006) and Chaudhuri and Chattopadhyay (1990). The soil samples were air dried at room temperature and was crushed and sieved using a 0.5 mm sieve. The concentration of heavy metals like Copper (Cu), Zinc (Zn), Lead (Pb) and Cadmium (Cd) of the soil samples were estimated by XRFS in the laboratory of, Geological Survey of India, Salt Lake, Kolkata.

Chironomid larvae, pupae, pupal exuviae and adults were preserved and stored in 70–90% ethyl alcohol. The phenol–balsam technique of Wirth and Marston (1968) was mainly adopted in preparation of microslides of material for study. The immature midge stages and adult chironomids were identified following Epler (1995) and Pinder and Reiss (1983). Deformities of chironomid larvae were evaluated after Warwick (1980) and Warwick and Tisdale (1988).

Deformity (% def.) was calculated with the following formula:-

$$\text{def} = \frac{\% \text{ Number of deformed larvae}}{\text{Total number of larvae examined}} \times 100$$

% deformity of particular structure in mouth parts of deformed larvae calculated as follows:- % def of particular structure =

$$\frac{\text{No. of larvae having deformed parts}}{\text{Total number of deformed larvae}} \times 100$$

The data of each site were subjected to statistical analysis to find out the correlation, regression coefficient and Principal Component Analysis (PCA). Statistical analysis was done by using software SPSS 17 and Minitab 16.

### Results and Discussion

Physical and chemical water and soil quality variables (means and standard deviations) of the three sites of rice field during 2009-2012 are presented in Table 1. These parameters varied in the three sampling sites. The mean water temperature was (1-2°C) higher in Rishra compared with the Khanakul and Serampore. The mean value of water pH in three sites ranges from 6.9-7.7. The Biological Oxygen Demand was higher in Rishra in compare to other two sites. Faria et al. (2007) demonstrated that water temperature and pH were higher in highly contaminated site compared to relatively less contaminated site. This indicates that Rishra site was more polluted than the other two sites.

**Table 1**

### Physico-chemical characteristics (Mean $\pm$ SD) of three sampling sites during 2009-2012

PARAMETERS	KHANAKUL			SERAMPORE			RISHRA		
	Jul	Aug	Sept	Jul	Aug	Sept	Jul	Aug	Sept
Humidity	70.1 $\pm$ 1.45	68.7 $\pm$ 1.64	68.2 $\pm$ 1.48	67.4 $\pm$ 1.71	66.8 $\pm$ 1.40	66.6 $\pm$ 1.35	68.5 $\pm$ 2.12	67.6 $\pm$ 1.35	66.7 $\pm$ 1.16
Air Temp.(°C)	27.8 $\pm$ 0.79	27.6 $\pm$ 0.52	26.6 $\pm$ 0.70	28.3 $\pm$ 0.95	28.1 $\pm$ 0.74	27.3 $\pm$ 0.82	30.2 $\pm$ 0.79	29.3 $\pm$ 0.95	28.2 $\pm$ 1.03
<b>Water Parameters</b>									
Temp.(°C)	24.7 $\pm$ 0.82	24.5 $\pm$ 0.71	23.6 $\pm$ 0.84	26.5 $\pm$ 1.08	25.9 $\pm$ 0.88	25.1 $\pm$ 0.99	26.3 $\pm$ 0.95	25.3 $\pm$ 0.82	24.9 $\pm$ 0.74
pH	6.9 $\pm$ 0.16	7.3 $\pm$ 0.24	7.5 $\pm$ 0.12	7.4 $\pm$ 0.36	7.3 $\pm$ 0.25	7.5 $\pm$ 0.13	6.9 $\pm$ 0.30	7.5 $\pm$ 0.42	7.7 $\pm$ 0.33
DO (mg/l)	7.7 $\pm$ 0.33	7.9 $\pm$ 0.13	8.1 $\pm$ 0.21	7.3 $\pm$ 0.24	7.5 $\pm$ 0.13	7.4 $\pm$ 0.36	6.6 $\pm$ 0.37	7.6 $\pm$ 0.33	7.4 $\pm$ 0.18

