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Fertilizer Industry Effluent Induced Biochemical Changes in Fresh water Teleost, *Channa striatus* (*Bloch*)

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Abstract The industrial activities pose threat to the life of aquatic organisms in many ways. This research communication presents an account of the impact of fertilizer industry effluent upon the levels of protein and the activity of lactate dehydrogenase (EC 1.1.1.28, LDH), a terminal key enzyme in glycolytic pathway, in different organs of a fresh water teleost fish, *Channa striatus* (*Bloch*). The fish exposed to different sublethal concentrations of fertilizer industry effluent (3.5, 4.7 and 7.0% v/v) equivalent to 1/20th, 1/15th and 1/10th of LC_{50} value (70% v/v) for varying treatment periods (96 h and 15 days) exhibited decrease in the level of protein (8–76%) in different organs of the effluent treated fish. At highest effluent concentration (7% v/v) treatment for short (96 h) or long (15 days) duration, the liver of the fish registered significant ($p < 0.001$) decrease (62–76%) in protein content as compared to control, whereas other organs of the fish showed only 38–52% decrease in the level of protein. The industrial effluent also caused marked reduction in the activity of LDH in different fish tissues when compared to the control. The treatment of fish with 7% effluent concentration for 96 h caused 78% decrease ($p < 0.001$) in the LDH activity in fish muscle whereas after 15 days the effect was maximum in fish brain as it exhibited 86% decrease ($p < 0.001$) in LDH activity as compared to control. The effect of effluent on the activity of LDH and protein content in different body tissues of the fish was dependent on concentration and duration of exposure. The significant reductions in the activity of LDH and level of protein in fish tissues due to treatment with the fertilizer industry effluent indicated the possibility of impairments in energy metabolism and protein turnover, respectively, in *C. striatus.*

Keywords Fertilizer industry effluent · Lactate dehydrogenase (LDH) · Protein · Energy metabolism

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Introduction

The rapid industrial growth through out the world in general and in India in particular due to alarming rise in human population has caused tremendous environmental contamination. The aquatic environment is affected by different types of chemicals toxic to the organisms that originate from both natural and anthropogenic sources (Kopecka 2006). The fertilizer industry has been one of the major sources of aquatic pollution in India. The effluents from fertilizer industries are so often disposed into water reservoirs such as lakes, ponds, rivers and ocean without any pretreatment or partial treatment. The fertilizer industry effluents consist of certain toxic components such as heavy metals, nitrates and ammonia which might be responsible for causing metabolic impairment in the aquatic organisms which could even lead to their death (Ekweozor et al. 2001; Bobmanuel et al. 2006).

Some toxicity tests have been performed for evaluating the effect of various types of chemicals and harmful heavy metals on aquatic organisms (Wel and Welling 1989). The alteration in various physiological and biochemical parameters of an aquatic animal due to exposure of

different toxicants has been shown to be directly or indirectly related to the behavior, immune system, neurotransmissions, energy metabolism and reproduction of the organism (Gopal et al. 1992; Sharma et al. 1993a, b; Kumari et al. 1997; Ekweozor et al. 2001; Adeyemo 2005).

Accumulation of the environmental pollutants and toxicants has been shown to cause alterations in the activities of many enzymes concerning to cellular energy metabolism (Niwelinski 1990; Claireaux and Dutil 1992; Sebert et al. 1993; Almeida et al. 1995). Lactate dehydrogenase (EC 1.1.1.28, LDH), a cytoplasmic biomarker in the glycolytic pathway, which catalyzes a reversible reaction of reduction of pyruvate into lactate (Pyruvate + NADH + $H^+ \leftrightarrow$ Lactate + NAD^+) plays an important role in the regulation of glycolysis. It is an extensively studied carbohydrate metabolizing enzyme which is crucial for normal cellular functions. The inhibition in the activity of this enzyme induced by pollutants has been shown due to changes in the conformation of active site (Valarmathi and Azariah 2002). The degree of alternations in the activity of any cellular enzyme and the level of protein have been shown to depend primarily on the magnitude, chemical nature of toxicant and duration of exposure (Singh and Sharma 1998).

