



ISSN 2320-7078

JEZS 2014; 2 (2): 93-101

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Received: 11-04-2014

Accepted: 03-05-2014

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Impact of industrial effluent on water quality and benthic macro invertebrate diversity in fresh water ponds in Midnapore district of west Bengal, India

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ABSTRACT

A study on the impact of industrial effluent discharge on the distribution and biodiversity of benthic macro-invertebrates and water quality of a fresh water pond, in Gokulpur, West Bengal, India near Tata metaliks (a metal refinery) was carried out during April 2009-March 2010. Two ponds were chosen for this study of which one was used as control. Comparing the physico-chemical parameters in two ponds it was seen that pond G had higher hardness, total suspended solid, pH, phosphate, hydrogen sulphide, cyanide, heavy metals like lead, cadmium and mercury. The total no of macro-benthic taxa and their overall richness indices and diversity indices were higher at pond in Santipukur, (44 taxa) than pond in Gokulpur (22 taxa). The pond S was dominated by Ephemeroptera, Hemiptera where as other order found in small quantities included Coleoptera, Diptera, Molluscs, Crustacea and Odonata. On the other hand pond G was mainly dominated by tolerant Diptera and Hemiptera. The relationship between physico-chemical parameter and macrobenthic data were investigated by Pearson correlation analysis. This statistical analysis showed that richness and diversity indices in pond G were mainly influenced by water hardness, total suspended solid, phosphate, hydrogen sulphide, lead and cyanide. It was also seen that a lot of species that were present in pond S were absent in pond G. CCA ordination biplot showed the presence of benthic macro-invertebrates in pond G was due to their strong and positive correlation with environmental variables.

Keywords: Benthic macro-invertebrates, Biodiversity, Industrial effluent, Physico-chemical parameters,

1. Introduction

Ponds are an exceptional fresh water resource around the world and represent 30% of global surface area of standing water. Ponds exhibit a particularly high biodiversity than other fresh water habitat and have a high potential for ecosystem function and services. Despite these impressive features, ponds face many threats from a variety of human activities like agricultural intensification, pollution, development, over-abstraction of water for human use, land drainage, inappropriate or lack of management etc. Today, most of the water bodies including ponds are heavily polluted by industrial wastes. Many of the industries, discharge huge quantities of chemically or thermally polluted water which disturbs the biodiversity of these fresh water habitats. Benthic macro-invertebrates (>0.55 mm in diameter) which are main faunal component and play an important role in nutrient recycling and energy flow in aquatic ecosystem, are primarily affected by this polluted industrial effluent. The biodiversity of these benthic macro-invertebrates diversity is also higher in fresh water ponds. But it is regretted that being an important part of fresh water resources, pond biodiversity is a neglected portion and have been little studied compare to other fresh water habitat. In India, benthic macro-invertebrate diversity of ponds has been studied by several authors [14], [19] have investigated the seasonal variation and diversity of benthic macro-invertebrates in fresh water ponds. [15, 16, 2, 3, 10, 18] have studied faunal diversity and community structure of aquatic insects in fresh water ponds. [15] have studied the role of aquatic insects in the evaluation of water quality of fresh water ponds. But how this benthic macro-invertebrate diversity of fresh water ponds has been affected by industrial effluent was not studied so far. In the present paper, some of the basic observations on the benthic macro-invertebrate's diversity with relation to water quality of two fresh water ponds in the district Midnapore, West Bengal have been presented.

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2. Materials and Methods

One of the study site is situated in Midnapore town (22°25'N, 87°17'E), near Gopegarh, the pond is locally called Santipukur (Pond S). This pond is naturally maintained (depth 5ft area 3acre) and is surrounded by forested area. Other study site is situated in Gokulpur (22°46'N, 87°50'E) near a metal refinery, Tata Metalics (Pond G). This pond is also naturally maintained (depth 3 ft area 4.5acre) but effluent from metal refinery reaches into this pond.

Benthic macro invertebrates were collected every month from April'09 to March'10 between 7.00 a.m. to 8.00 a.m. Collection was made by using D-frame dip net (mesh opening 500 µm) from four sites at four corner of the pond. Collected samples then shorted and stored in 70% ethyl alcohol in samples bottles. Identification of benthic macro invertebrates were made by using taxonomic keys following the methods of [2, 3]. Identification of species was confirmed in the Central Entomological Laboratory of Zoological Survey of India, Kolkata. Water samples were also collected in every month for physico-chemical analyses and all the parameter were analyzed in Department of zoology, Raja N.L.Khan Women's College, Vidyasagar University by using standard methods prescribe for water quality assessment [1].