BOD (mg/l)	4.5± 0.33	4.0± 0.18	4.0± 0.20	5.8± 0.32	4.0± 0.23	3.9± 0.23	5.0± 0.19	4.0± 0.20	4.4± 0.32
EC in $\mu$ simens/cm	139.50±8.43	135.00±1.089	132.25±1.486	430.25±3.842	431.25±3.572	406.75±4.252	1101.75±5.949	1113.00±4.949	1094.50±7.283
HCO <sub>3</sub> <sup>-</sup> (ppm)	107.25±4.86	107.25±3.20	110.00±5.89	173.25±1.031	173.50±5.26	173.75±1.060	449.75±11.18	454.75±5.0	455.75±9.03
SO <sub>4</sub> <sup>2-</sup> (ppm)	0.93±0.25	1.06±0.41	1.05±0.36	7.12±1.02	7.76±0.86	7.43±0.43	17.91±3.13	18.47±2.90	18.03±2.62
NO <sub>3</sub> <sup>-</sup> (ppm)	0.16±0.05	0.10±0.08	0.11±0.08	0.30±0.05	0.29±0.05	0.28±0.06	0.38±0.07	0.34±0.06	0.35±0.09
Cl <sup>-</sup> (ppm)	29.75±3.69	30.25±1.71	31.00±2.94	42.50±4.80	44.00±3.47	43.50±5.07	70.50±4.20	70.50±1.73	72.00±2.94
Total Hardness(ppm)	126.25±7.50	128.75±6.40	127.75±6.85	190.75±6.50	191.50±3.00	192.50±5.00	427.50±8.66	426.75±9.54	429.50±11.00
Ca <sup>++</sup> (ppm)	26.50±1.73	26.75±2.06	27.50±1.00	53.50±5.50	53.25±6.50	53.50±5.20	156.00±10.99	159.00±10.68	159.75±7.14
Mg <sup>++</sup> (ppm)	14.60±1.42	14.55±1.32	14.45±1.71	16.80±1.76	16.63±1.88	16.30±2.45	11.18±2.89	11.43±3.07	11.20±3.71
Na <sup>+</sup> (ppm)	1.76±0.53	1.77±0.55	1.92±0.70	6.41±0.93	6.32±0.80	6.55±0.63	21.11±0.75	21.19±0.72	21.16±0.73
K <sup>+</sup> (ppm)	0.58±0.39	0.62±0.42	0.70±0.66	3.82±0.87	3.80±0.81	3.62±1.20	10.82±1.88	10.84±2.26	10.52±2.04
PO <sub>4</sub> <sup>-3</sup> (ppm)	0.08±0.07	0.11±0.08	0.09±0.09	0.27±0.05	0.30±0.07	0.25±0.12	0.80±0.13	0.92±0.21	0.95±0.27
SiO <sub>2</sub> (ppm)	1.57±0.58	1.63±0.55	1.32±0.47	2.22±0.97	2.36±0.84	2.13±1.06	12.32±1.56	12.56±1.50	12.26±1.71
TDS (ppm)	83.25±14.22	82.75±12.66	85.25±12.39	278.00±1.874	280.25±1.808	282.00±1.521	689.00±18.01	692.00±7.44	694.00±10.23
F <sup>-</sup> (ppm)	0.42±0.12	0.43±0.14	0.44±0.08	0.58±0.12	0.57±0.12	0.56±0.10	0.58±0.07	0.58±0.11	0.54±0.15
<b>Soil Parameters</b>									
SiO <sub>2</sub> (%)	17.00±0.84	17.33±0.65	28.15±22.63	30.46±2.19	30.36±1.79	39.46±17.57	65.19±1.83	65.85±1.29	65.01±3.16
Cu (ppm)	33.00±3.37	32.50±4.80	33.50±3.51	38.00±4.32	38.25±3.86	38.50±1.00	64.00±4.55	63.00±8.29	63.75±1.50
Zn (ppm)	76.25±4.19	76.75±2.99	78.50±3.32	85.75±3.09	84.75±3.10	85.75±3.40	217.50±4.12	217.25±2.50	217.75±3.40
Pb (ppm)	29.50±2.08	29.00±2.16	29.00±1.41	40.25±1.70	40.00±2.70	38.50±3.00	79.00±2.58	79.50±2.38	78.50±2.08
Cd (ppm)	0.08 ± 0.02	0.09 ± 0.03	0.12 ± 0.04	0.11 ± 0.04	0.12 ± 0.03	0.17 ± 0.05	0.17 ± 0.07	0.23 ± 0.05	0.28 ± 0.04

A total of 13408 larvae comprising 6 taxa (Table 2) taken from three sampling sites of rice agro-ecosystem represent 4 taxa in Khanakul

(16.96%), 6 taxa, Serampore (27.84%) and 4 taxa in Rishra (55.2%) during July 2009 to September 2012.