There are only few reports available regarding evaluation of the toxicity of effluents (waste waters) on various commercial fish species in general (Wai-Ogosu 1987; Ojuola and Onuoha 1987). However, the information regarding assessment of the toxicity of fertilizer industry effluents and its impact on various biochemical indices particularly in *Channa* species, a teleost fish commonly found to inhabit the fresh water ponds and rivers of India have not received much attention.

In present communication the effect of fertilizer industry effluents on the protein content and activity of LDH in different tissues of *Channa striatus*, has been studied by exposing the fish to varying sublethal concentrations of the effluent for short (96 h) and long (15 days) treatment durations. The results obtained from the present study reflect the ability of fertilizer industry effluent to significantly induce certain biochemical changes in *C. striatus*. The information could be used as predictive biomarkers of aquatic pollution which may be exploited for management and monitoring of environmental stress in the aquatic ecosystem (Ekweozor et al. 2001; Bobmanuel et al. 2006).

Materials and Methods

All chemicals and reagents used in this study were extra purity grade and obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA) and Scientific Research Labs-India unless otherwise stated.

The healthy fresh water fish, *C. striatus* (length 5–7 cm, weight 18–20 g) were obtained from a local fish market and treated with potassium permanganate solution (0.5% w/v) for 2 min to remove any dermal adherent. The fish were acclimated for seven days in a rectangular glass aquarium (50 L) containing tap water at room temperature (26 ± 1.2°C) with food ad libitum under standard laboratory conditions. The aquarium was cleaned and replenished with fresh water after every 24 h. The aquarium system was equipped with water pump, flow meter and UV-light sterilizer. The fertilizer industry effluent from a local major industrial unit, Indian Farmer Fertilizer Co-operative popularly known as IFFCO, Phulpur at Allahabad, India was collected in a 50 L plastic container from the common outlet, which drain effluent to the disposal point.

The physico-chemical characteristics of fertilizer industry effluent were analyzed according to APHA (1975) (Table 1). The test medium (water) properties were also regularly analyzed (Table 2). The water temperature was maintained at 18 ± 0.5°C and water quality was monitored

Table 1 Physico-chemical characteristics of fertilizer industry effluent

S. no	Parameters	Values	Permissible limits	Units
1	Color	Grayish yellow	Clear[a]	–
2	Odor	Ammonical smell	Free[a]	–
3	pH	8.9 ± 0.1	6.5–8.0[b]	Unit less
4	Temperature	30.2 ± 0.3	35[a]	°C
5	Conductivity	380 ± 3.5	400[a]	μMho/cm
6	Salinity	1.38 ± 0.02	NA	mg/L
7	Total alkalinity	64.6 ± 1.7	NA	mg/L
8	Hardness	688.3 ± 7.3	NA	mg/L
9	Free CO_2	48.4 ± 1.1	NA	mg/L
10	DO	3.7 ± 0.05	5[a]	mg/L
11	BOD	14.4 ± 0.23	50[a]	mg/L
12	Chloride	758.6 ± 6.9	NA	mg/L
13	Sulfate	110.2 ± 2.1	NA	mg/L
14	Ammonia	4.8 ± 0.3	0.2[a]	mg/L
15	Sodium	72.4 ± 1.8	NA	mg/L
16	Potassium	10 ± 0.2	NA	mg/L
17	Zinc	0.82 ± 0.03	5.0[b]	mg/L
18	Chromium	0.97 ± 0.02	0.1[b]	mg/L

The fertilizer industry effluent was obtained from IFFC0, Phulpur, Allahabad, India in a plastic container as mentioned in Materials and Methods. The physico-chemical parameters were analyzed in the laboratory using standard procedures

NA Not available

[a] Federal Environmental Protection Agency (FEPA 1991)

[b] Central Board for the Prevention and Control of water pollution (CPCB 1983)

Table 2 Analysis of some physico-chemical properties of test water

S. no	Parameters	Values	Permissible limits	Units
1	Color	Colorless	Clear[a]	–
2	Odor	Odorless	Unobjectionable[a]	–
3	pH	6.9 ± 0.1	6.5 to 8.5[a]	–
4	Temperature	18.0 ± 0.5	NA	°C
5	Conductivity	281.0 ± 3.5	NA	µMho/cm
6	Total alkalinity	48.0 ± 0.7	200[a]	mg/L
7	Hardness	140.61 ± 4.3	300[a]	mg/L
8	DO	7.4 ± 0.2	NA	mg/L
9	Sulphate	2.0 ± 1.2	200[a]	mg/L
10	Chloride	23.3 ± 0.5	250[a]	mg/L
11	Sodium	21.4 ± 0.7	75[b]	mg/L
12	Potassium	0.79 ± 0.02	12.0[b]	mg/L
13	Zinc	0.25 ± 0.01	15.0[b]	mg/L
14	Ammonia	ND	0.50[b]	mg/L
15	Nitrate	ND	45.0[b]	mg/L
16	Nitrite	ND	0.10[b]	mg/L