Statistical analysis--Species richness, faunal diversity and species evenness of benthic macro invertebrates were calculated by Margalef's Index, Simpson's index of diversity, Shannon-Wiener Index and Equitability index [13]. All the calculation of diversity indices, pearson's correlation coefficient between diversity indices and environmental variables and canonical correspondence analysis were made by PAST software.

3. Result and Discussion

The results of the physico-chemical parameters of the two ponds are presented in Table- 1. It was seen that all the parameters studied i.e. temperature, pH, hardness, total suspended solid, dissolve oxygen, free carbon di-oxide, salinity, BOD5, primary productivity, inorganic nitrogen, phosphorus, organic carbon in soil revealed significant variation at both ponds in different seasons. Water temperature range from 14-29.5 °C in pond S whereas 17.5-28 °C in pond G. pH, salinity, hardness and total suspended solid was found to be higher in pond G at all seasons than pond S. pH in pond S range from 6.5-7 whereas pond G it was 7.5-8.5 i.e. pond G was highly basic. Water hardness found in pond S at summer season 30 mg/l whereas maximum hardness found in pond G at rainy season 1120 mg/l. Total suspended solid was also minimum in pond S at summer season 22 mg/l but maximum value exhibited in pond G in rainy season 2151 mg/l. Organic carbon and fluoride concentration also higher in pond G which was 7.42% and 5.8 mg/l respectively. But primary productivity by phytoplankton and other fresh water green algae was higher in pond S and highest value seen in spring season 915.6 mgC/m³/hr and lowest value found in pond G in rainy season 84.38 mgC/m³/hr, inorganic nitrogen and phosphorus concentration was higher in pond G than pond S. It was seen that highest value of free carbon di-oxide found in summer season in pond G but average value of free carbon di-oxide was higher in pond S and average value of BOD5 was higher in pond G. Dissolve oxygen which play significant role in fresh water biodiversity was not very high in both ponds. Its range is between 2.8-6 mg/l in pond S and in pond G it was 2.8-6.4 mg/l during study time (7 a.m-8a.m).

Some toxic substances like hydrogen sulphide, sulphate, cyanide, heavy metals like mercury, lead, cadmium also present in pond G.

There variations in different seasons were presented in Table-2. In fresh water ecosystem cadmium, lead, and mercury are most toxic heavy metals and according to USEPA 2005 maximum permissible limit of cadmium is 0.00066-0.002 mg/l. But in the present study these three heavy metals exceeded the permissible limit. Highest value of mercury found in spring season 0.003 mg/l and highest value of cadmium found in summer season 0.03 mg/l. Although highest concentration of lead found in rainy season 0.92 mg/l but in all other seasons concentration of lead was relatively high than maximum permissible limit. Continuous exposure of hydrogen sulphide caused mortality in aquatic animals as low level as 0.002 ma/l but in pond G its level was strikingly high in all seasons ranging from 0.98 mg/l-134.1 mg/l.

3.1 Benthic macro-invertebrates: In study time a total of 1044 individuals belonging to 30 families and 44 species of benthic macro-invertebrates collected from pond S and in pond G a total of 1227 individuals belonging to 20 family and 22 species were collected. The abundance of benthic macro- invertebrates differ among two ponds and among different seasons. Most dominant taxa in pond S were Ephemeroptera and Hemiptera whereas pond G most dominant taxa were Hemiptera and diptera. Major changes were observed in the community structure of the macro-invertebrates fauna between the seasons in both ponds. In pond S higher abundance of benthic macro-invertebrates was found in spring season and lower abundance in winter season where as in pond G higher abundance of benthic fauna found in spring season and lower abundance in rainy season.

Species composition and distribution of benthic macro-invertebrates in two ponds showed differences between them which based on the presence or absence of some species. According to table-3 some genera like *Baetis*, *Micronecta*, *Nyschia*, *Hydrovatus*, *Hydroglyphus*, *Amphiops*, *Globaria*, *Enochrus*, *Paracymus*, *Hydrochus*, *Pseudagrion*, *Ischnura*, *Bezzia*, *Mochlonyx*, *Gabbia*, *Melanoides*, *Terebia*, *Indoplanorbis*, *Lymnaea*, *Tubifex*, *Macrobrachium*, *Pseudocandona*, *Lynceus*, *Hydrachna* were absent in pond G although they were present in pond S. Only four genus *Diplonchus*, *Podura*, *Anax* and *Caliagrion* that were present in pond G were absent in pond S.