**Table 2**  
**Different taxa of Chironomid larvae collected from three sampling sites (A-Khanakul, B-Serampore and C-Rishra)**

Taxa	2009			2010			2011			2012		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Chironomus circumdatus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Chironomus javanus</i>	-	+	+	-	-	+	-	+	+	-	+	+
<i>Dicrotendipes pelochloris</i>	+	+	-	+	-	-	+	-	-	+	+	-
<i>Einfeldia sp.</i>	-	-	+	-	+	+	-	+	-	-	+	+
<i>Kiefferulus calligaster</i>	+	+	+	-	+	+	-	+	+	+	+	-
<i>Microchironomus tener</i>	+	+	-	+	+	-	+	-	-	+	+	-

Morphological deformities were studied in 6 taxa of chironomid larvae of three main rice agro-ecosystems in Hooghly District. Out of the total deformity the highest percentage of

deformities (53%) was found in Rishra, whereas 28% and 19% occurred in Serampore and Khanakul respectively (Fig 2).

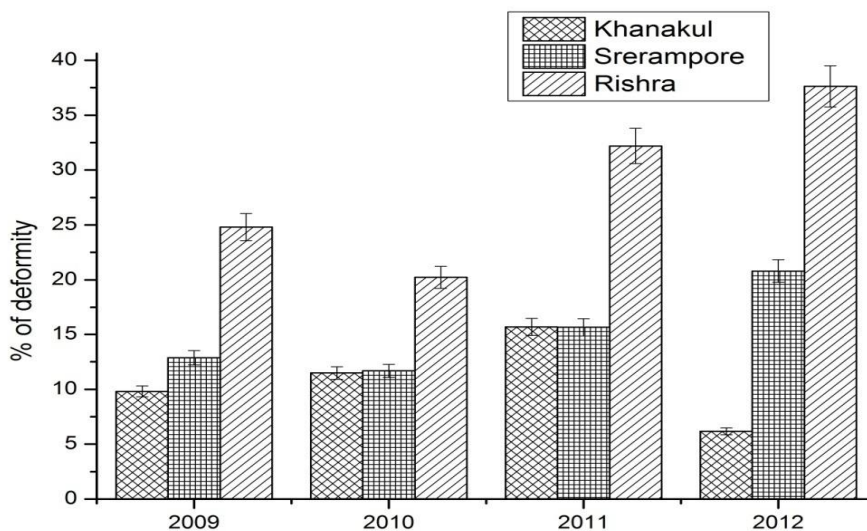


Fig. 2. Percentage of deformity incidence in three sampling sites.

Antenna five segmented, with third segment usually shorter than the fourth and bears number of mechanoreceptors and chaemoreceptors (Warwick 1985). The ratio of 5 segmented normal antennae was 25 (20-

32): 7.7 (6.50-7.90): 2.4 (1.90-2.70): 2 (1.80-2.20): 1.5 (1.30-1.80), basal antennal segment 0.08 (0.07-0.10, n=15) long and 0.025 (0.020-0.028, n=15) wide, ring organ 0.008 (0.007-0.009, n=14) in diameter situated at the lower half of

basal segment, lauterborn organ of 0.007 (0.006-0.01, n=14) long with ring of hair like structure on the distal rim of second antennal segment. The antennal deformities observed in larvae of the sites showed various types of changes which

included the loss of individual segment, appearance of new structures and additional segment and displacement or total loss of ring and lauterborn organs at times (Fig 3).