The physico-chemical parameters were analyzed in the laboratory using standard procedures

NA Not available, *ND* not detected

[a] Bureau of Indian Standards (BIS)

[b] World Health Organization (WHO 1984)

daily for pH (6.9 ± 0.1), electric conductivity (281 ± 3.5 µMho/cm), total alkalinity (48.0 ± 0.7 mg/L), hardness (140.61 ± 4.3 mg/L), dissolved oxygen (7.4 ± 0.2 mg/L) and also for certain ions (Table 2).

Serial dilutions of the respective effluents were prepared on a volume to volume (%) ratio basis with dechlorinated tap water (Effiok 1993). The LC_{50} value for fertilizer industry effluent was determined by exposing the fish, *C.striatus*, with different concentrations of the effluent (v/v) under constant laboratory conditions for 96 h. The percent mortality was recorded and LC_{50} value was computed from the probit curve. The LC_{50} was found to be 70% (v/v) (data not shown). The mortality of the fish, however, increased with increase in temperature by 2°C, i.e. up to 20°C from 18°C.

For evaluation of the effect of the effluent, the fish were divided into four different groups: each containing ten fish. The fish were exposed to sublethal concentrations of effluent (1/20th, 1/15th and 1/10th of LC_{50}) for two different exposure periods, i.e. 96 h and 15 days in aquarium each of 20 L capacity. Every treatment was replicated thrice. No mortality of the fish occurred in control (without effluent) as well as in the experimental aquaria during the treatment period. The aquaria were aerated daily for 6 h.

At the end of treatment period, the surviving fish were sacrificed by decapitation, dissected and tissues (liver, muscle, gills, kidney, heart, brain) were isolated from control as well as the experimental fish. The tissues were thoroughly washed in normal saline and stored at 4°C to be used within 2 h. The tissues were homogenized (10% w/v) in 50mM Tris HCl, pH 8.0 for 2 min (two strokes each of 1 min with intermittent cooling) by Potter–Elvehjam homogenizer using a Teflon-coated pestle under ice-cold condition. The homogenates were centrifuged at 800×*g* for 10 min at 6°C to remove large particulate material. The supernatants obtained were further centrifuged at 10,000×*g* for 30 min in a refrigerated centrifuge (6°C). Thus, the clear supernatants collected were used for estimation of protein and assaying the activity of enzyme. The volume of the supernatants was recorded after each centrifugation step.

The activity of lactate dehydrogenase (EC1.1.1.28, LDH) in the cell-free extracts of fish tissues was monitored by a NADH-linked spectrophotometric assay following the method of Horecker and Kornberg (1948) with slight modification. The reaction mixture (3 mL) contained 33 mM Tris HCl buffer, pH 7.4, 100 mM KCl, 2.4 mM NADH, 50 mM sodium pyruate and 50–100 µg enzyme protein. The rate of oxidation of NADH to NAD^+ (or reduction of pyruvate to lactate) at 340 nm was monitored using UVD-Model double beam spectrophotometer. All assays were performed in triplicate. The change in absorbance was recorded up to 3 min at interval of 30 s at 24 ± 1°C. All samples were corrected for background oxidation of NADH. The activity of LDH was expressed in nmol min^{-1} mg^{-1} of protein or units/g wet weight of tissues. The molar extinction coefficient of NADH at 340 nm (6.22×10^3 M^{-1} cm^{-1}) was used for calculating the enzyme activity.

The protein content in cell-free extracts of different fish tissues was determined by the method described by Lowry et al. (1951); using bovine serum albumin (BSA) as a standard protein.

The values are expressed as mean ± SEM. The significance of the impact of the fertilizer industry effluent treatment upon levels of protein and activity of LDH was analyzed using one way ANOVA with Tukey's test.