Diversity indices of macro-invertebrates obtained in two ponds in different seasons were shown in table-4. For measuring biodiversity we select simpson index, shannon wiener index and measuring species richness and species equitability the margalef's index and evenness index were used. The result showed that the species richness and species diversity values were higher in pond S in all season than pond G. It also observed that in pond S higher species richness during spring season and higher species diversity found in summer but in pond G higher species richness and higher species diversity both are found in spring. The correlation between Shannon wiener diversity index and mergalef's index with some selected physico-chemical parameters (i.e. total suspended solid, hardness, phosphate, lead, hydrogen sulphide and cyanide) in pond G are shown in table- 5. It was observed that all physico chemical parameter were negatively correlated with species diversity and species richness indices.

The CCA (Canonical Correspondence Analysis) ordination biplot showed how the macro-invertebrate species composition in pond G depended on the environmental variables. Six environmental variables were used in ordination procedure. Fig 1 showed the result of ordination of aquatic insect species with respect to environmental variables. Abbreviation list of the aquatic insect

species in CCA diagram shown in table 6. The colored lines in the diagram show the direction of influence of environmental variables on faunal assemblages. The longer the line, the stronger the

influence. According to this hardness, phosphate, hydrogen sulphide and cyanide had higher influence on benthic macro-invertebrates in pond G.

Table 1: Variation of physico-chemical parameter at the two study sites for the period of study (April 2009-March 2010).

		Summer Season	Rainy Season	Autumn Season	Winter Season	Spring Season
Water Temperature (⁰ C)	Pond S	29.5	28	23.5	14	26.5
	Pond G	28	28	24	17.5	27.5
pH	Pond S	7.0	6.5	7.0	6.5	7.0
	Pond G	8.5	8.5	7.5	8.5	8.5
Dissolved Oxygen (mg/l)	Pond S	3.6	3.2	6	4.4	2.8
	Pond G	2.8	4.0	3.2	6.4	6.0
Primary productivity (mgC/m ³ /hr)	Pond S	783.33	500	334.38	333.33	915.6
	Pond G	333.33	84.38	165.63	375.38	312.5
BOD5 (mg/ml)	Pond S	4	6.4	1.2	8.4	3.6
	Pond G	7.2	8.0	5.6	7.6	7.2
Free CO₂ (mg/ml)	Pond S	11	8.8	6.6	6.6	11
	Pond G	13.2	0	8.8	0	4.4
Salinity (ppt)	Pond S	0.08	0.1	0.06	0.06	0.08
	Pond G	0.10	0.1	0.07	0.08	0.17
Hardness (mg/l)	Pond S	30	166	26	44	36
	Pond G	316	1120	282	350	552
Organic Carbon in soil (%)	Pond S	2.82	1.92	2.85	2.46	3
	Pond G	3.21	3.21	0.15	7.42	1.68
Inorganic nitrogen (mg/l)	Pond S	<0.1	<0.1	0.86	0.86	2.06
	Pond G	1.28	0.40	5.11	5.84	0.82
Phosphorus as PO₄ (mg/l)	Pond S	0.76	0.44	<0.003	0.15	0.11
	Pond G	0.42	1.63	0.03	0.57	0.34
Total suspended solid (mg/l)	Pond S	22	34	22.2	44.7	45
	Pond G	971	2151	1271	806	171
Fluoride (mg/l)	Pond S	0.46	0.36	0.25	0.50	0.25
	Pond G	3.0	2.70	2.87	3.70	5.8

Table 2: Variation of toxic substances in pond [G] (April 2009-March 2010).

Toxic substances	Summer Season	Rainy Season	Autumn Season	Winter Season	Spring Season
Hydrogen sulphide (mg/l)	10.71	134.1	3.40	10.45	0.98
Sulphate (mg/l)	66.0	195	102	235	326
Mercury (mg/l)	0.001	<0.001	<0.001	<0.001	0.003
Lead (mg/l)	0.53	0.92	0.07	0.28	0.08
Cadmium (mg/l)	0.03	0.01	<0.01	<0.01	<0.01
Cyanide (mg/l)	0.13	0.14	0.15	0.24	0.08

Minimum detection limit of Mercury- 0.001 mg/l, Minimum detection limit of Lead-0.05 mg/l, Minimum detection limit of Cadmium-0.01 mg/l,

Table 3: Summary of the species composition, distribution and abundance of benthic macro-invertebrates at the two study sites for the period of study (April 2009-March 2010).