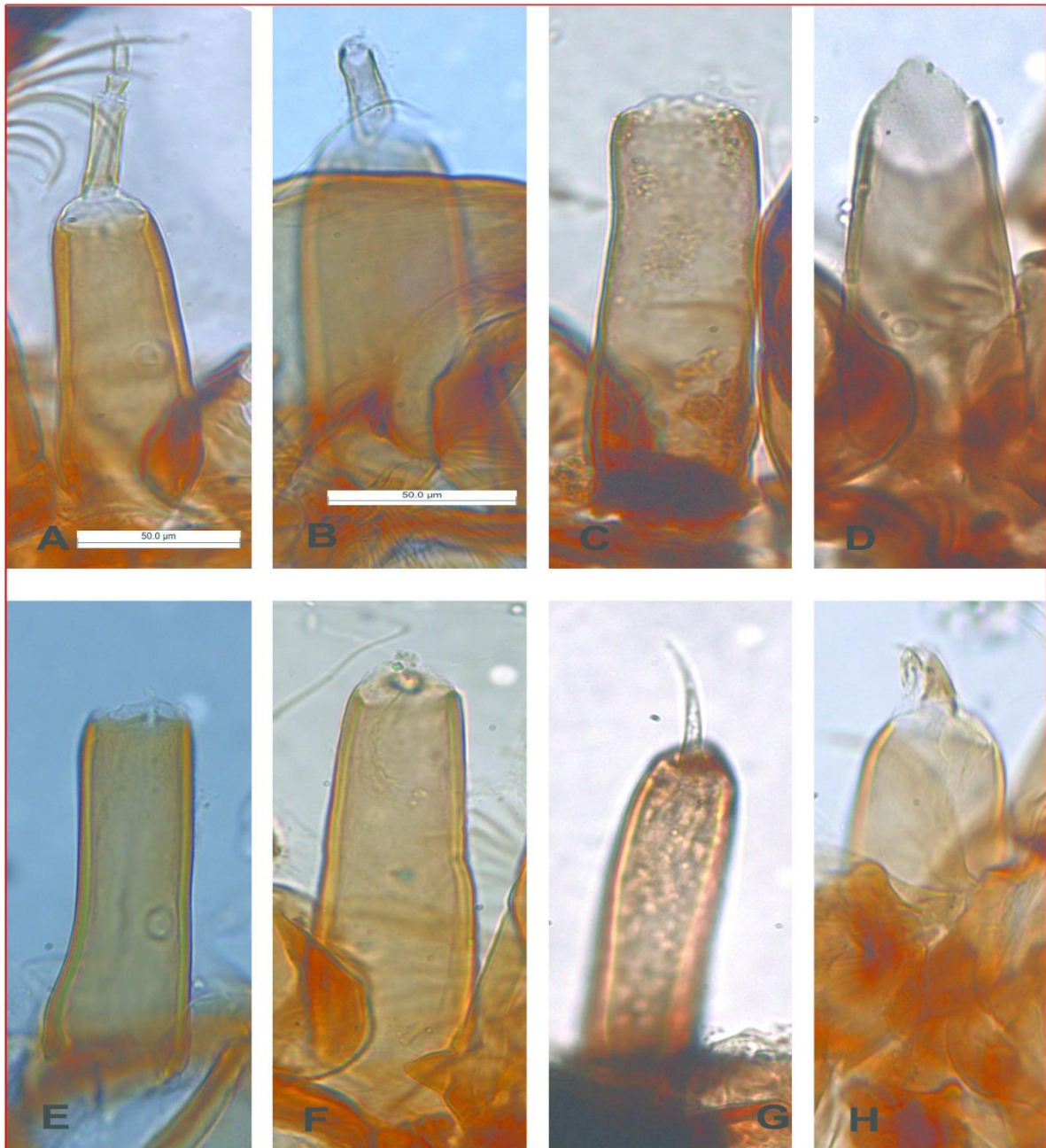


Fig. 3. A-Normal Antenna, B-H Deformed Antenna

**Types and indexing of severity of antennal deformities**

Severities of antennal deformities of larvae of the different sampling sites were not similar to establish the effect of

pollutants and to quantify the subjective severity in numerical figures. Geometric increase of points allocated to types of severity following Warwick (1985) has been summarized in the table 3.

**Table 3**  
**Categorization and allocation of points for different types of antennal deformities**  
**(Warwick 1985).**

Group	Type of deformities	*IMR <sub>(mentum)</sub> points
I	Basic classification categories (BCC)	1, 2, 4
	(a) Loss of genuine segment	1, 2, 4, 8, 16, 32
	(b) Presence of questionable segment	64
II	Reduction of length (LR)	1, 2, 3, 4, 5, 6, 7, 8
	Displacement of Ring organ (Ro D)	
III	Displacement of Lauterborn organ (Lo D)	1, 2
IV	Displacement of accessory blades (Ab D)	1, 2, 3
V	Fusion of apical with basal segments (FA)	1, 2, 3
VI	Presence of unknown abnormal structures (AS)	2
VII		1, 2

\*IMR= index of morphological response

From the principal component analysis, it has been demonstrated that Cd has profound effects on the deformities of *C. circumdatus* larvae. However, the water quality parameter such as DO, EC and BOD has no direct effect on such

deformation in Khanakul rice field sampling site. On the other hand, Serampore and Rishra rice field samples showed almost similar observation with some deviation in water quality parameters.