Results and Discussion

The values for different physico-chemical characteristics obtained after analysis of the fertilizer industry effluent employed in the present study are shown in Table 1. The effluent exhibited higher concentrations of ammonia (24 times) and chromium (about ten times) as compared to their respective permissible limits. The effluent was slightly more alkaline than the permissible limit as set by CPCB (1983), which may be due to presence of excess

ammonia in the effluent. The presence of odour and colour into the effluent may be due to the presence of ammonia and the transition elements and/or their complexes, respectively. The physico-chemical characteristics of test water are demonstrated in Table 2. The values of the parameters of test water were found to be within the permissible limits as set by Bureau of Indian Standards and World health organization (WHO 1984). The toxicity of the effluent on the fish tissues could be attributed to the impact of certain heavy metals and ammonia present in it. The reports available from early studies indicated that xenobiotics metabolism in fish takes place at a slower rate than mammalian systems (Wheelock et al. 2005). Recently, it has been made clear that the metabolism of xenobiotics in fish is different from that in mammals (Whyte et al. 2000; Fulton and Key 2001; Bello et al. 2001; Wheelock et al. 2005). However, there is little information available on the effect of fertilizer industry effluent on the aquatic organisms such as fish, which find easy access to interact with and also ingest components of the effluent due to their surfacing activity. Further, the increased mortality of fish in the present study due to increase in water temperature may be attributed to the increased uptake of effluent components and reduced level of dissolved oxygen in the medium (Ananthakrishnan and Kutty 1974; Powell and Fielder 1982). The fertilizer industry effluents toxicity may be influenced by several environmental factors such as temperature, pH, CO_2, oxygen and many other elements. These factors may influence behavior and certain biochemical indices of the fish, *C. striatus*, by acting in synergistic, antagonistic or simple additive manner (Henry et al. 1998; Yadav et al. 2005; Bobmanuel et al. 2006). A high conductivity value reflects the presence of excess concentration of dissolved ion present in industrial effluent (Chukwu 1993) but the conductivity value recorded in this study were slightly below the limit set by FEPA (1991). Excess concentration of ionized and/or unionized ammonia has been reported to be a major pollutant present in the industrial effluent. The unionized ammonia has been reported to be toxic to aquatic organisms including fish (Thurston et al. 1978; Kormakik and Cameron 1981; Kuma et al. 1983; Ekweozor et al. 2001). It was also shown to produce impairment of cerebral energy in the fish, *O. niloticus* and a hybrid catfish (Walker and Schenker 1970).

In order to evaluate the effect of fertilizer industry effluent on the status of protein in different key organs of the fish, *C. striatus* were exposed to varying sublethal concentrations of the effluent (3.5, 4.7 and 7.0% v/v) for two different treatment periods (96 h and 15 days). The effluent treatment caused apparent changes in the level of protein in certain tissues of the fish. The results presented in Table 3 showed the average protein concentration in various tissues of the control fish in the following order: gills > liver > brain > muscle > kidney > heart. The protein content in control fish tissues as estimated were: 48.65 ± 3.57, 45.16 ± 2.45, 44.04 ± 1.42, 30.25 ± 2.05, 28.30 ± 1.00, 16.74 ± 2.16 mg/g wet weight in gills, liver, brain, muscle, kidney and heart, respectively. The level of protein decreased in the tissues of *C. striatus* due to exposure with industrial effluent at all sublethal concentrations (3.5%, 4.7% and 7%) tested with two treatment periods (96 h and 15 days). At 4.7% and 7% concentrations of effluent for 96 h exposure duration, the level of protein decreased very sharply in the fish brain was significantly different at $p < 0.05$ and $p < 0.01$ relative to control than in other tissues (Table 3). The protein level reduced in all fish tissues at a higher sublethal concentration of industrial effluent i.e.7%; the order of decrease in protein level was different from that obtained when fish was exposed to a lower concentration (3.5%) of industrial

Table 3 Effect of fertilizer industry effluent on the protein content of different tissues (mg/g wet weight) of *C. striatus* exposed for 96 h