Order/ Class	Family	Genus and Species	Summer Season Pond Pond		Rainy Season Pond Pond		Autumn Season Pond Pond		Winter Season Pond Pond		Spring Season Pond Pond	
			[S]	[G]	[S]	[G]	[S]	[G]	[S]	[G]	[S]	[G]
Ephemeroptera	Leptophlebiidae	<i>Habrophlebia sp.</i>	X	4	15	X	2	9	10	1	5	5
	Baetidae	<i>Baetis sp.</i>	X	X	70	X	8	X	52	X	19	X
Hemiptera	Belostomatidae	<i>Diplonychus rusticus</i> (Fabricius).	X	13	X	X	X	8	X	X	X	20
	Corixidae	<i>Micronecta scuttellaris</i> <i>scuttellaris</i> (Stal)	3	X	3	X	4	X	102	X	15	X
		<i>Corixa sp.</i>	3	2	13	X	5	20	12	4	12	14
	Gerridae	<i>Metrocoris sp.</i>	4	1	3	X	2	X	1	X	1	2
	Mesoveliidae	<i>Mesovelia sp.</i>	1	X	7	1	3	6	X	X	2	10
	Nepidae	<i>Ranatra gracilis</i> Dalas.	2	2	1	X	X	4	X	1	10	14
	Notonectidae	<i>Anisops sp.</i>	1	35	35	X	8	54	70	55	8	7
		<i>Nychia marshalli.</i>	X	X	20	X	X	X	23	X	15	X
	Pleidae	<i>Paraplea sp.</i>	4	5	2	0	5	5	X	34	36	10
	Vellidae	<i>Microvelia sp.</i>	X	X	1	3	X	X	8	14	70	68
Coleoptera	Noteridae	<i>Canthydrus laetabilis</i> (walker)	7	X	1	X	X	X	X	X	2	X
		<i>Canthydrus rifsemmai</i> (Regimbert)	5	X	2	X	X	X	X	X	1	X
		<i>Canthydrus luctuosus</i> (Aube)	2	X	X	X	1	X	X	X	X	2
		<i>Laccophilus parvulus parvulus</i> (Aube)	9	X	X	X	3	X	X	X	1	2
		<i>Hydrovatus sp.</i>	2	X	3	X	7	X	2	X	2	X
	Dytiscidae	<i>Hydroglyphus flammulatus</i> (Sharp)	1	X	X	X	X	X	X	X	1	X
		<i>Amphiops pedestris</i> (Sharp)	X	X	1	X	1	X	X	X	2	X
		<i>Globaria sp.</i>	X	X	X	X	1	X	X	X	X	X
	Hydrophilidae	<i>Enochrus esuriens</i> (Walker)	1	X	1	X	X	X	1	X	X	X
		<i>Paracymus evanescens</i> (Sharp)	2	X	3	X	X	X	X	X	4	X
		<i>Hydrochus binodosus</i> Mots.	1	X	2	X	2	X	1	X	1	X
		<i>Berosus fairmairei</i> Zaitz	12	X	3	X	X	X	X	2	4	X