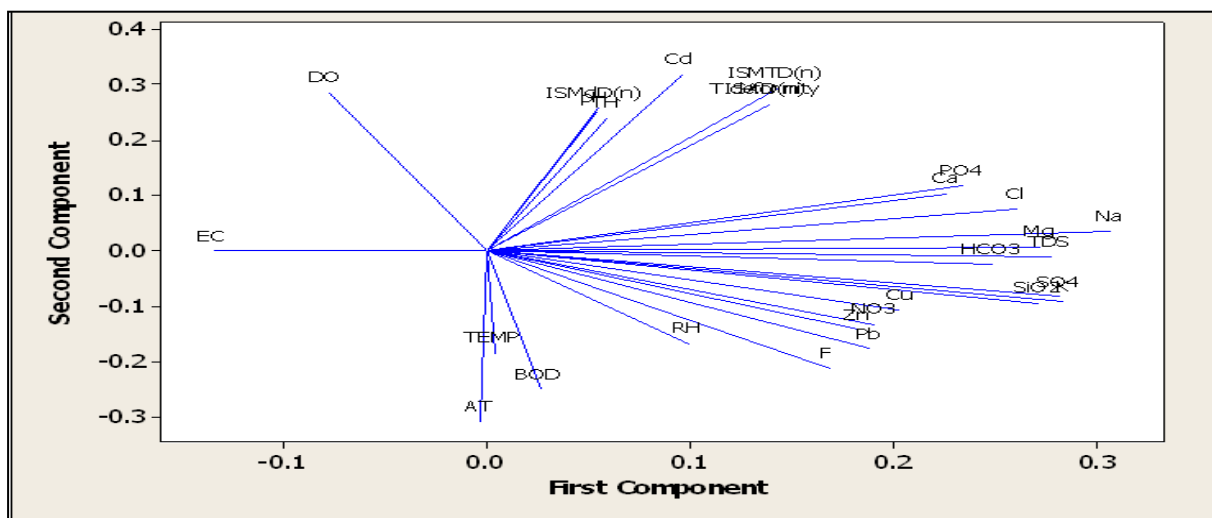


Fig. 4. Biplot of PCA showing the relationship of environmental parameters and index of severity of antennal deformity of *Chironomus circumdatus*

larvae collected from Khanakul rice field sampling site.

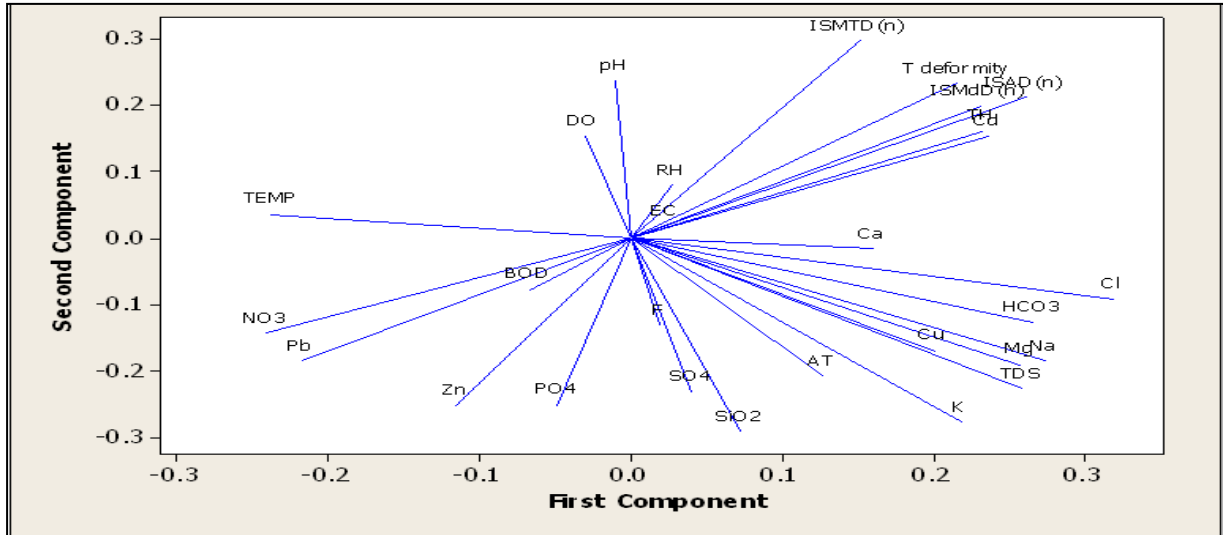


Fig. 5. Biplot of PCA showing the relationship of environmental parameters and index of severity of antennal