Tissue	Protein content (mg/g wet weight of fish tissues)[a,b,c]			
	Fertilizer industry effluent (% v/v)			
	0	3.5	4.7	7.0
Muscle	30.25 ± 2.05	27.01 ± 1.51^{NS} (–10.71)	23.64 ± 2.21^{NS} (–21.85)	18.65 ± 0.84* (–38.34)
Liver	45.16 ± 2.45	34.90 ± 2.11^{NS} (–22.71)	24.50 ± 2.25** (–45.74)	17.11 ± 1.76*** (–62.11)
Heart	16.74 ± 2.16	15.36 ± 0.73^{NS} (–8.24)	12.02 ± 1.78* (–28.19)	9.43 ± 1.06* (–43.66)
Brain	44.04 ± 1.42	34.34 ± 0.76^{NS} (–22.02)	25.33 ± 1.55* (–44.75)	22.37 ± 0.48** (–49.43)
Kidney	28.30 ± 1.00	24.84 ± 1.44^{NS} (–12.22)	20.12 ± 1.75^{NS} (–28.90)	15.86 ± 0.36* (–43.95)
Gill	48.65 ± 3.57	40.36 ± 1.03^{NS} (–17.04)	28.82 ± 1.49** (–40.76)	24.49 ± 1.51** (–49.64)

NS Not significant ($p > 0.05$)

[a] Each value represents the mean ± SEM of three independent experiments

[b] Values in parentheses are percent change over control

[c] Significance of data is shown in superscripts. The groups similar or significantly different from the control at ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) are analyzed using one way ANOVA, with Tukey's test

Table 4 Effect of fertilizer industry effluent on the protein content of different tissues (mg/g wet weight) of *C. striatus* exposed for 15 days

Tissue	Protein content (mg/g wet weight of fish tissues)[a,b,c]			
	Fertilizer industry effluent (%, v/v)			
	0	3.5	4.7	7.0
Muscle	28.62 ± 3.14	25.50 ± 2.49^{NS} (–10.90)	22.31 ± 1.25^{NS} (22.04)	13.69 ± 1.52* (–52.16)
Liver	47.50 ± 0.92	25.42 ± 1.82** (–46.48)	22.02 ± 1.57** (–53.64)	11.19 ± 0.75*** (–76.23)
Heart	15.49 ± 1.99	14.92 ± 2.55^{NS} (–3.03)	12.82 ± 0.28^{NS} (–16.59)	8.85 ± 0.73^{NS} (–42.86)
Brain	42.55 ± 0.80	32.38 ± 0.77^{NS} (–23.90)	23.20 ± 1.44*** (–45.47)	18.74 ± 1.01*** (–55.95)
Kidney	28.75 ± 1.50	24.14 ± 2.11^{NS} (–16.03)	19.50 ± 0.93^{NS} (–32.27)	14.58 ± 3.01^{NS} (–49.28)
Gill	48.88 ± 0.19	37.27 ± 1.61^{NS} (–23.75)	28.19 ± 1.64* (–42.32)	24.41 ± 1.00* (–50.06)

NS Not significant ($p > 0.05$)

[a] Each value represents the mean ± SEM of three independent experiments

[b] Values in parentheses are percent change over control

[c] Significance of data is shown in superscripts. The groups similar or significantly different from the control at ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) are analyzed using one way ANOVA, with Tukey's test

effluent; the values being in following order: liver > gills > brain > kidney > heart > muscle. The results presented in Table 3 indicated that heart ($p > 0.05$) and kidney ($p < 0.05$) as well as brain ($p < 0.01$) and gills ($p < 0.01$) showed similar trend of reduction in protein content at higher concentration (7% v/v) of industrial effluent for 96 h treatment period.

The level of protein further decreased in different fish tissues when exposed to industrial effluent for a longer incubation period (15 days). The results presented in Table 4 demonstrated that the protein content in fish gills ($p < 0.05$), muscle ($p < 0.05$), brain ($p < 0.001$) and liver ($p < 0.001$) were significantly reduced by 50%, 52%, 56% and 76%, respectively, at higher concentration (7%) as compared to control. The results indicated that the industrial effluent at low concentration (3.5%) and short (96 h) exposure duration could exert less toxicity effect (Table 3) as compared to that at higher concentration (7%) for a long (15 days) treatment period (Table 4). The gradual accumulation of toxicants present in the industrial effluent has been shown to cause drastic reduction in the protein content progressively in the fish tissues (Roger 1980). The decreased trend in protein content as observed in the present study may be due to inhibition of its biosynthesis (Tripathi and Verma 2004) or enhanced degradation of protein and metabolic utilization of the ketoacid into gluconeogenesis pathway for the synthesis of glucose (Tilak et al. 2005) under the fertilizer industry effluent induced stress.