Collembola	Poduridae	<i>Podura aquatica</i> (Linneaus)	X	1	X	X	X	X	X	X	X	X
Odonata	Libellulidae Aeshnidae Coenagrionidae	<i>Urothermis signata</i>	2	1	X	X	X	1	X	4	4	2
		<i>Anax sp.</i>	X	X	X	X	X	X	X	2	X	1
		<i>Enallagma sp.</i>	1	1	8	X	2	1	X	3	5	X
		<i>Pseudagrion sp.</i>	X	X	5	X	X	X	X	X	2	X
		<i>Ischnura sp.</i>	X	X	3	X	1	X	X	X	X	X
		<i>Caliagrion sp</i>	X	2	X	X	X	2	X	4	X	5
Diptera	Chironomidae	<i>Chironomus sp.</i>	1	53	3	X	14	2	4	295	12	17
	Ceratopogoni- dae	<i>Bezzia sp.</i>	X	X	1	X	X	X	1	X	X	X
	Stratiomyidae	<i>Stratiomys sp.</i>	X	X	1	1	X	X	X	X	2	2
	Culicidae	<i>Culex sp.</i>	X	19	2	X	2	2	2	304	62	4
	Chaboridae	<i>Mochlonyx sp.</i>	X	X	2	X	X	X	X	X	3	X
	Syrphidae	<i>Chrysogaster sp.</i>	X	8	X	X	X	1	9	43	X	X
Molluscs	Viviparidae	<i>Bellamyia bengalensis f.typica</i> (Lamarck)	4	3	2	X	4	1	X	2	4	3
	Bithyniidae	<i>Gabbia orcula Frauenfeld</i>	1	X	X	X	X	X	X	X	X	X
	Thiaridae	<i>Melanoides tuberculata</i> (Mueller)	1	X	X	X	X	X	X	X	4	X
		<i>Terebia lineate</i> (Gray)	X	X	X	X	2	X	X	X	3	X
	Bullinidae	<i>Indoplanorbis exustus</i> (Deshayes)	1	X	X	X	X	X	X	X	2	X
	Lymnaeidae	<i>Lymnaea sp.</i>	X	X	X	X	X	X	X	X	2	X
Annelids	Tubificidae	<i>Tubifex tubifex</i>	2	X	X	X	2	X	5	X	3	X
Crustacea	Palaemonidae	<i>Macrobrachium sp.</i>	X	X	2	X	2	X	X	X	X	X
	Candonidae	<i>Pseudocandona sp.</i>	8	X	X	X	12	X	X	X	X	X
	Lynceidae	<i>Lynceus sp.</i>	X	X	X	X	10	X	X	X	5	X
Arachnids	Hydrachnidae	<i>Hydrachna sp.</i>	X	X	15	X	1	X	X	X	2	X
No of species			26	15	30	3	25	14	16	15	35	18
No of individuals			81	150	230	5	104	116	303	768	326	188

X denoted absence of respective species.

Table 4: Diversity of Benthic macro-invertebrates at study sites

Diversity Indices	Summer Season		Rainy Season		Autumn Season		Winter Season		Spring Season	
	Pond [S]	Pond [G]	Pond [S]	Pond [G]	Pond [S]	Pond [G]	Pond [S]	Pond [G]	Pond [S]	Pond [G]
No of Species	26	15	30	3	25	14	16	15	35	18
No of Individuals	81	150	230	5	104	116	303	768	326	188
Simpson's Index Of Diversity (1-D)	0.941	0.796	0.864	0.7	0.939	0.743	0.796	0.686	0.894	0.833
Shannon Wiener Index (log)	4.212	2.787	3.659	1.371	4.182	2.627	2.756	2.11	3.954	3.25
Mergalef Richness Index	5.689	2.794	5.333	1.243	5.168	2.735	2.62	2.10	5.875	3.24
Evenness Index	0.896	0.713	0.746	0.865	0.901	0.689	0.689	0.54	0.771	0.780

Table 5: Correlation of Diversity Indices with different physicochemical parameters in pond G

Diversity Indices	Total suspended Solid	Hardness	Phosphate	Lead	Hydrogen Sulphide	Cyanide
Shannon Wiener Index (log)	-0.86233•	-0.6722***	-0.84001•	-0.77349***	-0.85038***	-0.49634***
Mergalef Richness Index	-0.8466•	-0.7179***	-0.8804**	-0.8055•	-0.8774**	-0.4553***

•P<0.05 level of significance, ** P<0.02 level of significance, *** P< 0.1 level of significance