deformity of *Chironomus circumdatus* larvae collected from Serampore rice field sampling site.

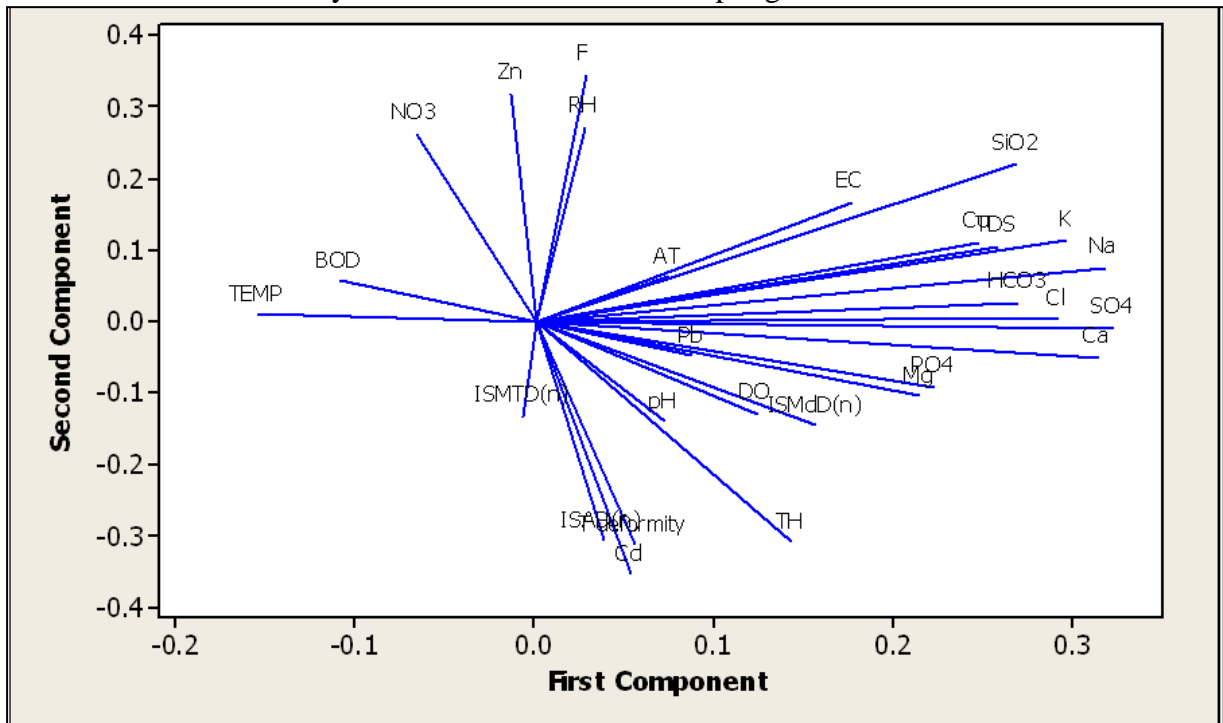


Fig. 6. Biplot of PCA showing the relationship of environmental parameters and index of severity of antennal

deformity of *Chironomus circumdatus* larvae collected from Rishra rice field sampling site.



### Conclusion

The occurrence of antennal deformities in the rice field of industrial region was relatively higher compared to non-industrialized agricultural areas. The antennal deformities of chironomid larvae are considered as indicators of environmental stress caused by water pollution. This study illustrates the use of chironomid deformities as tool for environmental degradation. The identified deformities are indicative of certain environmental stresses on the studied habitats and could provide as an empirical tool for their assessment. Based on bio-monitoring assessment, the study identifies the perturbations occurring in the rice field that are detrimental to inhabiting organisms, thus necessitating appropriate steps for improvement of water quality.

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