Table 5 Effect of fertilizer industry effluent on the activity of lactate dehydrogenase LDH, units/g wet weight) in different tissues of *C. striatus* exposed for 96 h

Tissue	Activity of LDH (units/g wet weight of fish tissues)[a,b,c]			
	Fertilizer industry effluent (%, v/v)			
	0	3.5	4.7	7.0
Muscle	53.25 ± 1.00	28.48 ± 1.17** (–46.51)	16.90 ± 1.10*** (68.26)	11.81 ± 0.38*** (–77.82)
Liver	33.82 ± 2.5	16.24 ± 1.80** (–51.98)	12.95 ± 1.58** (–61.70)	11.05 ± 0.84*** (–67.32)
Heart	40.91 ± 2.00	36.89 ± 1.46^{NS} (–9.82)	32.84 ± 0.83^{NS} (–19.72)	26.17 ± 0.80* (–36.03)
Brain	87.93 ± 2.36	62.89 ± 2.50* (–28.47)	39.55 ± 2.30** (–55.02)	33.56 ± 1.42*** (–61.83)
Kidney	27.00 ± 1.50	25.23 ± 1.99^{NS} (–6.56)	21.76 ± 1.44^{NS} (–19.40)	13.23 ± 2.38* (–59.33)
Gill	142.65 ± 1.91	85.79 ± 1.63* (–39.85)	76.71 ± 2.07* (–46.22)	55.91 ± 1.63** (–60.81)

Unit of enzyme activity is expressed as μmoles sodium pyruvate reduced/ml/ min

NS Not significant ($p > 0.05$)

[a] Each value represents the mean ± SEM of three independent experiments

[b] Values in parentheses are percent change over control

[c] Significance of data is shown in superscripts. The groups similar or significantly different from the control at ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) are analyzed using one way ANOVA, with Tukey's test

Table 6 Effect of fertilizer industry effluent on the activity of lactate dehydrogenase (LDH, Units/g wet weight) in different tissues of *C. striatus* exposed for 15 days

Tissue	Activity of LDH (Units/g wet weight of fish tissues)[a,b,c]			
	Fertilizer industry effluent (%, v/v)			
	0	3.5	4.7	7.0
Muscle	57.34 ± 1.01	26.02 ± 3.10*** (–54.62)	13.79 ± 0.93** (–75.95)	11.98 ± 1.72*** (–79.10)
Liver	35.20 ± 3.05	32.07 ± 1.06^{NS} (–8.89)	20.90 ± 0.73* (–40.62)	10.89 ± 0.85*** (–69.60)
Heart	37.43 ± 3.00	28.60 ± 2.76^{NS} (–23.59)	20.90 ± 2.63^{NS} (–44.08)	14.92 ± 3.12^{NS} (–60.13)
Brain	88.49 ± 0.53	46.50 ± 3.08** (–47.45)	22.90 ± 2.01*** (–74.12)	12.45 ± 2.17*** (–85.93)
Kidney	26.23 ± 2.26	11.78 ± 1.10** (–6.56)	8.71 ± 1.28*** (–19.40)	4.56 ± 0.41*** (–59.33)
Gill	127.60 ± 2.44	63.30 ± 2.64*** (–50.62)	43.44 ± 2.85** (–65.95)	36.13 ± 2.49*** (–71.68)

Unit of enzyme activity is expressed as μmoles sodium pyruvate reduced/ml/min

NS Not significant ($p > 0.05$)

[a] Each value represents the mean ± SEM of three independent experiments

[b] Values in parentheses are percent change over control

[c] Significance of data is shown in superscripts. The groups similar or significantly different from the control at ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) are analyzed using one way ANOVA, with Tukey's test