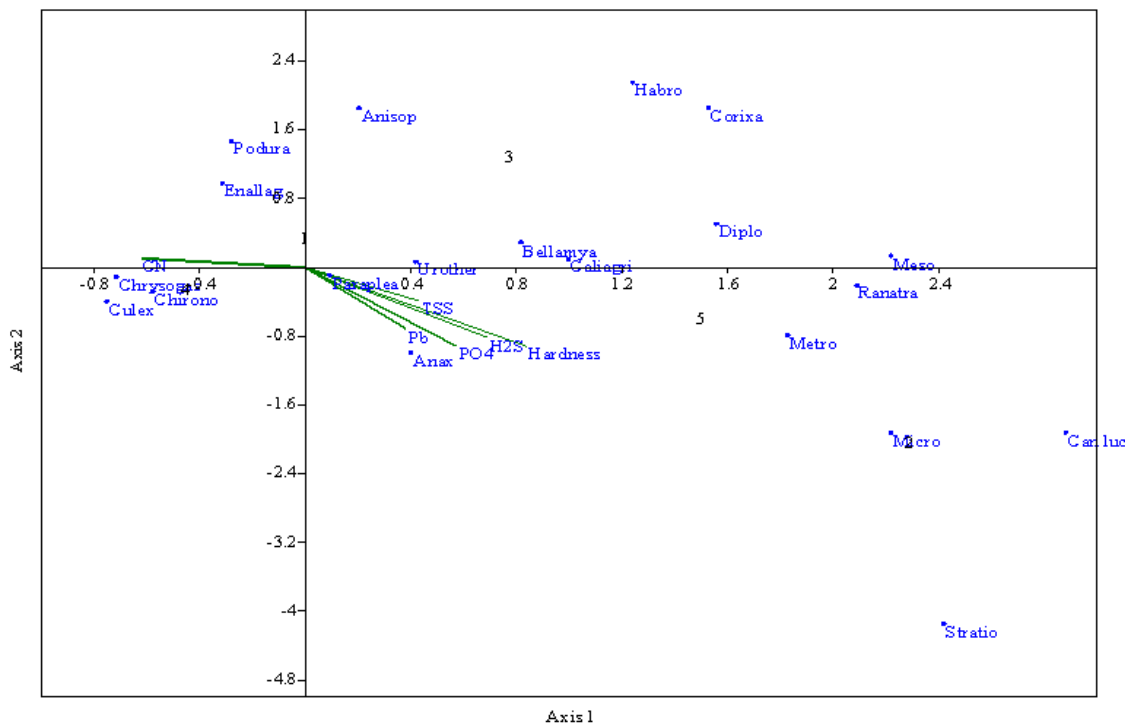


Fig 1: Canonical Correspondence Analysis of faunal assemblage with environmental variables in pond G. The eigenvalues of axis 1 and axis 2 were 0.48 and 0.27, respectively which explain 57% and 31.6% species.

Abbreviation list of the aquatic macro-invertebrate species in CCA Habro-*Habrophlebia* sp. (Ephemeroptera), Paraplea-*Paraplea* sp., Anisop-*Anisops* sp., Corixa-*Corixa* sp., Diplo-*Diplonychus rusticus*, Metro-*Metrocoris* sp, Ranatra-*Ranatra gracilis* Dalas, Meso-*Mesovelina* sp., Micro-*Microwelia* sp. (Hemiptera), Anax-*Anax* sp., Urother-*Urothermis signata*, Calia-*Calia* sp., Enallag-*Enallagma* sp. (Odonata), Bellamya-*Bellamya bengalensis* (Molluscs), Can luc-*Canthydrus luctuosus* (Coleoptera), Podura-*Podura aquatica* (Collembola), Chirono-*Chironomus* sp., Chrysogas-*Chrysogaster* sp., Culex-*Culex* sp., Stratio-*Stratiomys* sp. (Diptera)

The physico-chemical condition, species diversity and number of taxa encountered during the period of this study point clearly the degradation of water quality in pond G. Species richness and species diversity indices at two ponds also appear to respond the water quality deterioration at pond G. High species diversity at pond S was associated with unimpacted or unpolluted condition while lower species richness and species diversity often signified environmental stress due to human activities.

The assemblages and distribution of the benthic macro invertebrates frequently change in response to pollution stress in predictable ways. This is the basis of the development of biological criteria to evaluate anthropogenic influences^[18]. It is summarized the responses into three distinct categories: reduced diversity, increased domination by a single or group of opportunistic species, and reduced individual size. In pond G absence of a lot of species like *Baetis* (Ephemeroptera), *Micronecta*, *Nyschia* (Hemiptera), *Hydrovatus*, *Hydroglyphus*, *Amphiops*, *Globaria*, *Enochrus*, *Paracymus*, *Hydrochus* (Coleoptera), *Pseudagrion*, *Ischnura* (Odonata), *Bezzia*, *Mochlonyx* (Diptera), *Gabbia*, *Melanoides*, *Terebia*, *Indoplanorbis*, *Lymnaea* (Molluscs), *Tubifex* (Annelids), *Macrobrachium*, *Pseudocandona*, *Lynceus* (Crustacea), *Hydrachna* (Arachnids) and increased domination by some tolerant species like *Chironomus*, *Culex*, *Chrysogaster*, *Diplonchus* etc supported the above two responses^[11]. In pond G which is situated at Gokulpur near a metal refinery (Tata Metalics) was polluted from effluents release from that metal refinery. Studying the physico-chemical parameters in two ponds it was seen that in pond G have higher hardness, total suspended solid, phosphate, hydrogen sulphide, lead and cyanide which also showed a strong negative correlation with species richness and species diversity indices.

Total suspended solid comprised of fine particulate matter with a diameter less than 62 μm ^[23] and contain inorganic and organic substances. We all know that higher suspended solid decreases the rate of photosynthesis by reducing penetration of light and temperature but benthic macro-invertebrates are also greatly affected by suspended solid. It damage exposed respiratory organs of benthic macro-invertebrates and causing their dislodgment^[11]. A number of studies had shown that increased suspended solid associated with an increase in invertebrate drift or migration^[5]. For grazing invertebrates^[7] demonstrated that suspension of clay size particle can be trapped by epilithic periphyton and reduce its attractiveness for grazing macro invertebrates. For filter feeding invertebrates (e.g. crustacean, cladocera, copepoda), high level of suspended can clog gills and gut, reducing feeding efficiency and therefore reducing growth rates, stressing and even killing of these organism. So, higher suspended solid reduce abundance and richness of benthic macro-invertebrates. Chironomids which are known as tolerant species are also sensitive to suspended solid^[12]. So, in pond G reduced benthic macro-invertebrates even chironomid larvae in rainy and autumn season may be due to high concentration of suspended solid.

Most freshwater lakes, streams, and ponds have a natural pH in the

range of 6 to 8. pH 7 is called neutral pH and lower than pH 7 is acidic and higher than pH 7 is basic. The range of pH in pond G is 7.5-8.5. Higher pH causes the increase of water hardness. Water hardness is caused by the polyvalent metallic ions dissolved in water. In freshwater these are primarily calcium and magnesium. A commonly used classification of hardness is as follow (Sawyer, 1960): 0-75 mg CaCO₃/L is soft; 75-150 mg CaCO₃/L is moderately hard; 150-300 mg CaCO₃/L is hard, and 300 and up mg CaCO₃/L is very hard^[20]. According to the above mentioned classification, pond G has hard to very hard water which highly negatively affect the species richness and species diversity in that pond.

Phosphorus occurs naturally in rock formations in the earth's crust, usually as phosphate. Of high nutritive value to plants and animals, phosphates are used in fertilizers and as animal feed supplements. They are also used in the manufacture of industrial chemicals and pharmaceuticals and as detergent builders. High phosphate concentrations in surface waters may indicate fertilizer runoff, domestic waste discharge, or the presence of industrial effluents or detergents. Although phosphates from these sources are usually poly-phosphates or organically bound, all will degrade to "ortho" or reactive phosphates with time. If high phosphate concentrations persist, algae and other aquatic plant life will flourish eventually causing decreased dissolved oxygen levels in the water due to the accelerated decay of organic matter. According to^[20] surface waters that are maintained at .01 to .03 mg/l of total phosphorus tend to remain uncontaminated by algal blooms. But in the present study in pond G phosphorus level was much higher than the above level which causes eutrophication and death of aquatic macro-invertebrates due to lower level of dissolve oxygen.

Heavy metals, cadmium, lead, and mercury concentrations were high in pond G. According to IPCS,^[9] cadmium input to the aquatic environment is through discharge of industrial waste, surface runoff, and deposition. The average cadmium content of freshwaters is < 0.0001 to 0.00006 mg/L in unpolluted areas^[9]. The toxicity of metals to aquatic organisms is often modified by water hardness^[10]. The bioavailability of cadmium, lead and mercury in freshwater typically decreases with increasing hardness^[21]. Water hardness can have a major influence on cadmium toxicity to freshwater organisms^[17]. Cadmium is one of the most toxic heavy metals in the freshwater environment^[9]. According to USEPA,^[22] the limit values of cadmium for aquatic life range between 0.00066-0.002 mg/L. According to done measurements, the cadmium concentration was higher than the above mentioned values but due to higher hardness its toxicity and bioavailability may be much lower in pond G. According to IPCS,^[9] the toxicity of lead to aquatic organisms varies considerably depending on availability, uptake, and species sensitivity; generally, the earlier life stages are more vulnerable. The toxicity of inorganic lead is strongly dependent on environmental conditions such as water hardness, pH, and salinity. In communities of aquatic invertebrates, some populations are more sensitive (e.g. annelids and heavy metals induce autotomy of mucus production and mortality in annelids than others and community structure may be adversely affected by lead contamination. However, populations of invertebrates from polluted areas can show more tolerance to lead than those from non-polluted areas. According to USEPA,^[22] the limits of lead for aquatic life are between 0.0013-0.077 mg/L. According to the measurements, the lead concentration was higher than the upper limit although water hardness was also very high.