Keeping in view that carbohydrate metabolism is a major source of energy production in many fish species and the activity of LDH has been an easy target for the action of various xenobiotics, the impact of fertilizer industry effluent was studied on LDH activity in different body tissues of *C. striatus* by exposing the fish to three sublethal concentrations of effluent (3.5%, 4.7% and 7.0%) for 96 h and 15 days exposure durations. The data represented in Tables 5 and 6 indicated that the exposure of *C. striatus* to fertilizer industry effluent resulted in drastic reduction in the LDH activity. The enzyme activity was found to be highest in gills and lowest in kidney of the control fish. The fish tissues exhibited the following order of LDH activity: gills > brain > muscle > heart > liver > kidney; the values being 142.65 ± 1.91, 87.93 ± 2.36, 53.25 ± 1.00, 40.91 ± 2.00, 33.82 ± 2.50, 27.00 ± 1.50 μg^{-1} wet weight of tissues, respectively. However, the fish treatment with sublethal concentrations of industrial effluent caused reduction in enzyme activity, the effect being more pronounced when the fish were exposed with the higher concentration for longer period of exposure, i.e.15 days (Table 6) than at the lower concentration for shorter exposure period, i.e. 96 h (Table 5). The effluent at maximum concentration (7% v/v) for 96 h exposure period caused significant inhibition of LDH activity in muscle ($p < 0.001$), liver ($p < 0.001$), brain ($p < 0.001$), gills ($p < 0.01$) and kidney ($p < 0.05$) but only small change was recorded in heart ($p < 0.05$) relative to control. However, after 15 days treatment duration it exerted significant inhibitory effect on LDH activity in brain ($p < 0.001$), muscle ($p < 0.001$), gills ($p < 0.001$) and liver ($p < 0.001$) whereas it exhibited relatively small change in LDH activity in kidney ($p < 0.001$) as compared to other tissues of fish (Table 6). Interestingly, the increase in treatment duration from 96 h to 15 days could not further increase in the inhibition of LDH activity in kidney, whereas only marginal increase (about 2%) in the inhibition of enzyme activity was recorded in muscle and liver. However, the fish brain ($p < 0.001$) was maximally (about 86%) affected after prolonged treatment duration (15 days). Further, heavy metals in association with other chemicals present in the effluent may cause distortion in the cell organelles and inhibit the activity of various enzymes (Valarmathi and Azariah 2003), which may perturb the physiological state of the exposed animal. The heavy metals present in the fertilizer industry effluent in dissolved state are easily taken up by fish, which has been shown to cause damage at kidney and liver at low concentration. The early studies suggested that their higher concentration can be carcinogenic and teratogenic (O′Brien et al. 2003). Sastry et al. (1997) has reported the alteration in the LDH activity in the tissues of the fish, *C. punctatus*, exposed to Cu. Several other toxicants including pesticides (Singh and Sharma 1998) also have been shown to exert adverse effects on the activities of enzymes associated with carbohydrate metabolism (Helinmayer et al. 1970; Smart 1978) in different fish species.

The LDH activity in the tissues of *C. striatus* exposed to the fertilizer industry effluent reduced at all three concentrations of the effluent and at both treatment durations. The inhibition of enzyme activity in different organ of *C. striatus* was found to be largely dependent on the concentration of the fertilizer industry effluent and duration of exposure (present investigation). Some heavy metals such as Zn, Cr, Cu and Pb present in the fertilizer industry effluent can bind to certain proteins disrupting membrane

integrity, cellular metabolism and ion-transports (Lorz and Mcpherson 1971; Nussey 1998; Obodo 2002; Chen and Liao 2003), which may pose threat to the maintenance of homeostasis (Engel et al. 1981).

Some components present in the fertilizer industrial effluent interact together and produce toxicity to aquatic organisms for example the interaction between dissolve oxygen and elevated ammonia has been shown to alter the respiratory physiology of fresh water fish (Pickering and Pottinger 1987). These findings indicated that the inhibitory effect of industrial effluent on the activity of LDH may be mediated via formation of enzyme–inhibitor complex leading to impairment of carbohydrate metabolism (Panepucci et al. 1984, 1987; Copper and Somero 1990; Sharma and Gopal 1995).

In conclusion, the fertilizer industry effluent had deleterious impact in *C. striatus* particularly at high sublethal concentration by inducing significant alterations in the levels of protein and activity of LDH in different fish tissues. The toxicity of the effluent in fish was reflected in concentrations and duration dependent manner. Though, the fertilizer industry effluent at high concentration markedly affected all the fish organs tested but the magnitude of response varied from one organ to other after increasing the treatment period from 96 h to 15 days. The results from the present investigation may be useful for assessing early warning signals of industrial effluent poisoning and also as a bio-indicator for water quality control.

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