Hydrogen sulphide is a common but toxic metabolite formed in fresh water ponds. When aerobic bacteria breakdown excess feed and accumulated organic waste, oxygen is depleted, and the result is anaerobic zones where sulphate reducing bacteria will thrive,

resulting in the build up of hydrogen sulphide. Excess amount of hydrogen sulphide in fresh water can also be produced either by the decomposition of organic effluents from municipal sewage and many industries (4) or released directly in industrial effluents. According to USEPA continuous exposure of hydrogen sulphide causes mortality of aquatic animals, and level as low as 0.002ppm have shown to negatively affect of aquatic life. A common sign of a pond with hydrogen sulphide problems is black bottom sludge. Such black bottom sludge found in pond G. During the study time it was seen that in pond G has higher amount of hydrogen sulphide which may cause absence of different species of benthic macro-invertebrates in pond G.

The cyanide ion (CN⁻) is the predominant stable form of free cyanide above a pH of about 9.2. As the pH drops, increasing amounts of CN⁻ convert to hydrogen cyanide (HCN). The percentage of HCN continues to increase as the pH drops further, until at a pH of 7.0, about 99.5 percent of the cyanide exists as HCN. This HCN forms are highly toxic to humans and aquatic life if ingested (12). Fish and aquatic invertebrates are particularly sensitive to cyanide exposure. Invertebrates experience adverse nonlethal effects at 18 to 43 micrograms per liter free cyanide, and lethal effects at 30 to 100 micrograms per liter (although concentrations in the range of 3 to 7 micrograms per liter caused death in the amphipod *Gammarus pulex*). The sensitivity of aquatic organisms to cyanide is highly species specific, and is also affected by water pH, temperature and oxygen content, as well as the life stage and condition of the organism.

Although in pond G all these environmental variables were negatively correlated with diversity indices but not all macro-invertebrate species were negatively correlated with environmental variables. According to CCA biplot it was seen that in pond G most of the species were positively correlated. The CCA analysis also showed the macro-invertebrate species composition in pond G stood out because of their strong and positive correlation with environmental variables. The abundance of specific taxa such as *Habrophlebia* (Ephemeroptera), *Parapleia*, *Anisops*, *Corixa*, *Diplonchus*, *Metrocoris*, *Ranatra*, *Mesovelina*, *Microvelina* (Hemiptera), *Anax*, *Urothermis*, *Caliagrion* (Odonata), *Bellamyia* (Molluscs) and *Canthydrus*, *Laccophilus* (Coleoptera) were higher along axis-1 which represent higher hardness, phosphate, total suspended solid, Lead and Hydrogen sulphide. In contrast other species like *Baetis* (Ephemeroptera), *Micronecta*, *Nyschia* (Hemiptera), *Hydrovatus*, *Hydroglyphus*, *Amphiops*, *Globalaria*, *Enochrus*, *Paracymus*, *Hydrochus* (Coleoptera), *Pseudagrion*, *Ischnura* (Odonata), *Bezzia*, *Mochlonyx* (Diptera), *Gabbia*, *Melanoides*, *Terebia*, *Indoplanorbis*, *Lymnaea* (Molluscs), *Tubifex* (Annelids), *Macrobrachium*, *Pseudocandona*, *Lynceus* (Crustacea), *Hydrachna* (Arachnida) seemed more sensitive because these taxa only found in reference pond i.e. pond S. It was also seen that among all macro-invertebrate species *Diplonchus rusticus* (Hemiptera), *Anax* sp., *Caliagrion* sp., (Odonata) are able to develop and survive only industrially polluted pond. So, it is suggesting that these taxa are less sensitive to higher hardness, total suspended solid, phosphate, hydrogen sulphide and lead and these species may be identified as a bio-indicator species in industrial effluent rich pond.

4. Acknowledgement

The authors are thankful to the Head Dept. of Zoology, Vidyasagar University and Teacher in Charge, Raja N. L. Khan Women's college, Gope Palace, Paschim Midnapore for providing the laboratory facilities and to CPE (UGC) & DST for financial support to MS and PPC. Thanks are also due to Director, Zoological Survey of India, Kolkata, for arranging identification of

benthic macro-invertebrates.

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